How to decipher the structural secrets of RNA behind its translational efficiency?

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34th TBI Winterseminar - Bled
February 5th 2019
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In the Faller lab, we study the role that RNA translation plays in cancer models.
Eukaryotic RNA translation

1. Initiation
2. Elongation
3. Termination
4. Recycling

Adapted from Sokabe et al. Cold Spring Harb Perspect Biol 2018
Eukaryotic RNA translation

1. Initiation
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LETTER

mTORC1-mediated translational elongation limits intestinal tumour initiation and growth

doi:10.1038/nature13806

*Faller et al. Nature 2015
Our research questions:

A) Which RNAs are regulated at the level of translation elongation after APC loss?
B) What are the determinants of elongation changes?
Measuring translation: RiboSeq vs RNAseq

Ribo-seq:
- Nuclease digestion
- Monosome isolation
- RNA purification
- Ribosome footprints
- Library construction
- Deep sequencing
- Actively translated regions show strong 3-nt periodicity

RNA-seq:
- RNA purification
- Fragmentation
- Library construction
- Deep sequencing
- RNA-seq does not show 3-nt periodicity

Novel peptide identification

Translation efficiency = \frac{\text{Ribo-seq levels}}{\text{RNA-seq levels}}

Adapted from Hsu et al. 2016
RiboSeq read profiles

Fast elongation

Low ribosome density

High ribosome density

Slow elongation
RiboSeq vs ElongationSeq

Fast elongation

Low ribosome density

Slow elongation

High ribosome density

Harringtonine

$\Delta \tau$

Cycloheximide

0 sec

30 sec

60 sec

90 sec
Using both techniques, we can

(1) determine the transcripts that are regulated at the level of translational elongation

(2) predict ribosome stalling sites in different contrasts

(3) investigate elongation profiles across each transcript
Potential determinants of elongation speed and ribosome stalling

➔ tRNA availability
➔ RBPs and miRNA binding
Potential determinants of elongation speed and ribosome stalling

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➔ RNA secondary structure
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   ➢ structure enrichment test (?) for stalled regions
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2) Sequence logos can potentially reveal some RBP binding sites but
   ➢ Can we improve sequence logos with RNA structure features?
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3) Can we predict if ribosomal walking (RNA unwinding) can cause mRNA refolding?
   ➢ co-translational re-folding ??
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   - co-translational re-folding ??

4) How can we better estimate translational efficiency of transcripts using RNAseq with Ribo-seq and Elongation-seq data?
Would you like to help us to decipher the structural secrets of RNA behind its translational efficiency?

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Thank you