

## U2 snRNA

1 25 28 35 38 41 44 45 48 55  
*Drosophila* **A<sub>m</sub>**U CGCUUCUCG G CCUUAUG GCUAAG**G<sub>m</sub>**AU**C<sub>m</sub>**AA AGUG**Ψ**AG**Ψ**A**Ψ****C<sub>m</sub>****Ψ**G**Ψ****Ψ**CU**U<sub>m</sub>**AUCAGC**Ψ**UAACAUC UGAUAGUCCU [1-73]  
 # # #  
*Xenopus* **A<sub>m</sub>****U<sub>m</sub>**CGC**Ψ****Ψ**CUC**G<sub>m</sub>****G<sub>m</sub>**CC**Ψ**UUU**G<sub>m</sub>**GCUAAG**G<sub>m</sub>**AUC **AA<sub>m</sub>**-GUG**Ψ**AG**Ψ**A**Ψ****C<sub>m</sub>****Ψ**G**Ψ****Ψ**CU**U<sub>m</sub>**AUCAGU**Ψ**UAA**Ψ**AU**C<sub>m</sub>**UGAUACGUCCC [1-72]  
 #  
 Human **A<sub>m</sub>****U<sub>m</sub>**CGC**Ψ****Ψ**CUC**G<sub>m</sub>****G<sub>m</sub>**CC**Ψ**UUU**G<sub>m</sub>**GCUAAG**G<sub>m</sub>**AUC **AA<sub>m</sub>**-GUG**Ψ**AG**Ψ**A**Ψ****C<sub>m</sub>****Ψ**G**Ψ****Ψ**CU**U<sub>m</sub>**AUCAGU**Ψ**UAA**Ψ**A**Ψ****C<sub>m</sub>**UGAUACGUCCU [1-72]  
 1 2 6 7 11 15 19 25 30 34 37 40 43 47 54 58 61  
 12 39 41 44 60

We previously showed that termination of the reverse transcription reaction at 2'-O-methylated positions depends on the source of the enzyme (Deryusheva et al. 2012). AMV-RT from New England Biolabs, used in this study, is the best choice for testing modifications near C28. However, AMV-RT fails to stop at Gm19 and Cm61 in vertebrate U2. When we injected *in vitro* transcribed *Drosophila* U2 snRNA into *Xenopus* oocytes and looked for modifications, we found that Gm11/12, Gm25, Am30, Cm41 and Um48 were induced by the corresponding *Xenopus* guide RNAs; Gm19 and Cm62 (equivalent to position 61 in vertebrates) even if induced, would not produce stop signals and would not be detected in AMV-RT reactions.

Deryusheva, S.; Gall, J.G. Small Cajal Body-specific RNAs of Drosophila Function in the Absence of Cajal Bodies. *Mol. Biol. Cell* **2009**, *20*, 5250–5259.

Deryusheva, S.; Choleza, M.; Barbarossa, A.; Gall, J.G.; Bordonne, R. Post-transcriptional modification of spliceosomal RNAs is normal in SMN-deficient cells. *RNA* **2012**, *18*, 31–36.

Deryusheva, S.; Gall, J.G. Dual nature of pseudouridylation in U2 snRNA: Pus1p-dependent and Pus1p- independent activities in yeasts and higher eukaryotes. *RNA* **2017**, *23*, 1060–1067.