SMN complex-mediated regulatory mechanisms of U4 small nuclear ribonucleoprotein assembly

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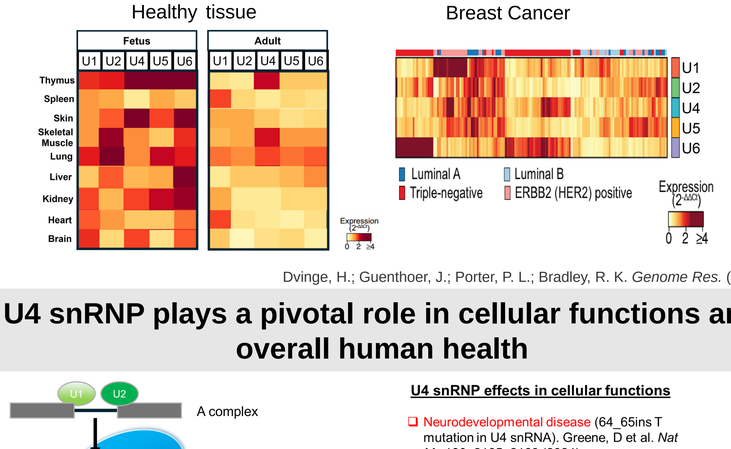
Abstract

The spliceosome is a macromolecular RNA-Protein complex that catalyzes splicing, a two-step reaction that converts precursor messenger RNA (pre-mRNA) into mature mRNA—an essential intermediate in gene-to-protein expression. Although spliceosome assembly requires equal stoichiometry of snRNPs, the abundance and repertoire of small nuclear ribonucleoprotein complexes (snRNPs) vary significantly in a tissue- and development-specific manner. Among snRNPs, U4 snRNP is crucial for spliceosome activation and regulation, yet given its critical role in ensuring splicing fidelity, function, and human health, the mechanism governing its biogenesis remain poorly understood.

Here, we examined the interaction between U4 snRNP-specific proteins and the SMN complex, a multi-component RNP chaperone responsible for assembling an Sm core on each snRNA and ensuring the stability of snRNP biogenesis. Depletion of U4 snRNP-specific snRNP27 reduced U4 snRNA levels drastically and impaired its interaction with the SMN complex. Moreover, our findings reveal that it not only reduces U4 snRNP assembly but also affects the assembly of other snRNPs. These results suggest that snRNP-specific proteins regulate snRNP abundance by either promoting canonical snRNP assembly or stabilizing snRNPs, a process mediated by the SMN complex. Given the role of U4 snRNP-specific proteins in spliceosome stability, dysregulation might contribute to splicing diseases, and it could be considered a potential target for the further investigation of RNA processing disorders.

Diversity in snRNP abundance and repertoire across healthy and diseased tissues



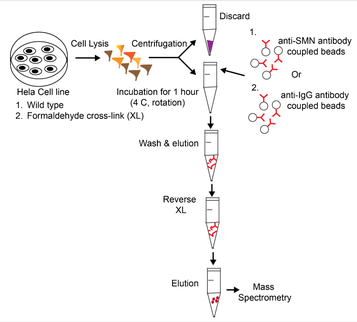
Dvinge, H.; Guenthoer, J.; Porter, P. L.; Bradley, R. K. *Genome Res.* (**2019**)

U4 snRNP plays a pivotal role in cellular functions and overall human health

U4 snRNP effects in cellular functions

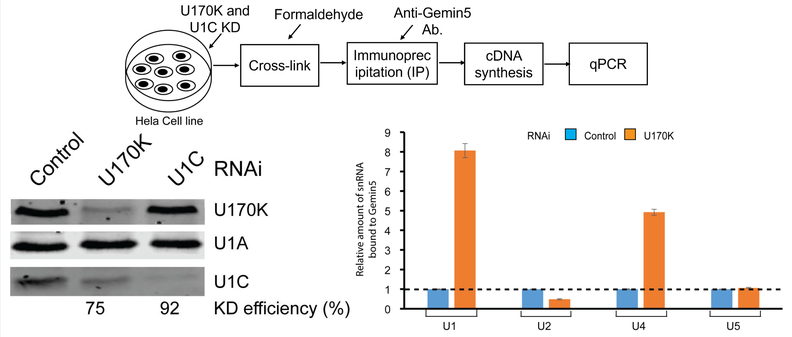
- Neurodevelopmental disease (64_65ins T mutation in U4 snRNA). Greene, D et al. *Nat Med* **30**, 2165–2169 (2024)
- Premature cleavage and polyadenylation (PCPA). Feng Q et al. PNAS Jul 2;121(27):e2406710121
- Defects in 5' splice site positioning (M141 mutation in snRNP27). Zahler AM et al. RNA. 2018 Oct;24(10):1314-1325
- Load alternative 5' splice site (H765 mutation in SART1). Sarka K. et al. RNA. 2024 May 16;30(6):695-709
- Retinitis Pigmentosa (mutations in PRPF3, PRPF4, PRPF31). Krausová M. et al. Seminars in Cell & Developmental Biology 79 (2018) 92–102

Interaction of U4 snRNP-specific proteins with RNP chaperon Survival Motor Neuron (SMN) Protein complex

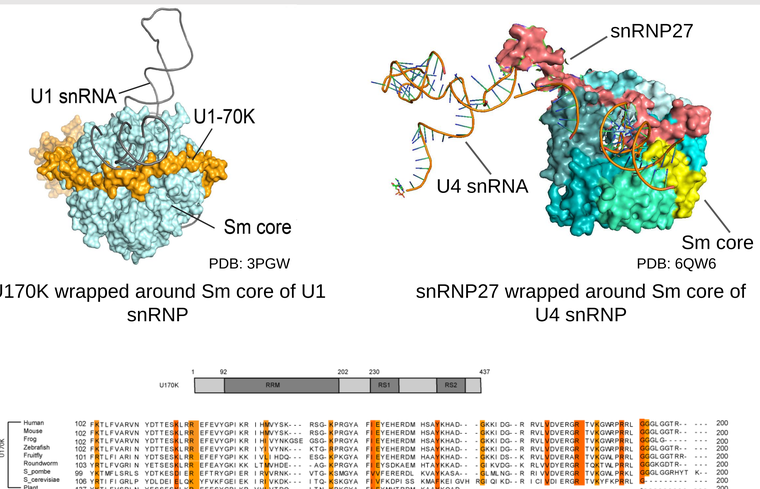


snRNP	SMN-No-XCLIP	SMN-XCLIP
U1	U170K	U170K
	U1A	
U2	U2A'	U2A'
	U2B"	
		SF3A1
		SF3B1
		SF3B3
U4	PRPF4	
		NHP2L1
U5	EFTUD2	

Knockdown (KD) of the U1 snRNP-specific protein U1-70K stalls snRNAs at the SMN complex factor Gemin5.

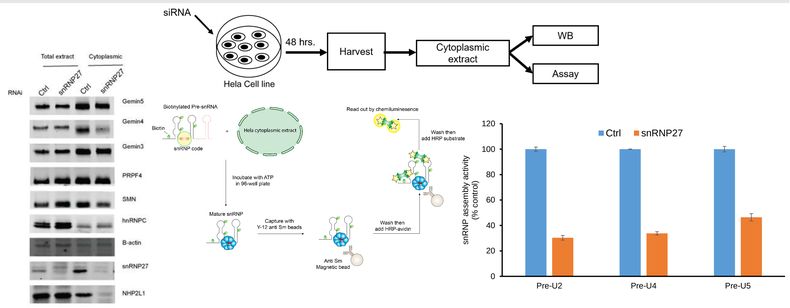


The U1 snRNP protein U1-70K and the U4 snRNP protein SNRNP27 wrap around the Sm core in a similar manner and share sequence similarity

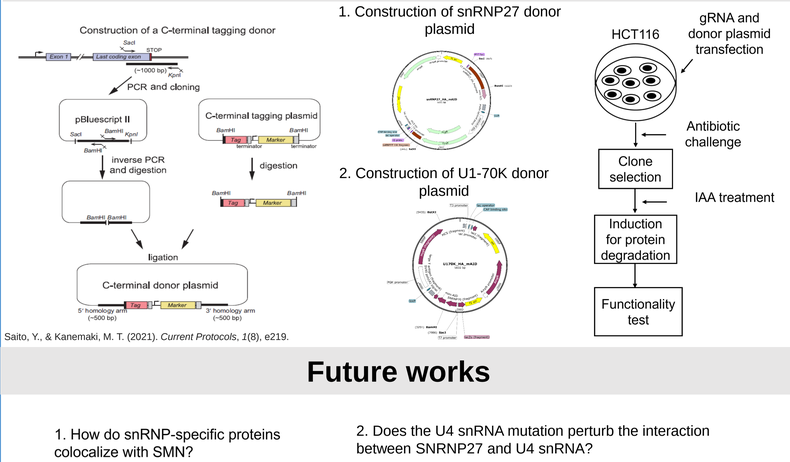


Simossis VA, Heringa J. Nucleic Acids Res. (2005)

snRNP27 depletion reduces U4 Sm core assembly and affects others as well by reducing Gemin4 in the cytoplasm



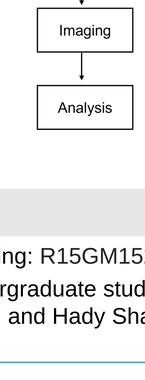
Development of an inducible degradation system for SNRNP27 and U1-70K to assess the mechanism of U4 snRNP assembly



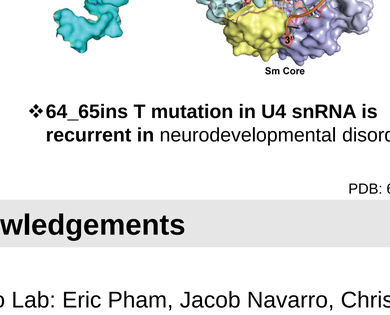
Saito, Y., & Kanemaki, M. T. (2021). *Current Protocols*, *1*(8), e219.

Future works

1. How do snRNP-specific proteins colocalize with SMN?



2. Does the U4 snRNA mutation perturb the interaction between SNRNP27 and U4 snRNA?



❖**64_65ins T mutation in U4 snRNA is recurrent in** neurodevelopmental disorder.

PDB: 6QW6

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