A compendium of RNA-binding motifs for decoding gene regulation

Ray D., et al. Nature, 2013, vol. 499, pp. 172-177

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Introduction

RNA-binding proteins (RBPs)

- Are proteins binding to double or single stranded RNA in cells.
- Regulate numerous aspect of co- and post-transcriptional gene expression
    - RNA splicing, capping, polyadenylation, mRNA export, etc.

Figure 1

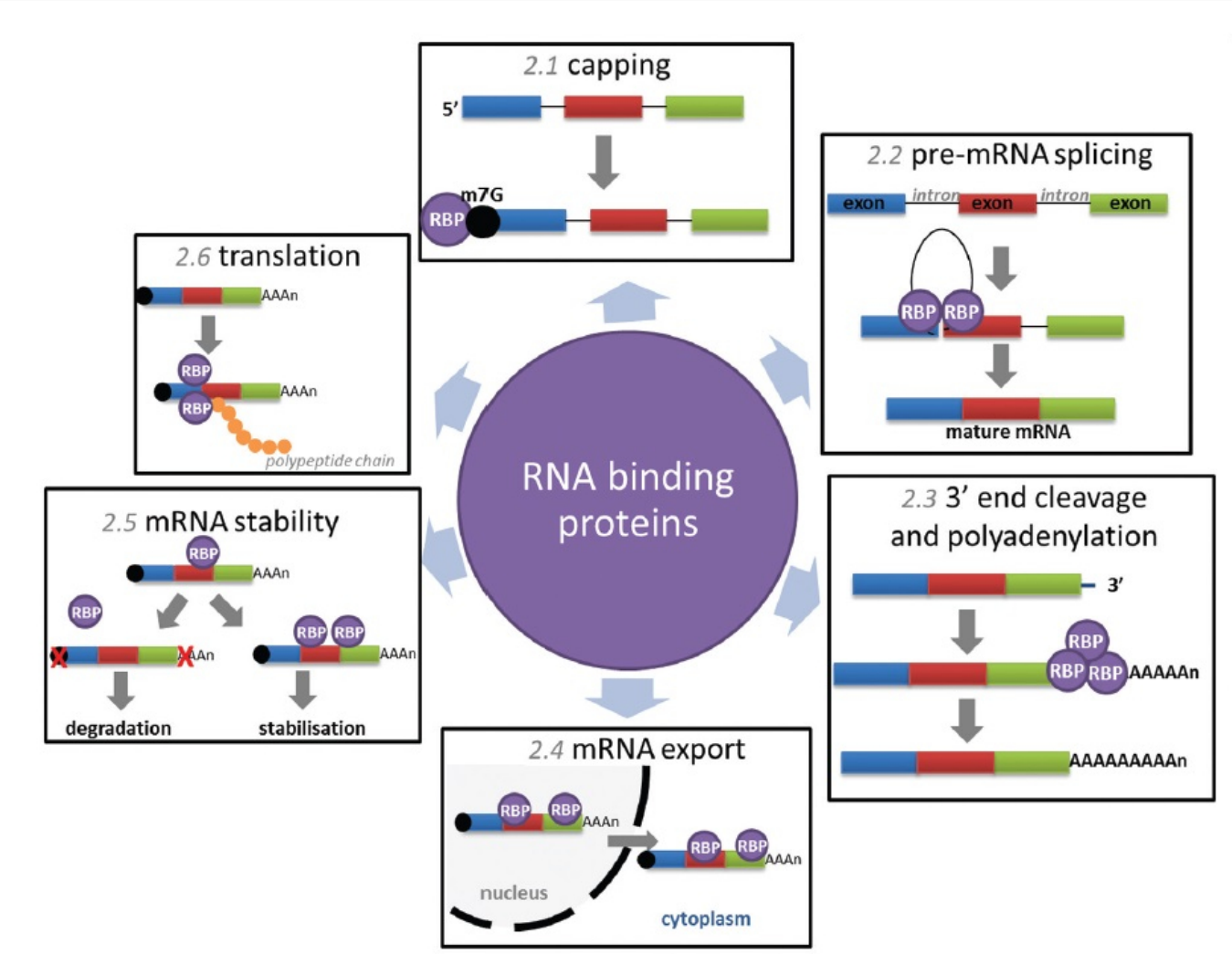


Figure 1: Sutherland JM., et al. Asian J Androl 2015

RNA-binding domains (RBDs)

Functions

- Recognize RNA: bind short, single-stranded RNA sequences, or structured RNAs.

Types

- RNA recognition motif, hnRNP K-homology (KH), zinc finger domains, etc.

Post-transcriptional regulation

- Contributes substantially to gene expression across human tissues.

However,

- Lack of motifs for the vast majority of RBPs across all branches of eukaryotes.
  - Due to higher flexibility of the RNA-protein interface for major types of BRPs;
  - Example: only 15% of human RBD-containing proteins have known RNA-binding motifs.

This paper: identifies binding motifs for a broad range of RBPs

Methods

RNAcompete experiments

- An *in vitro* method for the analysis of RNA binding preferences of hundreds of RBD-containing RBPs, from diverse eukaryotes.
- Rely on binding reaction between RBD and RNA-binding motifs.
    - An RBP is incubated in a complex pool of RNAs by affinity selection.
    - The pool contains ~240,000 short RNAs, divided into two halves for internal cross-validation purpose.
- The associated RNAs are interrogated by microarray and computational analyses.

Figure 2

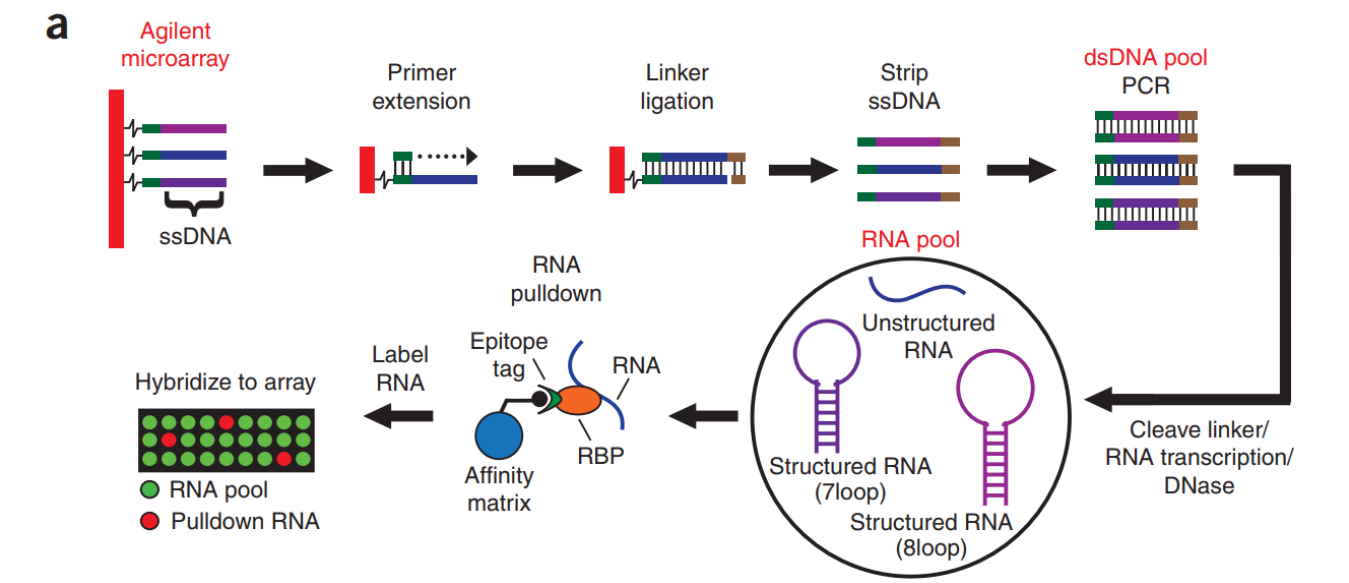


Figure 2: Ray D., et al. Nature 2009, Fig 1

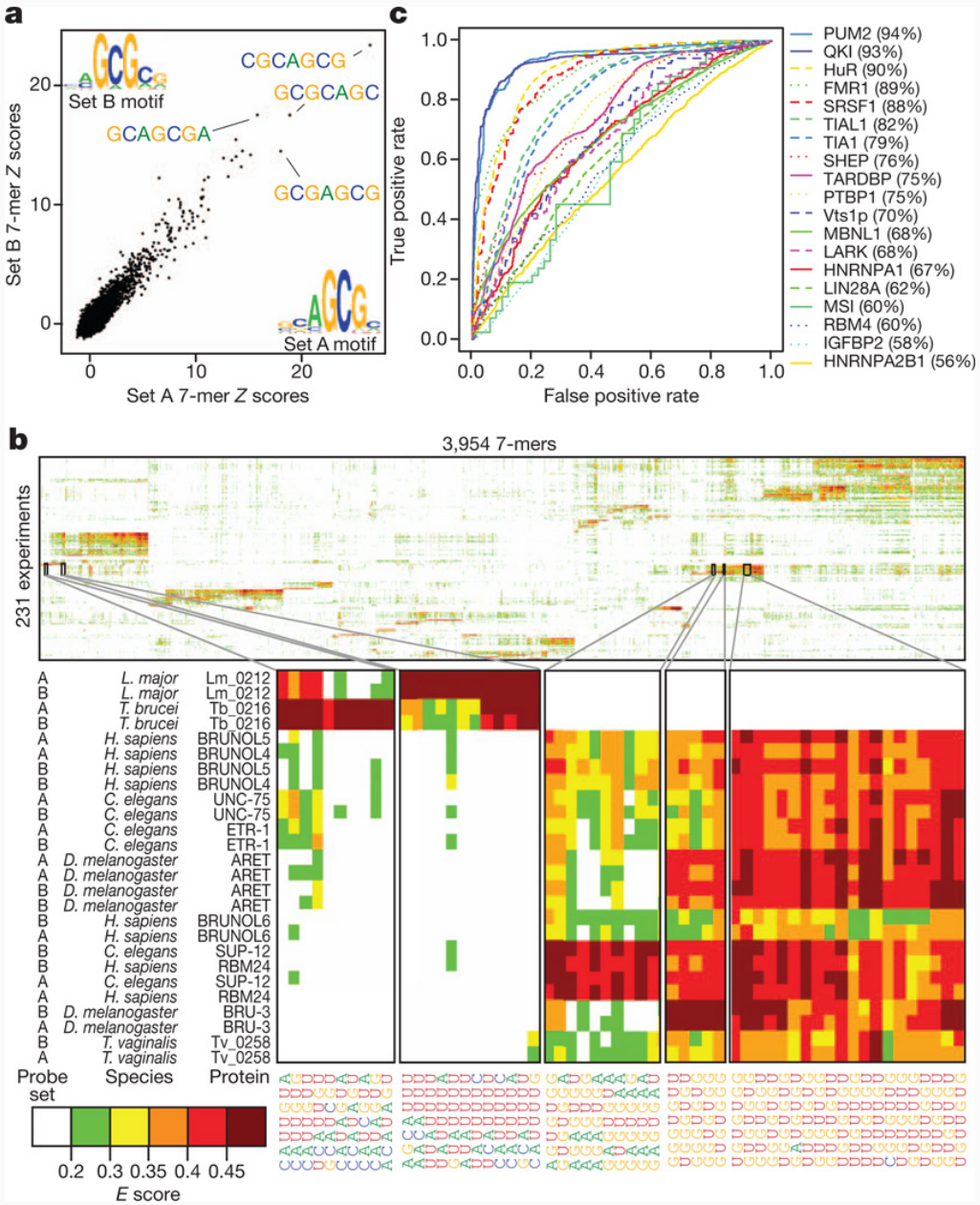
Results

Large-scale analysis of RBPs

Determined sequence preferences for 207 RBPs (from 193 unique RBP-encoding genes), 85 from human.

- Most RBDs fundamentally recognize and bind ssRNA, requires rarely on RNA second structure.
  - Fig. 1a: Z score and motifs for ZC3H10 (no previously known motif)
- Highlight specificity and diversity of RBP sequence preferences.
  - Fig. 1b: E score (enrichment score, Berger MF, et al., Nature Biotech 2006)
- The RNAcompete motif substantially outperforms the literature motif by AUROC analysis.
  - Fig. 1c: AUROC

Figure 3


a
Set B motif
CGCAGCG
GCGCAGC
GCAGCGA
GCGAGCG
Set A motif
Set B 7-mer Z scores
Set A 7-mer Z scores
c
True positive rate
False positive rate
PUM2 (94%)
QKI (93%)
HuR (90%)
FMR1 (89%)
SRSF1 (88%)
TIAL1 (82%)
TIA1 (79%)
SHEP (76%)
TARDBP (75%)
PTBP1 (75%)
Vts1p (70%)
MBNL1 (68%)
LARK (68%)
HNRNPA1 (67%)
LIN28A (62%)
MSI (60%)
RBM4 (60%)
IGFBP2 (58%)
HNRNPA2B1 (56%)
b
3,954 7-mers
231 experiments
Probe set
Species
Protein
E score

Conservation of ancient motifs

- Groups of ancient RBP families retain closely related sequence preferences.
  - A2BP1/RBFOX1, BRUNO/ARET
  - all RBPs in the SUP12–RBM24–RBM38 cluster prefer similar (G+U)-rich sequences.
- Subtle differences between more distantly related proteins are found.
  - family members from fungi, protists and algae maintained the presumed ancestral CAC core-recognition specificity17, but differ in their preferenceforflanking nucleotides

Figure 4

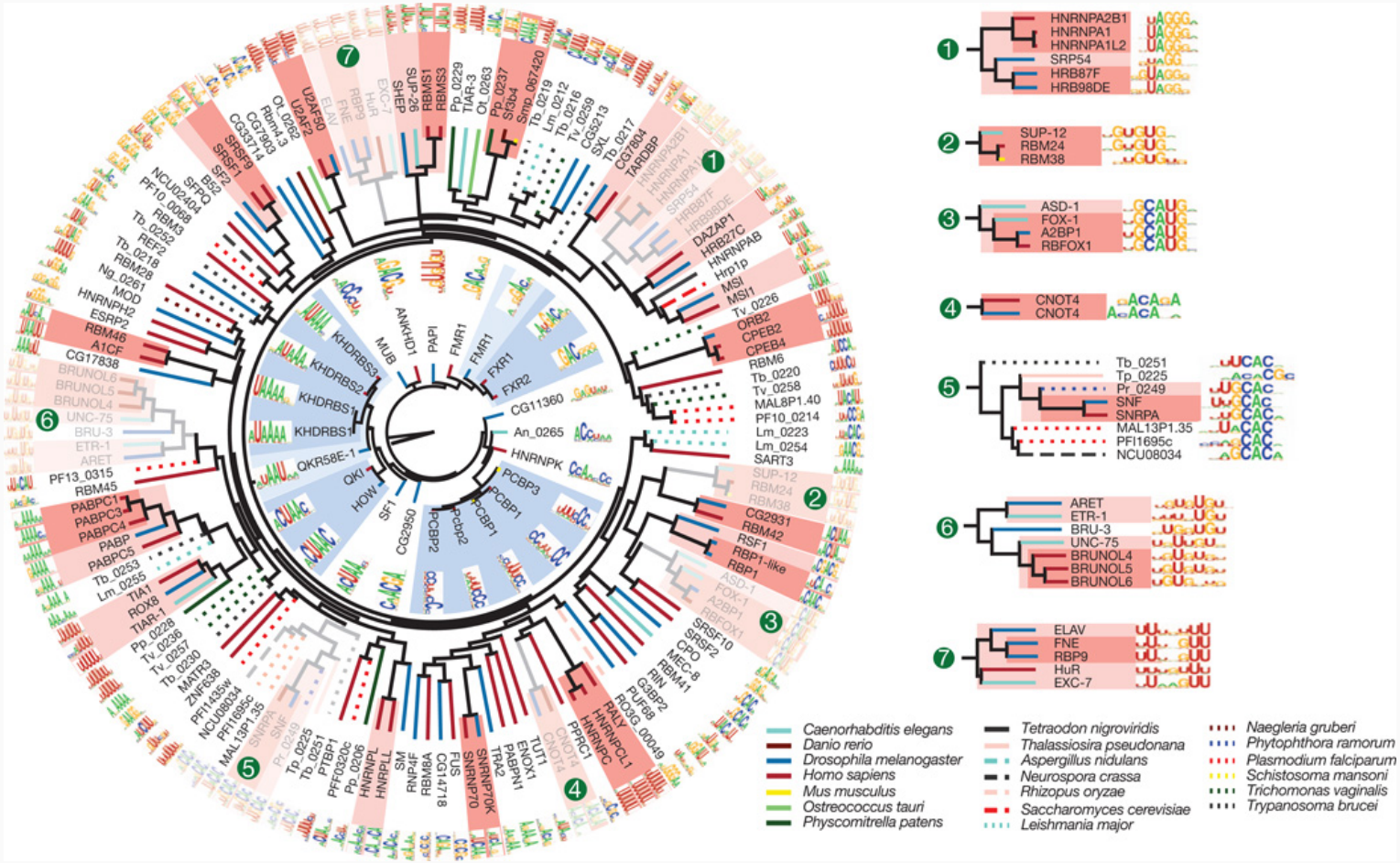


Figure 4: Ray D., et al. Nature 2013, Fig 2

Figure 5

- Amino acid sequence identity higher than ~70% yields very similar motifs
- RNAcompete data captured 57% of all human RBPs contained multiple RBDs, assuming 70% sequence identity
- Validation of motifs predicted for proteins at 61–96% amino acid identity

Figure 5

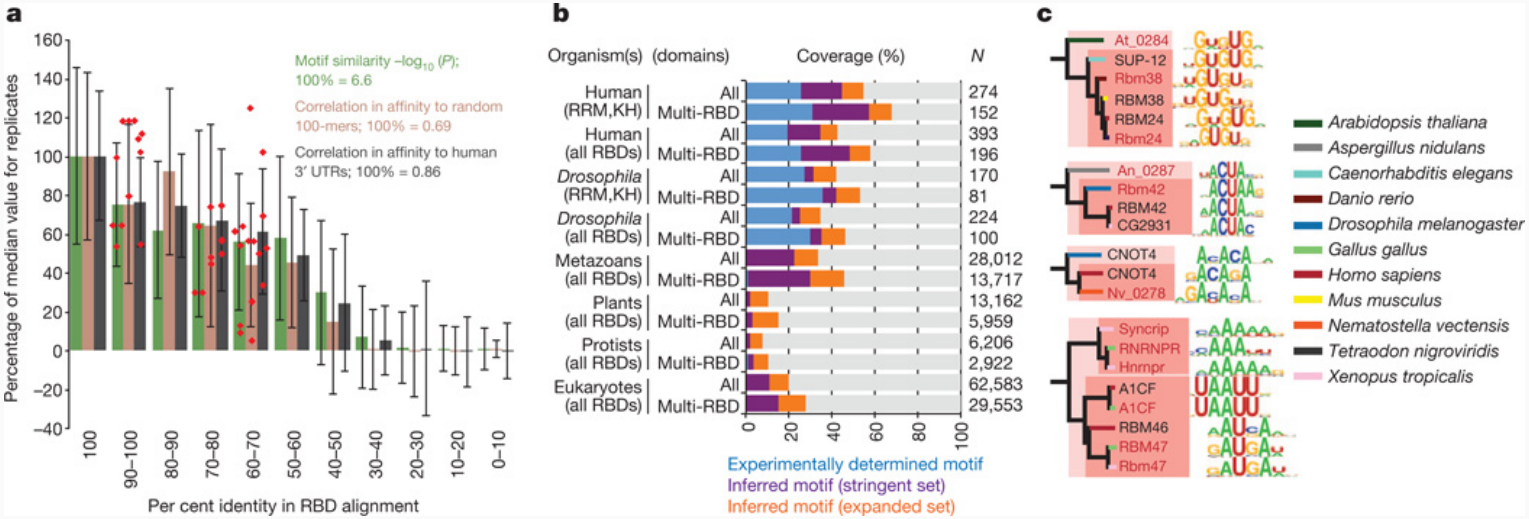


Figure 5: Ray D., et al. Nature 2013, Fig 3

Sequence conservation of motif matches

- Motifs for most RBP families display significant conservation in one or more of the three regions examined.

Figure 6

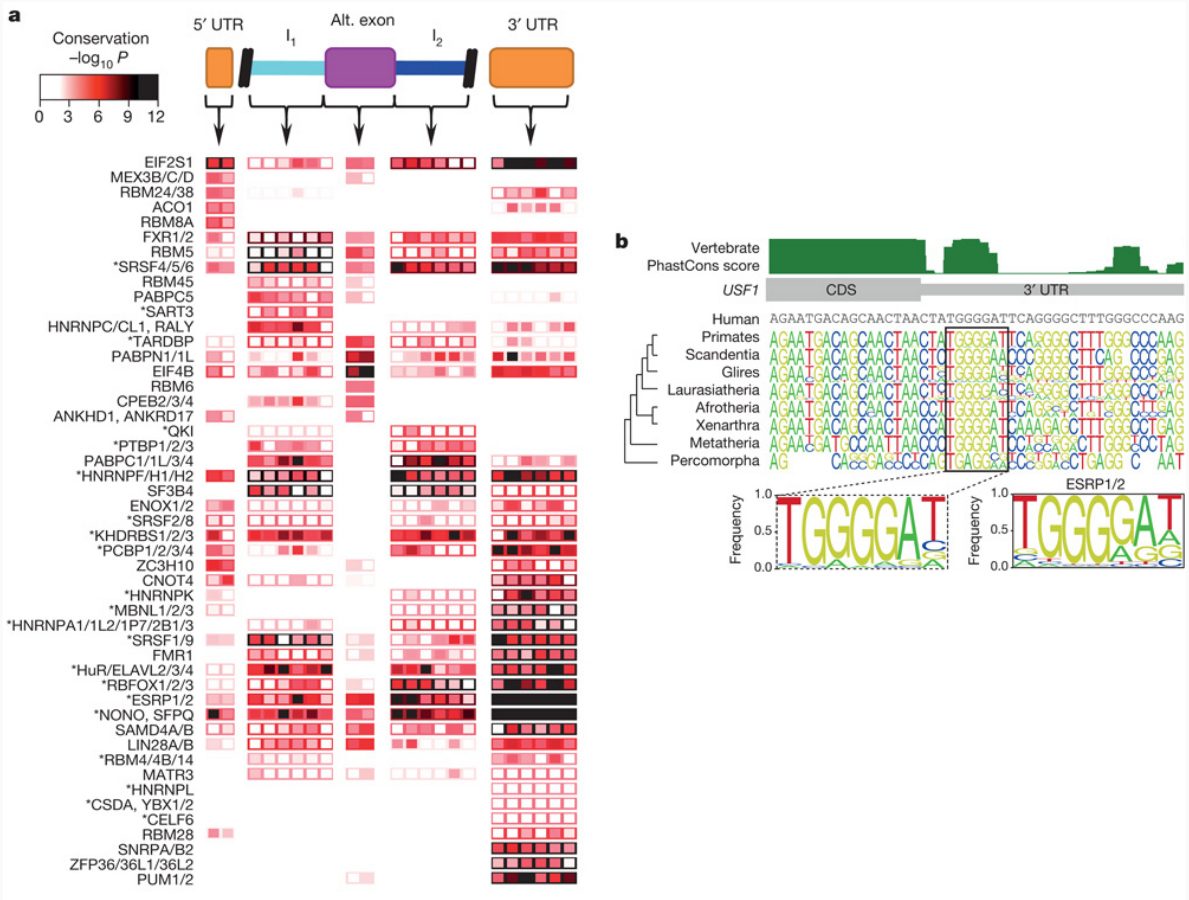


Figure 6: Ray D., et al. Nature 2013, Fig 4

Insights into RBP multi-functionality

- Role of RBPs in mRNA stability: positive/negative regulator
    - For example: RBFOX1 positively regulates mRNA stability/stabilizes its predicted mRNA targets
- Reduction of the stability of RBFOX1 targets may affect nervous-system-specific processes
    - Levels of RBFOX1 in the brains of individuals with autism is associated with changes in alternative splicing of exons

Figure 7

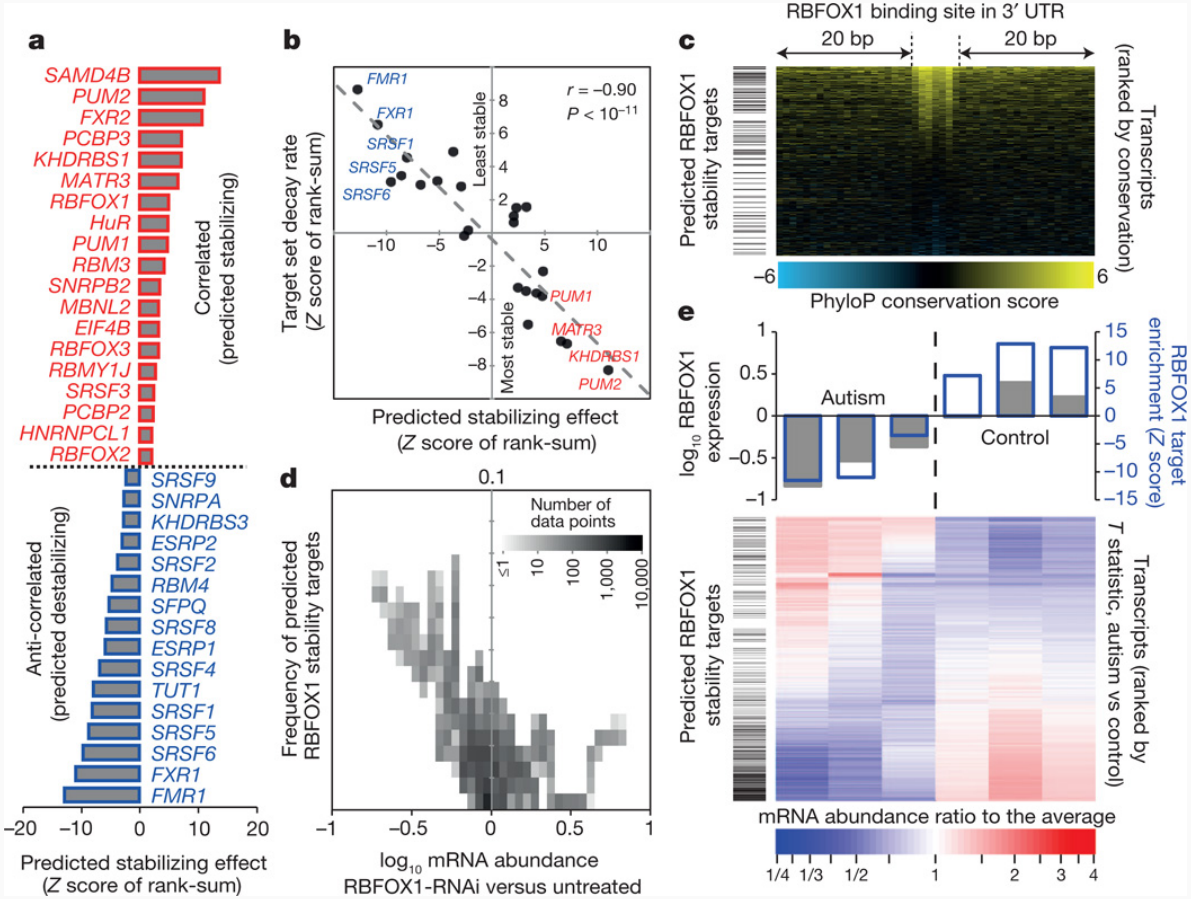


Figure 7: Ray D., et al. Nature 2013, Fig 5

Discussion

Significance

- The resulting motifs represent an unprecedented resource for the analysis of post-transcriptional regulation across eukaryotes;
- provide insight into the function and evolution of both RBPs and their binding sites;
- reveal broad linkages among different post-transcriptional regulation processes;
- uncover an unexpected role for a splicing factor in the control of transcript abundance that is mis-regulated in autism.