REVIEWER: LIBOR KRÁSNÝ, PHD

Institute of Microbiology
Academy of Sciences of the Czech Republic
Vídeňská 1083
142 20 Prague
Czech Republic
e-mail krasny@biomed.cas.cz
Tel. +420 241 063 208

Reviewer's report of the Thesis **Molecular principles of translation reinitiation in mammals** by Mgr. Vladislava Hronová.

The Thesis focuses on eukaryotic translation in mammals and yeast, bringing new information about reinitiation of this process after translation of short upstream reading frames (uOFS) that precede the main, regulated ORF.

The Thesis consists of Review of the Literature, Aims, Results in the form of original publications and reviews, and Discussion. The Review of the Literature section provides sufficient information about eukaryotic translation with a special focus on what is known about uORFs in this process.

The Results consist of four research papers, of which VH is the first author/coauthor in two cases, and three review articles. The articles are in well-respected peer-reviewed journal with high impact factors.

In this review I will focus mainly on the two studies where VH is the first author/coauthor.

The first of these two studies, **Structural integrity of the PCI domain of eIF3a/TIF32 is required for mRNA recruitment to the 43S pre-initiation complex**, analyzed in detail a subunit of eukaryotic initiation factor 3 (eIF3), eIF3a/Tif32. The study was performed with the yeast model system. VH showed that specific amino acid residues from the second PCI domain of this subunit interact with mRNA and assist in mRNA loading onto the 43S preinitiation complex. This study advanced our understanding of the molecular details of the translation machinery during translation initiation.

Question 1: *RNA binding properties of a/TIF32 were studied using DAD4 mRNA, one of the smallest naturally occurring RNAs in yeast. Have you (or your colleagues) tested other RNAs and, perhaps, determined their affinity for a/TIF32? Would it be informative if RNA was pulled out of cell lysates with a/TIF32 and sequenced to determine the best binders, and identify, genome-wide, the RNA determinants (sequence, secondary structure) of this interaction?*

The second of these two studies is **Does eIF3 promote reinitiation after translation of short upstream ORFs in mammalian cells?** This study focuses on human cells, and shows that the mechanism of translation reinitiation is conserved between yeast and humans. Specifically, VH demonstrated that the sequences flanking uORF1 of human ATF4 and their interaction with eIF3 play the same role as the corresponding sequences from the homologous uORF1 of yeast GCN4, the best studied gene with respect to reinitiation.

Question 2: *Figure 1 shows predicted secondary RNA structures. Is there experimental evidence (at least for the human variant) that these RNAs are folded as predicted?*

Question 3: *Do mouse, cow, pig etc. RNA structures function with the human eIF3?*

Question 4: *45% of mRNA have uORFs in mammals (concluding remarks in this paper). Do these RNA sequences flanking these uORFs, share some commons characteristics, such as similar predicted secondary structures, at least between genes with similar level of expression?*

Question 5: *This paper and also the review* ***Please do not recycle!*** *mention that ~45% of mammalian mRNAs possess uORFs. However, in the Abstract of the very same review you state that "…the* ***majority*** *of eukaryotic mRNAS contain only one coding sequence…" and that " there are* ***several exceptional*** *mRNAs that carry short open reading frames upstream of the main coding sequence…". This is a contradiction as 45% does not appear to be "several". What is the rationale?*

In summary, the Thesis brings new insights into our understanding of translational initiation and reinitiation in yeast and human cell lines. The quality of the Thesis is high, the text flows well and it was a pleasure to read it. I recommend this Thesis to be classified as passed.

Finally, I wish Vlaďka all the best in her further professional career.

Prague, 31 January 2018 Libor Krásný