

RNA - protein interactions in mRNA decay

A case study on TTP and HuR in AMD

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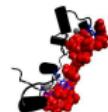
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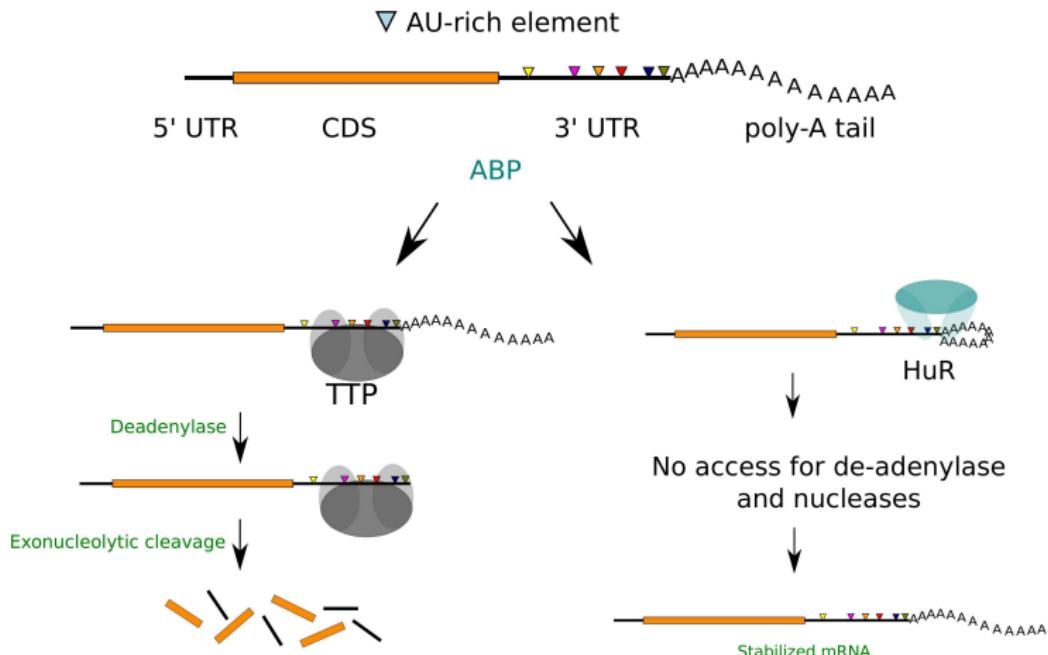


Research Platform

Decoding mRNA decay in inflammation

Interdisciplinary project funded by the University of Vienna

ARE Mediated Decay (AMD)



Open Questions

- How do ABPs choose their target(s)?
- Do RNA elements dictate function in AMD?
- What makes the difference (sequence and/or structure)?
- Different binding site == different decay rate?
- **Do ABPs compete for RNA motifs?**

Exact mechanisms of AMD and ARE-ABP interaction remain unclear

Biological system

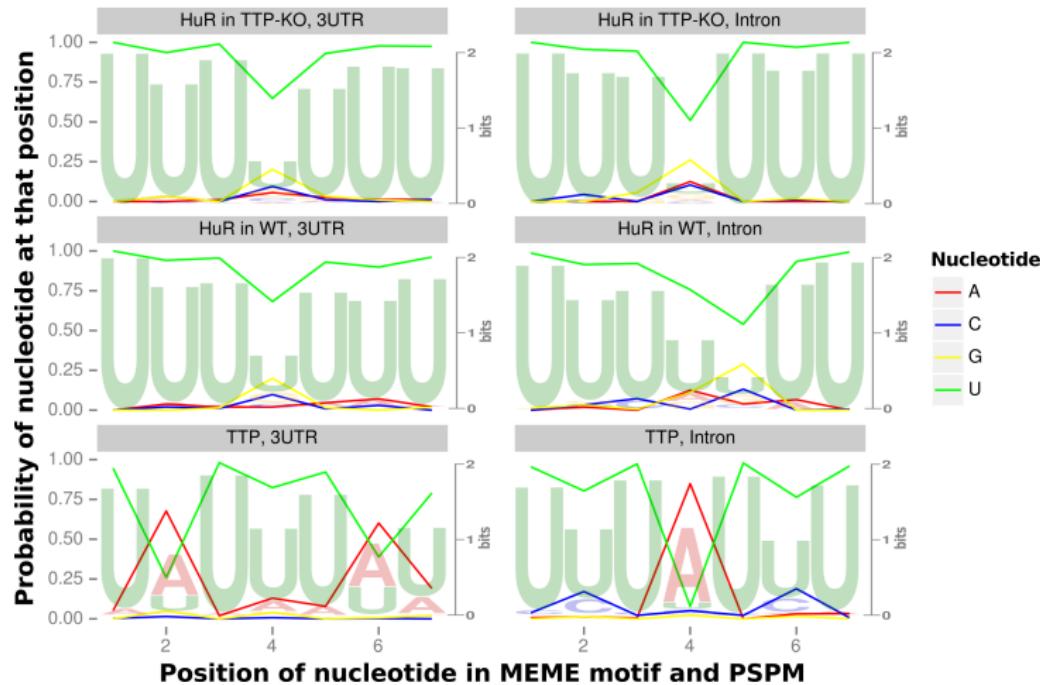
- Primary mouse macrophages
 - LPS induces inflammatory response
 - No overexpression needed
- $TTP^{+/+}$ (WT) and $TTP^{-/-}$ (KO) mouse
- PAR-iCLIP based dataset
- RNA-Seq without crosslink

(Vitaly Sedlyarov, Kovarik Lab)

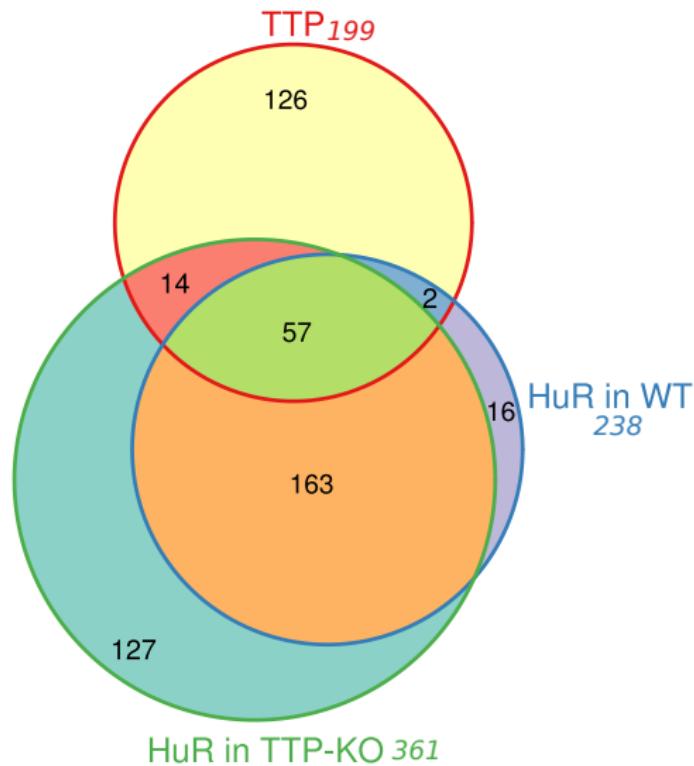
Binding preferences

- TTP
 - TTP binds predominantly to 3'UTR exons
 - Surprisingly high read counts in CDS introns
 - Function of intronic binding unclear (sponge?)
- HuR
 - HuR binds predominantly to 3'UTR exons independently of TTPs presence

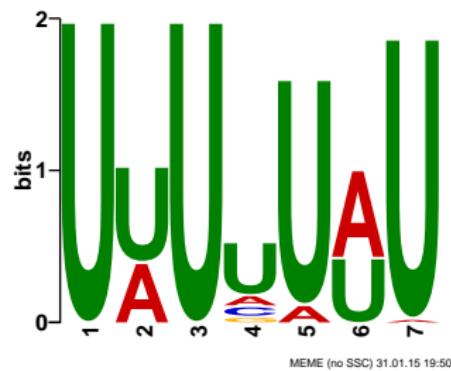
MEME



TTP/HuR 3'UTR target genes



Overlap sequence motif



Modeling binding cooperativity by constraint folding

- Calculation of cooperativity free energy via constraint folding
- A pair of binding sites is used as constraint

$$\Delta G_{0 \rightarrow 1} = \Delta G^1 - \Delta G^0 - RT \times \ln \left(\frac{c_1}{K_{D,1}} \right)$$

$$\Delta G_{2 \rightarrow 12} = \Delta G^{12} - \Delta G^2 - RT \times \ln \left(\frac{c_1}{K_{D,1}} \right)$$

$$\Delta\Delta G = \Delta G_{0 \rightarrow 1} - \Delta G_{2 \rightarrow 12} = G^1 + G^2 - G^{12} - G^0$$

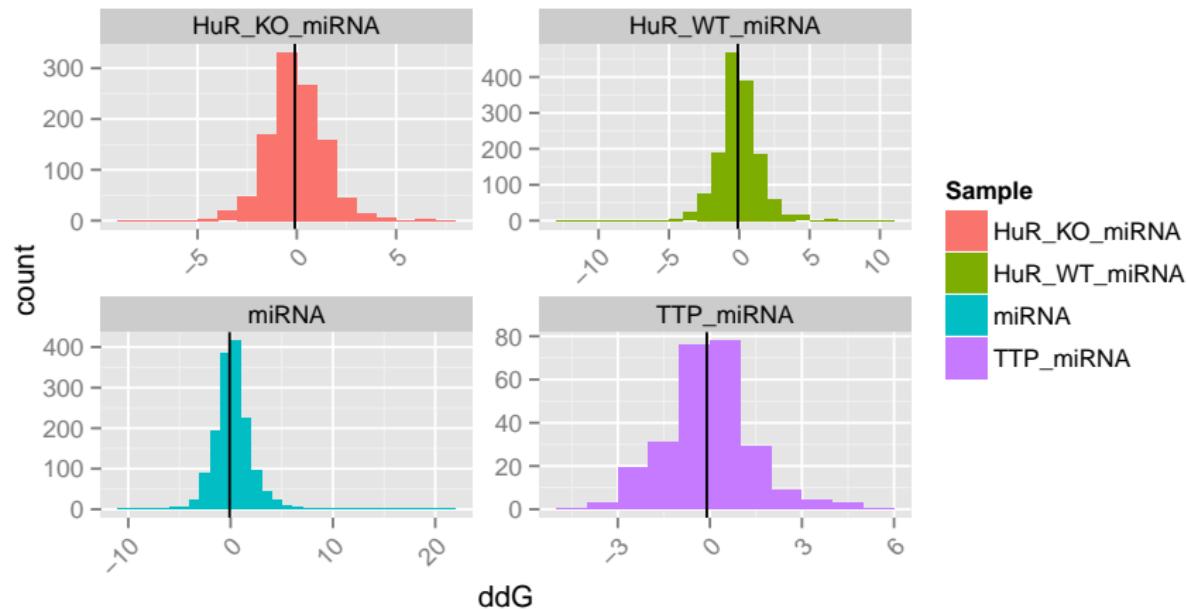
Lin et al. 2015, RNA structure generates natural cooperativity between single-stranded RNA binding proteins targeting 5' and 3' UTRs

Procedure

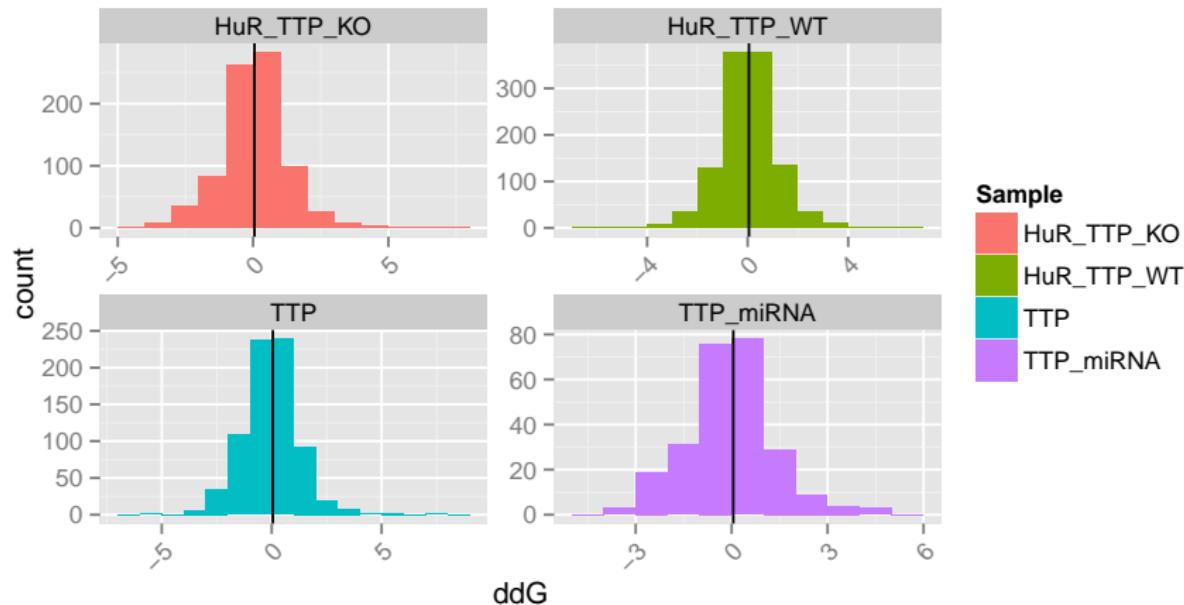
- Non-overlapping pairs of binding sites with minimal distance of 10nt
- Folding of 3'UTR with/without these bs as constraints
- Additionally Ago-CLIP derived miRNA bs as constraints

Lu et al. 2014, ELAVL1 Modulates Transcriptome-wide miRNA Binding in Murine Macrophages

Un/Cooperative binding



Un/Cooperative binding



Summary

- In general we see only a small effect
- ABPs bind ssRNA, so this is not completely surprising
- Top targets among cooperative list
- TTP itself is one of the RNAs where miRNA-27 binding has negative effect

Acknowledgments

TBI

Ivo
Andrea
Xtof
Colleagues

MFPL

Pavel Kovarik
Vitaly Sedlyarov

Leipzig

Peter Stadler
Steve Hoffmann
Christian Otto
Stefan Bernhart

Freiburg

Rolf Backofen
Daniel Maticzka
Steffen Heyne
Fabrizio Costa



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