

LANDSCAPE AND VARIATION OF RNA SECONDARY STRUCTURE ACROSS THE HUMAN TRANSCRIPTOME

Y. Wan et al. (2014)

INTRODUCTION

THE STUDY

Transcriptome-wide study of RNA secondary structure (RSS) in a family trio (mother, father and son)

- RSS landscape of mRNA
- Influence of RSS on gene regulation
- Effects and importance of SNVs on RSS

OVERVIEW

1. Background to RSS

2. How to technically assess RSS

3. How to measure RSS

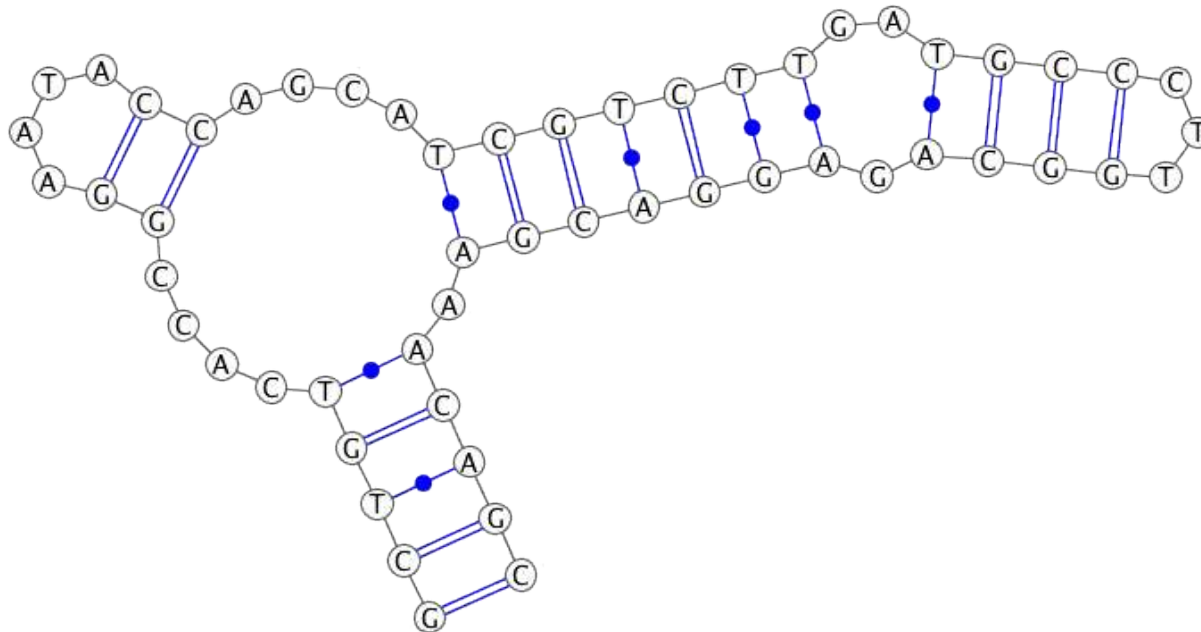
4. How to study effects of RSS

5. Results & Lookout

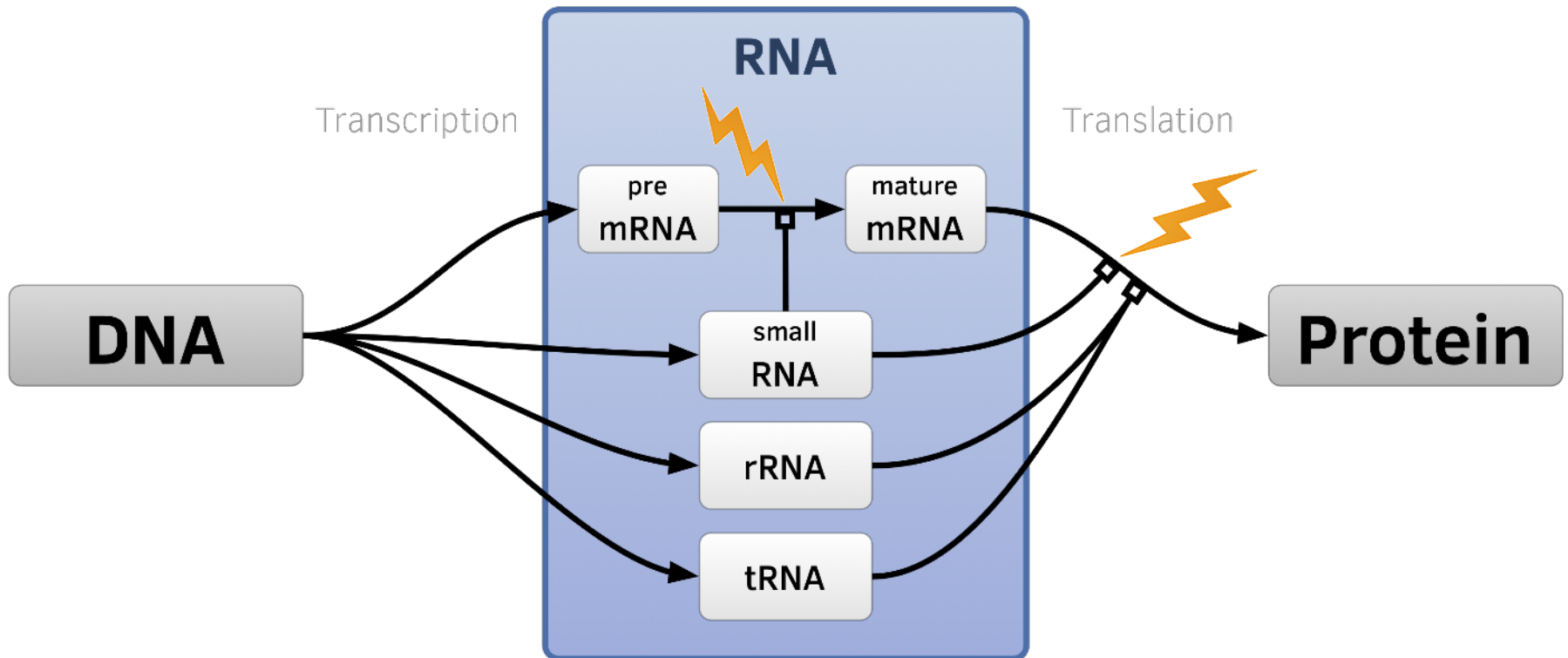
BACKGROUND

RNA SECONDARY STRUCTURE

Sequence → **Secondary Structure** → Tertiary Structure



INFORMATION FLOW



INFLUENCE OF RSS

- Gene regulation
 - Accessibility of miRNA
- Splicing
 - Accessibility of spliceosome
- Folding
 - Guidance after translation

METHODS

WORKFLOW

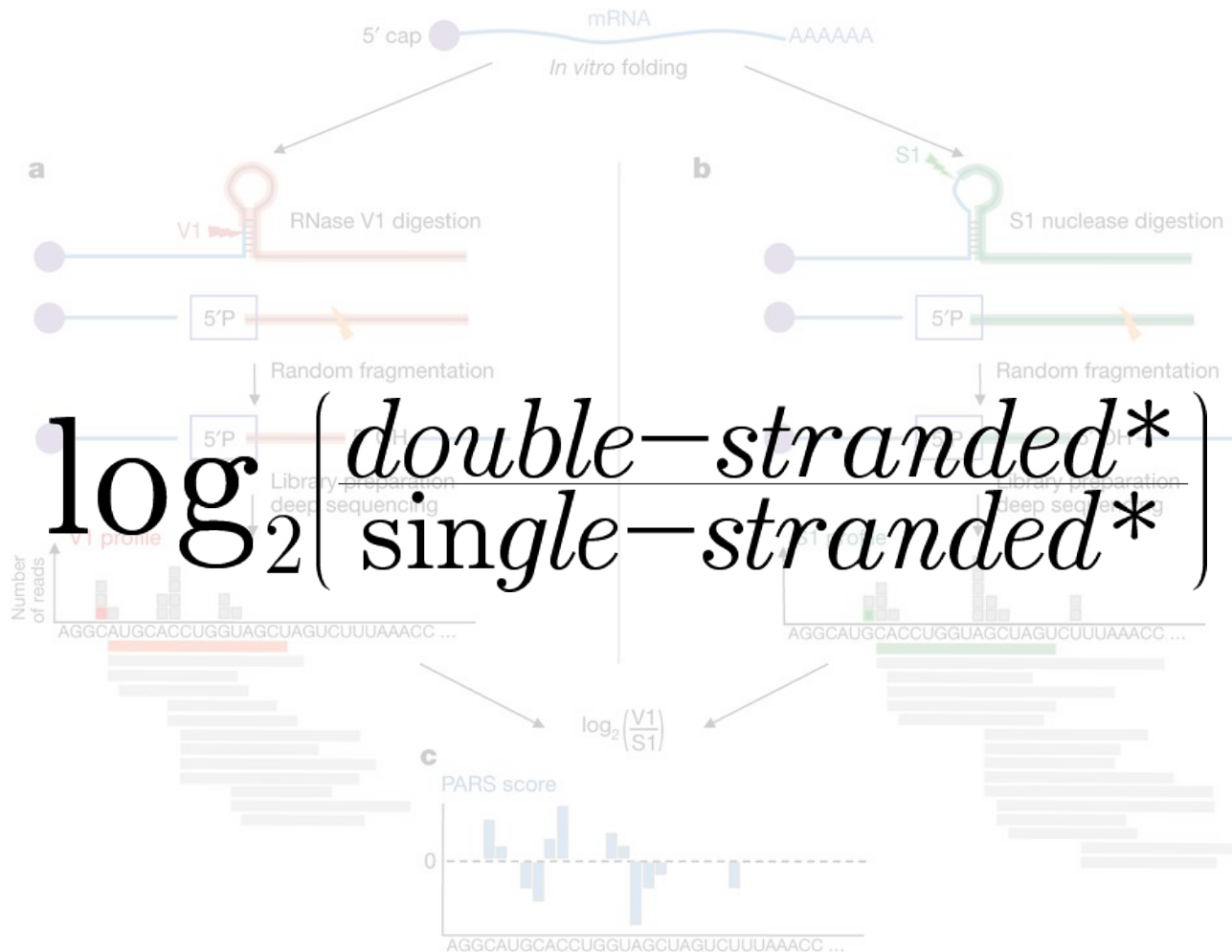
Wet laboratory

1. RNA extraction
2. RNA structure probing
3. Library preparation
4. Deep Sequencing

Dry laboratory

1. Read mapping to human transcriptome
2. PARS score
3. Structural differences score
4. Impact of SNVs on RSS

PAIRWISE ANALYSIS OF RNA STRUCTURE (PARS)

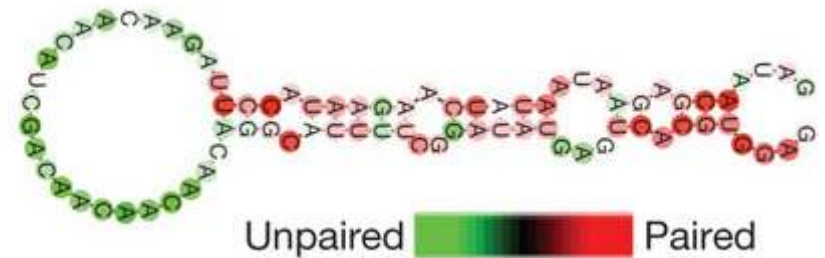


* Number of normalised mapped reads for RNase V1 and S1 nuclease

PARS SCORE

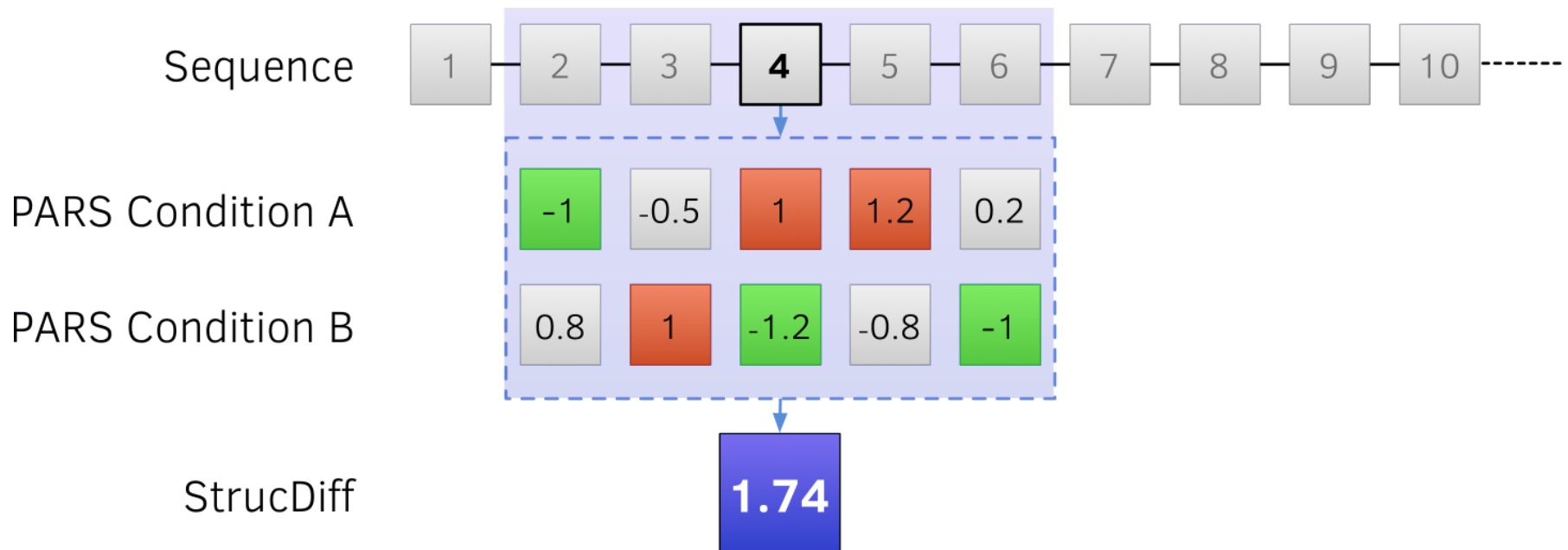
Less Structure = more unpaired = score < 0

More structure = more paired = score > 0



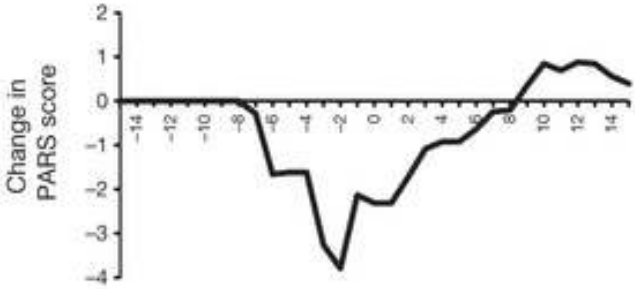
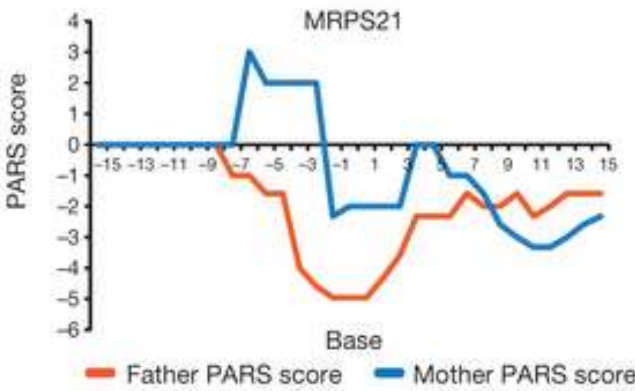
STRUCTURAL DIFFERENCE SCORE

Normalised, absolute difference between PARS score of 2 conditions in a 5 bp window.



STRUCTURAL DIFFERENCE SCORE

Father: TCTCTTTTCTTCTCTATGCGAGGATTTGGAC
 Mother: TCTCTTTTCTTCTCTGCGAGGATTTGGAC



Father: TCTCTTTTCTTCTCT**A**TGCGAGGATTTGGAC
 Mother: TCTCTTTTCTTCTCT**C**TGCGAGGATTTGGAC

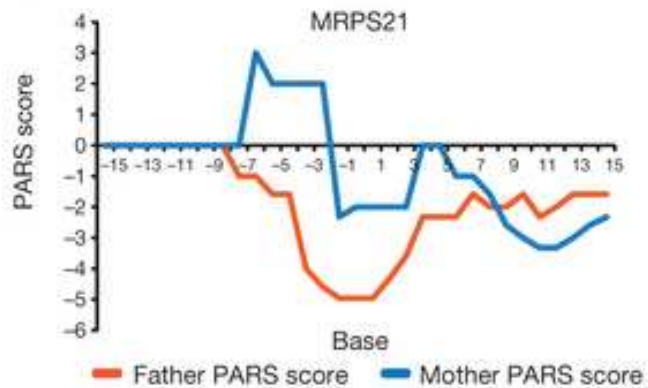
RIBOSNITCHES

SNV that might significantly alter RSS are called riboSNitches.

1. Structural Difference Score > 1
2. P-value < 0.05
3. FDR less than 0.1
4. Local read coverage > 10 and at least 3 single / double-stranded reads in 11 bp window

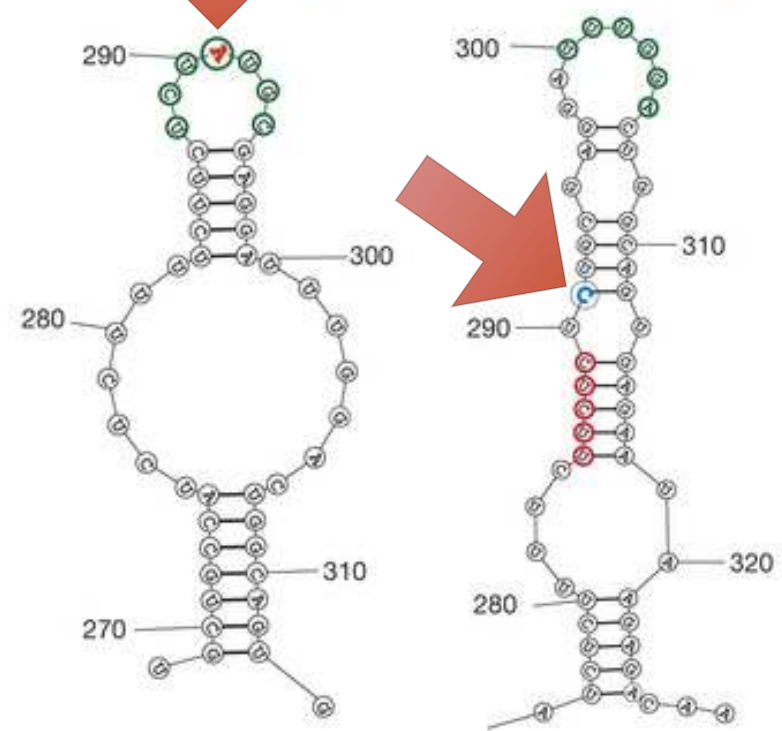
RIBOSNITCHES

Father: TCTCTTTTCTTCTCTATGCGAGGATTTGGAC
 Mother: TCTCTTTTCTTCTCTGCGAGGATTTGGAC



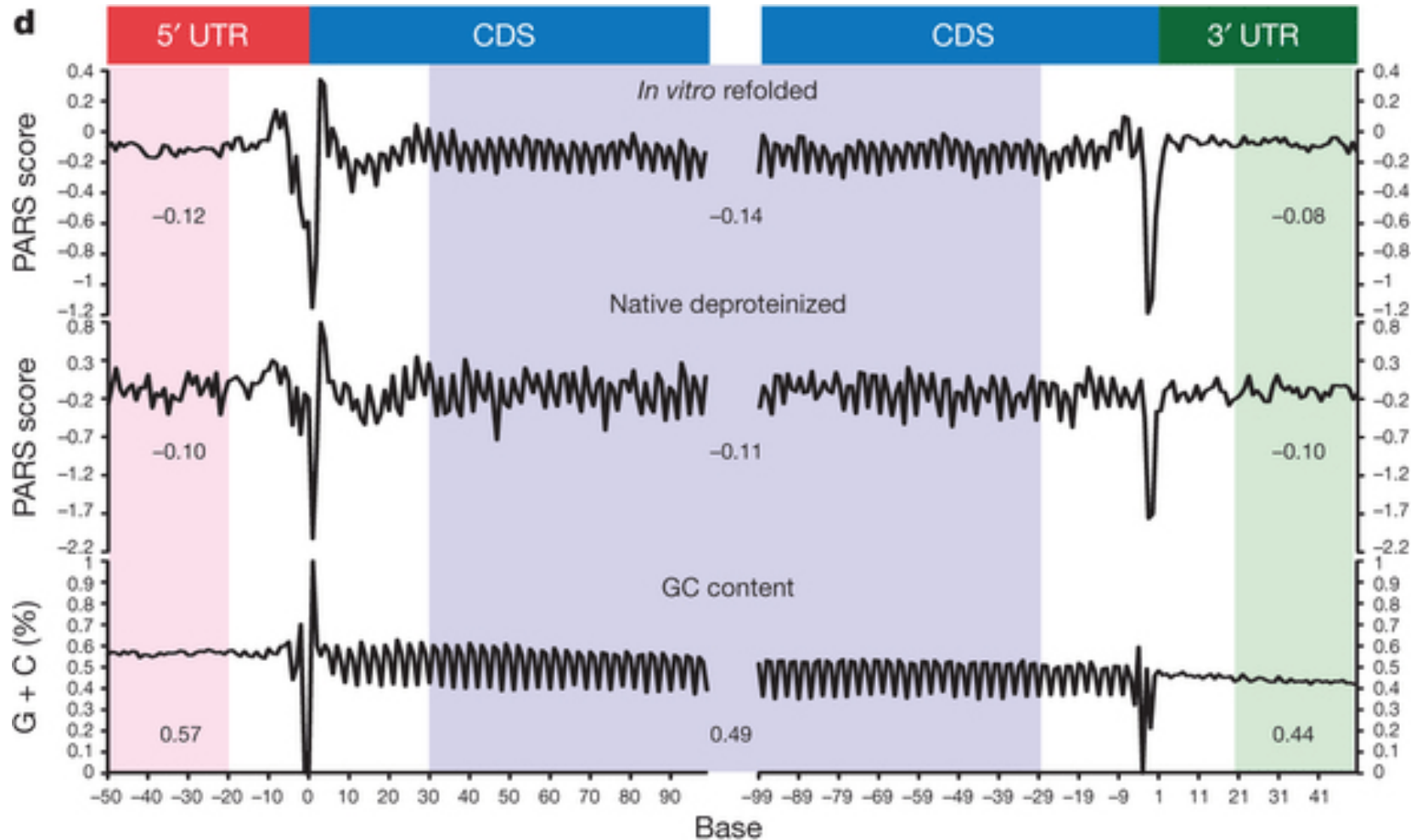
Father: TCTCTTTTCTTCTC**A**TGCGAGGATTTGGAC
 Mother: TCTCTTTTCTTCTC**C**TGCGAGGATTTGGAC

MRPS21 Seqfold Father (A) allele MRPS21 Seqfold Mother (C) allele



RESULTS

RSS LANDSCAPE (1)

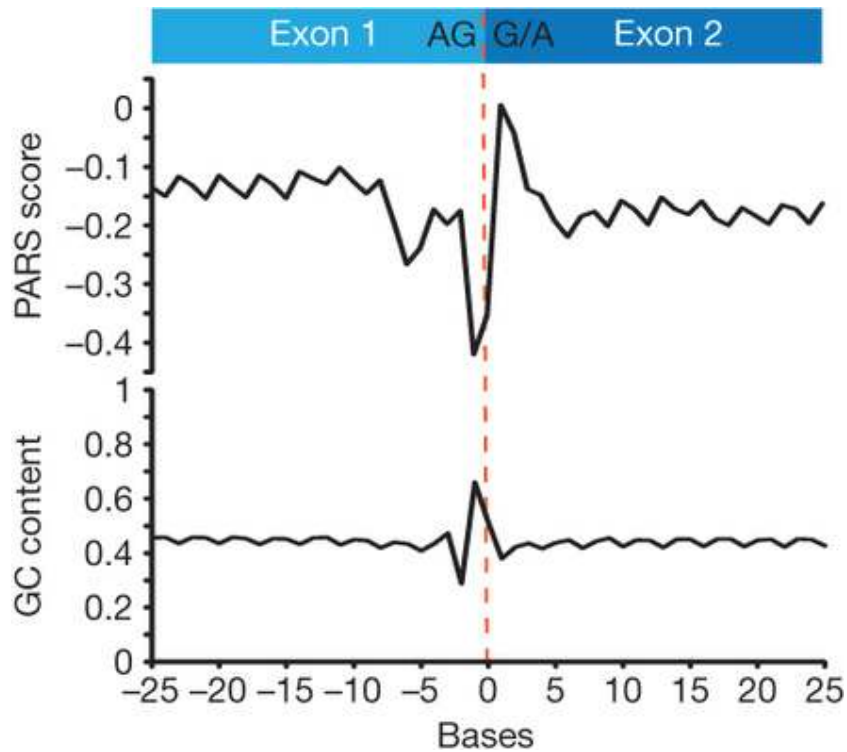


RSS LANDSCAPE (2)

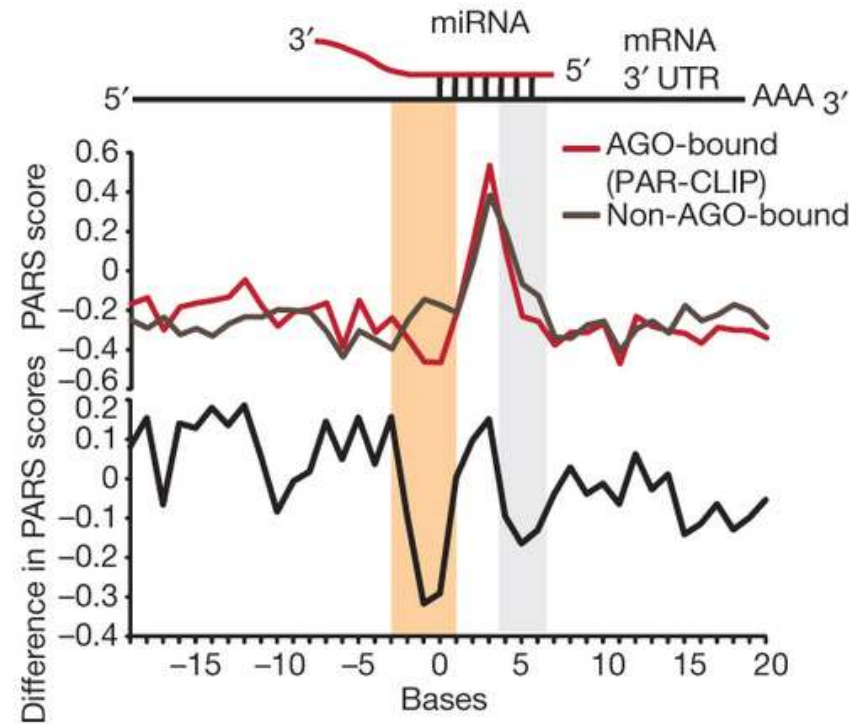
- High similarity re-folded vs native deproteinised
 - RSS mainly determined by sequence
- Highly structured RNA have less variations
 - RSS important for RNA function

POST-TRANSCRIPTIONAL REGULATION (1)

Splicing



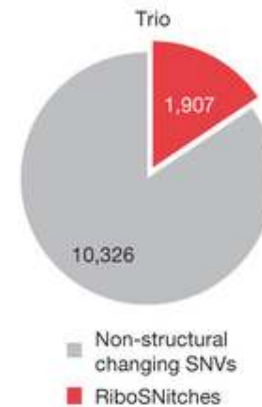
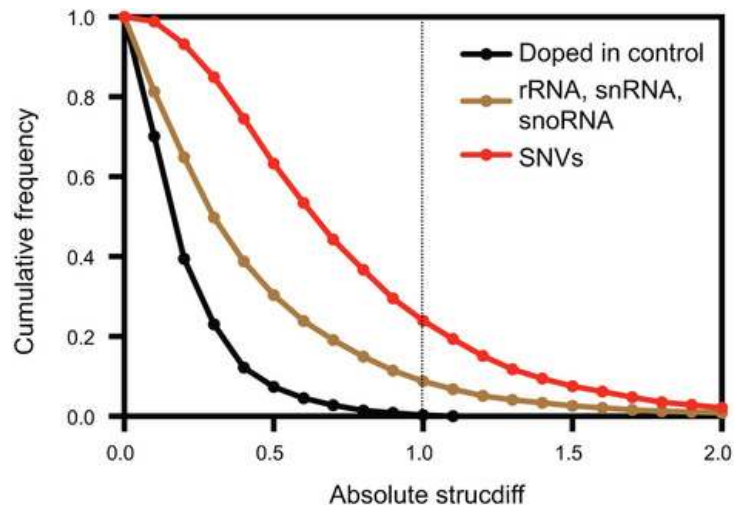
miRNA-related AGO binding



POST-TRANSCRIPTIONAL REGULATION (2)

- Unique RSS signature around exon-exon junctions
- Structural accessibility drives miRNA binding possibility
 - miRNA binding drives AGO / RISC binding
 - Gene silencing

RIBOSNITCHES



- The more RSS is functionally important the less riboSNitches were found!
- RiboSNitches influes gene regulation, splicing and diseases

DISCUSSION

DISCUSSION

- Native deproteinised \neq in vivo
 - Issues raised earlier still not addressed
 - 18S RNA PARS score only agrees to 50%
 - 9 of 14 tRNA structures predicted incorrectly
- Secondary \neq tertiary structure
- PARS scores useful for folding algorithms (SeqFold)
- Importance of SNVs in non-coding regions

SUMMARY

SUMMARY

- RSS determines accessibility of RNA
 - Translation and gene regulation
 - Splicing
- PARS: method for genome-wide study of RNA secondary structure
- SNVs can alter RSS & function of RNA (riboSNitches)

THANK YOU!

Questions?

APPENDIX

RECOMMENDED READING

1. Mauger & Weeks (2010) Toward global RNA structure analysis. [PMID:21057487](#)
2. Lokody (2014) RNA: riboSNitches reveal heredity in RNA secondary structure. [PMID:24535248](#)
3. [What do your riboSNitches tell about you?](#) (2014)
4. Wan et. al. (2014) Landscape and variation of RNA secondary structure across the human transcriptome. [PMID:24476892](#)
5. Kertesz & Wan et. al. (2010) Genome-wide Measurement of RNA Secondary Structure in Yeast. [PMID:20811459](#)
6. Ouyang et al. (2013) SeqFold: genome-scale reconstruction of RNA secondary structure integrating high-throughput sequencing data. [PMID:23064747](#)

* Ordering: easy to hard to additional

STRUCTURAL DIFFERENCE SCORE

Z-score: $(\text{StrucDiff} - \text{mean}) / \text{s.d. of pos. control}^1$

P-value: Estimated likelihood that a 5-base average permuted² PARS structural difference is greater than the 5-base average of a SNVs PARS structural difference.

FDR: Multi-hypothesis in R using p.adjust

¹ Tetrahymena ribozyme acts as positive control

² 1000 permutations of abs. PARS score differences of transcript containing SNVs

STUDY: RSS & GENE REGULATION

- Argonaute (AGO) is an important catalyst in RISC
- RISC is responsible for gene silencing
- Predict miRNA target sites and assess PARS score
- Compare true and false AGO-binding sites
- Compare real AGO-binding with iCLIP
- Study repression of mRNA with high and low miRNA accessibility in HeLa cells

STUDY: RIBOSNITCH

- Predict riboSNitches *in silico*
- Experimentally confirm using RNA structure probing (e.g. SHAPE)
- Compare PARS score derived RSS with experimental data

RIBOSWITCH VS SNITCH

Riboswitch: mRNA binding sites which cause a significant structural and functional change of the mRNA.

RiboSNitch: a SNV / SNP that putatively alters the RSS significantly and might point us to a possible Riboswitch.