

# The role of U1 snRNP-specific proteins for post-transcriptional regulation of pre-mRNAs



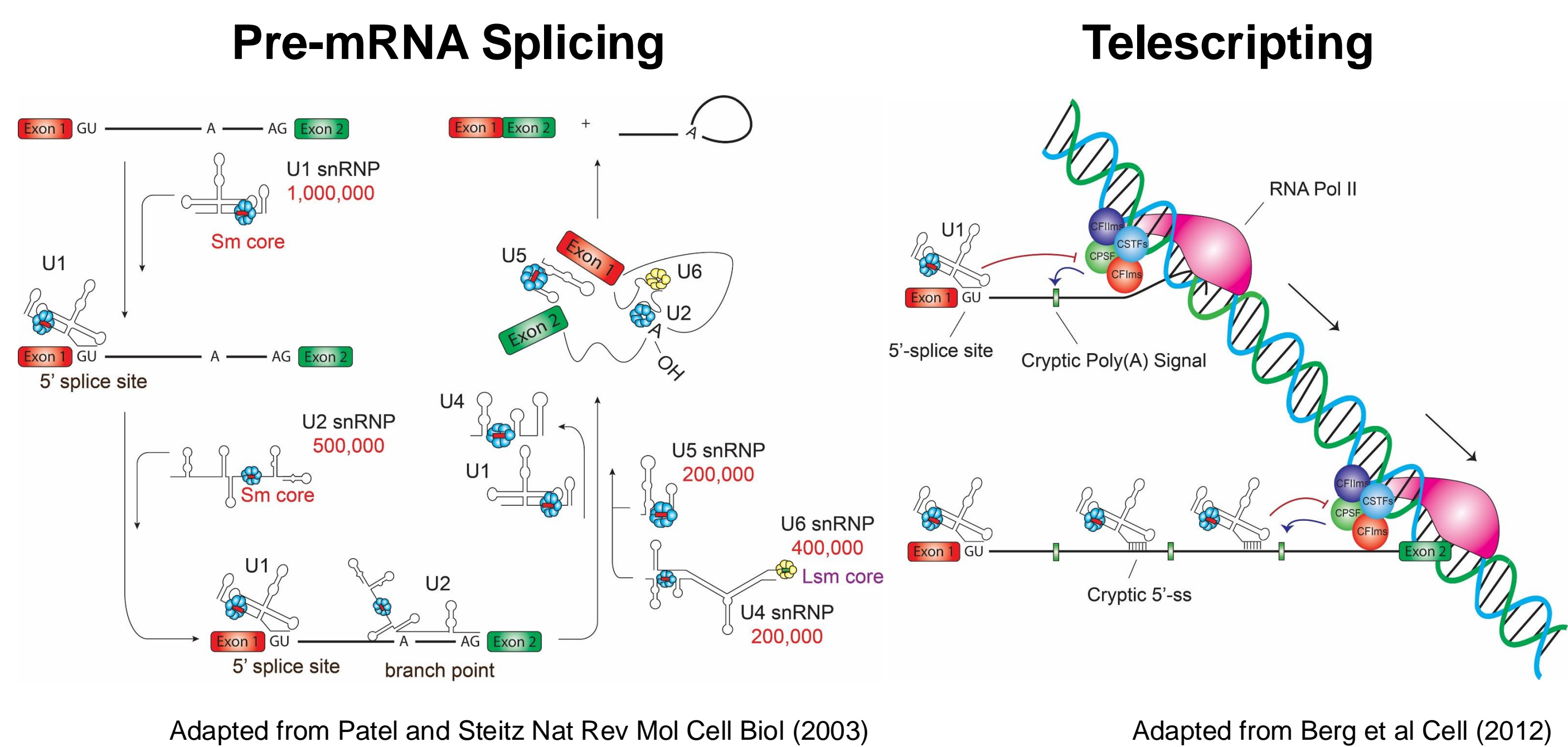
Duc Minh Ngu, Yae-lin Irene Kim, Haritha Asokan Sheeja, He Dong, and Byung Ran So

Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019

## Abstract

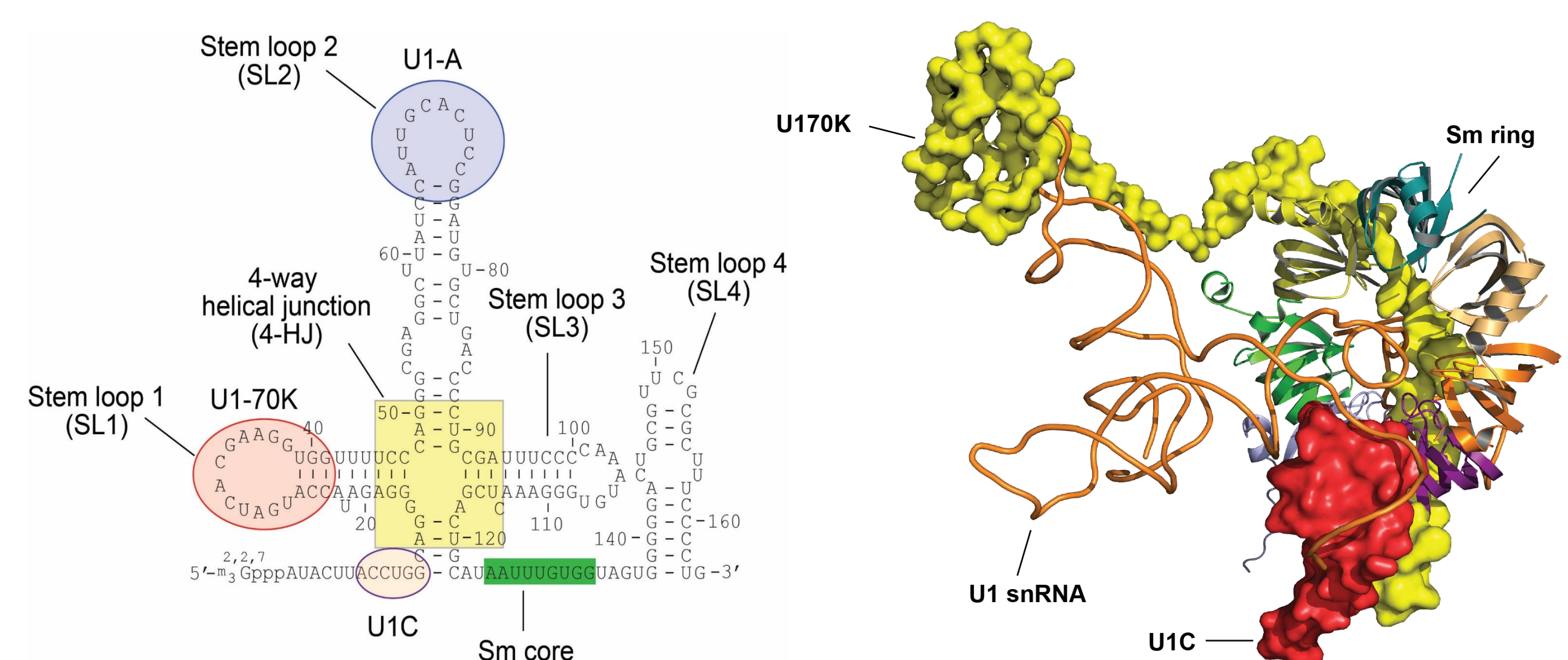
In eukaryotes, precursor messenger RNA (pre-mRNA) undergoes multiple post-transcriptional processes to produce mature mRNA, a crucial intermediate in the flow of genetic information from DNA to protein expression. Small nuclear ribonucleoproteins (snRNPs) play a key role in spliceosome-mediated pre-mRNA splicing, facilitating the joining of coding exons and the removal of non-coding introns. U1 snRNP is one of the most abundant in human (HeLa) cells and is essential for both splicing and preventing premature transcription termination, a process known as telescripting. To investigate the functional role of U1 snRNP-specific proteins in U1 snRNP assembly, we established an *in vitro* U1 snRNP reconstitution system and found that U1C, a U1 snRNP-specific protein that stabilizes U1 snRNP binding to pre-mRNAs, is essential for U1 snRNP assembly. Additionally, we developed a dual-luciferase reporter system to monitor splicing and telescripting using engineered U1 snRNPs with alternative pre-mRNA bindings. Furthermore, we initiated an *in vitro* system to detect liquid-liquid phase separation, utilizing the disordered and low complexity domains of U1 snRNP-specific proteins in their interaction with SMN complex during snRNP biogenesis. Our ongoing studies aim to elucidate that the role of U1 snRNP-specific proteins in U1 snRNP assembly and their broader interactions in splicing and telescripting regulation.

## Roles of U1 snRNP in post-transcriptional gene regulation



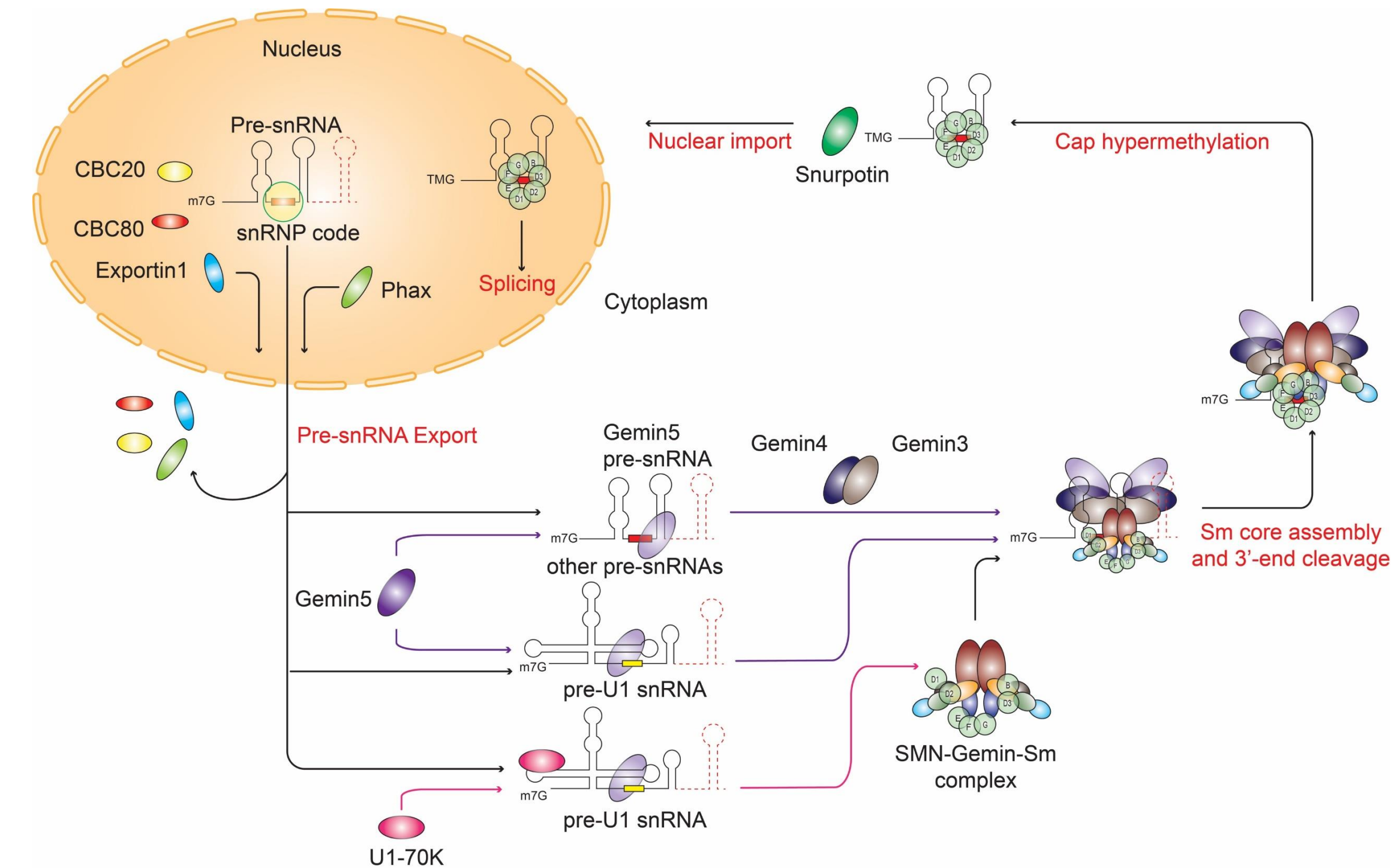
- U1 snRNP recognizes 5'-splice site of pre-mRNA during the splicing process.
- U1 snRNP inhibits premature cleavage and polyadenylation of pre-mRNA (telescripting).

## U1 snRNP contains 3 specific binding proteins



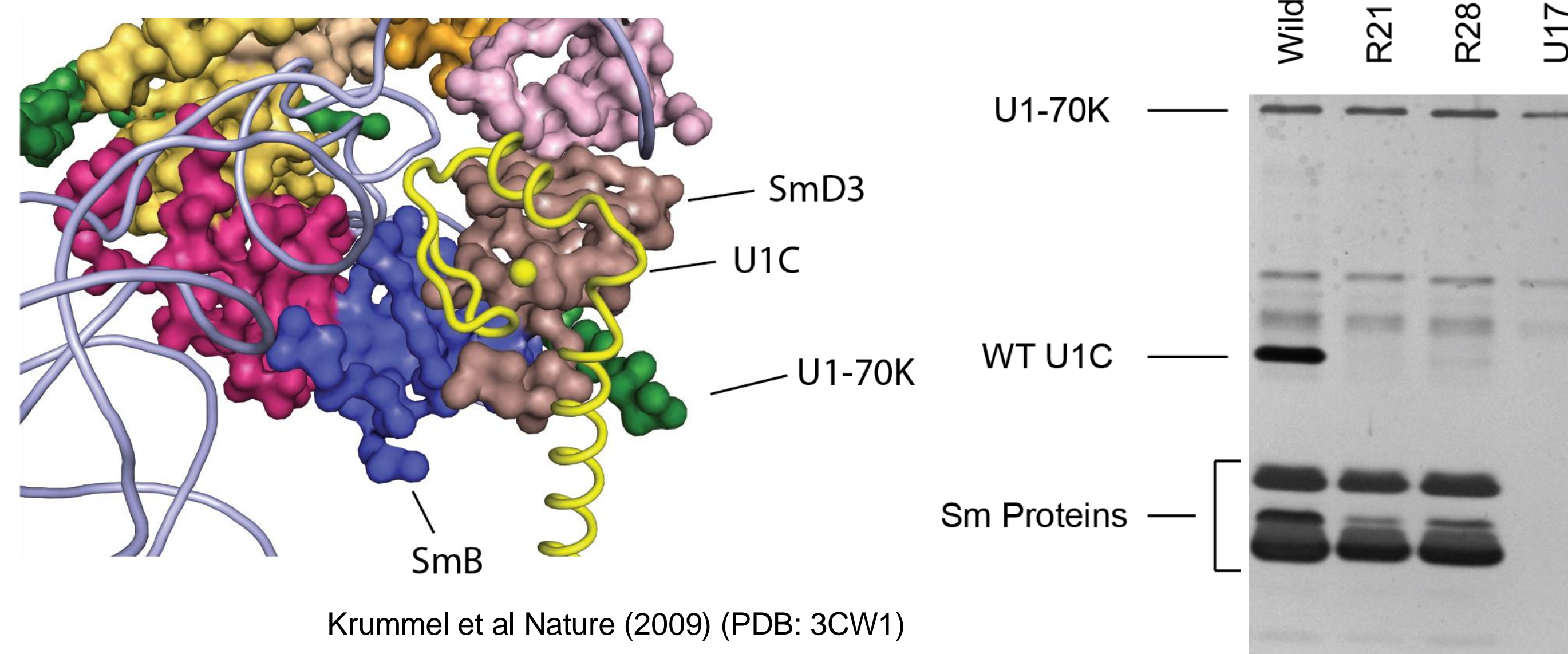
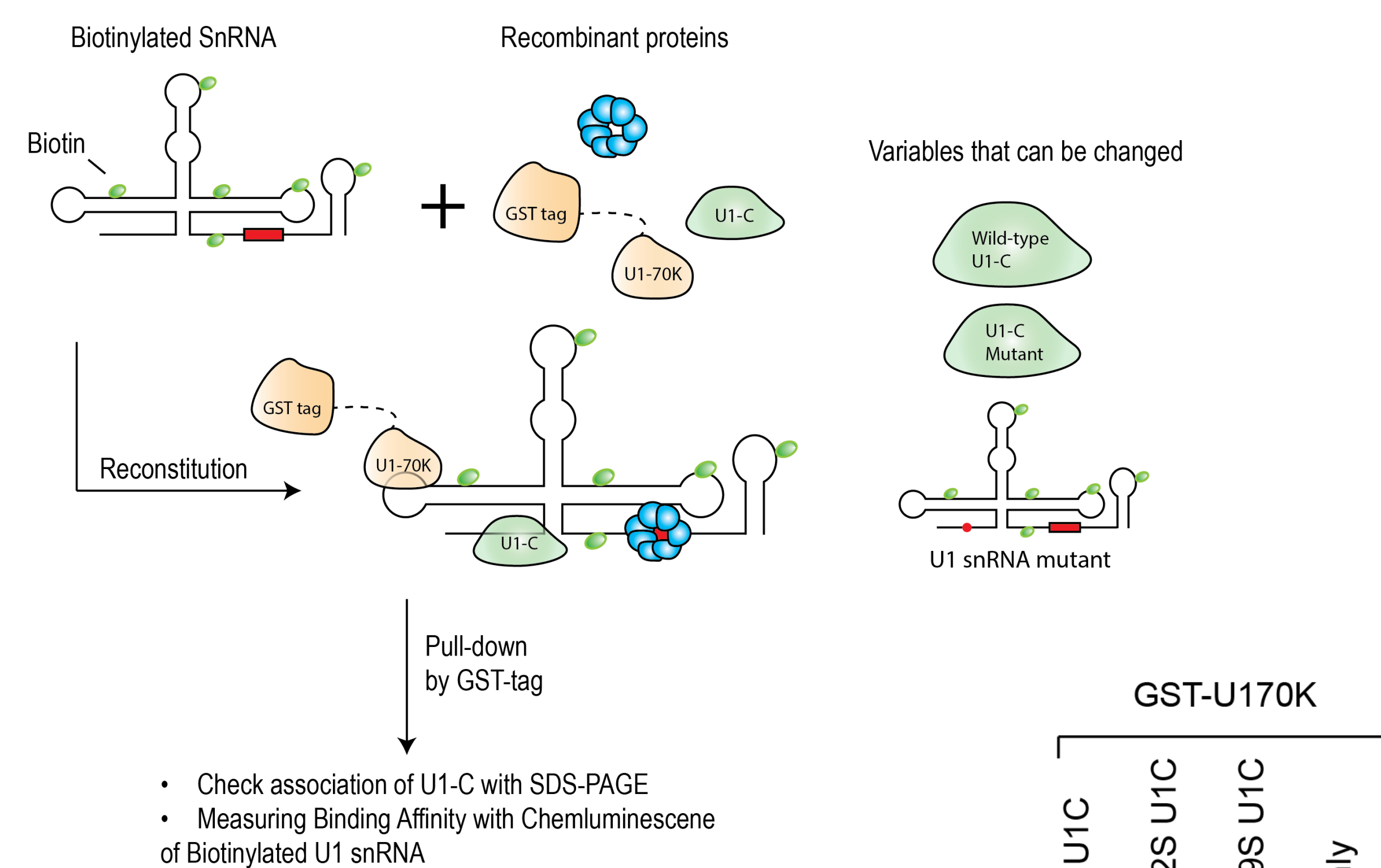
Kondol et al eLife (2015) (PDB: 4PJO)  
Weber et al EMBO J. (2010) (PDB: 3PGW)

## SMN complex-mediated U snRNP biogenesis



U1 snRNA association with the SMN complex is mediated by U1-70K and Gemin5.

## In vitro reconstitution of U1 snRNP

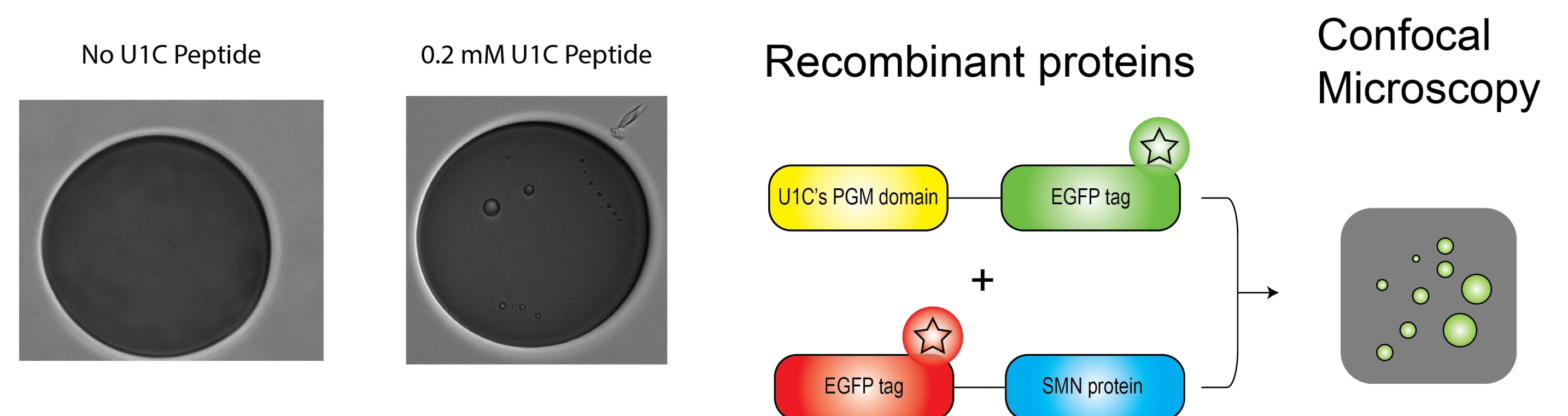


- U1C's basic residues are required to associate with U170K and Sm proteins
- U1C might interact with SMN-Gemin complex to close the Sm-ring of U1 snRNP during biogenesis

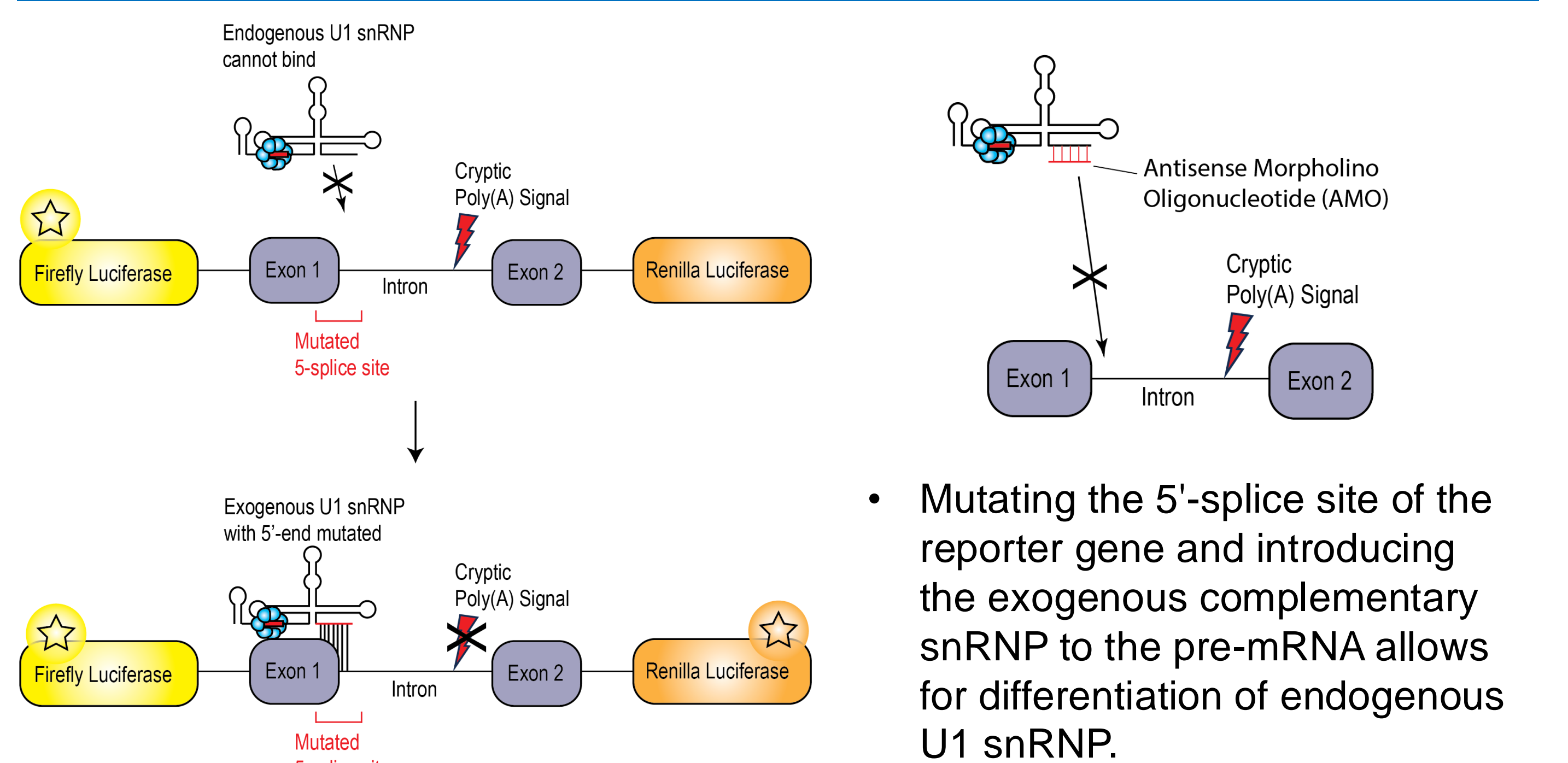
## Liquid-liquid phase separation of U1 snRNP proteins

70 KIPPTFSSAP PPPAGAMIPP PPSLPGPPRP  
100 GMPPAPHMGG PPMPPMMGPP PPGMPPVGPA  
130 PGM<sup>R</sup>PPMGGH MPMPGPMPM <sup>R</sup>PPA<sup>R</sup>PMMPV  
150 TRPGMT<sup>R</sup>PDR

- Recombinant proteins with fluorescence tags will mix PEG 8000 for droplet formation and visualized under confocal microscopy



## Dual Luciferase Reporting system design



## Conclusions

- U1C's basic residues are required for its association with U1 snRNP.
- The Dual Luciferase Reporter system will be used for *in vivo* study of monitoring splicing and telescripting in cell.
- Droplet formation assay can be used to study the association of U1C with SMN complex promoting liquid-liquid phase separation.

## Acknowledgement

Univ of Texas Arlington  
Start Up Funds  
Research Enhancement Program

National Institutes of Health  
R15GM152936



National Institutes  
of Health



Chemistry and Biochemistry