Landscape and variation of RNA secondary structure across the human transcriptome

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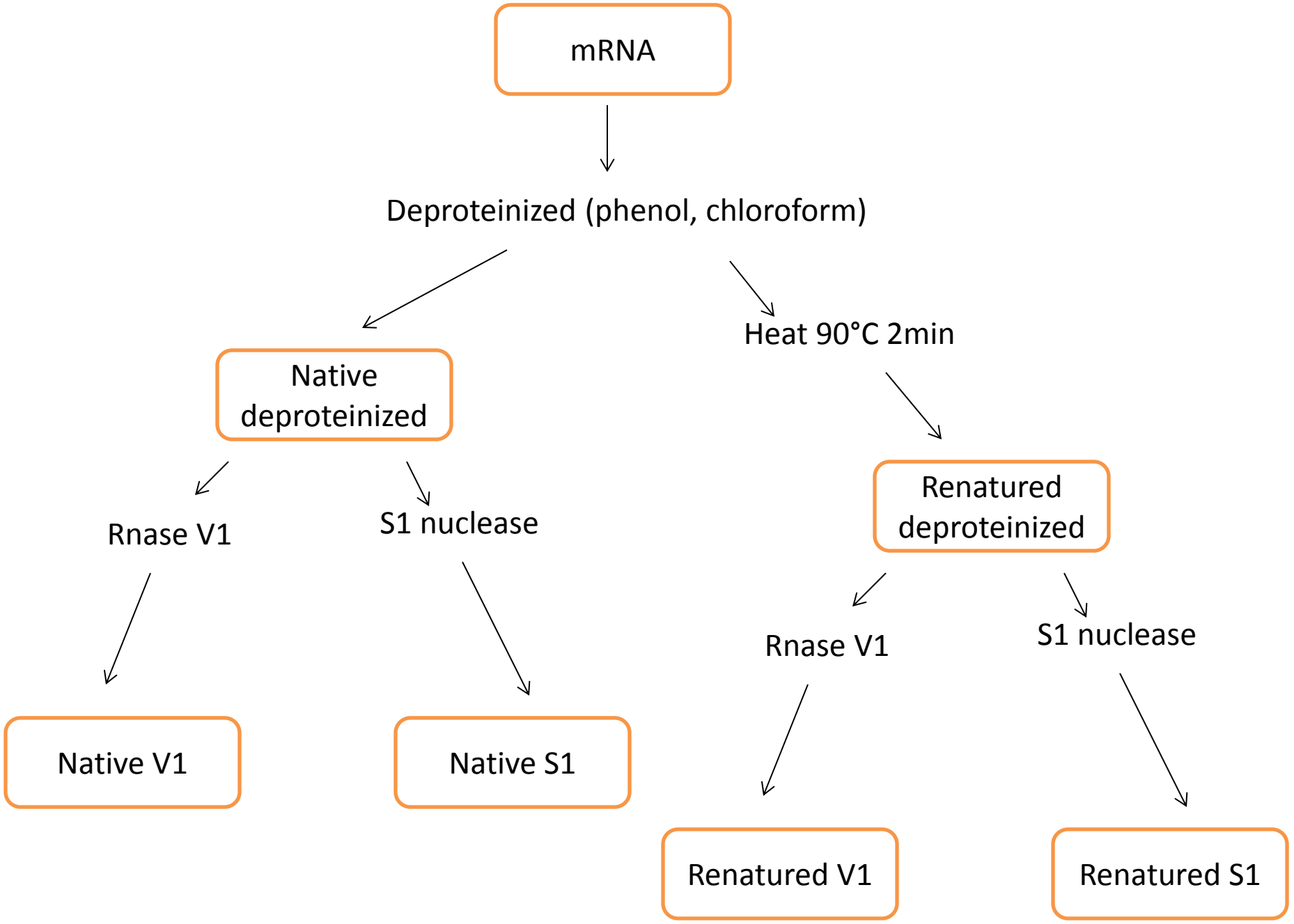
Introduction

- In parallel to the genetic code for protein synthesis, a **second layer of information** is embedded in all RNA transcripts in the form of RNA structure. RNA structure influences practically every step in the gene expression program

SAMPLE

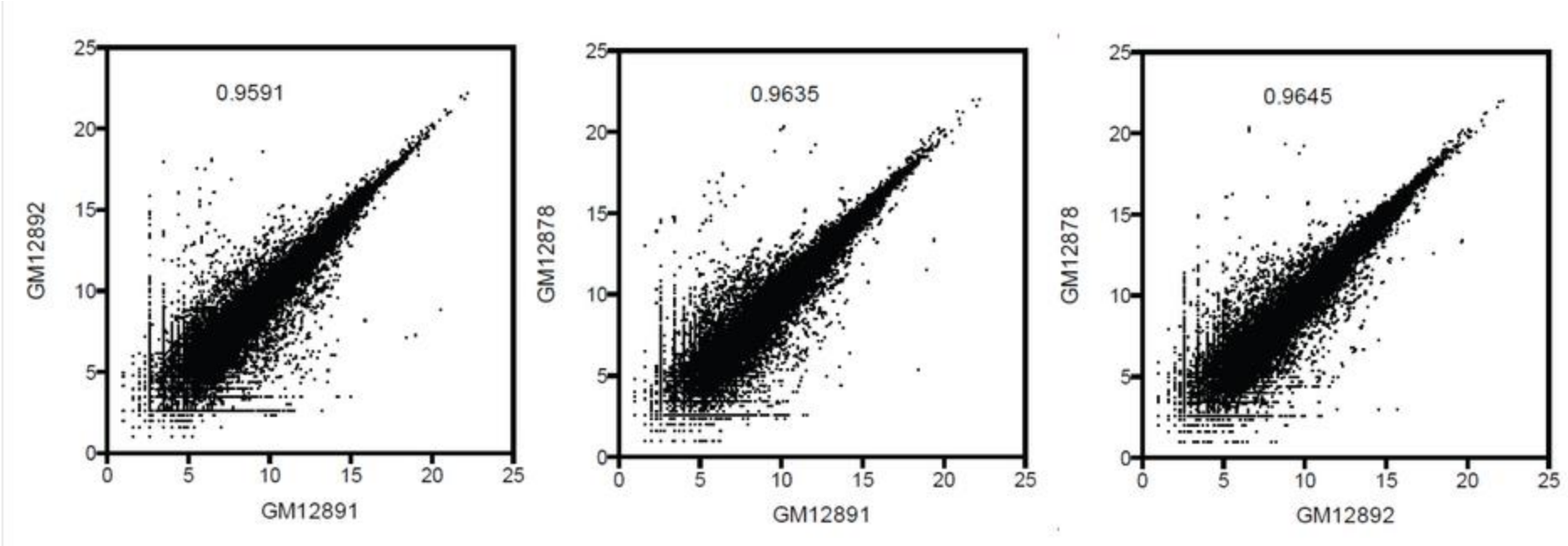
- Performed parallel analysis of RNA structure
  - Human lymphoblastoid cells (GM12878,GM12891,GM12892)
  - poly(A)+ RNA was obtained(mRNA)

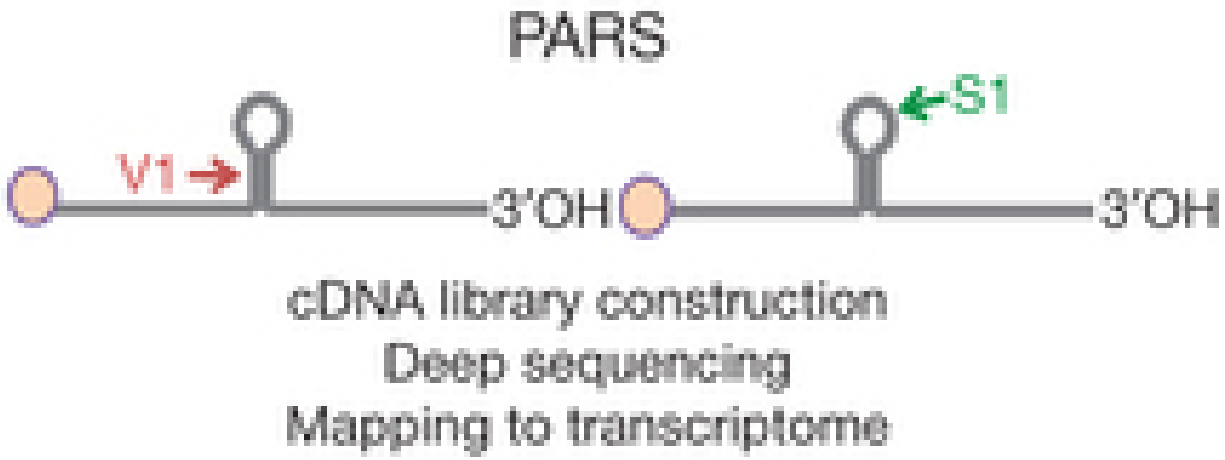

mRNA
Deproteinized (phenol, chloroform)
Native deproteinized
Heat 90°C 2min
Rnase V1
S1 nuclease
Renatured deproteinized
Native V1
Native S1
Rnase V1
S1 nuclease
Renatured V1
Renatured S1

Illumina sequencing and mapping

- Paired-End sequencing
- Raw reads were truncated to **50 bases** (51 bases from the 3’ end were trimmed)
- Mapped to human transcriptome with **Bowtie2**. Non-redundant transcripts from UCSC RefSeq and the Gencode v12 (hg19 assembly)
- They obtained **166- to 212-million mapped reads** for a Rnase v1 or S1 nuclease sample

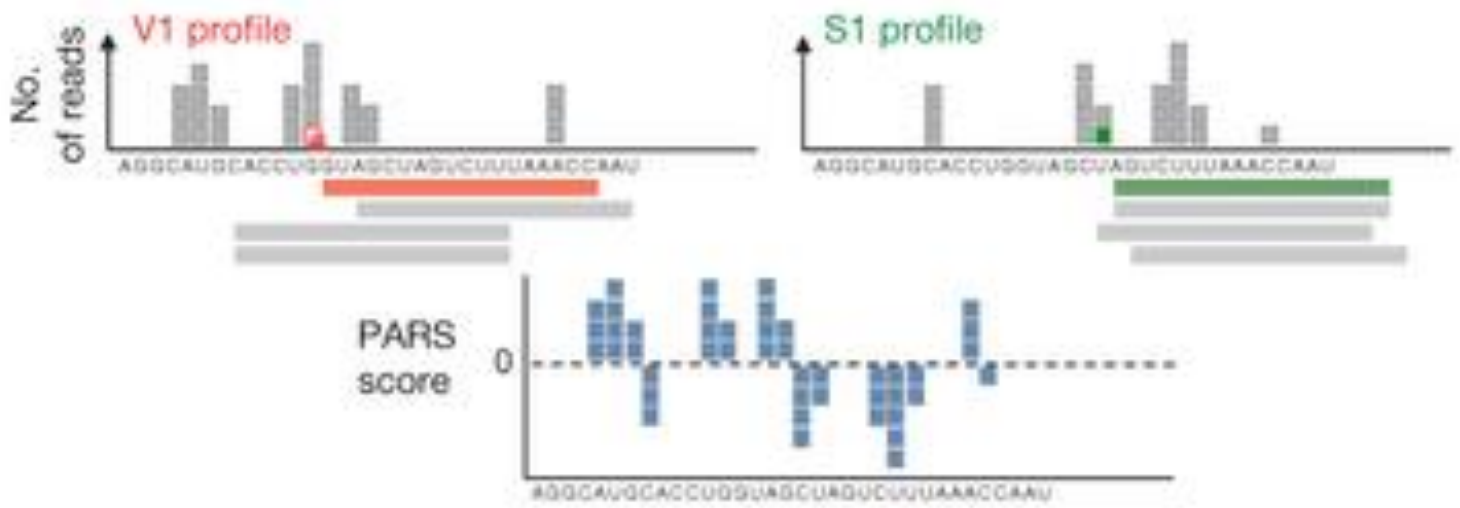
Transcript abundance




PARS
V1
S1
3'OH
3'OH
cDNA library construction
Deep sequencing
Mapping to transcriptome

PARS-score

- Parallel analysis of RNA structure (PARS)
- calculated the number of double-stranded reads and single-stranded reads that initiated on each base on an RNA.



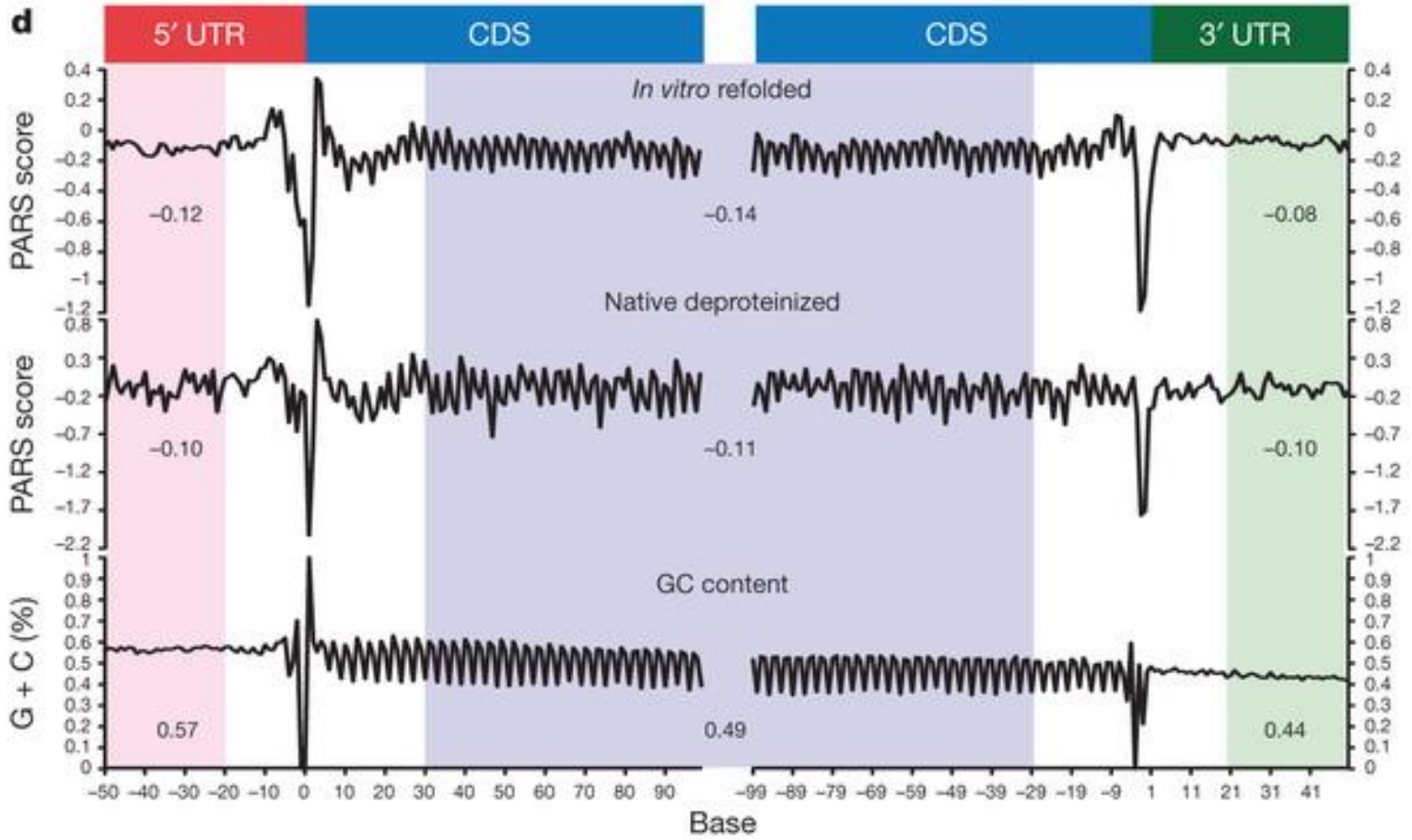
PARS-score

- The number of **double (V1)** and single stranded **reads (S1)** for each sequencing sample were then normalized by sequencing depth
- **Positive score** shows double stranded RNA
- **Negative score** shows single stranded RNA

$$\mathrm{PARS}_{i=1\ldots N} = \log_2(\mathrm{V1}_i + 5) - \log_2(\mathrm{S1}_i + 5)$$

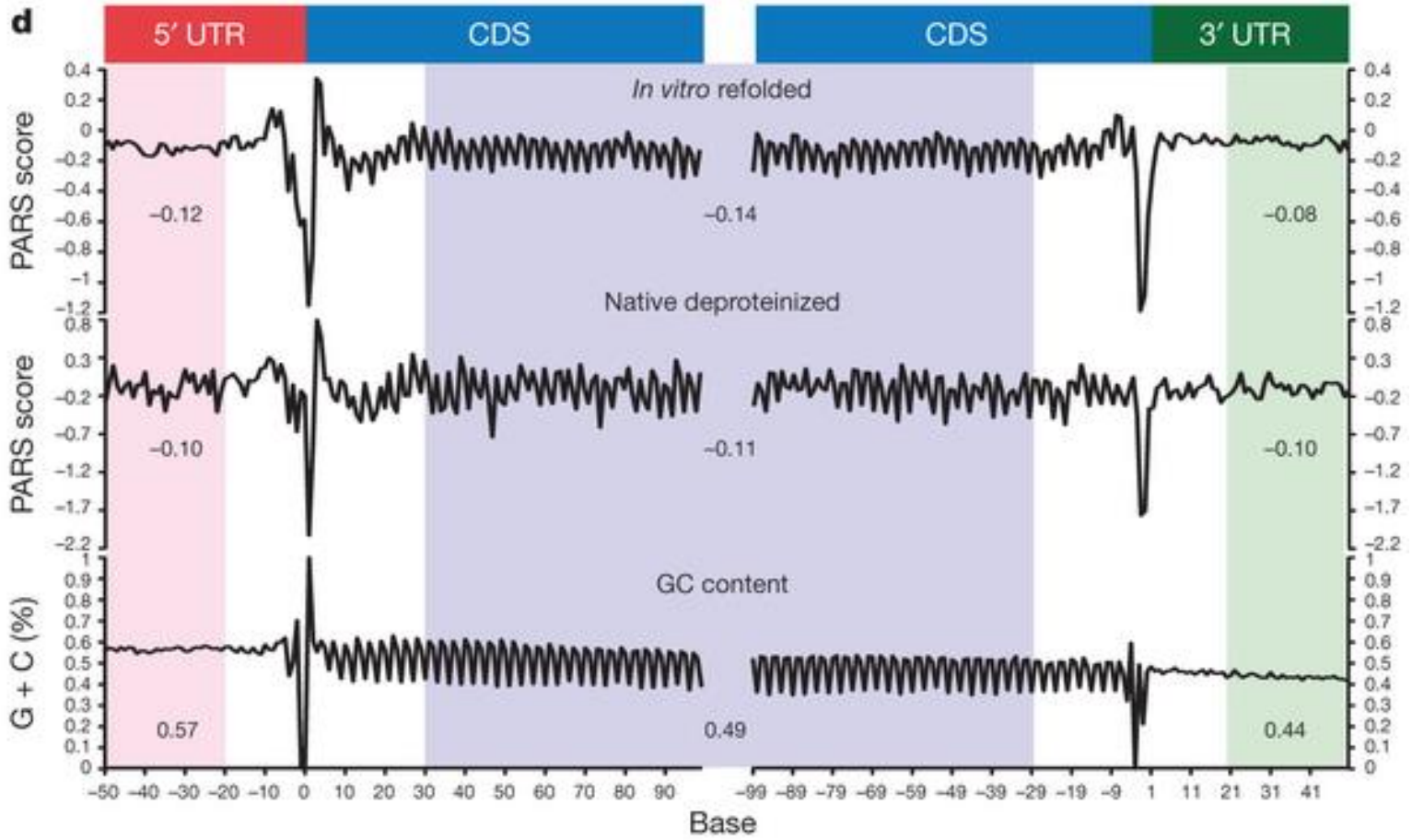
Result

- Both renatured and native mRNAs showed similar RSS features, suggesting that **RNA sequence is a strong determinant of RSS**



Result

- Metagene analysis show that, on average, CDS is more accessible near transational start site and stop codon



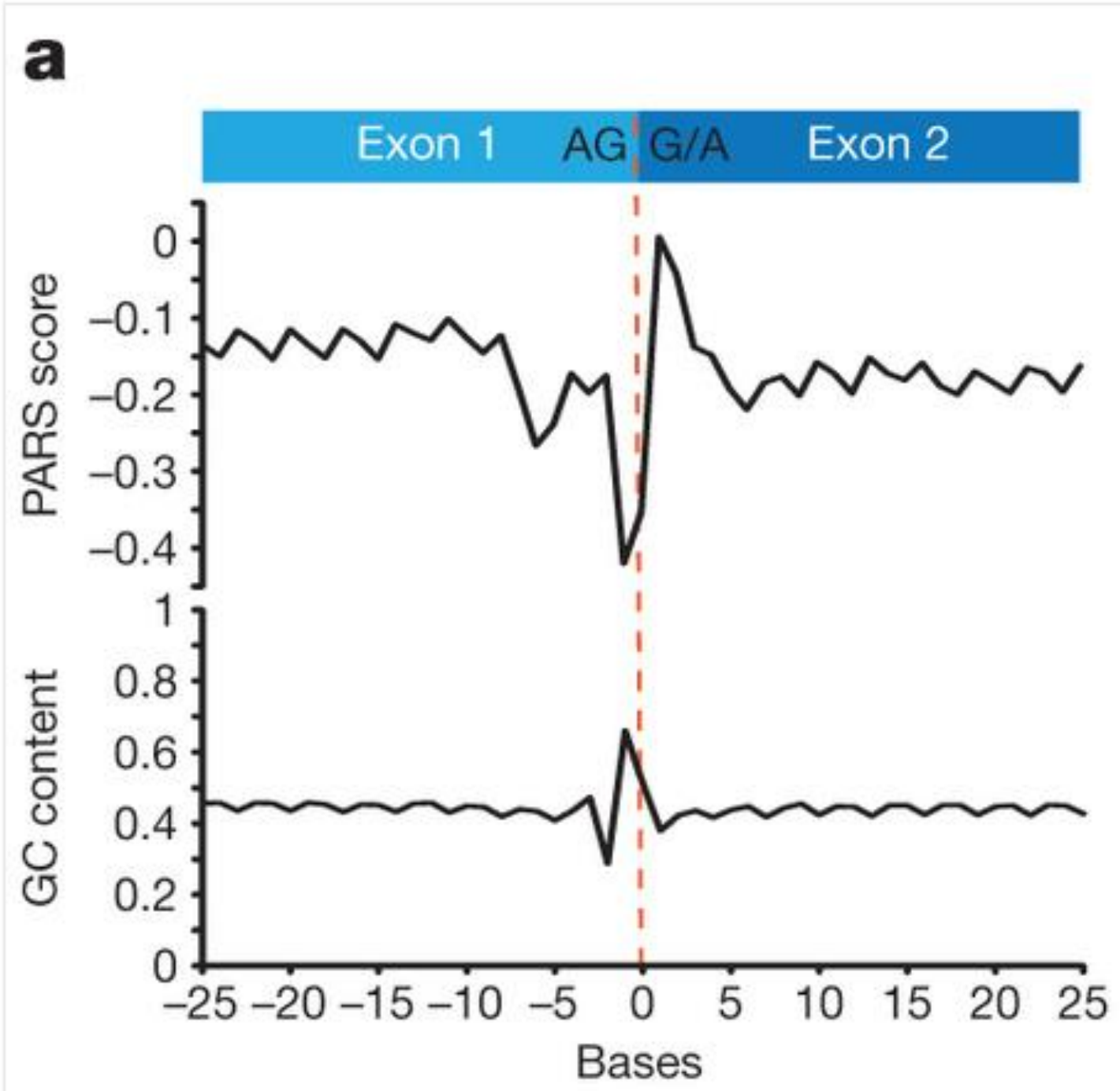
Result

- They also identified 583 (5.7%) consistently different regions between native deproteinized and renatured structure profiles, **providing candidate sites for regulation of RNA structure** *in vivo*

- They noted that 3.7% of bases (residing in 9.7% of transcripts) have both strong V1 and S1 reads, indicating the existence of **multiple mRNA conformations**

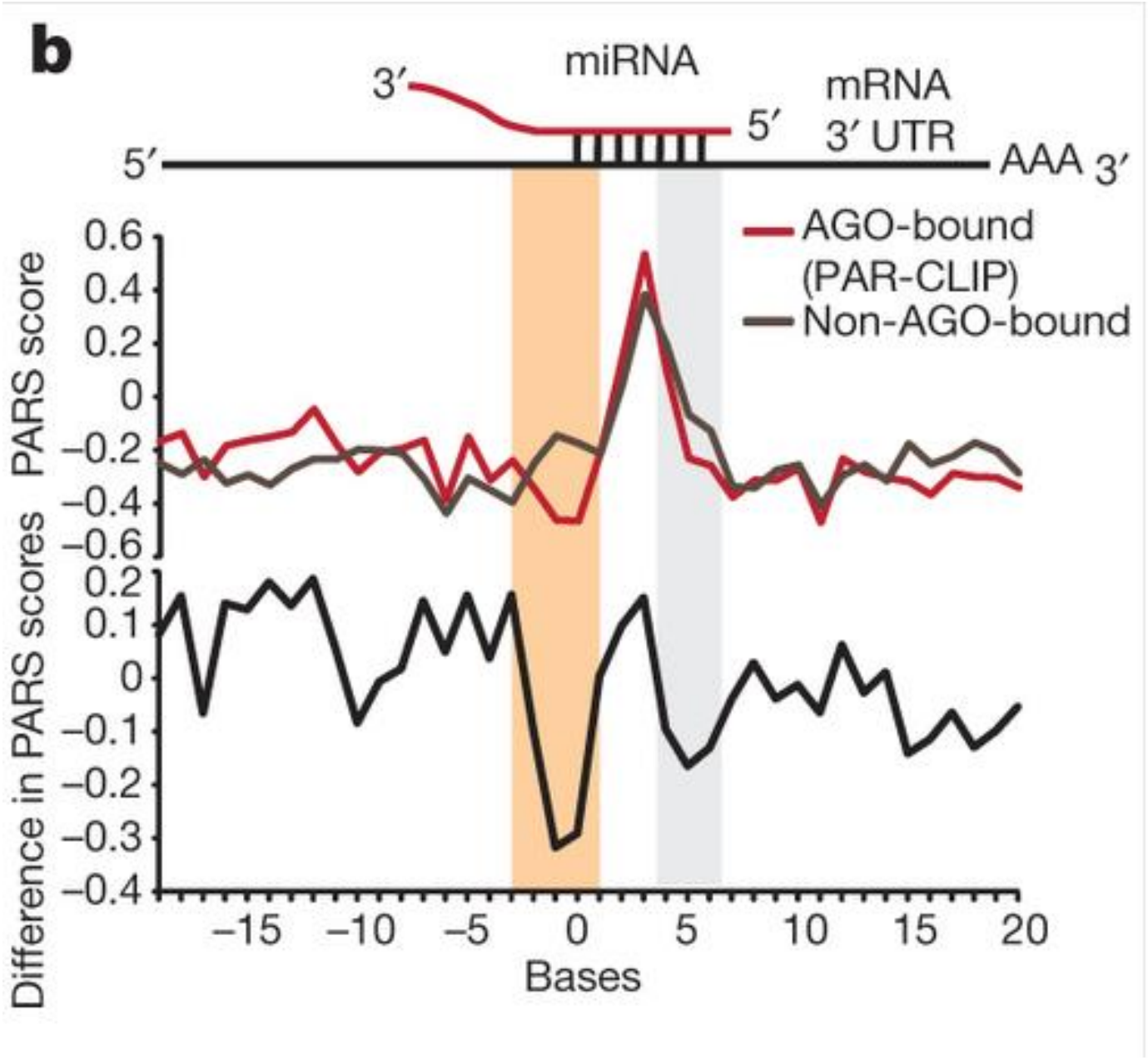
Result

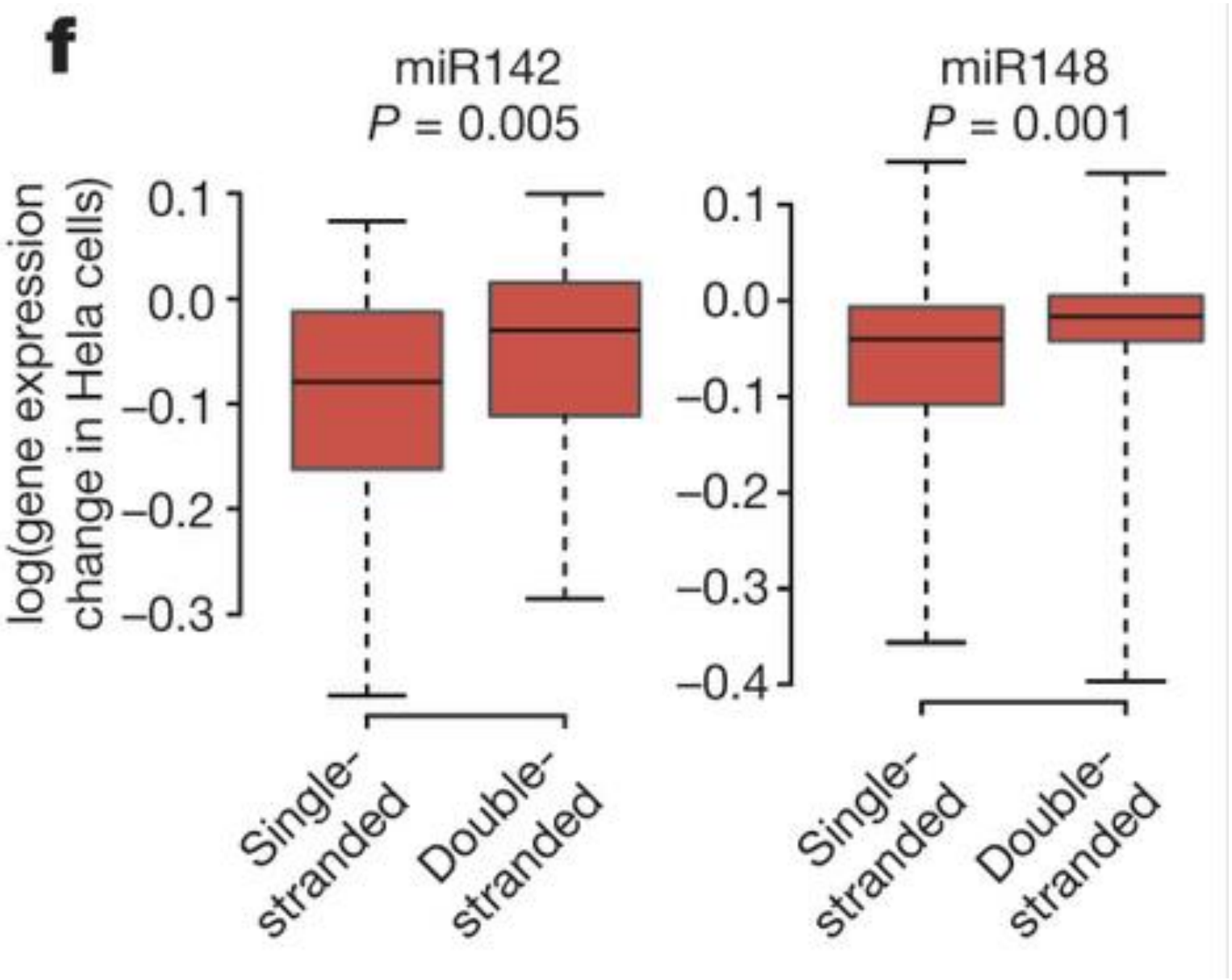
- They observed a unique asymmetric RSS signature at the **exon–exon junction** in both renatured and native deproteinized transcripts that is not simply explained by GC content. The terminal **AG dinucleotide** at the end of the 5′ exon tends to be **more accessible**, whereas the first nucleotides of **the 3′ exon are more structured**


a
Exon 1
AG
G/A
Exon 2
PARS score
0
−0.1
−0.2
−0.3
−0.4
GC content
1
0.8
0.6
0.4
0.2
0
−25 −20 −15 −10 −5 0 5 10 15 20 25
Bases

Rsults

- Analysis of RSS from renatured RNA around predicted miRNA targets revealed that true Argonaute **(AGO)-bound target sites** show strong structural accessibility from −1 to 3 nucleotides upstream of the miRNA-target site compared to predicted targets not bound by AGO


b
miRNA
3′
5′
mRNA
3′ UTR
5′
AAA 3′
AGO-bound
(PAR-CLIP)
Non-AGO-bound
PARS score
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
Difference in PARS scores
0.2
0.1
0
−0.1
−0.2
−0.3
−0.4
−15
−10
−5
0
5
10
15
20
Bases


f
miR142
P = 0.005
miR148
P = 0.001
log(gene expression change in Hela cells)
0.1
0.0
−0.1
−0.2
−0.3
−0.4
Single-stranded
Double-stranded
Single-stranded
Double-stranded

Part 2 riboSNitches

- Comparison of RNA structural landscapes between individuals revealed the **impact of diverse sequence variants on RNA structure**.

- SNVs that alter RNA structure, known as **‘riboSNitches’**

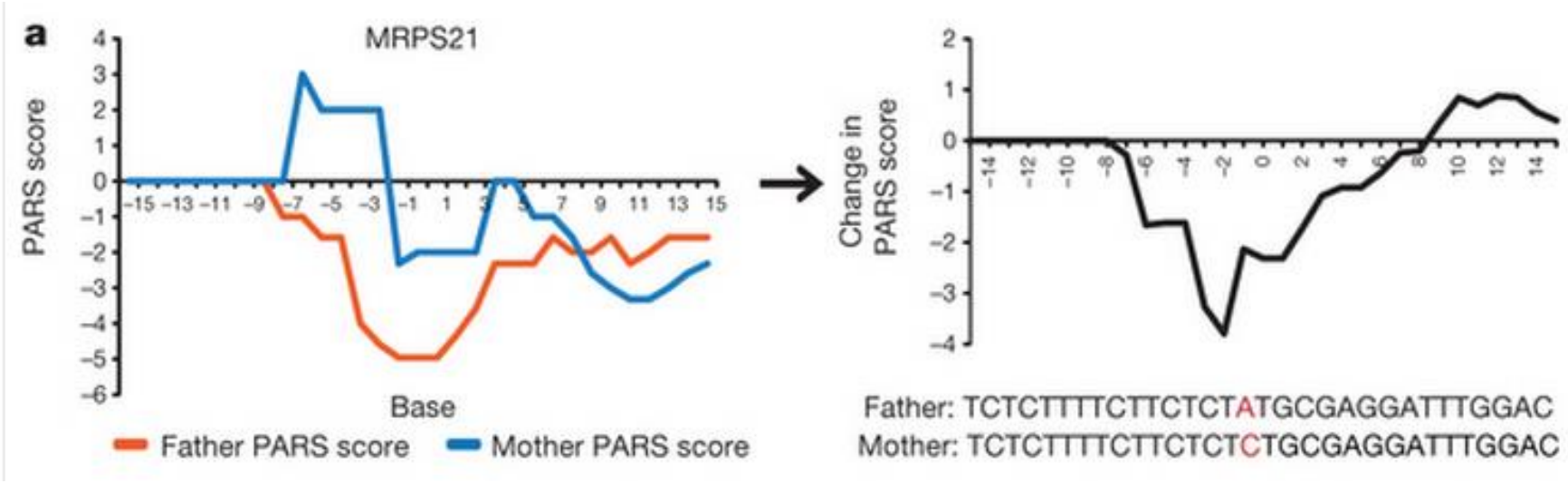
Part 2 riboSNitches

- Furthermore, **78.2%** of all structure changing bases lie in transcripts that contain either SNVs or indels, suggesting that sequence variation is important in shaping RSS variation in the human transcriptome

Part 2 riboSNitches

- To **pinpoint riboSNitches**, we calculated structure changes between each pair of individuals and selected SNVs that had large **PARS score differences, low false discovery rate** (FDR), **significant *P* value**, **and high local read coverage**

Part 2 riboSNitches



Result

- We found that **1,907** out of 12,233 (15%) SNVs switched RNA structure in the trio.
- They also **experimentally validated** nine riboSNitches using independent structure probing methods such as nucleases, selective 2′ hydroxyl acylation and primer extension (SHAPE) or dimethyl sulphate (DMS), and **confirmed the ability of PARS to discover riboSNitches**

Result

- Out of **172** parental homozygous riboSNitches, **117** (68%) were validated by allele-specific mapping in the child. **As only reads upstream of the riboSNitch can be uniquely mapped and detected, this is likely to be an underestimate**. We also observed a validation rate of 61% in native deproteinized samples of the child, indicating that the structural changes are biologically relevant *in vivo*

Result

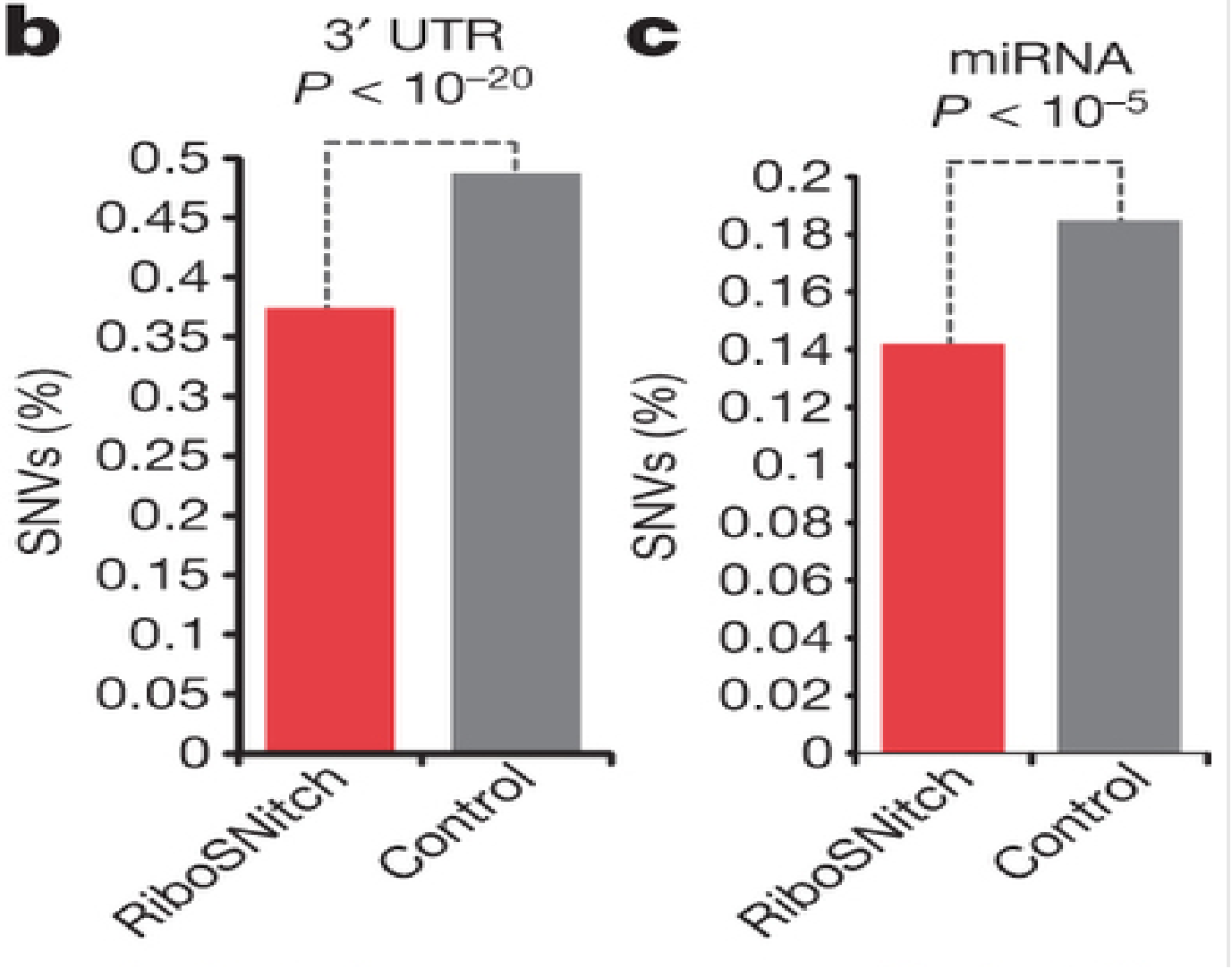
- Intersection with expression quantitative trait loci (eQTL) identified **211** riboSNitches that are associated with changes in gene expression.

sequence and context rules in riboSNitches

- **First**, riboSNitches that lie in double- or single-stranded regions tend to become more single- or double-stranded, respectively, after nucleotide change.
- **Second**, the nucleotide content of the riboSNitch is instructive of the direction of RSS change. Bases that undergo G/C to A/T changes tend to become more single-stranded, whereas bases that change from A/T to G/C tend to become more paired

sequence and context rules in riboSNitches

- **Third**, the structural context flanking SNVs influence their transition to become more single- or double-stranded
- **Fourth**, riboSNitches have fewer SNVs around them as compared to non-structure changing SNVs, suggesting that co-variation of some SNVs may help to maintain functional RNA structures



- riboSNitches are also significantly depleted at 3’ UTR compared to control (structurally synonymous SNV) and also around predicted miRNA target sites

RiboSNitches

- RiboSNitches may also influence gene regulation through splicing. Indeed, riboSNitches near splice junctions are associated with greater alternative splicing changes ,suggesting that **RNA structures could regulate splicing.**

Conclusion

- They identified unique RSS signatures that demarcate **open reading frames** and **splicing junctions**, **and define authentic microRNA-binding sites**.
- Majority of the RSS information is encoded within RNA sequence.
- Over **1,900** transcribed single nucleotide variants (approximately 15% of all transcribed single nucleotide variants) **alter local RNA** structure.
- They discovered **simple sequence and spacing rules** that determine the ability of point mutations to impact RSSs.
- Selective depletion of **'riboSNitches**' versus structurally synonymous variants at precise locations suggests selection for specific RNA shapes at thousands of sites, including 3'UTR, binding sites of microRNAs and RNA-binding proteins genome-wide.

Conclusion

- These results highlight the potentially broad contribution of RNA structure and its variation to gene regulation.

Tänan kuulamast!