

Towards a Functional Classification of ARE Protein Interactions

Jörg Hackermüller¹

joerg.hackermueller@pharma.novartis.com

Nicole-Claudia Meisner^{2,3}

nicole-claudia.meisner@pharma.novartis.com

Andras Aszodi¹

andras.aszodi@pharma.novartis.com

Manfred Auer^{2,3}

manfred.auer@pharma.novartis.com

Markus Jaritz¹

markus.jaritz@pharma.novartis.com

¹ Novartis Forschungsinstitut GmbH Vienna, Brunnerstr. 59, 1235 Vienna, Austria

² Novartis Drug Discovery Center/Innovative Screening Technologies Unit, Brunnerstr. 59, 1235 Vienna, Austria

³ Universität Salzburg, Institute of Genetics and General Biology, Hellbrunnerstr. 34, 5020 Salzburg, Austria

Keywords: mRNA stability, RNA secondary structure prediction, RNA-protein interaction

1 Introduction

The regulated stability of many short lived mRNAs depends on adenosine-uridine rich elements (AREs) as cis-elements in the mRNA, and on ARE-binding proteins as trans-acting factors. Many ARE containing mRNAs are disease relevant. A well-understood mechanism of how regulatory proteins specifically recognise AREs would provide a means to selectively boost or inhibit the expression of genes.

Selecting a certain ARE-protein interaction as a promising drug target requires knowledge about the interference of other AREs with the selected protein or of other proteins with the selected ARE respectively. Therefore we aim to provide a classification of AREs according to the proteins they interact with and the effect of the interaction on the fate of the mRNA in the cell. Complementary to this approach we group proteins that are most likely to bind to the same ARE by investigating the relatedness of ARE and RNA binding proteins. Recent efforts to cluster ARE mRNA sequences [1] do not satisfy our needs, as the sequences clustered together have diverse biological functions, are most probably bound by different trans-acting factors, and are regulated independently of each other.

We use the interaction between HuR (Hu antigen R, ELAVL1) and mRNA subsequences of early response genes as a model system to develop methods for the detection of signals which qualify an mRNA as most probably HuR bound. HuR is a well studied ARE-binding protein [2], the 3D Structure of a close homologue (HuD) is known [5]. This allows us to search for HuR related proteins on sequence and structure level.

2 Methods and Results

Common Signals in AREs Bound by HuR. The affinity between HuR and ARE was obtained using confocal single molecule spectroscopy. Fluorescence labeled ARE mRNA was titrated with HuR and the change in fluorescence anisotropy measured using 2D-FIDA (Fluorescence Intensity Distribution Analysis). HuR shows considerable differences in affinities for individual AREs. The

occurrence of the pattern NTTNNTTT, proposed in [5], partitions the set of AREs into two groups. The groups are clearly separated according to the mean affinities for HuR (p-value < 0.07).

For each ARE sequence the thermodynamic probability of RNA secondary structures within which the pattern NUUNNUUU occurs at least once in single-stranded conformation can be calculated with *RNAfold* [3]. The binding affinity to HuR correlates with this probability for the majority of AREs containing this pattern. Consequently binding of an ARE by HuR requires the pattern NUUNNUUU and is enhanced if the pattern is presented in a single stranded conformation.

ARE Binding HuR Analogues. Sequence similarity searches were performed with *PsiBlast* to identify distant homologues of HuR. Additionally a profile hidden markov model (HMM) was computed from a multiple alignment of all proteins with the same CATH classification as HuD. As *ClustalW* performed poorly on aligning these sequences, they were structure-structure aligned to HuD using *ProSup*[4]. The pairwise structure alignments were combined to a pseudo multiple sequence alignment. To monitor the detection efficiency of the searches all sequences of HuD's CATH class were included in the searched databases. Applied to a recent *Celera* protein database the HMM detected 103 protein sequences which were not detected using the *Pfam* HMM. According to the fraction of control sequences detected, the structure alignment based HMM outperformed *PsiBlast* and HMMs based on sequence alignments.

3 Discussion

To date the binding motif of HuR is unclear and allows no classification of AREs as HuR binding. We have shown that the ARE consensus motif for HuD binding proposed in [5] is also required for HuR binding. Sequences lacking this pattern are not bound efficiently by HuR. As Hu family proteins are known to bind single stranded RNA, we anticipated that the binding affinities would depend on the availability of the motif in a single stranded conformation. A correlation between the binding affinity and the probability of single stranded motifs in the secondary structure ensemble reveals that the base pairing potential of the ARE sequence is important for this interaction. However RNA secondary structure alone cannot explain the differences in affinities. Recapitulating, proper binding of HuR to an ARE requires the motif NUUNNUUU and a low base pairing potential. Our knowledge of this interaction is not yet sufficient to truly classify AREs as HuR bound, but permits the opposite: many candidate ARE sequences do not fulfill these requirements.

References

- [1] Bakheet, T., Frevel, M., Williams, B.R.G., Greer, W., and Khabar, K.S.A., ARED: Human AU-rich element containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins, *Nucleic Acids Research*, 29:246–254, 2001.
- [2] Brennan, C.M. and Steitz, J.A., HuR and mRNA stability, *Cellular and Molecular Life Sciences*, 58:266–277, 2001.
- [3] Hofacker, I.L., Fontana, W., Stadler, P.F., Bonhoeffer, L.S., Tacker, M., and Schuster, P., Fast folding and comparison of RNA secondary structures, *Monatsh.Chem.*, 125:167–188, 1994.
- [4] Lackner, P., Koppensteiner, W.A., Sippl, M.J., and Domingues, F.S., ProSup: A refined tool for protein structure alignment, *Protein Engineering*, 13(11):745–752, 2000.
- [5] Wang, X. and Tanaka Hall, T.M., Structural basis for recognition of AU-rich element RNA by the HuD protein, *Nature Structural Biology*, 8(2):141–145, 2001.