

# Mapping RNA-protein interactions

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# Methods used to study RNA-protein interactions

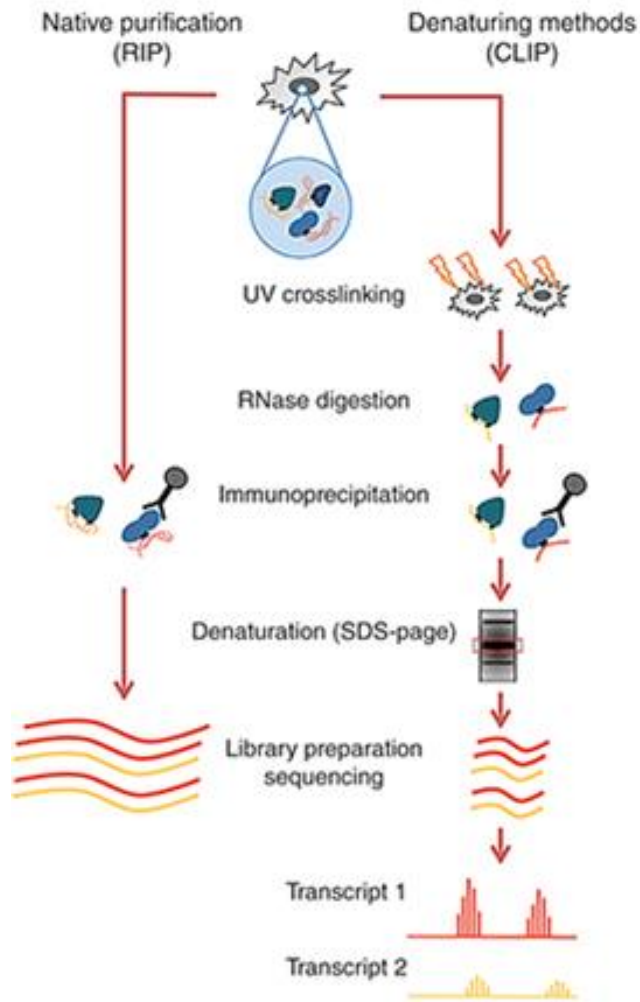
- **Low throughput methods**
  - Electrophoretic mobility shift assay (EMSA)
  - Fluorescent anisotropy/polarization
  - Förster resonance energy transfer (FRET)
  - Surface plasmon resonance (SPR)
  - Microscale thermophoresis (MST)
  - Stochastic optical reconstruction microscopy (STORM)

Permit analysis of only a few RNA-protein interactions at a time.

# Methods used to study RNA-protein interactions

- **High throughput methods->genome-wide identification of RBPs**
  - Based on next-generation sequencing, protein mass spectrometry and RBPs immunoprecipitation
  - 2 categories
    - Protein-centric approaches
    - RNA-centric approaches

# Protein-centric approaches – *in vivo*



Marchese et al, 2016

## RNA immunoprecipitation (RIP) (Tenenbaum et al, 2000)

- Protein-specific antibody
- Detection of RNAs
  - RIP-chip
  - RIP-seq
- Problems:
  - Non-specific interactions



<http://ulelab.info/>

## Cross-linking and immunoprecipitation (CLIP)

- Variants (depending on cross-linking and library preparation protocols)
  - HiTS-CLIP (high throughput sequencing of cDNA library) (Licatosi et al, 2008)
  - PAR-CLIP (photoactivable ribonuclease enhanced) (Hafner et al, 2010)
  - iCLIP (individual-nucleotide resolution) (Konig et al, 2011)
  - eCLIP (enhanced) (Van Nostrand et al, 2016)

Single nucleotide resolution

## Problems

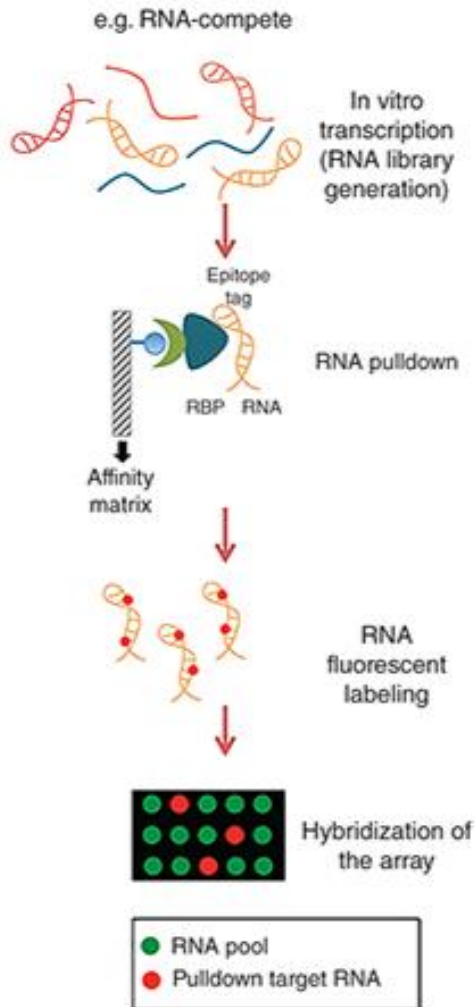
- False negatives
- Laborious
- UV-induced cross-linking:
  - Not all RBPs are amenable (Darnell, 2010)
  - Captures only direct RNA-protein interactions

# Protein-centric approaches – *in vivo*

## Biological insights

- **CLIP** (*Ule et al, 2003*)
  - Role of NOVA 1 and 2 in paraneoplastic opsoclonus-myoclonus-ataxia (POMA)
    - NOVA proteins regulate alternative splicing of RNAs encoding proteins of inhibitory synapses (GABA $\beta$ 2, GIRK2)
- **RIP-chip** (*Ince-Dunn et al, 2012; Lu et al, 2014*)
  - Targets of ELAVI family
    - Role in cancer pathogenesis and neurological disease

# Protein-centric approaches – *in vitro*



Marchese et al, 2016

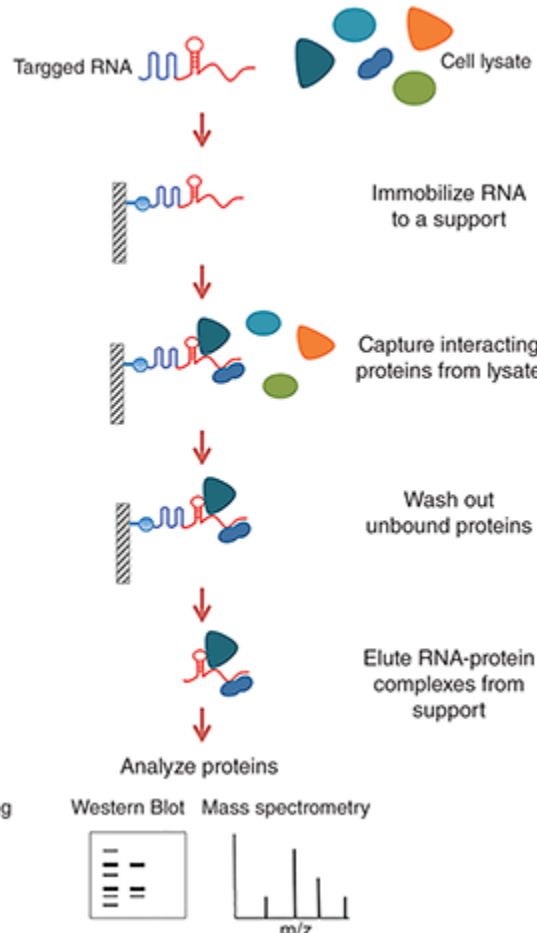
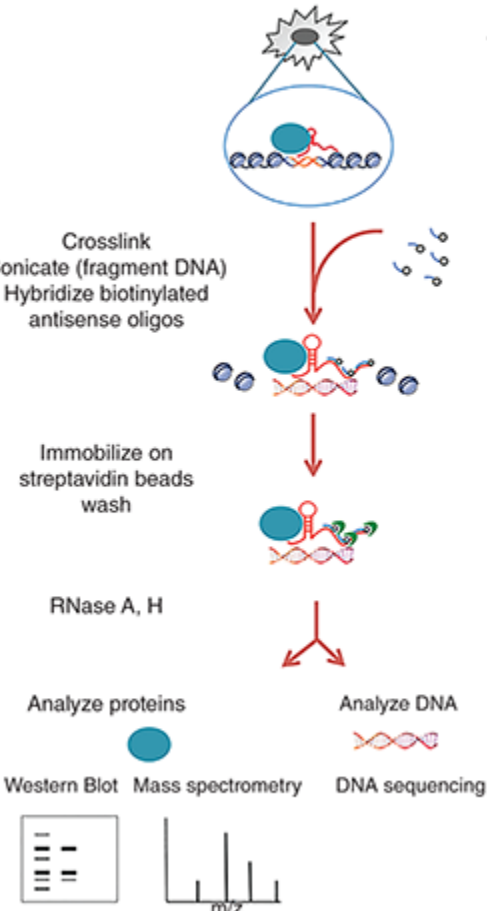
- Techniques:
    - Microarrays
    - Microfluidics
    - Fluorescent labeling
    - RNA seq
  - Variants
    - RNA-competete
      - Target protein immobilized on affinity matrix
      - Fluorescent labeling of RNAs
      - Hybridization to a microarray
    - SEQRS (selection high throughput sequencing of RNA and sequence specificity landscape)
    - RBNS (RNA Bind-n-Seq)
    - RNA-Map (Quantitative analysis of RNA on a massively parallel array)
    - HiTS-RAP (high-throughput sequencing RNA affinity profiling)
    - RNA-MITOMI
- High throughput sequencing
- In situ synthesis of RNA libraries in Illumina flow cell
- Visualization of multiple proteins simultaneously

# RNA-centric approaches

## RNA-centric methods

(c) *In vivo*

(d) *In vitro*



*Marchese et al, 2016*













- Identify RBPs targeting a single RNA of interest
  - Tagged RNAs
    - Affect secondary structure of RNA
  - Modified ribonucleotides (eg biotin, fluorescent dyes)
  - *In vivo*
    - MS2-BioTRAP
      - MS2-hairpin loop tagged RNA
      - Bacteriophage MS2 coat protein-protein tag
    - **ChIRP** (chromatin isolation by RNA purification)
    - **CHART** (capture hybridization analysis of RNA targets)
    - **RAP-MS** (RNA antisense purification)
- Noncoding-RNAs
- Cross linking, pull down of target RNA using complementary biotinylated oligodeoxyribonucleotides, reversal of cross linking, RNAseq+mass-spec
- *In vitro*
    - TRAP/RAT
    - RaPID
    - RiboTrap
    - RNA-assisted chromatography

# The current methods used to detect RNA-protein interactions are far from perfect

- Each method offers a partial/biased/inaccurate snapshot of protein-RNA interactions.



# Capturing the interactome of newly transcribed RNA

Xichen Bao<sup>1,2,24</sup>, Xiangpeng Guo<sup>1,2,24</sup>, Menghui Yin<sup>3,24</sup>, Muqddas Tariq<sup>1,2,4</sup>, Yiwei Lai<sup>1,2,4</sup>, Shahzina Kanwal<sup>1,2</sup>, Jiajian Zhou<sup>5</sup>, Na Li<sup>1,2,6</sup>, Yuan Lv<sup>1,2,4</sup>, Carlos Pulido-Quetglas<sup>7</sup>, Xiwei Wang<sup>1,2</sup>, Lu Ji<sup>5</sup>, Muhammad J Khan<sup>1,2,8</sup>, Xihua Zhu<sup>1,2</sup>, Zhiwei Luo<sup>1,2,4</sup>, Changwei Shao<sup>9</sup>, Do-Hwan Lim<sup>9</sup>, Xiao Liu<sup>10</sup>, Nan Li<sup>11</sup>, Wei Wang<sup>12</sup>, Minghui He<sup>13</sup>, Yu-Lin Liu<sup>14</sup>, Carl Ward<sup>1,2</sup>, Tong Wang<sup>15</sup>, Gong Zhang<sup>15</sup>, Dongye Wang<sup>1,2,16</sup>, Jianhua Yang<sup>17</sup>, Yiwen Chen<sup>18</sup>, Chaolin Zhang<sup>19</sup>, Ralf Jauch<sup>16</sup>, Yun-Gui Yang<sup>20</sup>, Yangming Wang<sup>21</sup>, Baoming Qin<sup>1</sup>, Minna-Liisa Anko<sup>22</sup>, Andrew P Hutchins<sup>23</sup>, Hao Sun<sup>5</sup>, Huating Wang<sup>5</sup>, Xiang-Dong Fu<sup>9</sup>, Biliang Zhang<sup>3</sup>  
& Miguel A Esteban<sup>1,2</sup>

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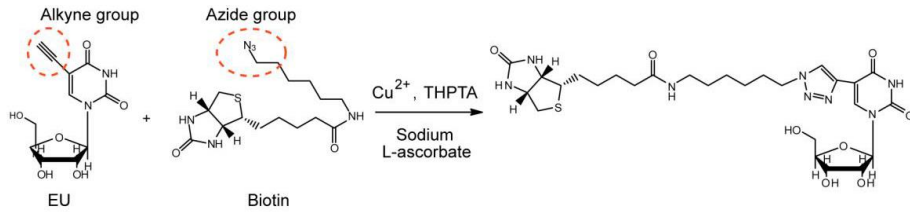
# Limitations of current methods used to map RNA-protein interactions

- Focus on polyadenylated (polyA) RNAs (mostly mRNAs) with oligo(dT)-coated beads (*Castello et al, 2012, Cell*).
- Problems:
  - PolyA tails: newly transcribed RNAs
  - Mature mRNAs: can be non-polyA or bimorphic
  - Mature non-polyA RNAs are a substantial fraction of transcribed sequences

# Method development

## Capture of the newly transcribed RNA interactome using click chemistry (RICK)

Click chemistry reaction for EU-RNA labeling with biotin-azide



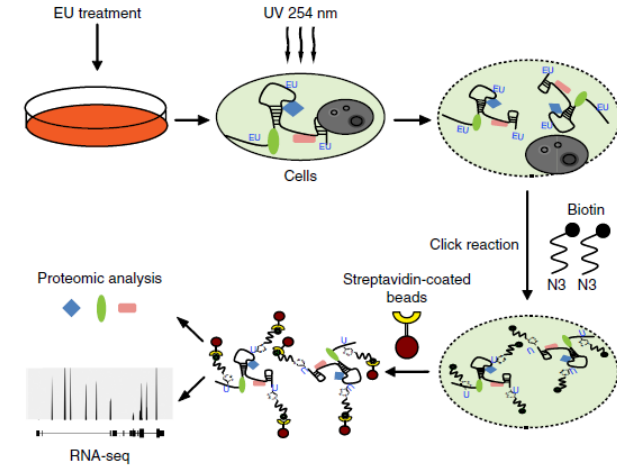
EU=ethynyluridine

Ligand: THPTA=tris(3-hydroxypropyltriazolymethyl)

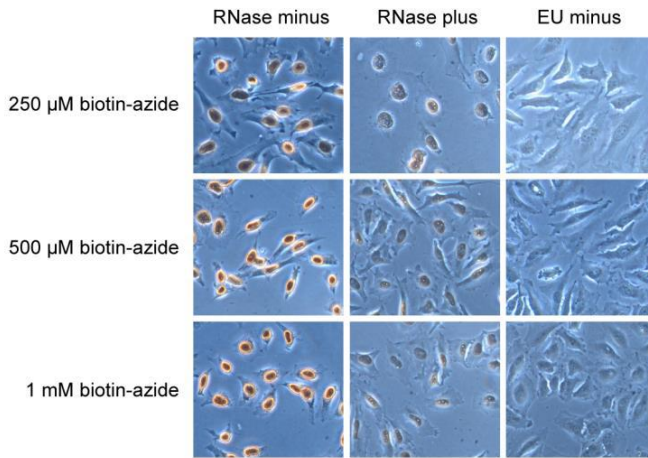
Label the 5' terminus of RNA

Catalysts:  $\text{Cu}^{2+}$ , sodium ascorbate

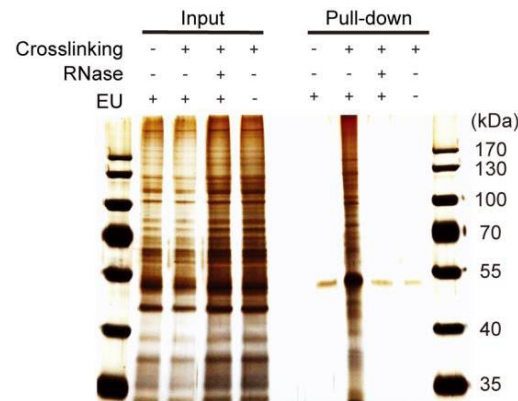
Schematic representation of the RICK procedure (16h treatment)



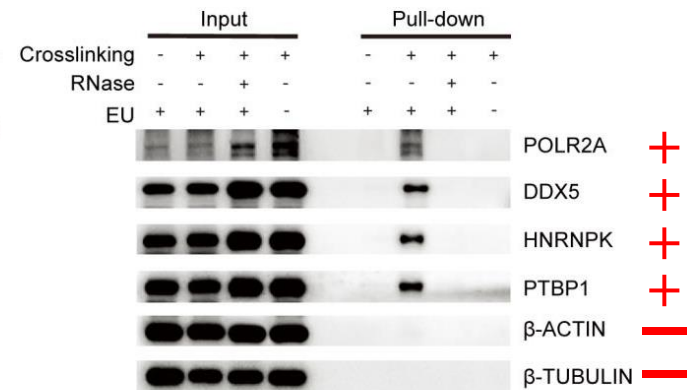
Visualization of EU incorporation (indicated by DAB staining) in HeLa cells



Confirmation of the specificity of the pull down (silver staining)



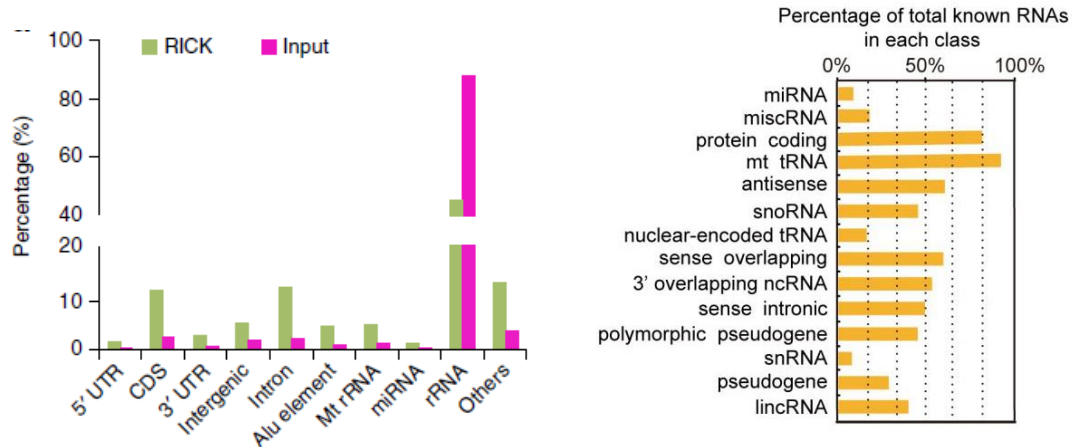
Western blotting to validate the capture of known RBPs by RICK



# Characterization of the method

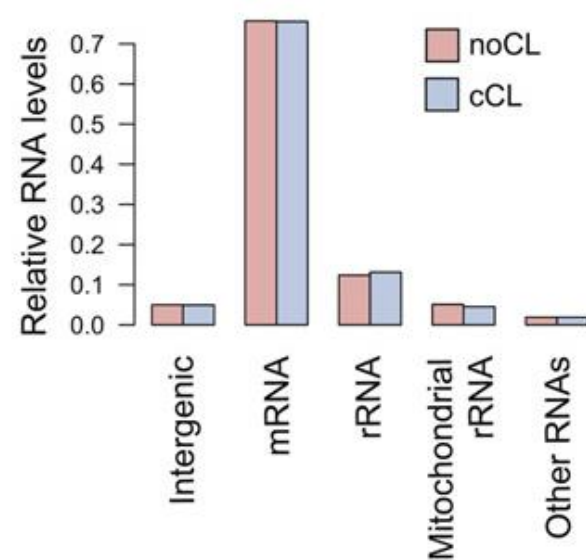
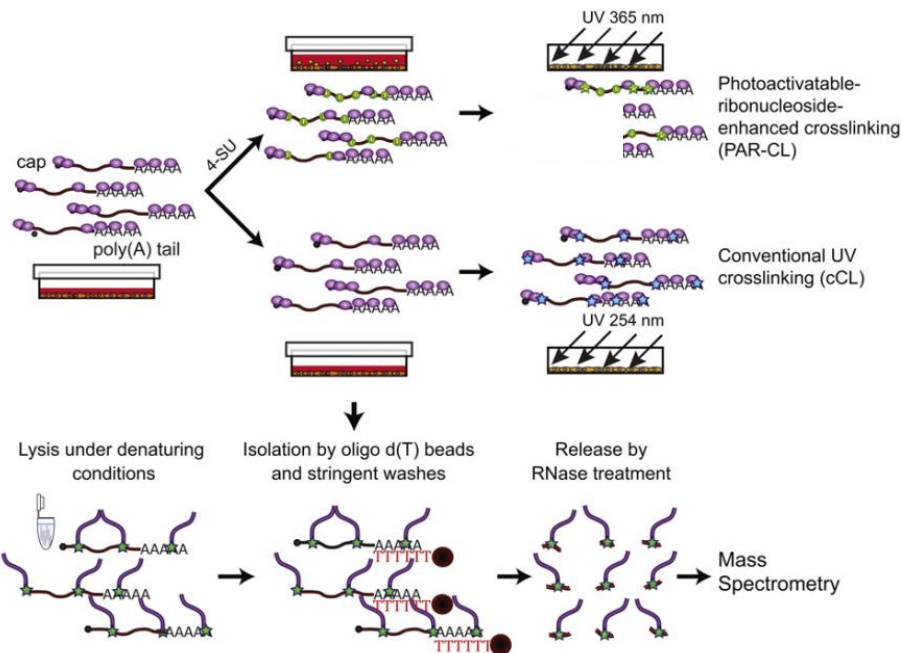
## Determination of RNA species captured by RICK

Distribution of RNA species isolated in a representative RICK experiment



RNA sequencing on a RICK pull-down sample

## Comparison with oligo(dT) isolation method

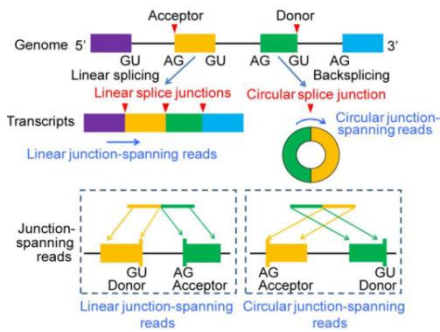


80% of captured RNAs were mRNAs

# Characterization of the method

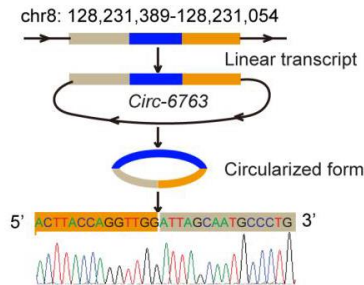
## Determination of RNA species captured by RICK

Non-polyA RNA species isolation with RICK vs oligo(Td) methods



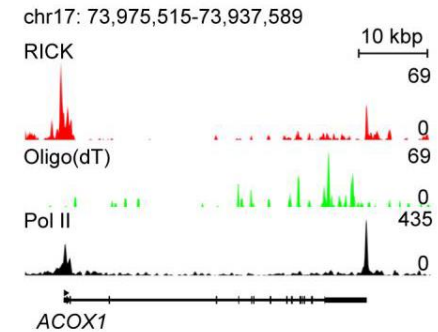
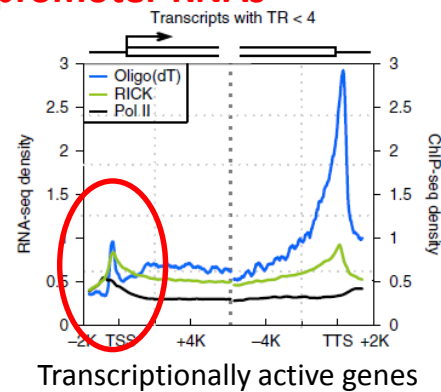
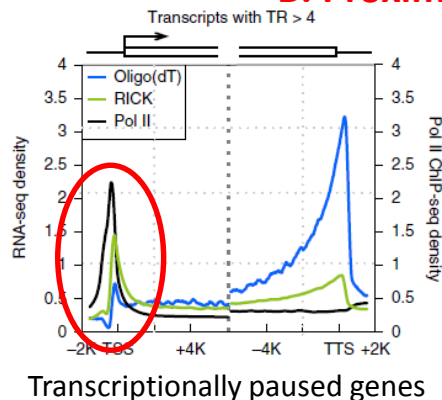
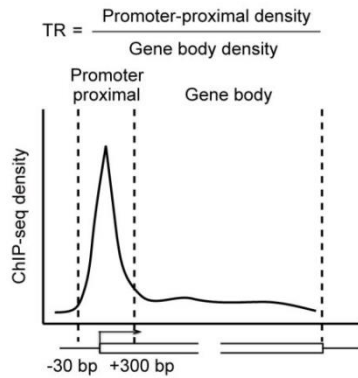
### A. Circular RNAs

Name	Coding gene	Read No. RICK/oligo(dT)	Sequence of joint site
Circ-6763	CCAT1	92/0	CCAGGTTGGG ATTAGCAATG
Circ-1903	RPPH1	23/0	GCCAGCGAAG GTGAGTTCCC
Circ-2154	MGA	14/0	TTTGTGAATG GGATGGGAAG
Circ-1557	CPSF6	10/0	TCGTTAGAAG GGAAGCTGAA
Circ-2327	IGF1R	7/0	TGCCAGAAA GTCTGCGGGC
Circ-3811	TET3	2/0	GATTGCGTGG GACAGGCTCA

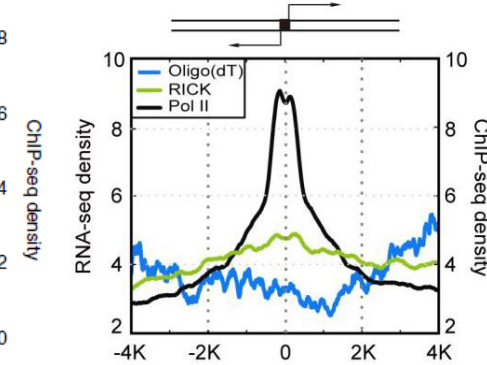
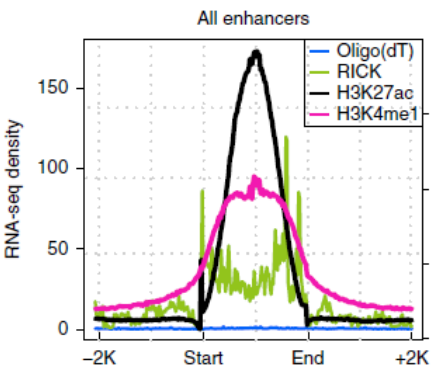


TR=traveling ratio

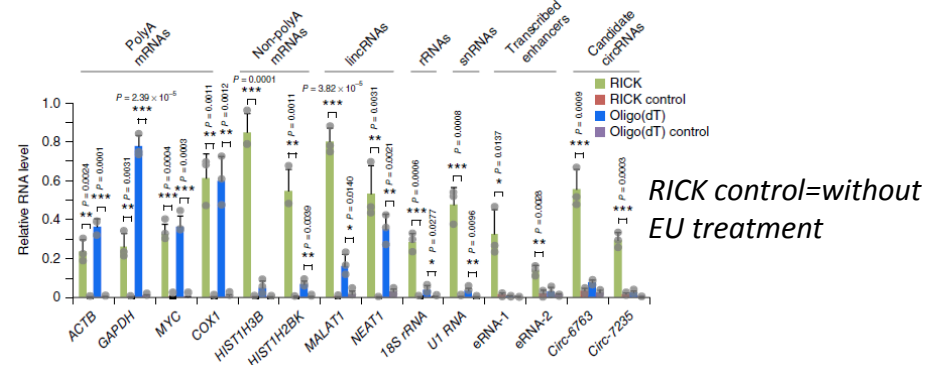
### B. Proximal promoter RNAs



### C. Enhancer RNAs



### Verification of results with qPCR

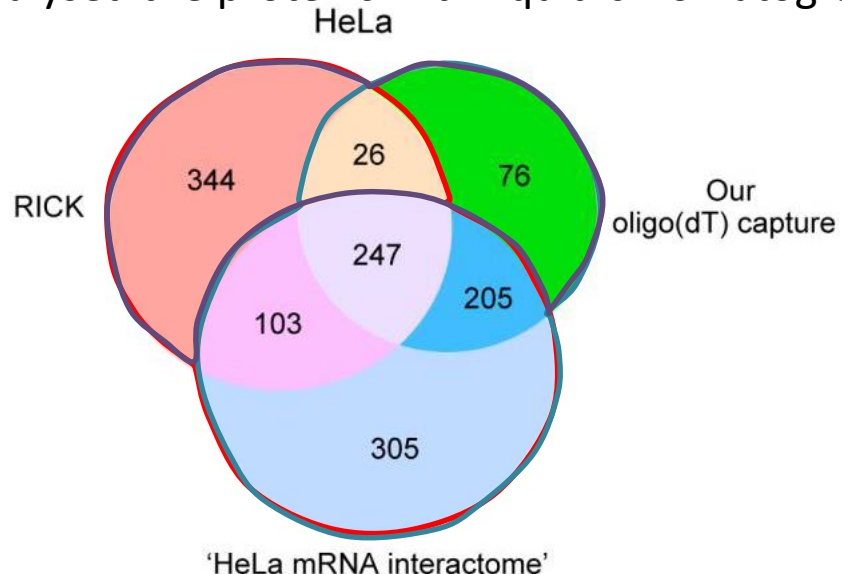




# Characterization of the method

## Characterization of proteins isolated by RICK

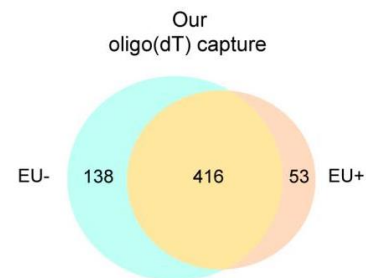
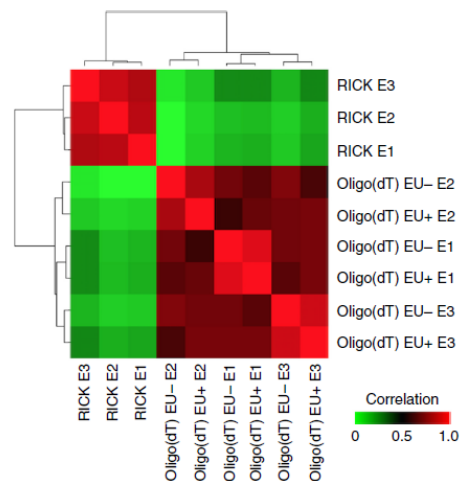
Analysed the proteins with liquid chromatography–tandem mass spectrometry (LC-MS/MS)



### Comparisons

- HeLa mRNA interactome by oligo(dT) capture Costello et al, 2012
- RICK by authors
- Oligo(dT) capture by authors

Oligo(dT) capture in the presence of EU to evaluate whether its incorporation affects RNA–protein interactions



RICK vs HeLa mRNA interactome (Castello et al, 2012):

- 720 RICK
- 860 mRNA interactome
- 350 in both datasets
- 370 only in RICK

RICK vs authors' oligo(dT) capture (excluding HeLa interactome):

- 26 overlap
- 344 "RICK-exclusive RBPs"

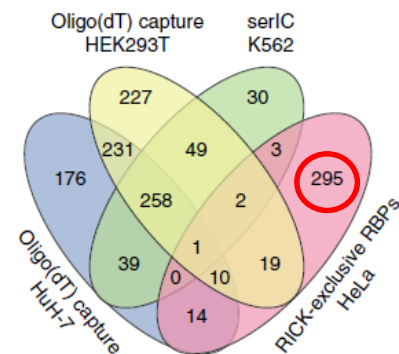
HeLa mRNA interactome vs authors' oligo(dT) capture:

- 81.6% overlap

### Comparison of the 344 RICK-exclusive RBPs with three reported human oligo(dT) capture studies

Most of these proteins (295, 85.8%) are truly unique to RICK = 'RICK unique RBPs'.

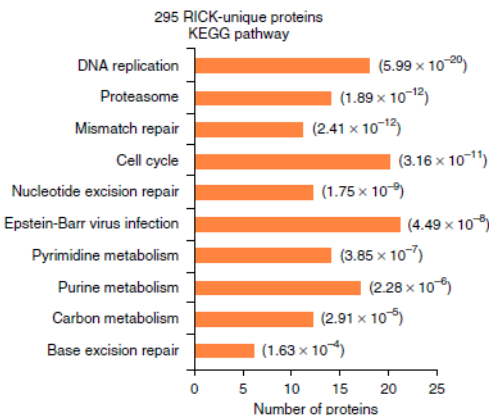
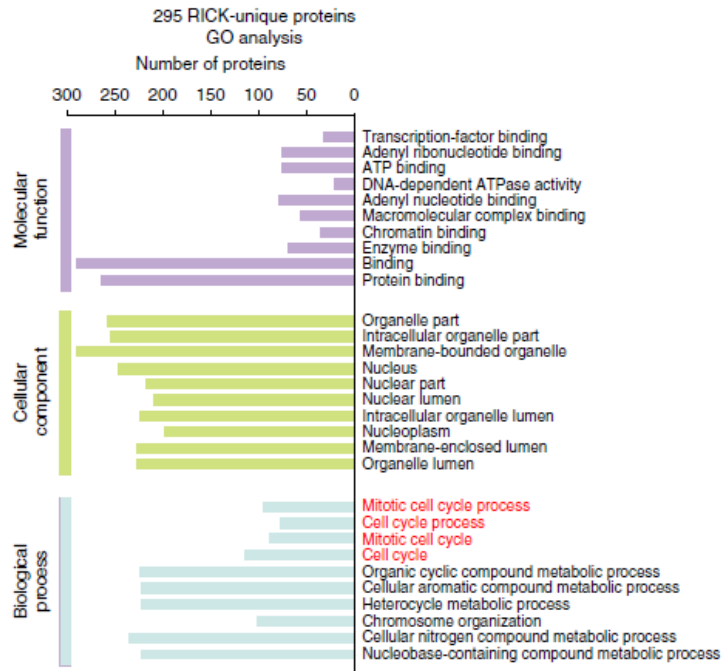
- Baltz et al, 2012 Mol Cell
- Beckmann et al, 2015 Nat Commun
- Conrad et al, 2016, Nat Commun



# Characterization of the method

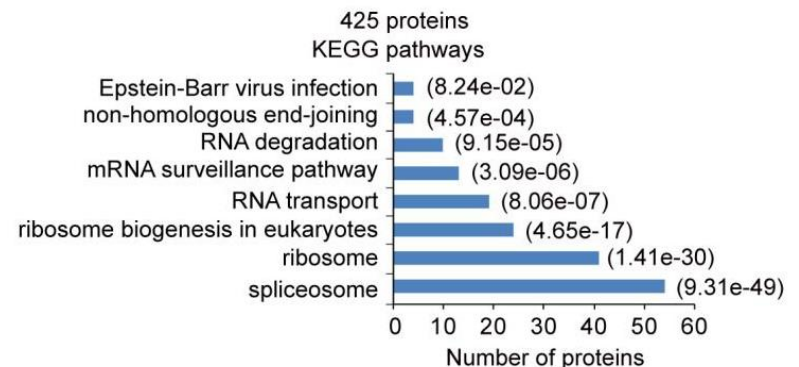
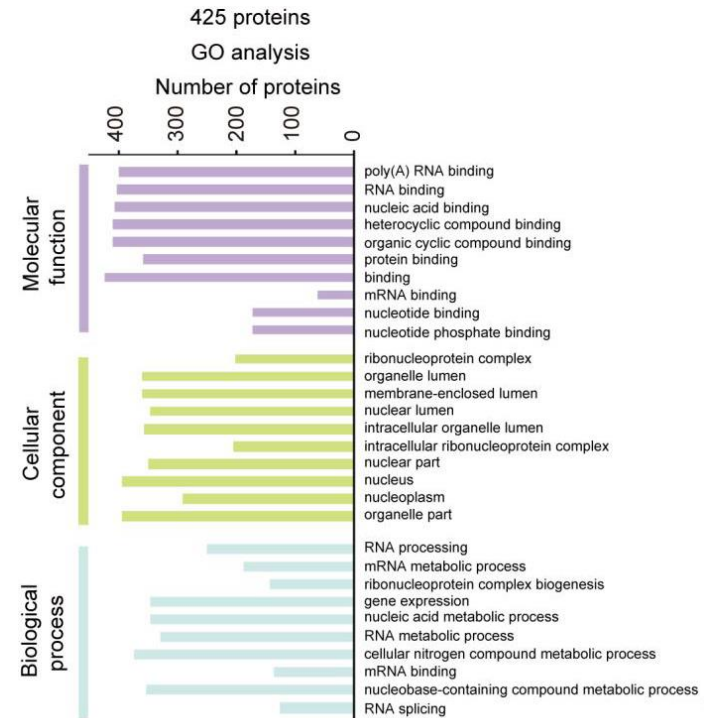
## Functional analysis of proteins isolated with RICK

Gene Ontology (GO) analysis of the 295 RICK unique RBPs, observing enrichment of biological processes related to “mitosis”



KEGG pathway analysis also included “cell cycle” ( $P = 3.16 \times 10^{-11}$ ) amongst the top ten most significantly enriched pathways

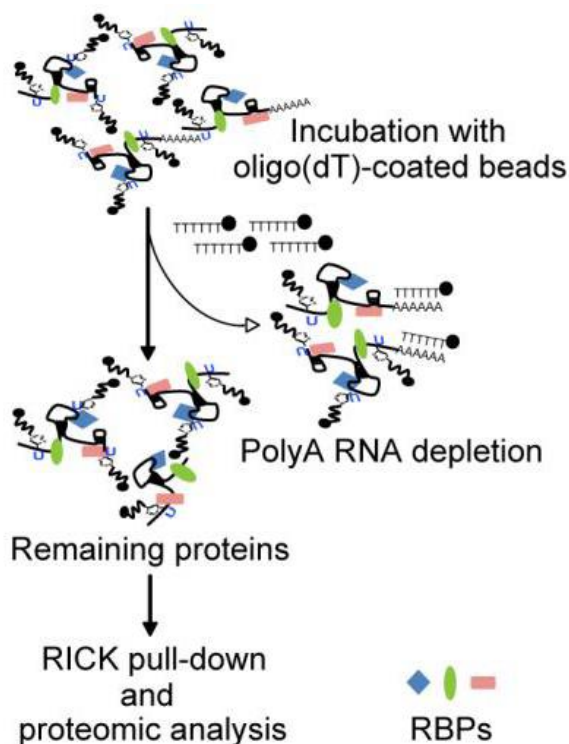
GO and KEGG analysis of the 425 proteins identified by RICK and present in oligo(dT) capture data sets showed mostly RNA-related processes



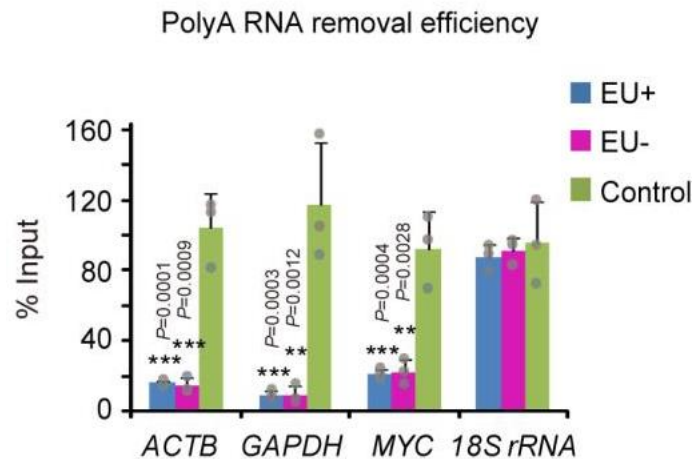
# Characterization of the method

## RICK identifies proteins with preferential binding to non-polyA RNAs

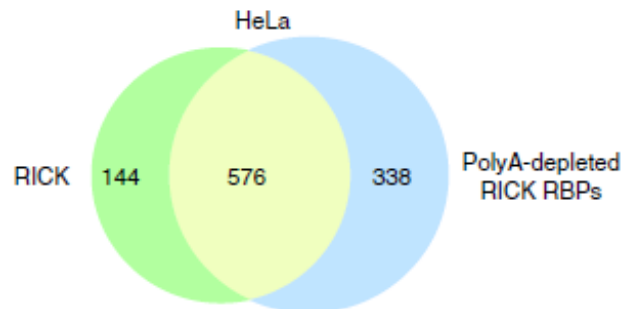
Added another step consisting of three consecutive rounds of incubation with oligo(dT)-coated beads to the standard RICK protocol



Confirmation of efficient removal of polyA RNAs by RT-qPCR, whilst the control 18S RNA remained unaffected



LC-MS/MS identified 914 high-confidence proteins ('polyA-depleted RICK proteins'). Of these 914 proteins, 576 overlapped with the 720 high-confidence proteins of the standard RICK procedure.



GO analysis: enrichment of biological processes related to mitosis



# Proof of concept experiments

## Characterization of RNAs interacting with **METTL1** and CDK1

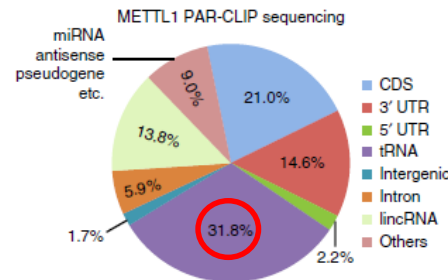
Study of the interacting RNAs of two RICK-unique RBPs, **METTL1** and CDK1 using PAR-CLIP sequencing

Two independent PAR-CLIP sequencing experiments for METTL1 showed extensive overlap in the captured RNAs, a large proportion of which were captured with RICK too.

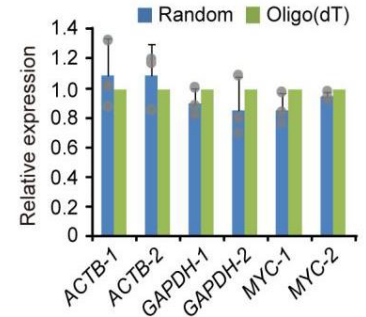
METTL1 PAR-CLIP sequencing



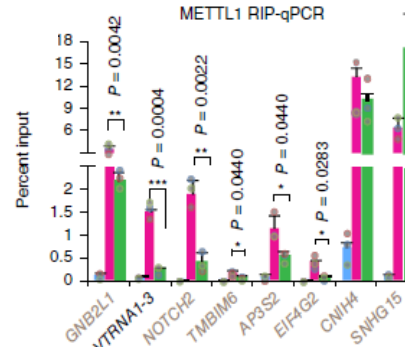
RNA-binding profile of METTL1



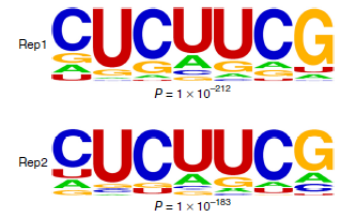
RIP-qPCR using random hexamers or oligo(dT) primers to discern whether METTL1 target RNAs have polyA tails



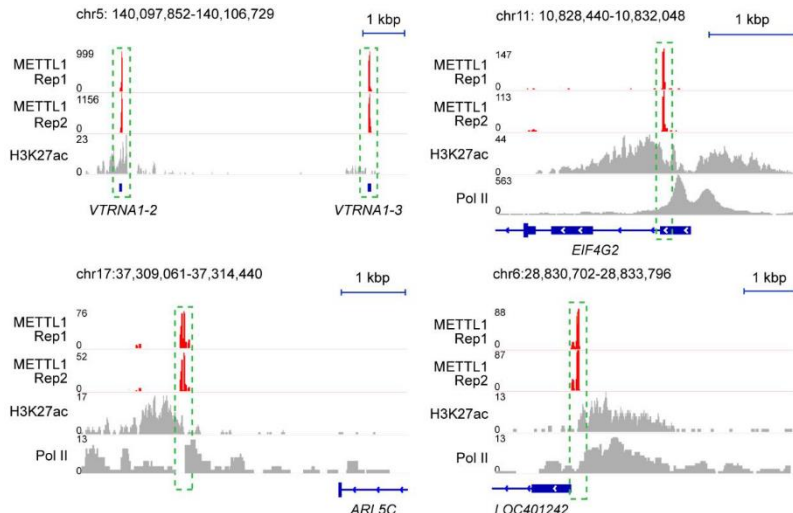
RIP-qPCR validated the interaction of METTL1 with the nonpolyA VTRNA1-3



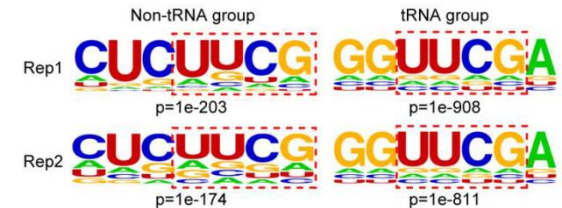
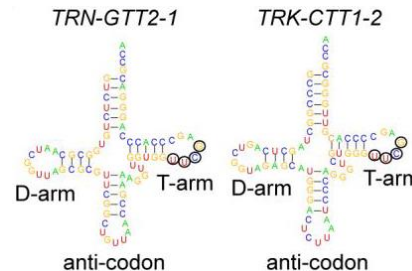
METTL1 binding motif



Sequencing tracks for METTL1  
(Rep1=1st replicate, Rep2=2nd replicate)



tRNA binding motifs

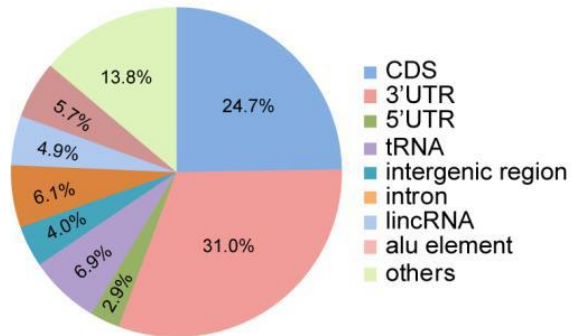


# Proof of concept experiments

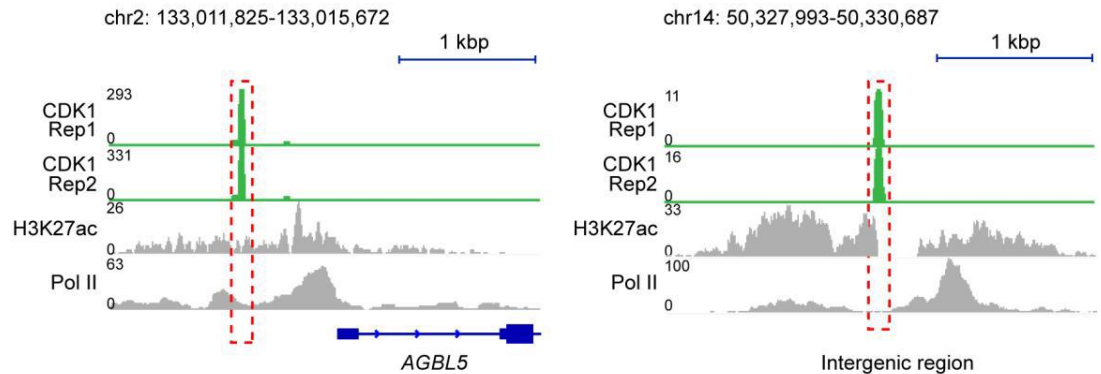
## Characterization of RNAs interacting with METTL1 and CDK1

Study of the interacting RNAs of two RICK-unique RBPs, METTL1 and CDK1 using PAR-CLIP sequencing

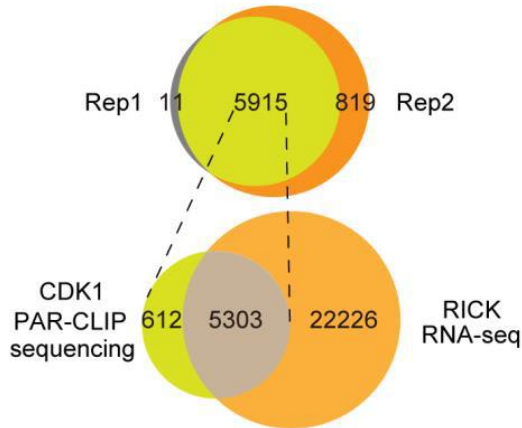
RNA-binding profile of CDK1



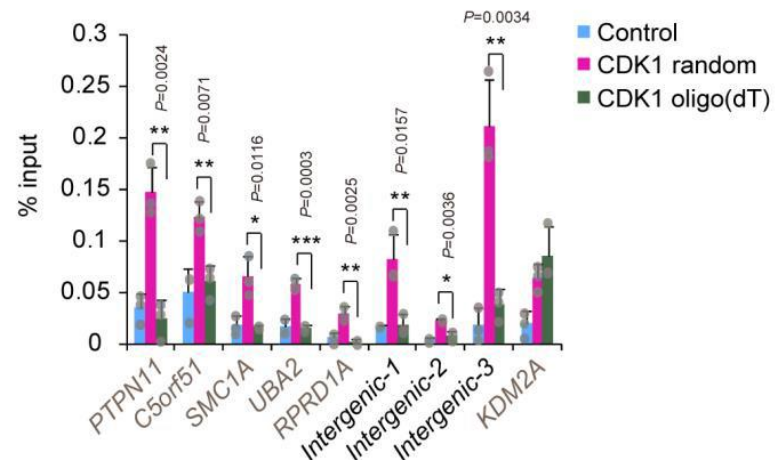
Sequencing tracks for two intergenic RNAs bound by CDK1



Comparison of results between PAR-CLIP sequencing and RICK RNA-seq



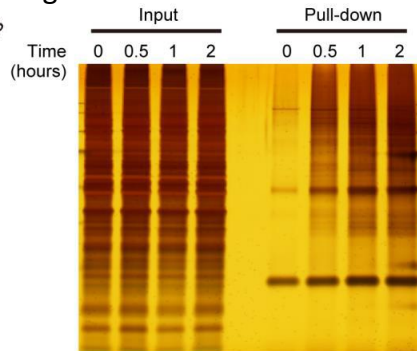
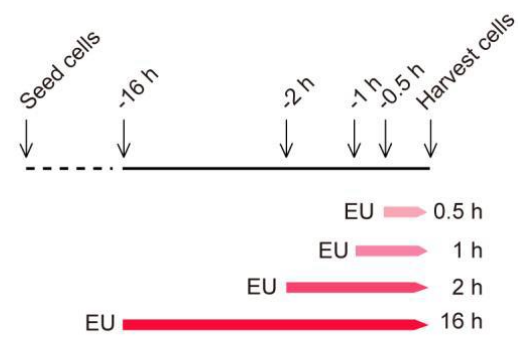
- RIP-qPCR confirmed the capture of selected intergenic RNAs identified in the sequencing
- Of six selected mRNA sequences, only one showed enrichment with oligo(dT) primers using RIP-qPCR



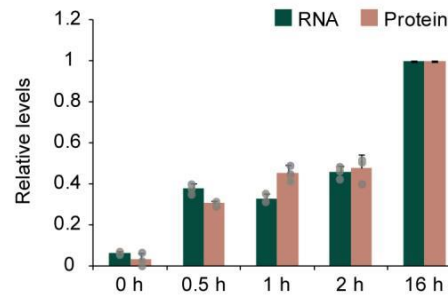
# Demonstration of applications for RICK

## Capture of the nascent RNA interactome using RICK

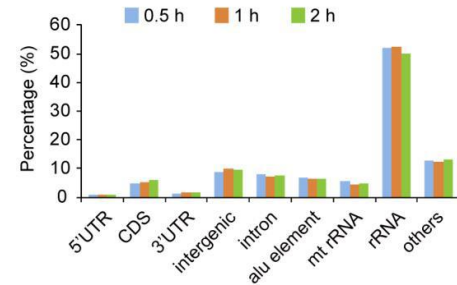
Protocol: Short EU labeling of HeLa cells



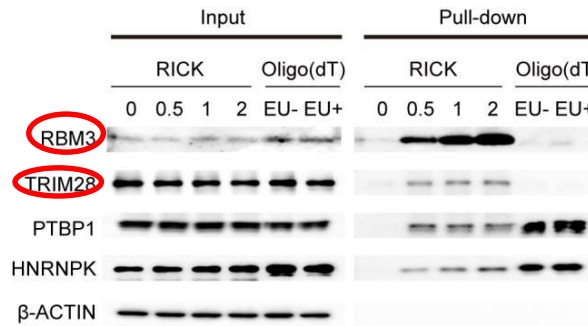
Short vs long RICK EU incubation



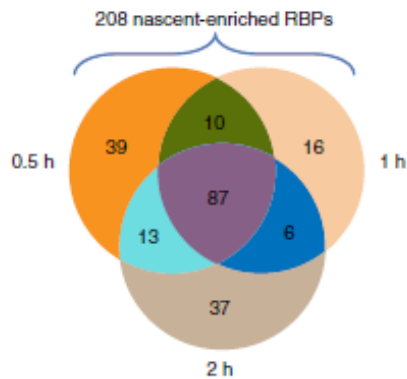
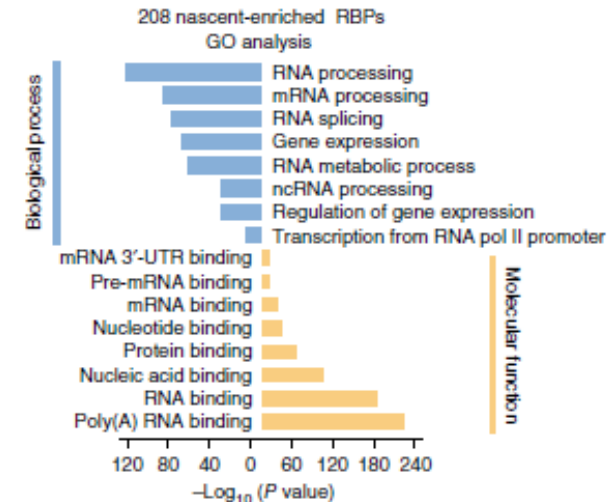
% of sequences corresponding to 5'UTR, CDS, and 3'UTR was reduced with the short-labeling RICK=accumulation of fewer mature mRNAs



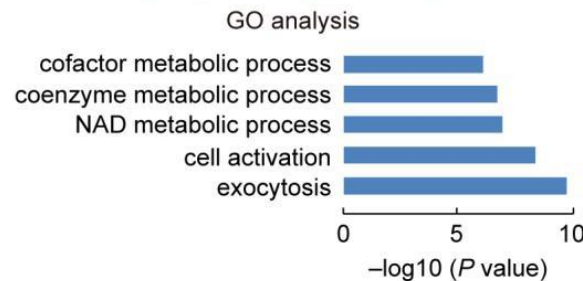
WB validation of two nascent-enriched RBPs



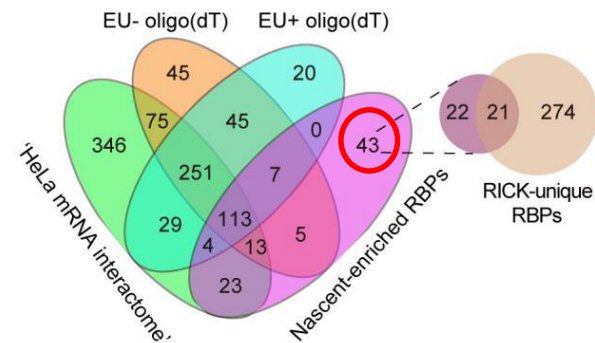
Enrichment in transcription and RNA metabolism



43 nascent-enriched RBPs



\*Nascent=emerging/primary RNAs



# Demonstration of applications for RICK

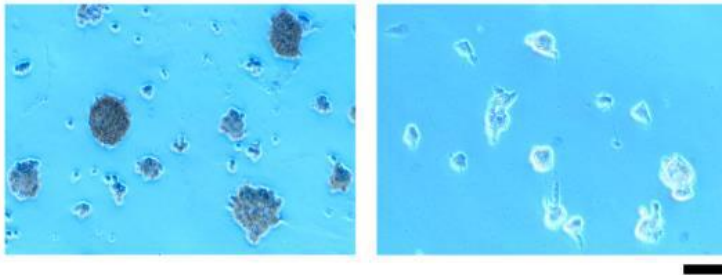
## Capture of the total RNA interactome of mESCs using RICK

Incorporation of EU in cells as shown by streptavidin-conjugated horseradish peroxidase

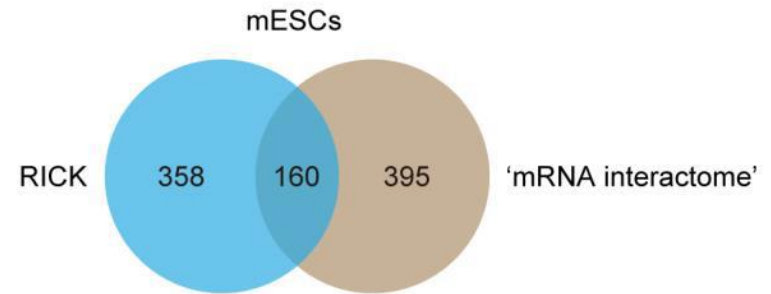
EU plus

EU minus

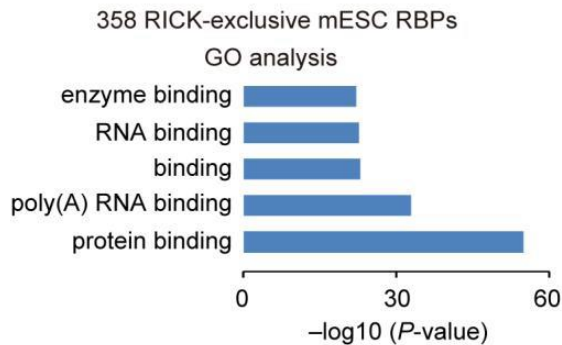
mESCs



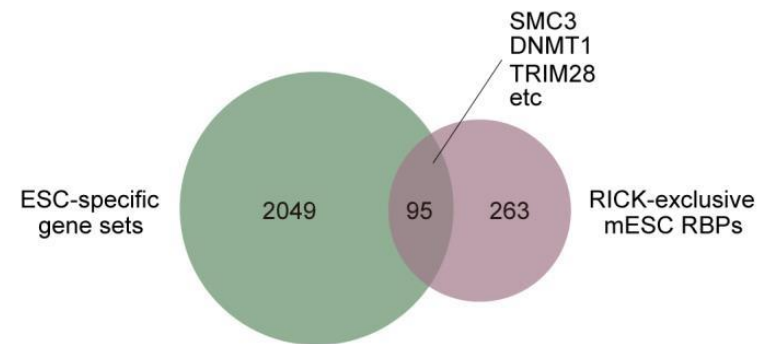
Overlap with mESC mRNA interactome (Kwon et al, 2013, Nat Struct Mol Biol)



GO enrichment of RNA-binding or polyA-RNA-binding terms or RICK-exclusive mESC RBPs



95 of those proteins were expressed at higher levels in mESCs than in differentiated cells




ESC-specific gene sets:

- Kwon et al, 2013 Nat Struct Mol Biol
- Wong et al, 2008 Cell Stem Cell
- Ben-Porath et al, 2008, Nat Genet
- Bhattacharya et al, 2004, Blood

# Conclusions

- Alternative to oligo(Td) beads pull-down that allows a more unbiased capture of RNA species because it doesn't depend on polyA tails
- RICK modifications can be used to broaden the applications of this technique
  - Short-labeling RICK
    - Link between metabolic enzymes and nascent RNAs
    - Interaction between RBPs and steady-state RNAs
    - Map dynamic changes during acute cell stimulation or stem cell fate transitions
    - Live animals: characterization of RBPs in mammalian organs or during development

# RNA–protein interaction detection in living cells

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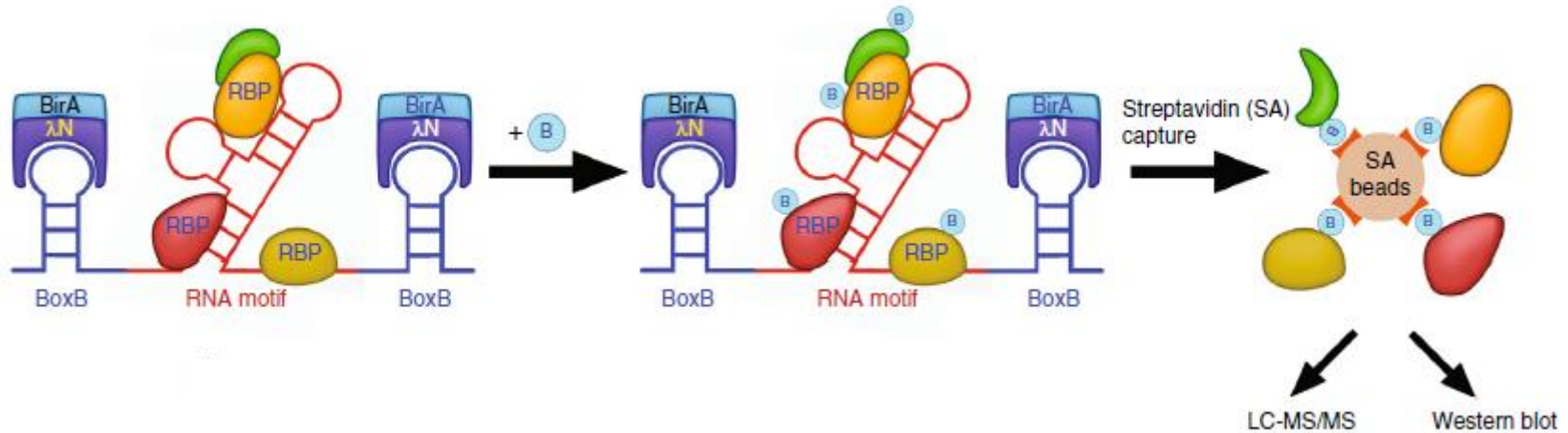
# Limitations of current methods used to map RNA-protein interactions

- Biases from postlysis protein reassortment
- Aim of the study
  - Direct labeling of proteins that bind to specific RNA motifs in intact live cells
- Proximity-dependent protein labeling



# Method development

## Development of RaPID (RNA-protein interaction detection)



### RNA component

BoxB stem loops flanking any RNA motif of interest.

### Protein component

22-amino-acid  $\lambda$ N peptide fused to the N terminus of the HA-BirA\* biotin ligase ( $\lambda$ N-HA-BirA\*).

### Procedure

BoxB stem loops recruit the RaPID protein



Biotinylation of proteins bound to the flanked adjacent RNA motif of interest



Streptavidin capture of motif-bound proteins for analysis by western blotting and mass spectrometry (MS).

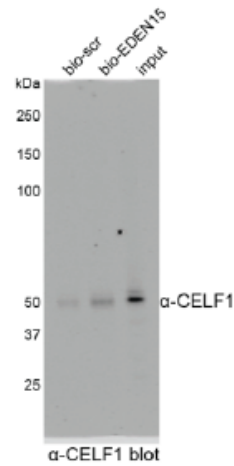


# Proof of concept experiment

## Validation of RaPID with known RNA-protein interactions

EDEN15:  
UG-rich RNA sequence  
known to bind CELF1

Conventional biotinylated  
RNA pulldown



4-fold enrichment of  
CELF1 proteins over  
scrambled controls

RaPID-Western

Expression of RaPID RNA and protein components  
in HEK cells in biotin-containing media

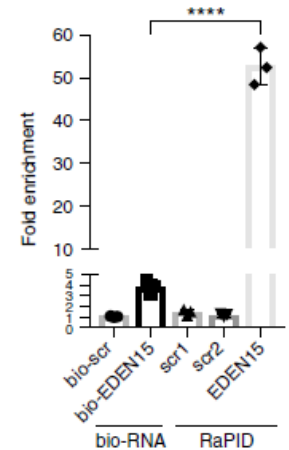


Pulldown of biotinylated proteins with streptavidin  
beads



Western Blotting

Conventional biotinylated  
RNA pulldown vs RaPID

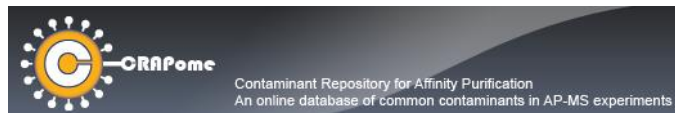


### RaPID-mass spectrometry (RaPID-MS)

Identification of true binding proteins:

- CRAPome filtering analysis
- Significance analysis of interactome (SAINT) threshold score of 0.9

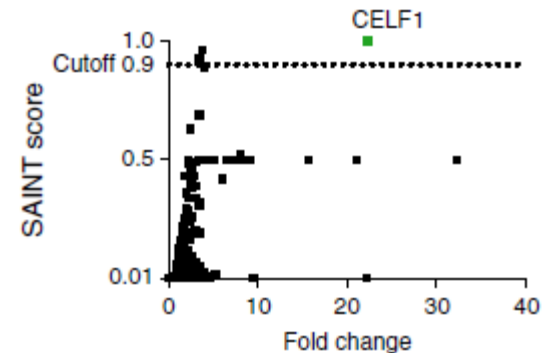
<http://crapome.org/>



The CRAPome: a contaminant repository for affinity  
purification-mass spectrometry data

Dattatreya Mellacheruvu<sup>1,2</sup>, Zachary Wright<sup>2</sup>, Amber L Couzens<sup>3</sup>, Jean-Philippe Lambert<sup>3</sup>, Nicole A St-Denis<sup>3</sup>, Tuo Li<sup>4</sup>, Yana V Miteva<sup>4</sup>, Simon Hauri<sup>5</sup>, Mihaela E Sardiou<sup>6</sup>, Teck Yew Low<sup>7,8</sup>, Vincentius A Halim<sup>7-9</sup>, Richard D Bagshaw<sup>3</sup>, Nina C Hubner<sup>10</sup>, Abdallah al-Hakim<sup>3</sup>, Annie Bouchard<sup>11</sup>, Denis Faubert<sup>11</sup>, Damian Fermin<sup>1</sup>, Wade H Dunham<sup>3,12</sup>, Marilyn Goudreault<sup>3</sup>, Zhen-Yuan Lin<sup>3</sup>, Beatriz Gonzalez Badillo<sup>3</sup>, Tony Pawson<sup>3,12</sup>, Daniel Durocher<sup>3,12</sup>, Benoit Coulombe<sup>11,13</sup>, Ruedi Aebersold<sup>5,14</sup>, Giulio Superti-Furga<sup>15</sup>, Jacques Colinge<sup>15</sup>, Albert J R Heck<sup>7,8</sup>, Hyungwon Choi<sup>16</sup>, Matthias Gstaiger<sup>5</sup>, Shabaz Mohammed<sup>7,8,17,18</sup>, Ileana M Cristea<sup>4</sup>, Keiryn L Bennett<sup>15</sup>, Mike P Washburn<sup>6,19</sup>, Brian Raught<sup>20,21</sup>, Rob M Ewing<sup>22,23</sup>, Anne-Claude Gingras<sup>3,12</sup> & Alexey I Nesvizhskii<sup>1,2</sup>

RaPID LC-MS/MS



Confirmation of RaPID's ability to identify known RNA-protein  
interactions in living cells

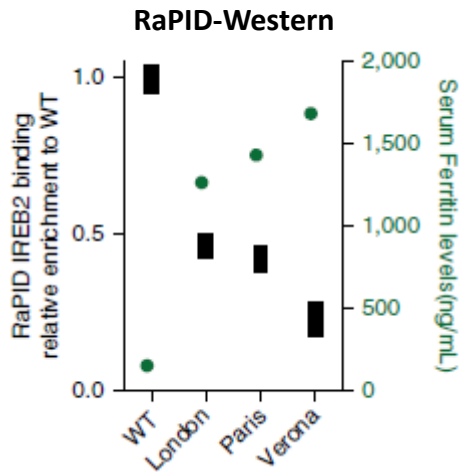
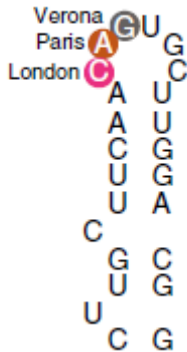
# Proof of concept experiment

## RNA–protein interaction information

Using RaPID to study the pathogenesis of human disease

- **Hereditary Hyperferritinemia- Cataract Syndrome (HHCS)**
  - Disorder characterized by increased serum ferritin levels and early-onset cataracts
  - Iron responsive element (IRE) RNA motif in the L-ferritin (*FTL*) gene transcript altered by point mutations
- **IRE binding proteins**
  - IREB2 and IRP1 (IREB1/ACO1)
  - Regulate the translation/stability of target transcripts in the iron metabolic pathway.

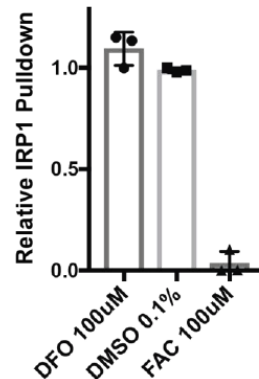
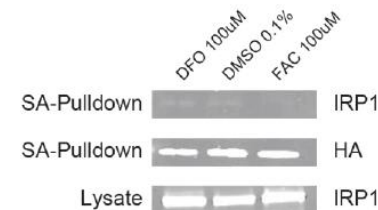
L-ferritin (*FTL*)  
gene mutations



- Significant IREB2 enrichment over scrambled controls
- Disease associated IRE mutants had lower fold IREB2 binding enrichment compared to wild type IRE
- Binding loss correlated with increased serum ferritin levels seen in specific HHCS patient mutations

### Effect of iron levels on the ability of IREB2 and IRP1 to bind with IRE

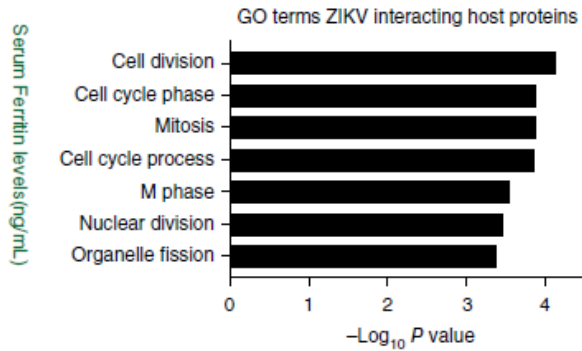
- Evaluation of the interaction between IRP1 and the IRE by RaPID-WB
- DMSO and Deferoxamine (DFO) treatment
  - IRE motif interaction with IRP1
- Ferric ammonium chloride (FAC): raises iron levels
  - There was no interaction detected between IRE and IRP1



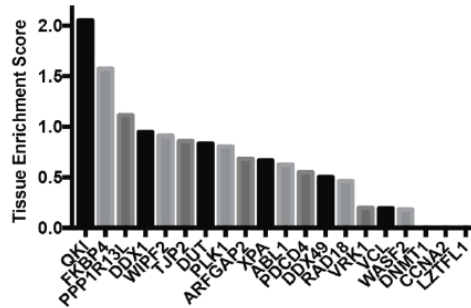
# Proof of concept experiment

## Identification of host proteins associated with ZIKV RNA

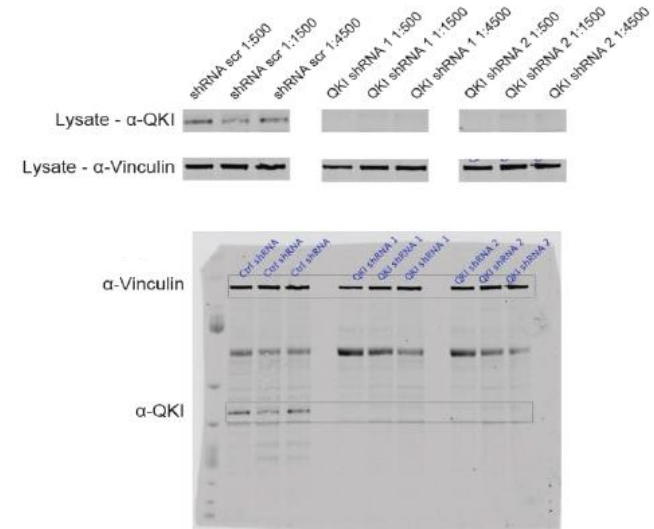
- To identify host proteins that bind ZIKV RNA, RaPID-MS was performed using ZIKV UTR sequences from the current epidemic strain (KU527068)
- Enrichment in cell cycle proteins



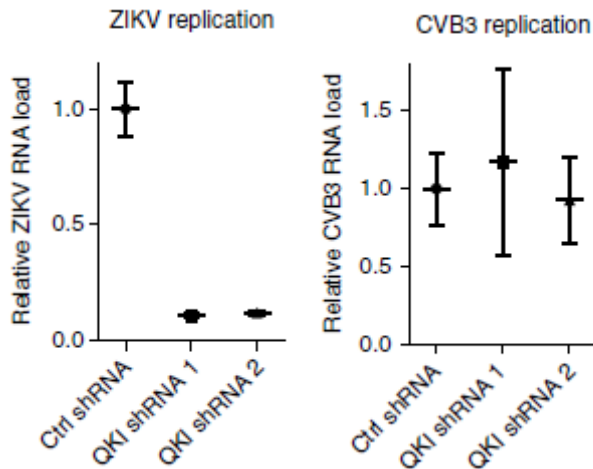
ZIKV UTR interacting proteins identified by RaPID were intersected with Tissue Protein Atlas.



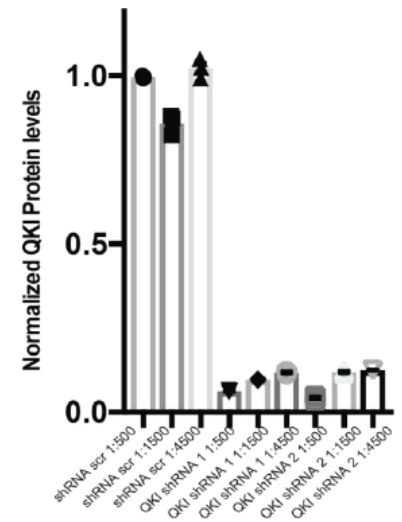
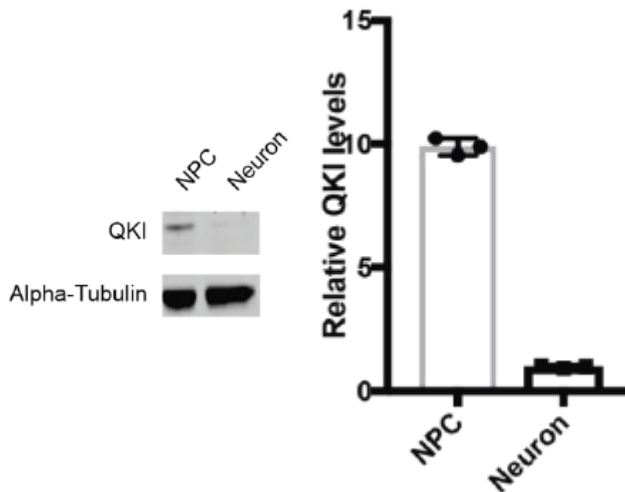
QKI depletion in the U87 Neuroblastoma cell line



QKI loss decreased ZIKV viral RNA levels by 90% without altering control Coxsackievirus B3 viral RNA levels



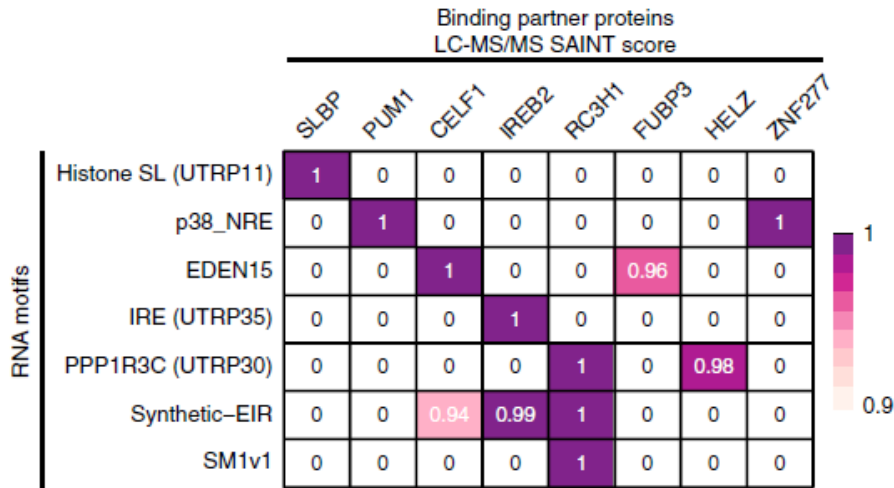
QKI protein levels decrease as neural progenitor cells (NPCs) differentiate to neurons



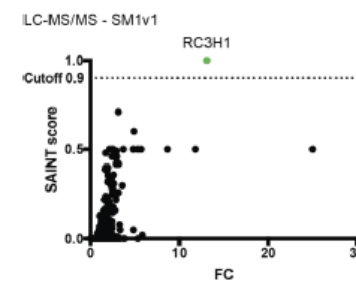
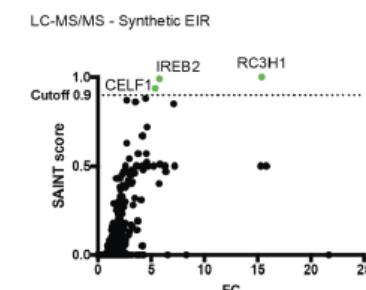
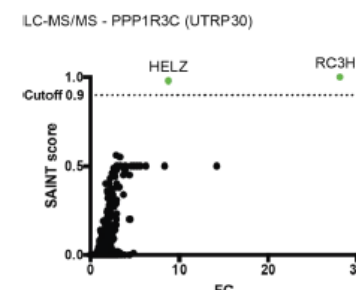
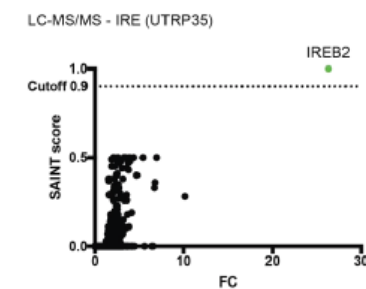
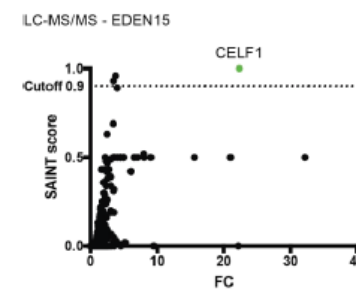
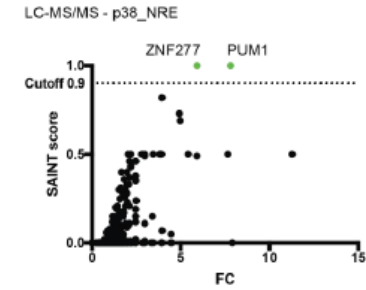
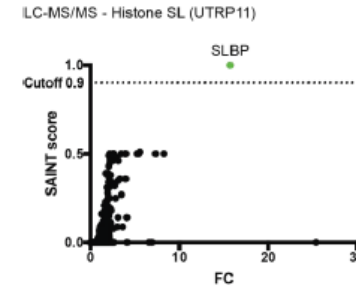
# Proof of concept experiment

## Characterization of synthetic RNA motifs

- RaPID-MS
- 3 representative conserved RNA motifs identified by a comparative analysis of 41 vertebrate genomes (Parker et al, 2011, Genome Res)
  - Histone SL-UTRP11
  - PPP1R3C-UTRP30
  - IRE-UTRP35
- Synthetic concatamer (Syn-EIR) consisting of the EDEN15, IRE and ROQ CDE motifs
  - Bind CELF1, IREB2 and RC3H1 proteins, respectively.



LC-MS/MS SAINT scores for each protein-RNA motif pair



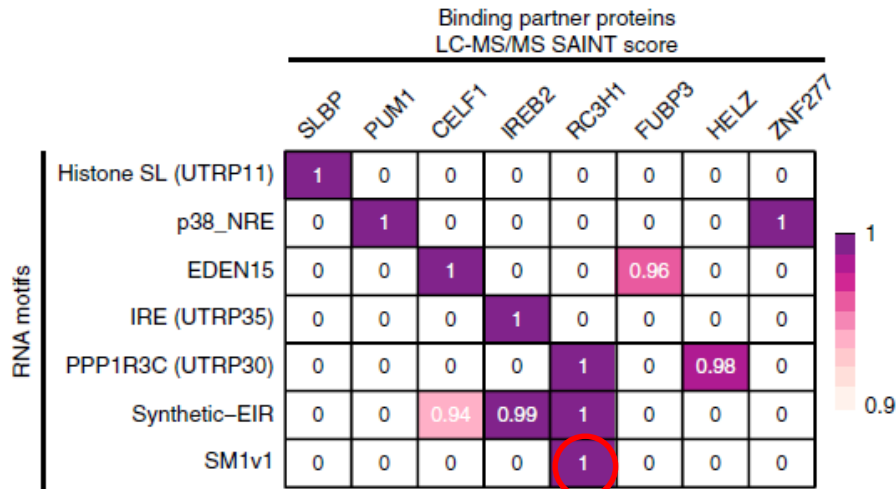
FC=fold change

# Demonstration of applications for RaPID

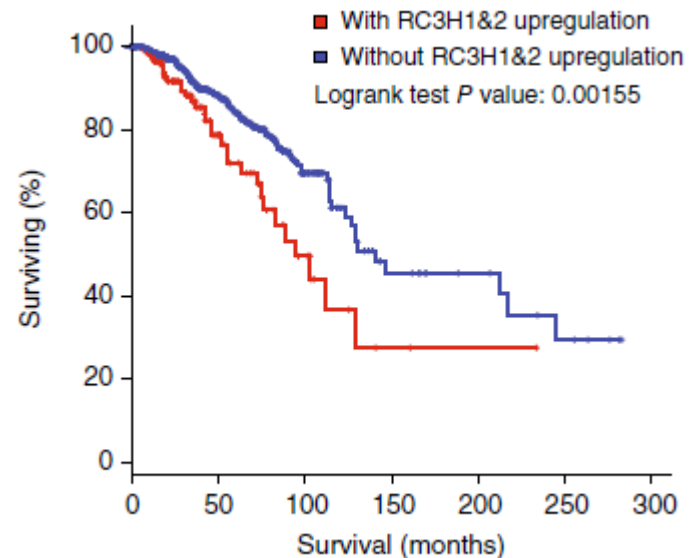
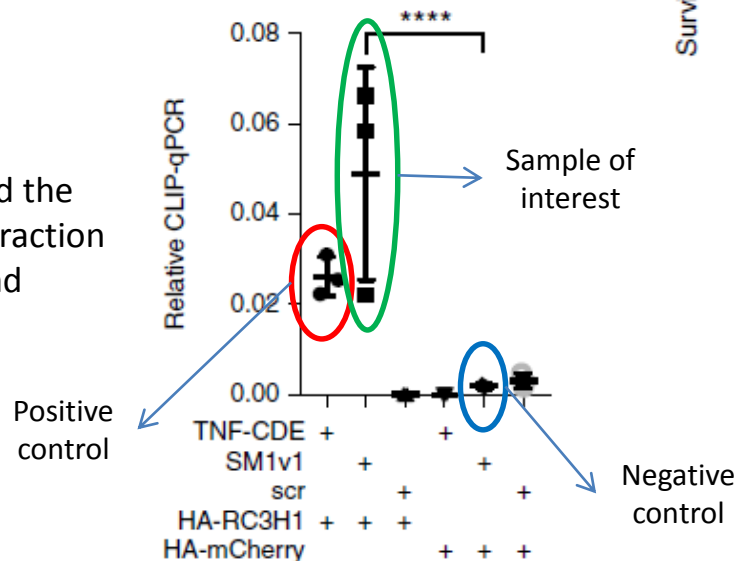
## RaPID-MS specificity and new RNA-protein interactions

Among new interactions identified by RaPID-MS is RC3H1 protein binding to the SM1v1 RNA motif

- Decreased levels of RNA transcripts bearing SM1v1 motifs in breast cancer patients with advanced tumours (Tavazoie et al, 2014, Cell Rep).
- Analysis of TCGA breast cancer data demonstrated that RC3H1 and RC3H2 mRNA upregulation correlates with poorer breast cancer survival.
- RC3H1 and its paralog RC3H2 are known RNA-binding proteins that promote RNA decay.



CLIP-qPCR demonstrated the direct nature of the interaction between SM1v1 RNA and RC3H1 protein.

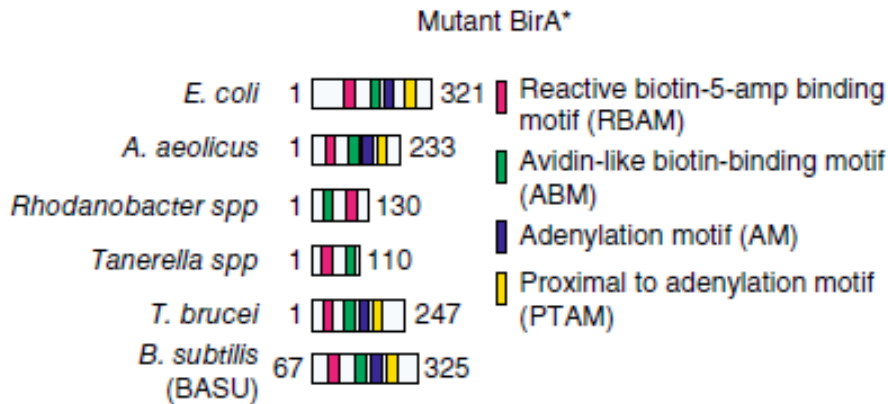


# Expanding the spectrum of the assay

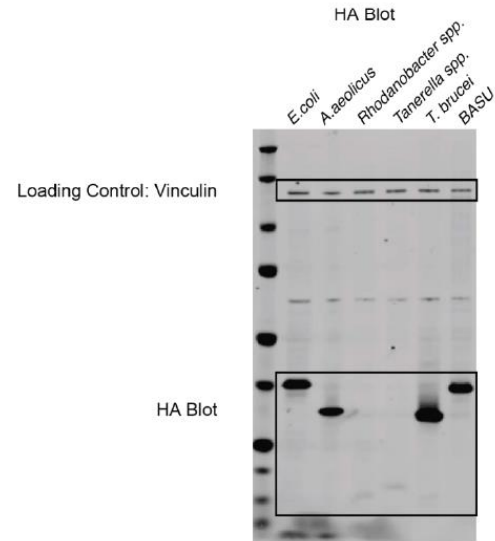
## Additional BirA\* proximity-labeling proteins

*Generation of a faster biotin ligase for RaPID*

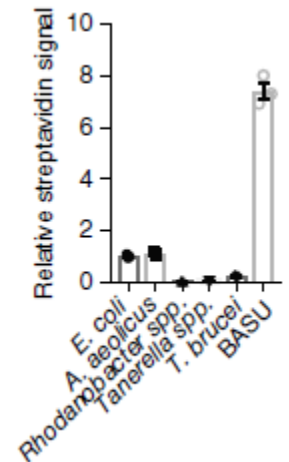
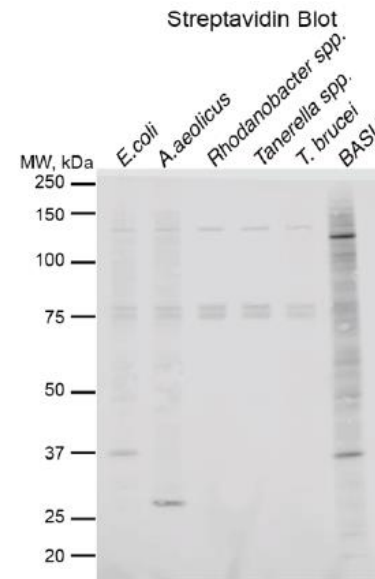
- *E. coli* BirA\* requires 16–18 h for optimal labeling.
- Aim: generation of a faster biotin ligase
- Structural and sequence comparison of *E. Coli* BirA\* to other biotin ligases in the UniProt database lead to the identification of four motifs:
  - **Reactive biotin-5-AMP binding motif (RBAM)**
  - Avidin-like biotin binding motif (ABM)
  - Adenylation motif (AM)
  - Proximal to adenylation motif (PTAM)



- BASU was assessed for labeling activity by exposing cells for 1 h in 200  $\mu$ M biotin media.
- BASU displayed substantially higher global streptavidin signal relative to other mutant BirA\*s



BirA\*s from *Rhodanobacter spp.* and *Tanerella spp.* expressed at a lower level than other BirA\*s



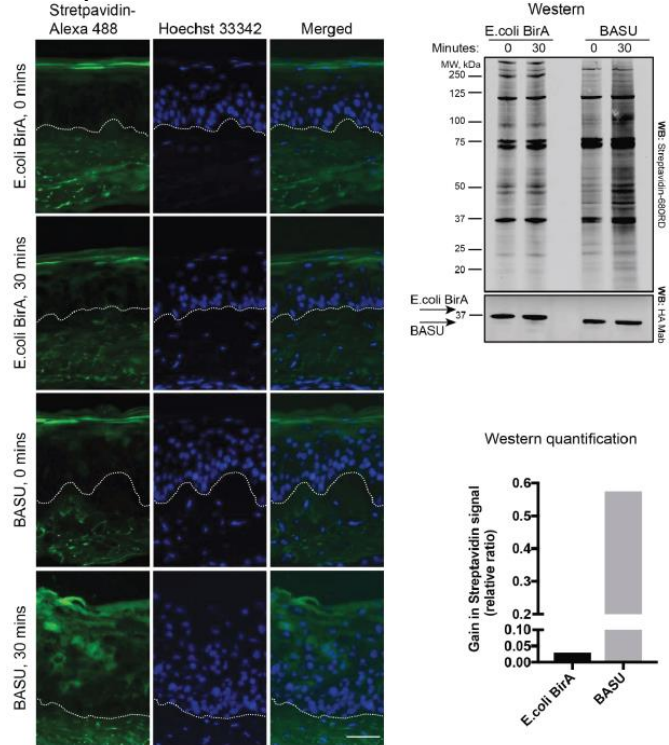


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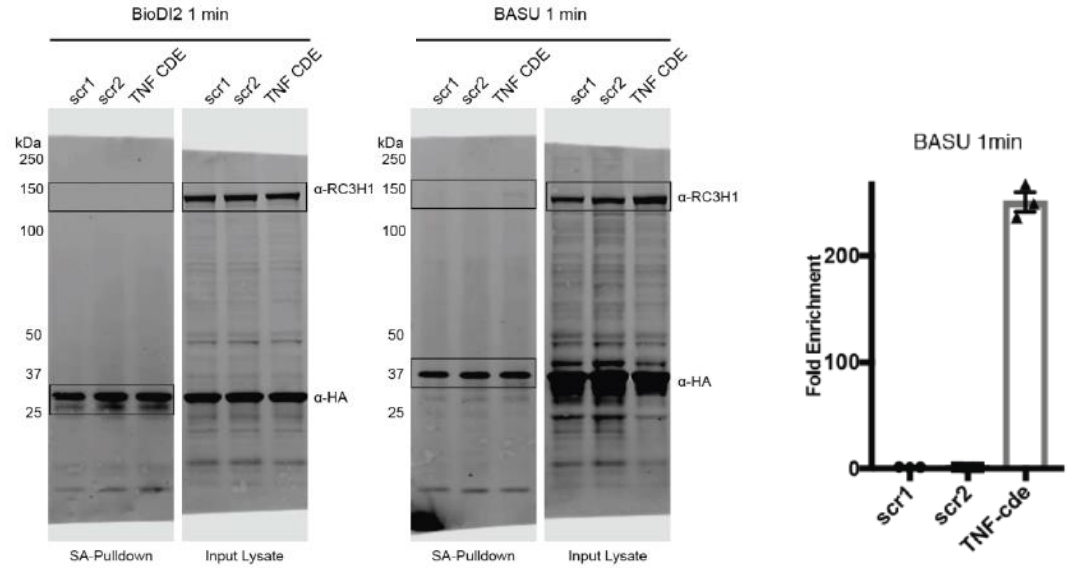
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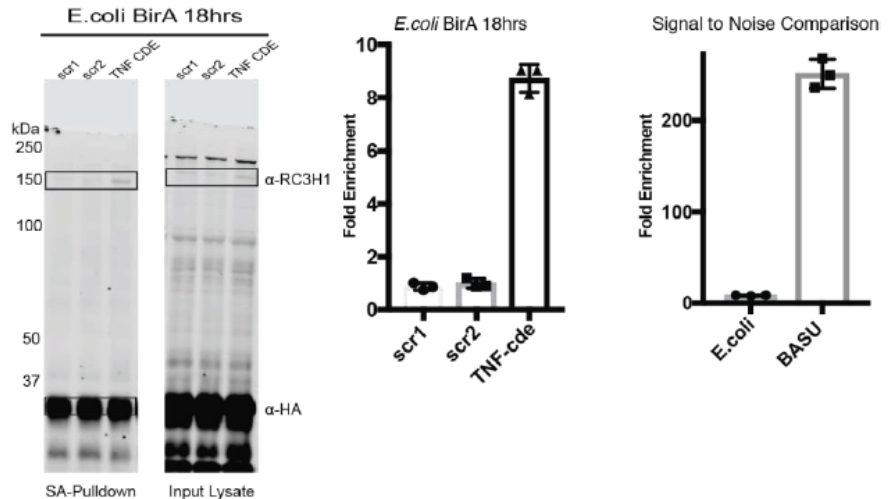
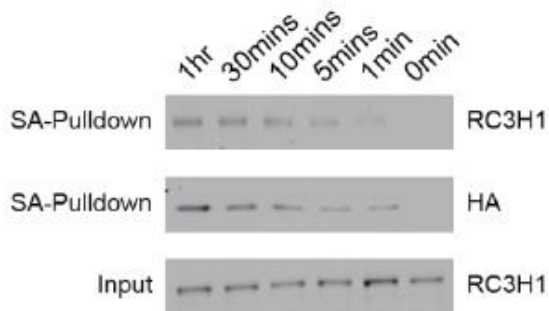
Comparison between *E. coli* BirA\* and BASU



To compare BASU to *E. coli* BirA\* and BioID2 in the context of RaPID, the RC3H1 protein-binding TNF-CDE RNA motif was used.



BASU-RaPID yielded a strong signal in as little as 1 min



# Conclusions

- **Advantages**
  - No need for formaldehyde cross-linking
  - Requires fewer cells
  - Motifs <50 nucleotides long -> short RNA sequences
  - Complement in vitro methods used to study protein-RNA interactions
  - BASU: offers the opportunity to monitor short timescale protein interactions in vivo
- **Limitations**
  - Biotin-ligase-based proximity proteomics are based on biotinylation of lysine residues of proteins, therefore not all proteins might be equally detected because lysine residues aren't equally exposed on their surface
  - RaPID can't distinguish between directly interacting proteins vs proteins that interact indirectly with RNA
  - Can't be used to study endogenous RNAs at their physiological concentrations

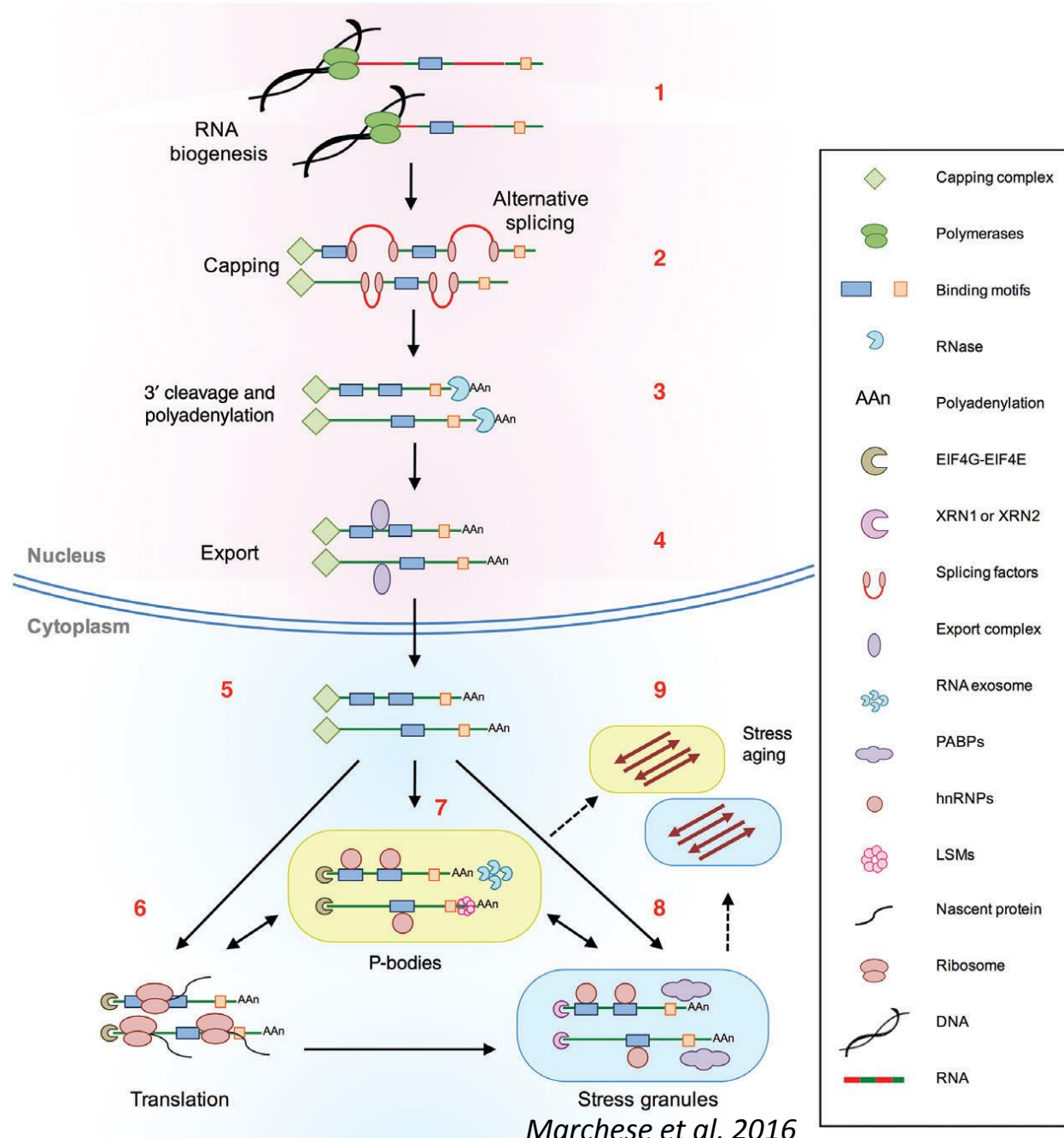


# General conclusions and commentary

- Both methods are robust and the papers are thorough with the appropriate controls and validation experiments
- Both papers demonstrate the types of potential applications for the methodologies they developed
- The first method might be more useful for high throughput screenings vs the second method for targeted validation of RNA-protein interactions



# Every aspect of RNA life involves protein binding



- miRNAs bind to Argonaute proteins to reach target mRNAs
- piRNAs bind to PIWI proteins to form silencing complexes to protect the germline genome from transposons
- Formation of membrane-less organelles (P-bodies, stress granules)
- Mutations in RNA-binding proteins cause human disease (neurodegeneration, cancer)