



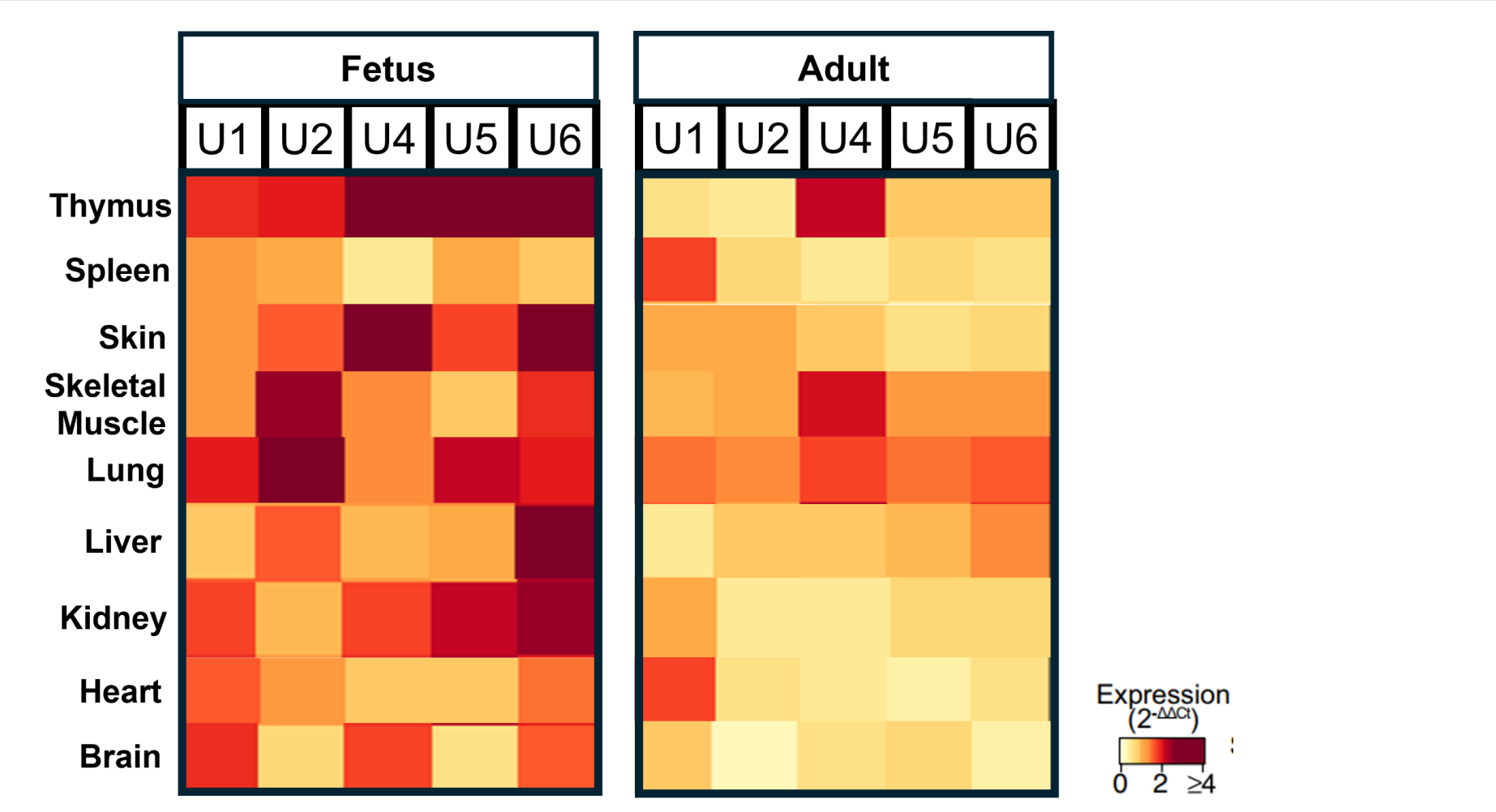
# Deciphering the snRNP Code: Regulatory Mechanisms of Small Nuclear Ribonucleoprotein Abundance and Repertoire

Sanat Myti, Nhi Nguyen, Jacob Navarro, Christian Peck, Duc Ngu, Ayesha Khan, and Byung Ran So  
Department of Chemistry and Biochemistry, The University of Texas at Arlington

## Abstract

Ribonucleoprotein complexes play a crucial role in post-transcriptional gene expression. Spliceosome is a macromolecular RNA:Protein (RNP) complex that facilitates splicing, producing full-length messenger RNAs. Comprising five small nuclear ribonucleoproteins (snRNPs) and over fifty splicing factors, this machinery organizes the assembly and catalysis required for the conversion of premature mRNA into mature forms. While the spliceosomes require equal stoichiometry of snRNPs for splicing, the abundance of snRNP vary significantly in a tissue- and development-specific manner. However, the molecular mechanisms by which cells regulate snRNP abundance and its repertoire remain poorly understood. This process involves the SMN complex, a multi-component RNP chaperone responsible for assembling an Sm core on each snRNA and ensuring the stability of snRNPs. Here, we examined the snRNP code and identified interactions between snRNP-specific proteins and the SMN complex. We measured the in vitro Sm core assembly activity on snRNAs and the abundance of native snRNPs in HeLa cells with snRNP-specific protein knockdown by siRNA transfection. Our findings reveal that some snRNP-specific proteins not only reduced canonical snRNP assembly, but also enhanced others. These results suggest that snRNP-specific proteins play a role in regulating snRNP abundance by either promoting snRNP assembly or stabilizing snRNPs, mediated by the SMN complex.

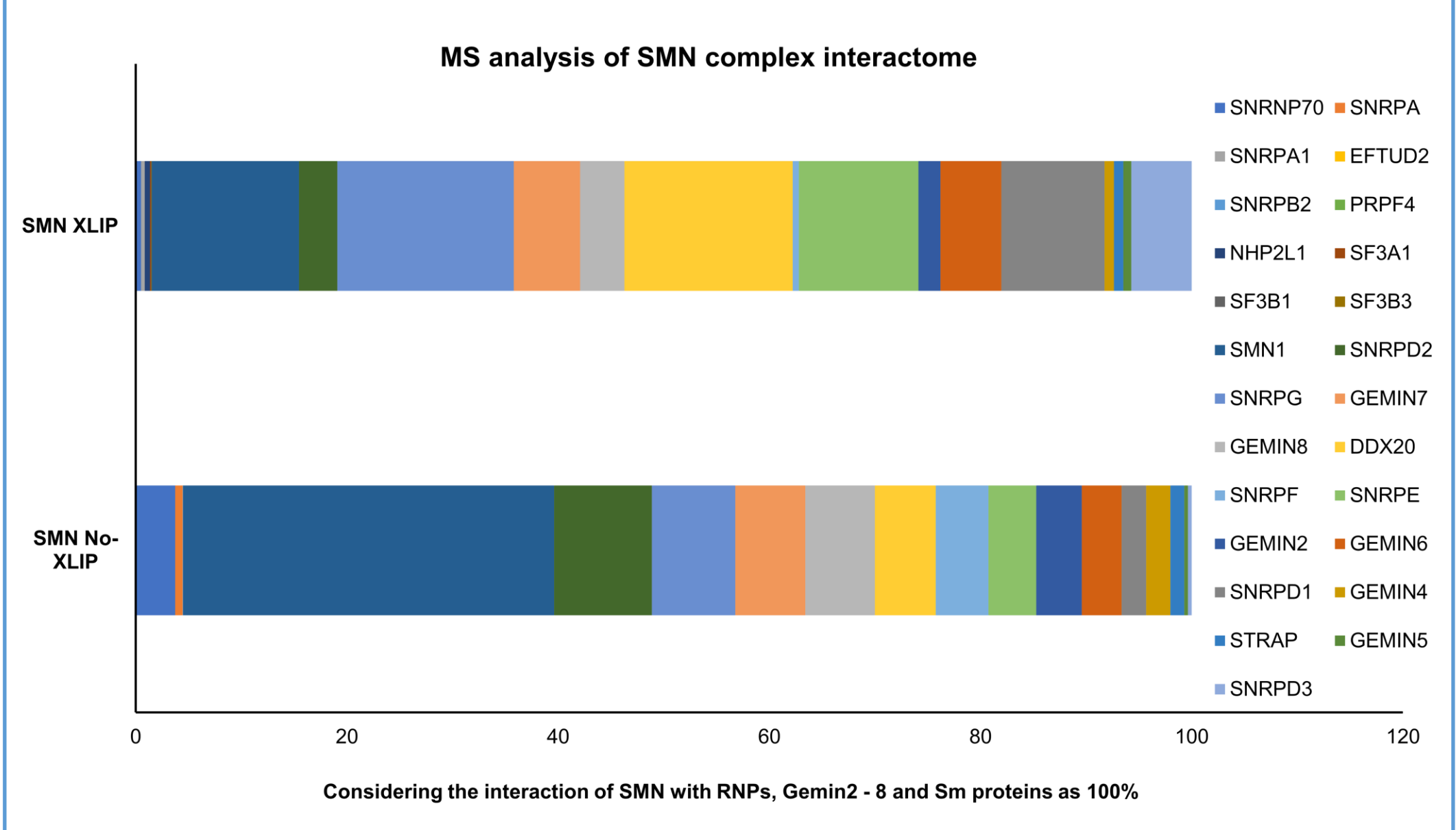
## Heatmap of relative snRNA abundance across different tissues



Heatmap of relative snRNA abundance across healthy tissues, represented as  $2^{-\Delta\Delta Ct}$ . For the calculation of  $\Delta Ct$ , the mean of the 7SK RNA, the signal recognition particle RNA (7SL) and 5S rRNA within each tissue was used as a reference.  $\Delta\Delta Ct$  was calculated relative to the median across individual snRNA within each cell line.

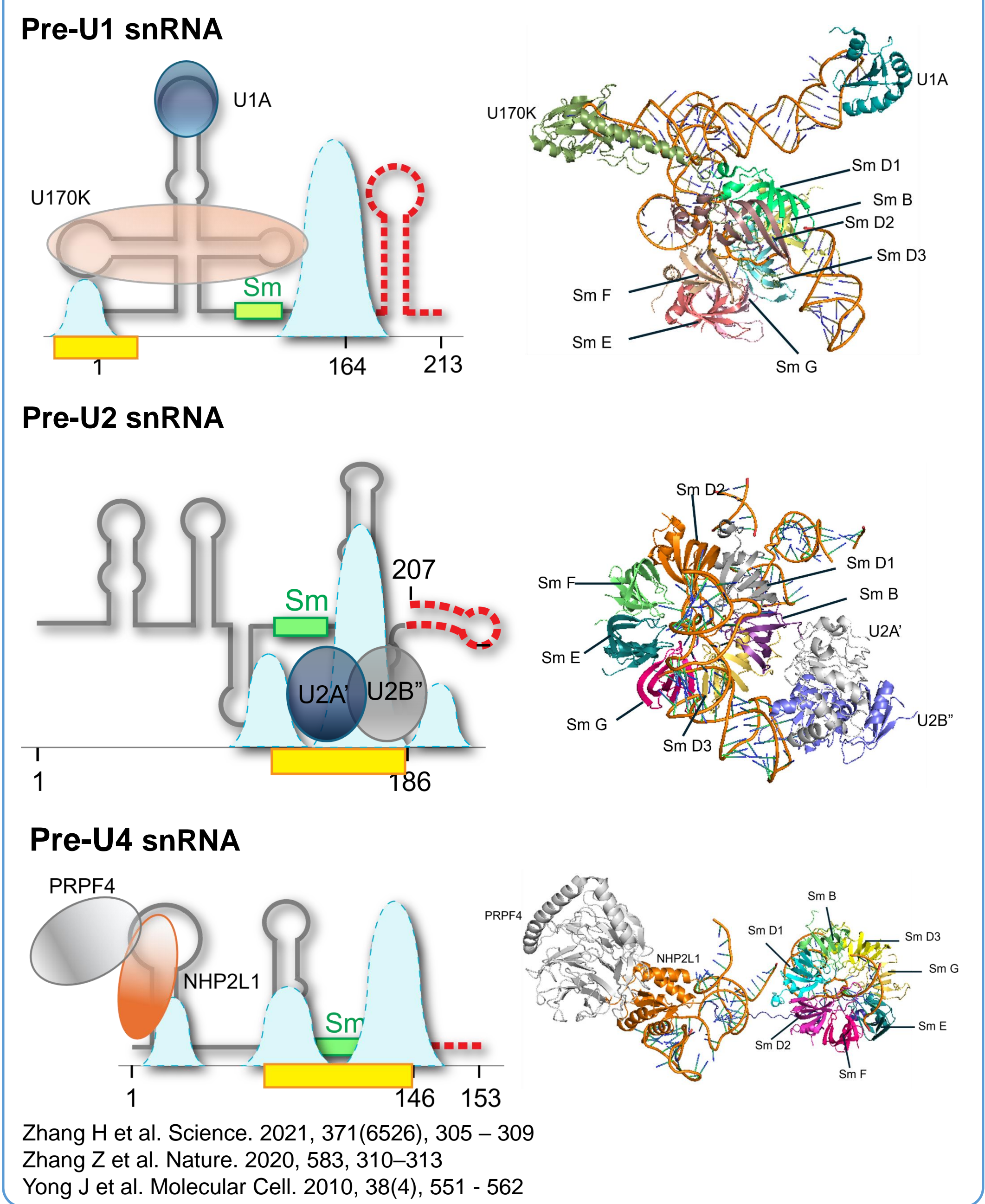
Adapted from Dvinge H et al. Genome Research (2019) 29:1591-1604

## SMN protein interacts with many snRNP-specific proteins



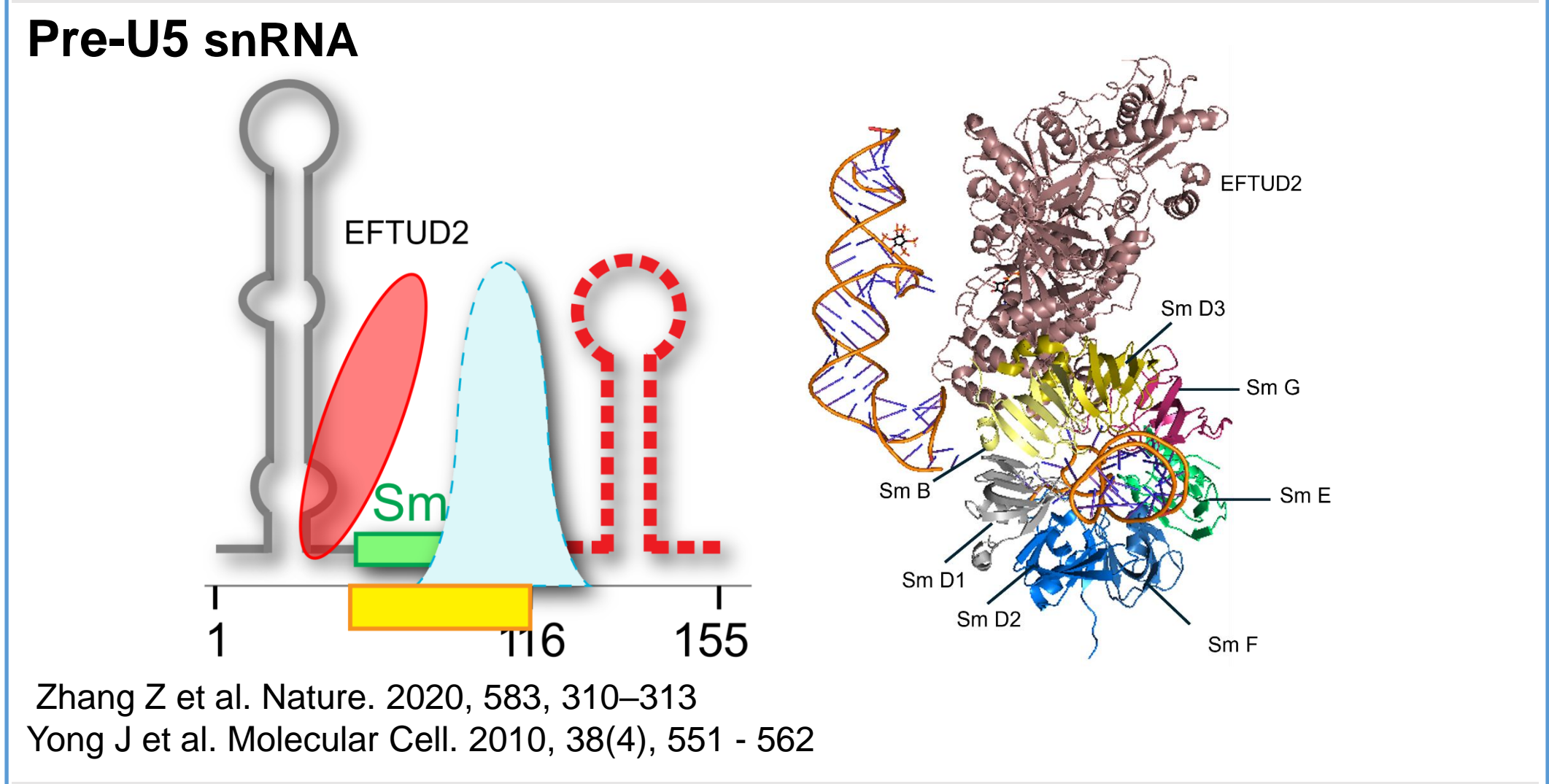
List of RNPs interact with SMN complex	
Protein names	Gene names
U2 small nuclear ribonucleoprotein A	SNRPA1
116 kDa U5 small nuclear ribonucleoprotein	EFTUD2
U2 small nuclear ribonucleoprotein B	SNRNP2
U4/U6 small nuclear ribonucleoprotein Prp4	PRPF4
NHP2-like protein 1	NHP2L1
Splicing factor 3B subunit 1	SF3B1
Splicing factor 3B subunit 3	SF3B3

## snRNP code recognized by Gemin5 and snRNP-specific proteins



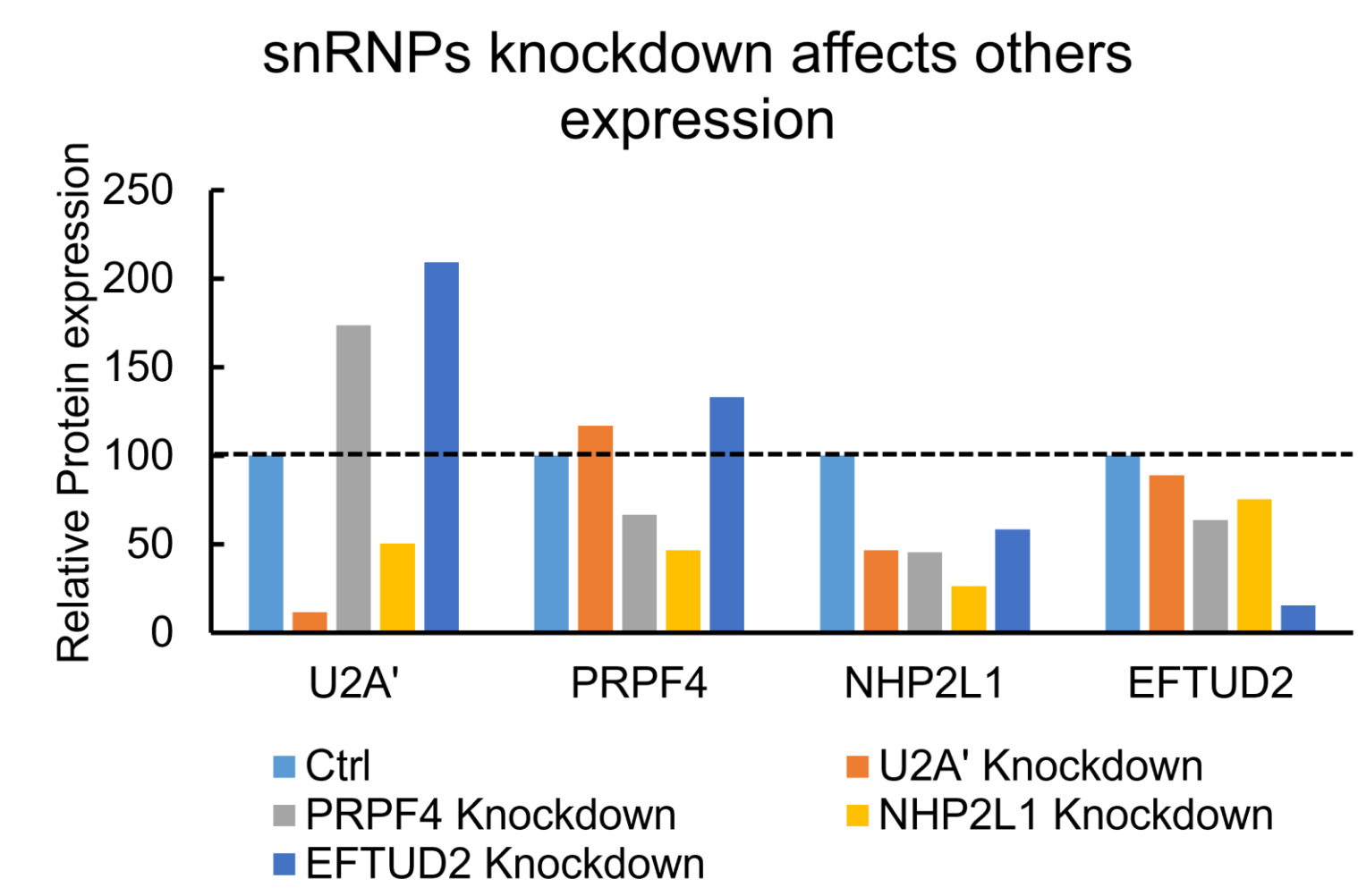
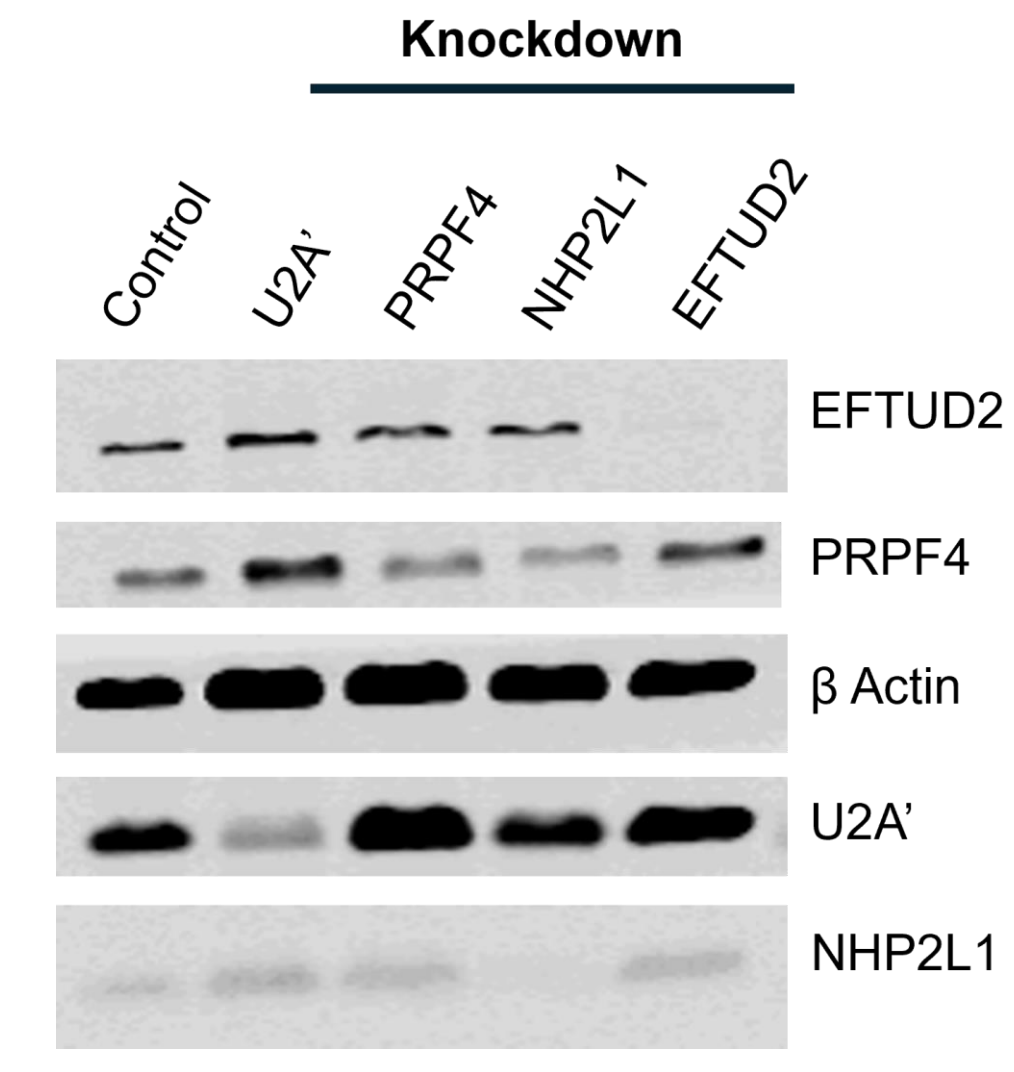
Zhang H et al. Science. 2021, 371(6526), 305 - 309  
Zhang Z et al. Nature. 2020, 583, 310-313  
Yong J et al. Molecular Cell. 2010, 38(4), 551 - 562

## snRNP code recognized by Gemin5 and snRNP-specific proteins (cont.)

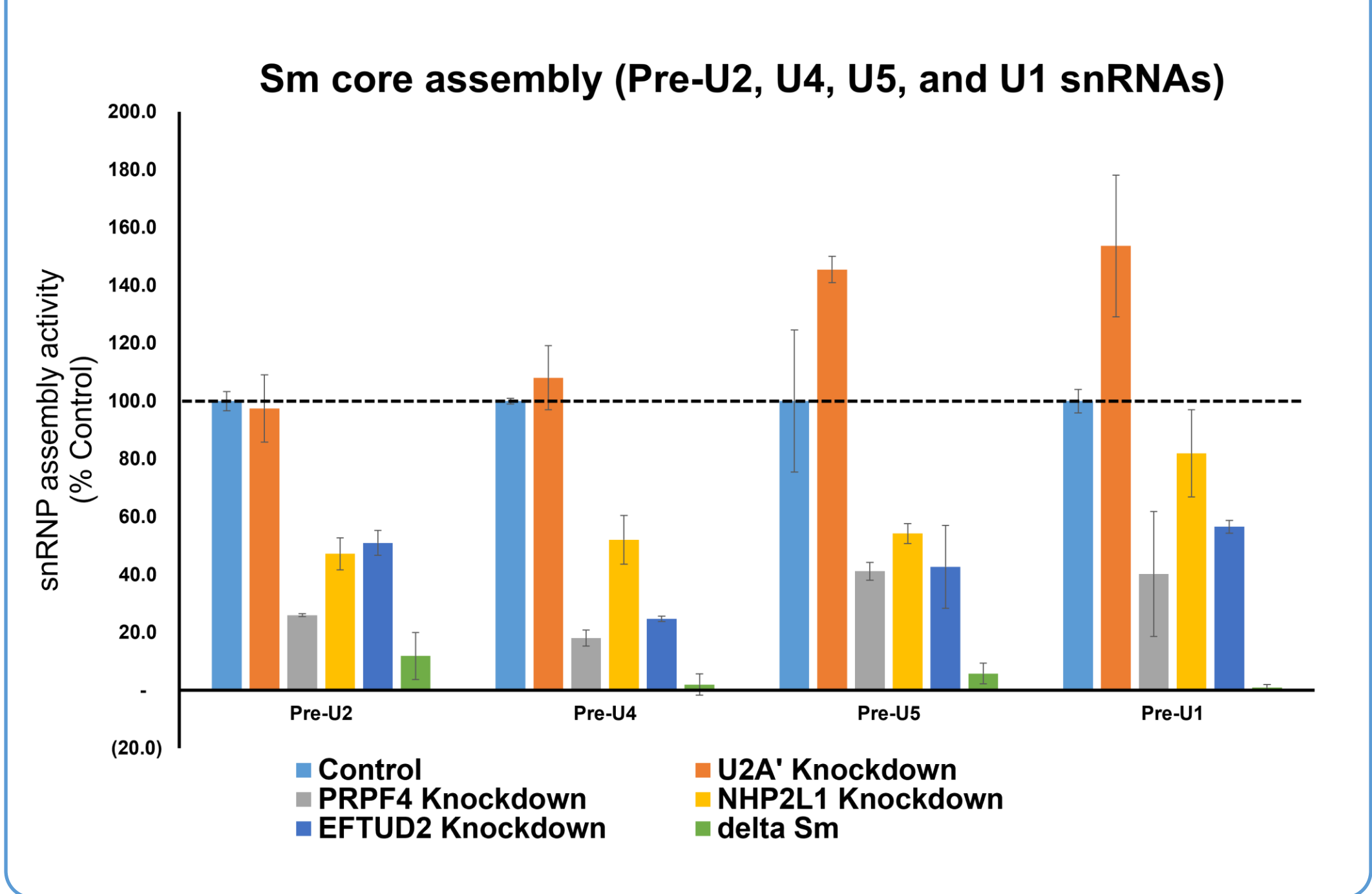


Zhang Z et al. Nature. 2020, 583, 310-313  
Yong J et al. Molecular Cell. 2010, 38(4), 551 - 562

## Knockdown of snRNP-specific proteins affects the expression of other snRNPs



## Knockdown of snRNP-specific proteins alter the Sm core assembly activity



## Arginine dimethylation of snRNP-specific proteins

Protein names	R site	Peptides	Prediction score
U2A'	219	NASTLAEVERLKGLLQSGQ	0.90707
U2A'	232	LLQSGQIPGRERRSGPTDD	0.947946
U2A'	234	QSQGIPGRERRSGPTDDGE	0.976974
U2A'	235	SGQIPGRERRSGPTDDGEE	0.923807
PRPF4	130	LFEGGPAERRERLRNLSV	0.907228
NHP2L1	91	FVRSKOALGRACGVSVPVI	0.860373
EFTUD2	803	AVVAQEPLHRGGQIIPTA	0.977171
EFTUD2	854	AVYTVLARRRHVHTQDAPI	0.879231

<http://bioinfo.icgeb.res.in/PRmePRed/>

## Future directions

- Investigate the impact of SMN interactome alteration with U170K knockdown.
- Identify the interaction between snRNP-specific proteins and the SMN complex and their role in facilitating Sm core assembly.
- Examine the abundance of snRNP-specific proteins in various tissues and their impact on the snRNP stability and repertoire.

## References

- Dvinge, H.; Guenther, J.; Porter, P. L.; Bradley, R. K. RNA Components of the Spliceosome Regulate Tissue- and Cancer-Specific Alternative Splicing. *Genome Research* 2019, 29 (10), 1591-1604. <https://doi.org/10.1101/gr.246678.118>.
- So, B. R.; Wan, L.; Zhang, Z.; Li, P.; Babiash, E.; Duan, J.; Younis, I.; Dreyfuss, G. A U1 SnRNP-Specific Assembly Pathway Reveals the SMN Complex as a Versatile Hub for RNP Exchange. *Nature Structural & Molecular Biology* 2016, 23 (3), 225-230. <https://doi.org/10.1038/nsmb.3167>.
- Yong, J.; Wan, L.; Dreyfuss, G. Why Do Cells Need an Assembly Machine for RNA-Protein Complexes? *Trends in Cell Biology* 2004, 14 (5), 226-232. <https://doi.org/10.1016/j.tcb.2004.03.010>.
- Will, C. L.; Luhrmann, R. Spliceosome Structure and Function. *Cold Spring Harbor Perspectives in Biology* 2010, 3 (7), a003707-a003707. <https://doi.org/10.1101/cshperspect.a003707>.
- Zhang, Z.; Will, C. L.; Bertram, K.; Dybkov, O.; Hartmuth, K.; Agafonov, D. E.; Hofele, R.; Urlaub, H.; Kastner, B.; Luhrmann, R.; Stark, H. Molecular Architecture of the Human 17S U2 SnRNP. *Nature* 2020, 583 (7815), 310-313. <https://doi.org/10.1038/s41586-020-2344-3>.
- Charenton, C.; Wilkinson, M. E.; Nagai, K. Mechanism of 5' Splice Site Transfer for Human Spliceosome Activation. *Science (New York, N.Y.)* 2019, 364 (6438), 362-367. <https://doi.org/10.1126/science.aax3289>.
- Yong, J.; Kasim, M.; Bachorik, J. L.; Wan, L.; Dreyfuss, G. Gemin5 Delivers SnRNA Precursors to the SMN Complex for SnRNP Biogenesis. *Molecular Cell* 2010, 38 (4), 551-562. <https://doi.org/10.1016/j.molcel.2010.03.014>.
- Zhang, S.; Aibara, S.; Vos, S. M.; Agafonov, D. E.; Luhrmann, R.; Cramer, P. Structure of a Transcribing RNA Polymerase II-U1 SnRNP Complex. *Science (New York, N.Y.)* 2021, 371 (6526), 305-309.

## Acknowledgement

**Collaborators**  
Dr. Zibiao Guo (North Texas Genomic Center)  
Dr. Kayunta Johnson-Winters (Univ of Texas Arlington)  
Dr. Jeongsik Yong (Univ of Minnesota)  
Dr. Eul Hyun Suh (Univ North Texas Health Center)



**Univ of Texas Arlington**  
Start Up Funds  
Research Enhancement Program



**National Institutes of Health**  
R15GM152936

**Chemistry and Biochemistry**