Kermanshah University Of Medical Sciences

In the name of GOD

# Journal Club Presentation

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Article

# Small RNAs are modified with N-glycans and displayed on the surface of living cells

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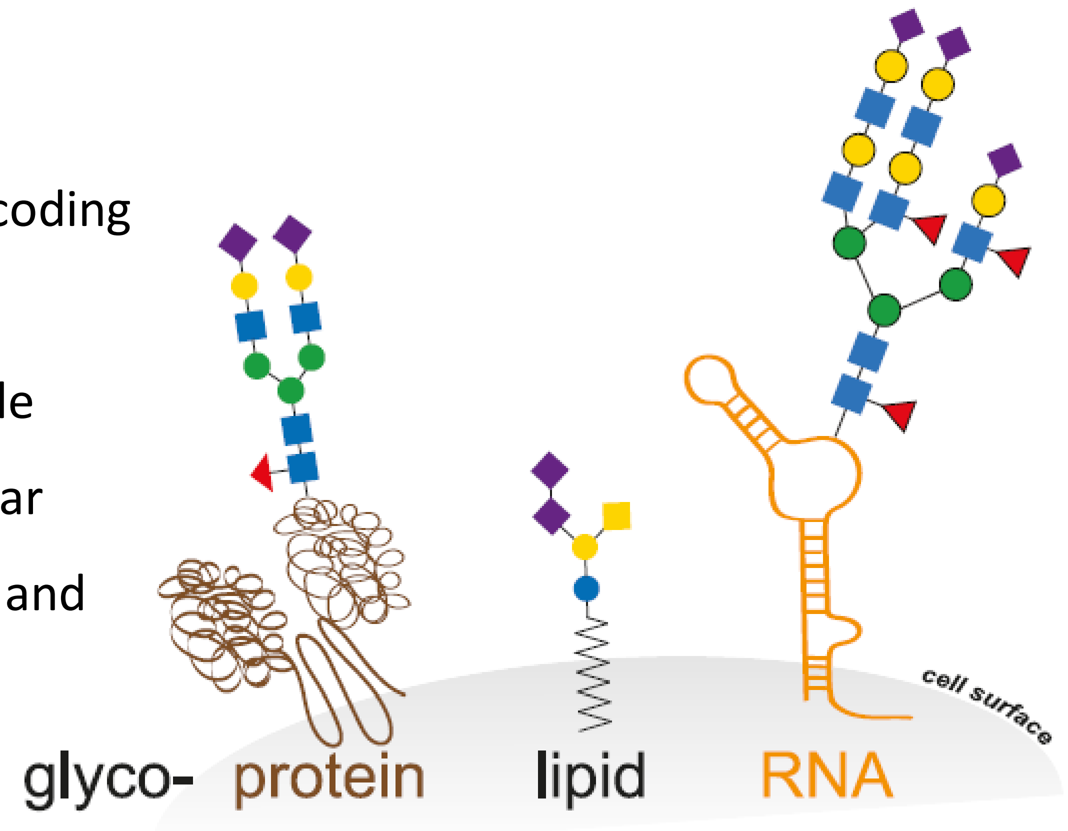
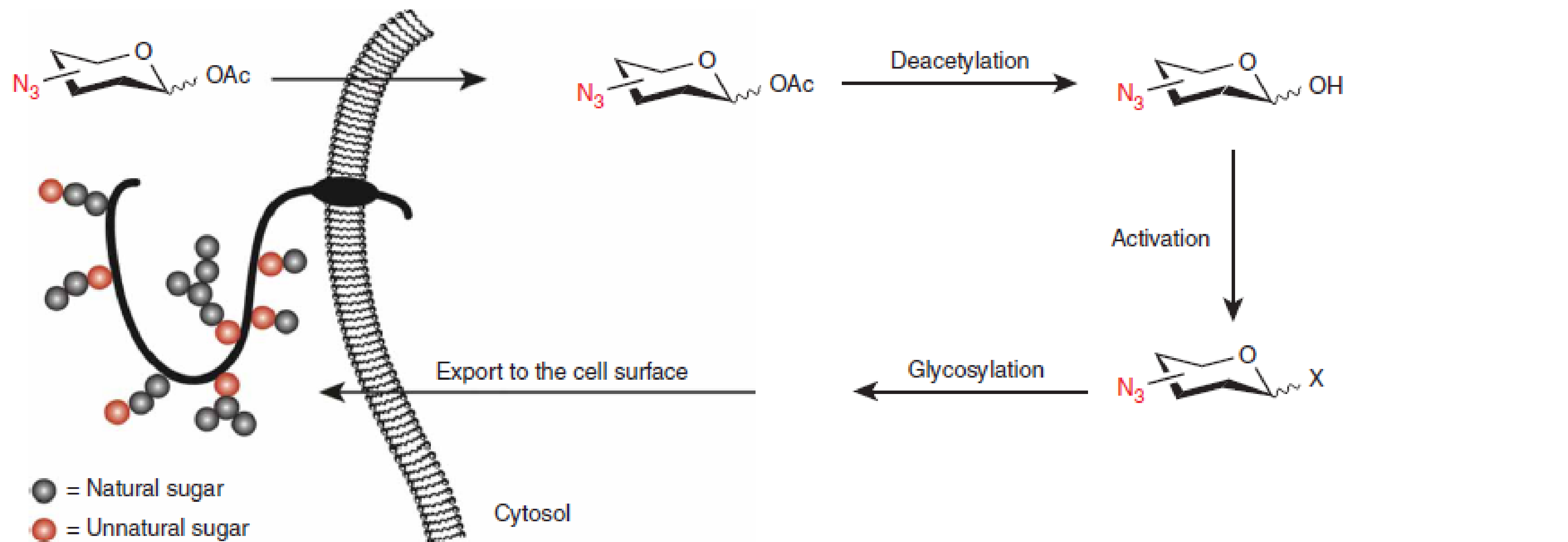
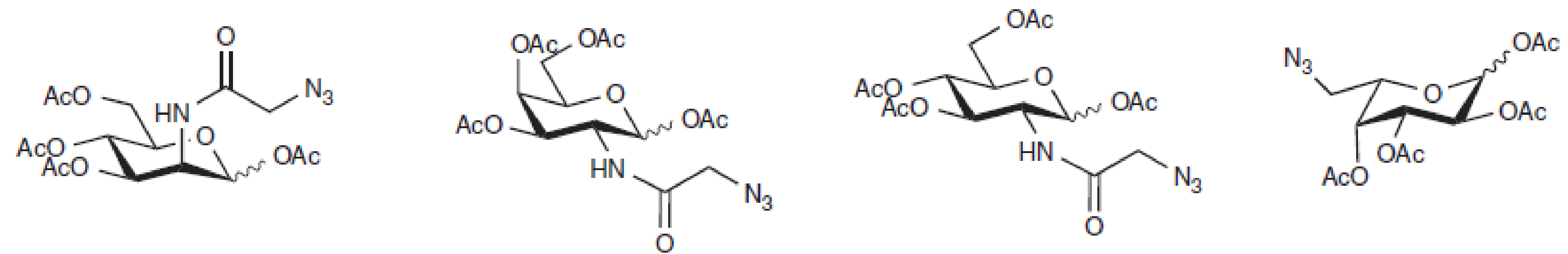
# INTRODUCTION:

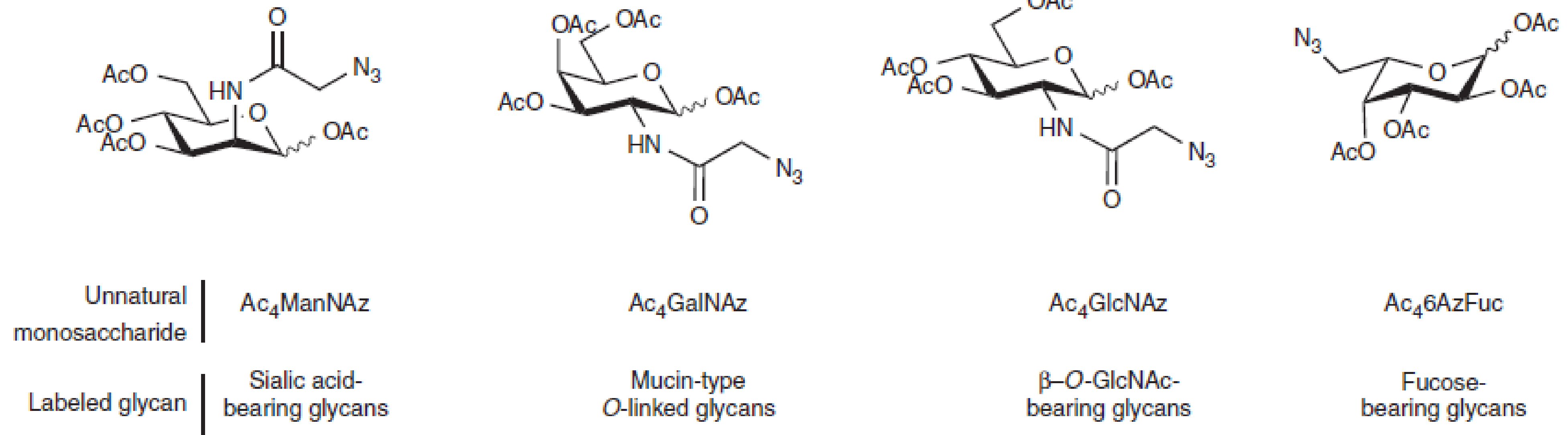
- ✓ Glycans are present in every cell studied to date across the kingdoms of life
- ✓ Glycans modify lipids and proteins to mediate inter- and intramolecular interactions across all domains of life.
- ✓ By chemical and biochemical approaches, defined small noncoding RNAs can be as  third scaffold for glycosylation.
- ✓ the cellular role for RNA is more complex than that of a simple messenger. For instance, RNAs function as scaffolds, molecular decoys, enzymes, and network regulators across the nucleus and cytosol

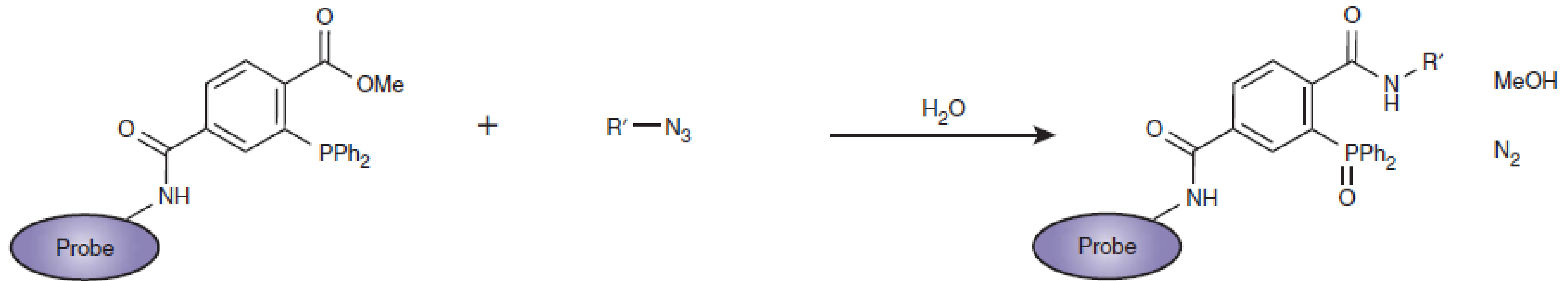





✓ In this strategy, metabolically label cells or animals with precursor sugars functionalized with a clickable azide group. Once incorporated into cellular glycans, the azidosugars enable bioorthogonal reaction with a biotin probe for enrichment, identification, and visualization.

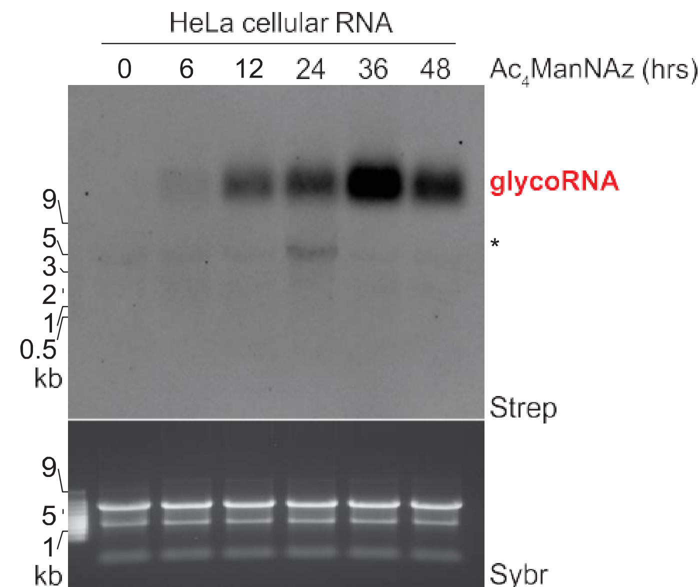


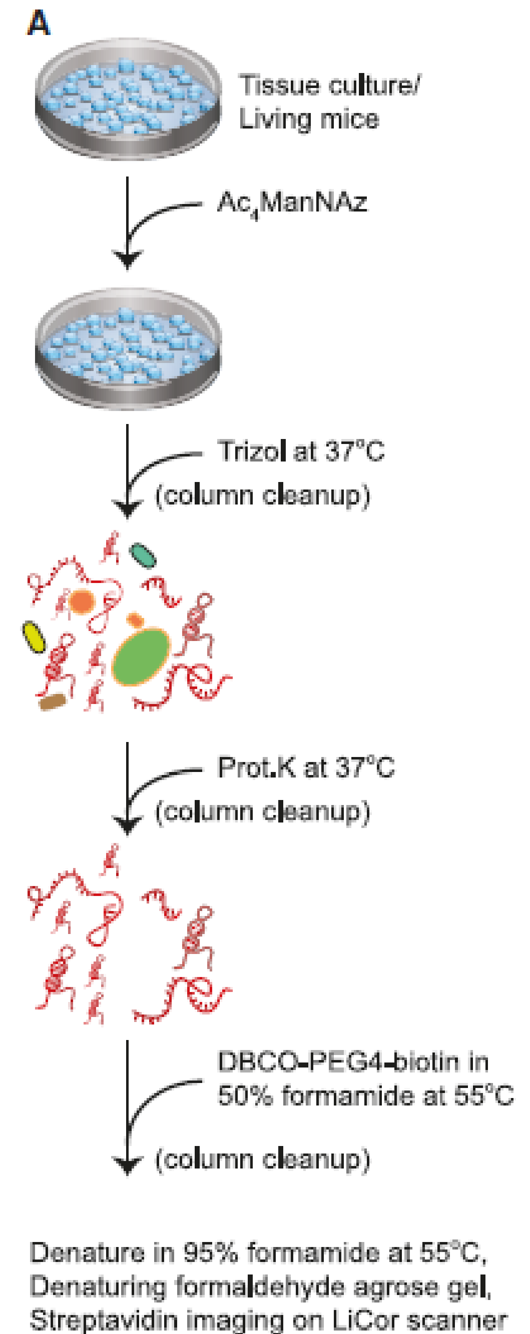




✓ In this strategy, we metabolically label cells or animals with precursor sugars functionalized with a click-able azide group. Once incorporated into cellular glycans, the azidosugars enable bioorthogonal reaction with a biotin probe for enrichment, identification, and visualization.

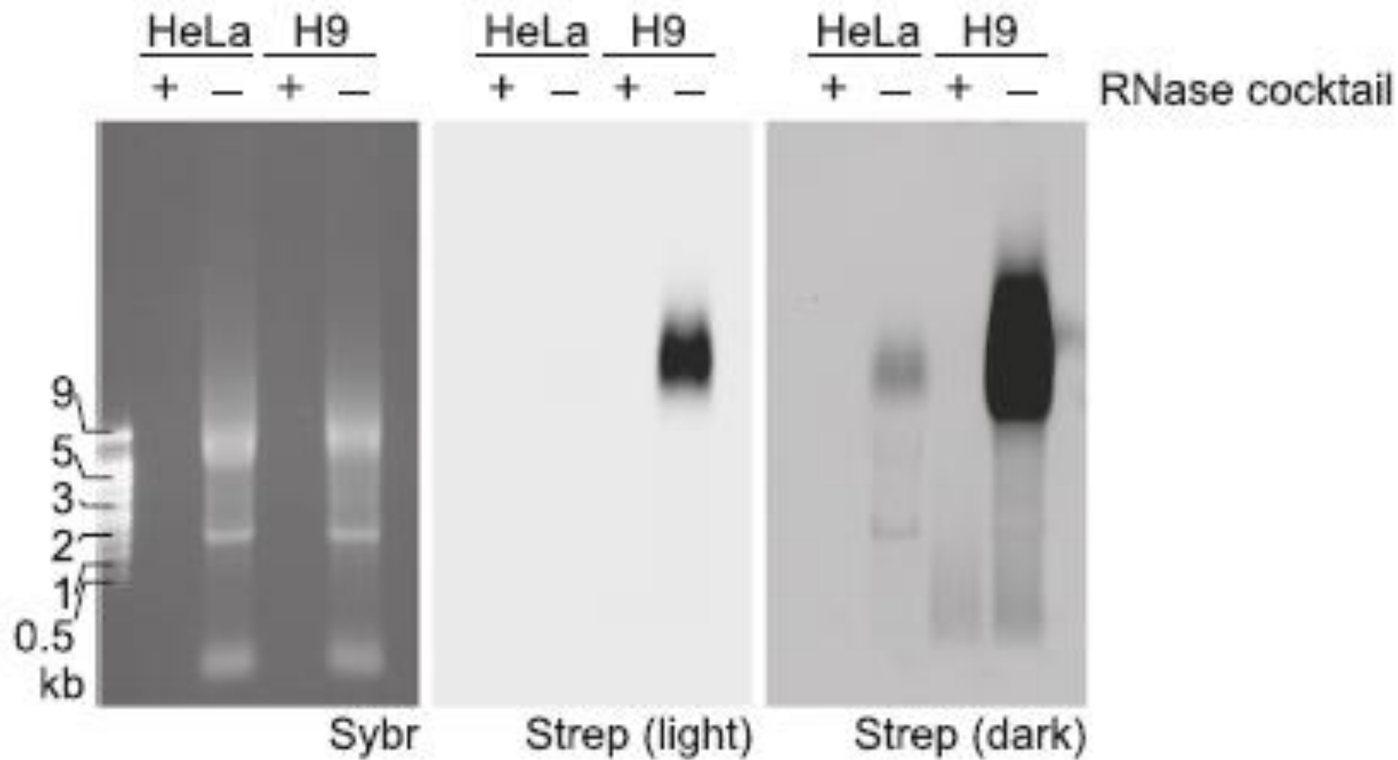
# A glycan metabolic reporter is incorporated into cellular RNA

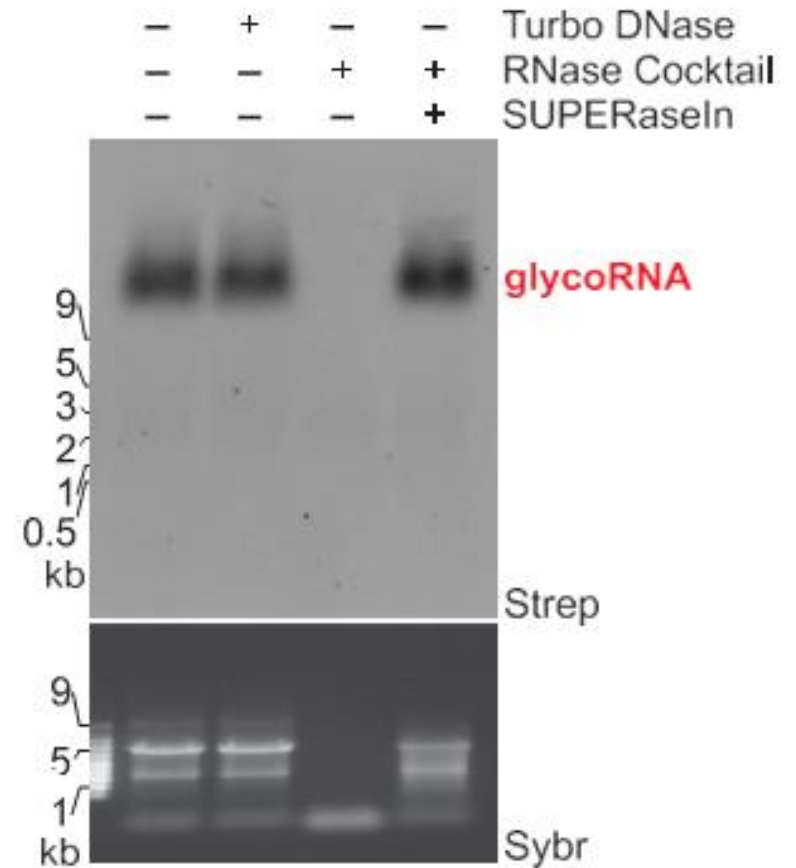
- ✓ First Hela cell treated by 100 mM Ac4ManNAz for up to 48 h and then RNA is extracted with warm TRIzol method.
- ✓ To visualize azide-labeled components, RNA samples added to dibenzocyclooctyne-biotin (DBCO- biotin) in denaturing conditions (50% formamide) at 55°C, subsequently separated by denaturing gel electrophoresis and analyzed by blotting



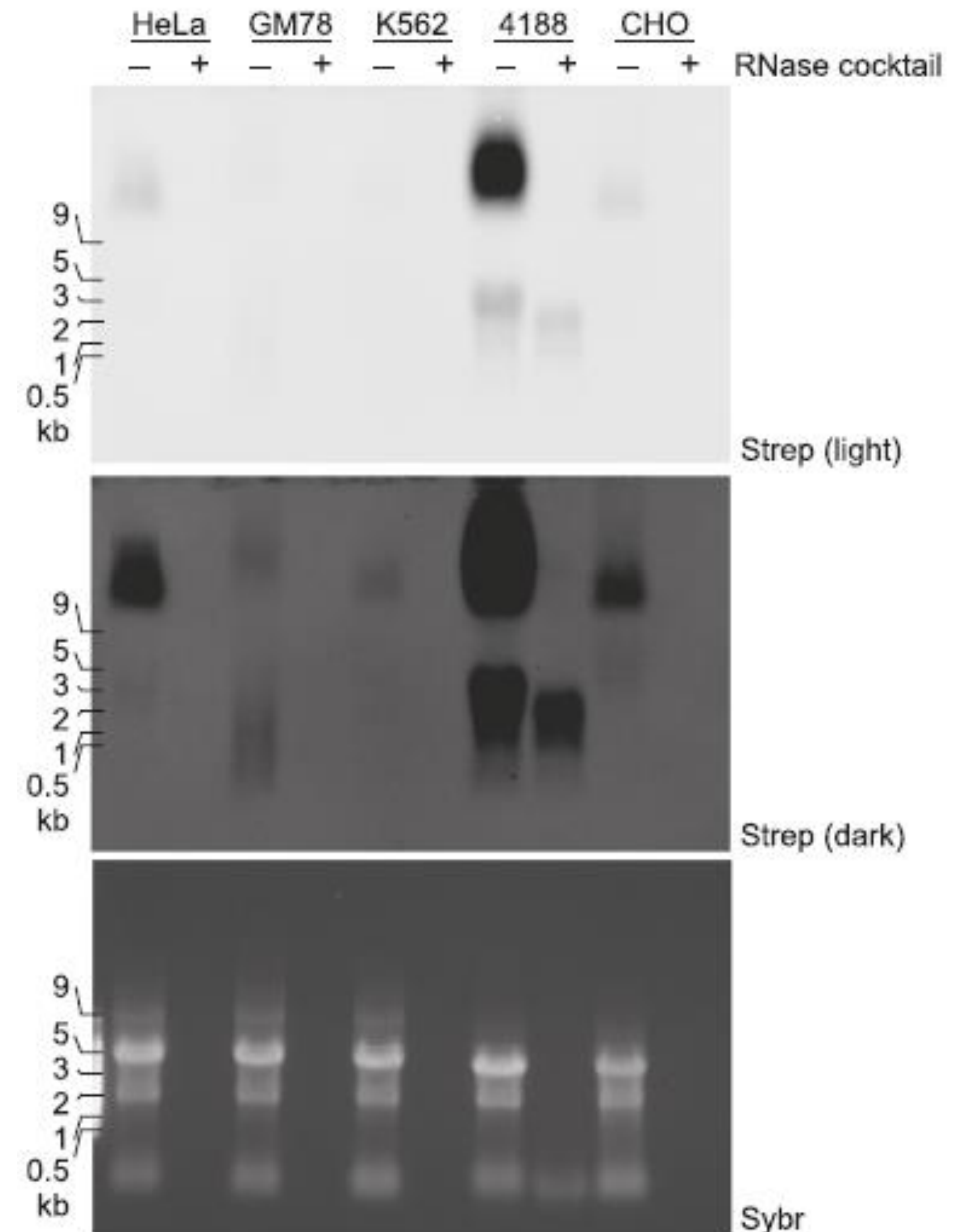



- ✓ treatment of RNA from Ac4ManNAz-labeled HeLa cells with DNase did not affect the glycoRNA signal, whereas treatment with an RNase cocktail efficiently digested the total RNA as well as the biotinylated glycoRNA

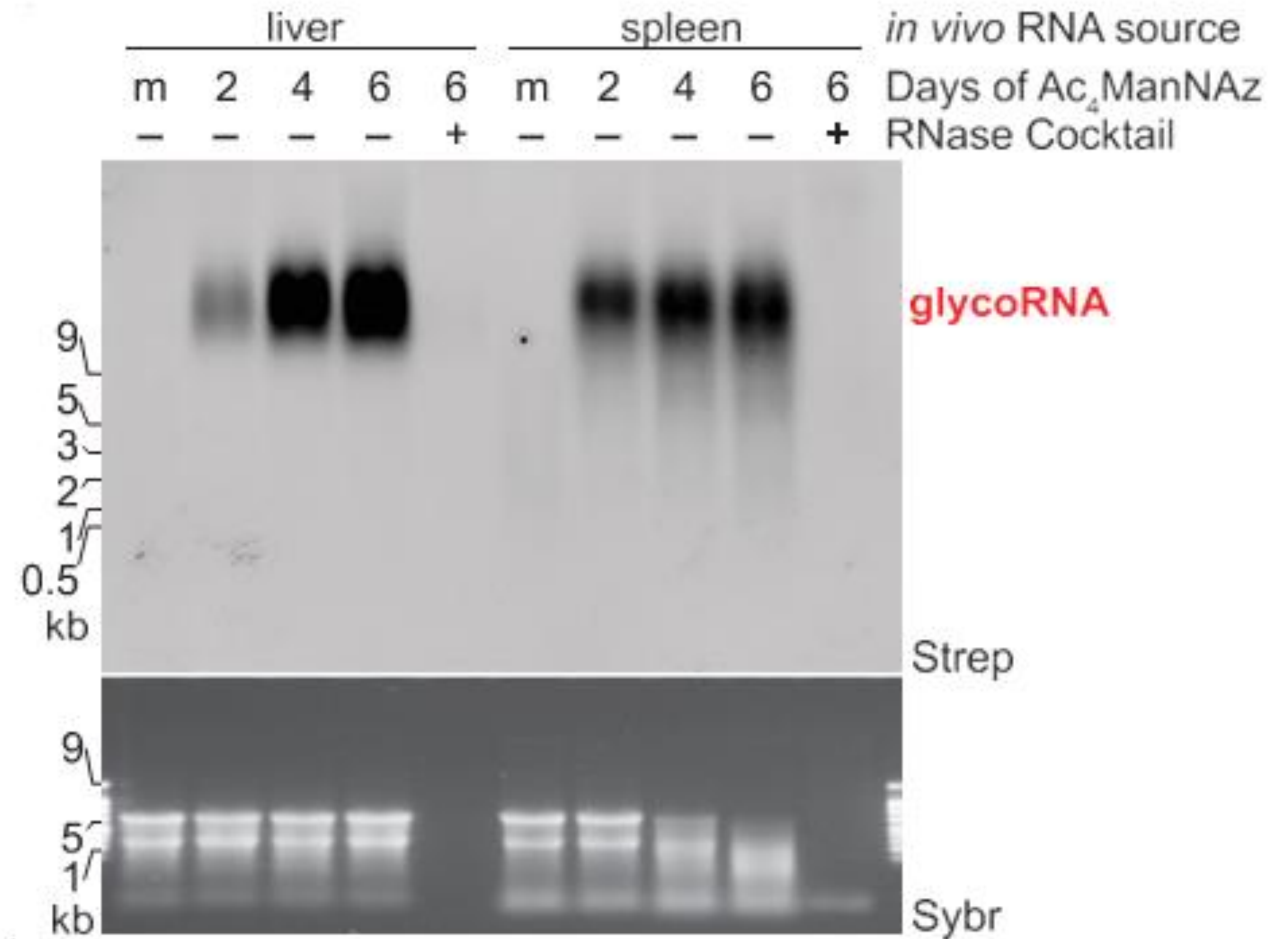


- ✓ Human embryonic stem cells (H9), a human myelogenous leukemia line (K562), a human lymphoblastoid cell line (GM12878), a mouse T cell acute lymphoblastic leukemia cell line (T-ALL 4188), and Chinese hamster ovary cells (CHO) all showed evidence of the presence of glycoRNA.
- ✓ H9 and 4188 cells showed significantly more labeling with Ac4ManNAz per mass of total RNA than other cell types

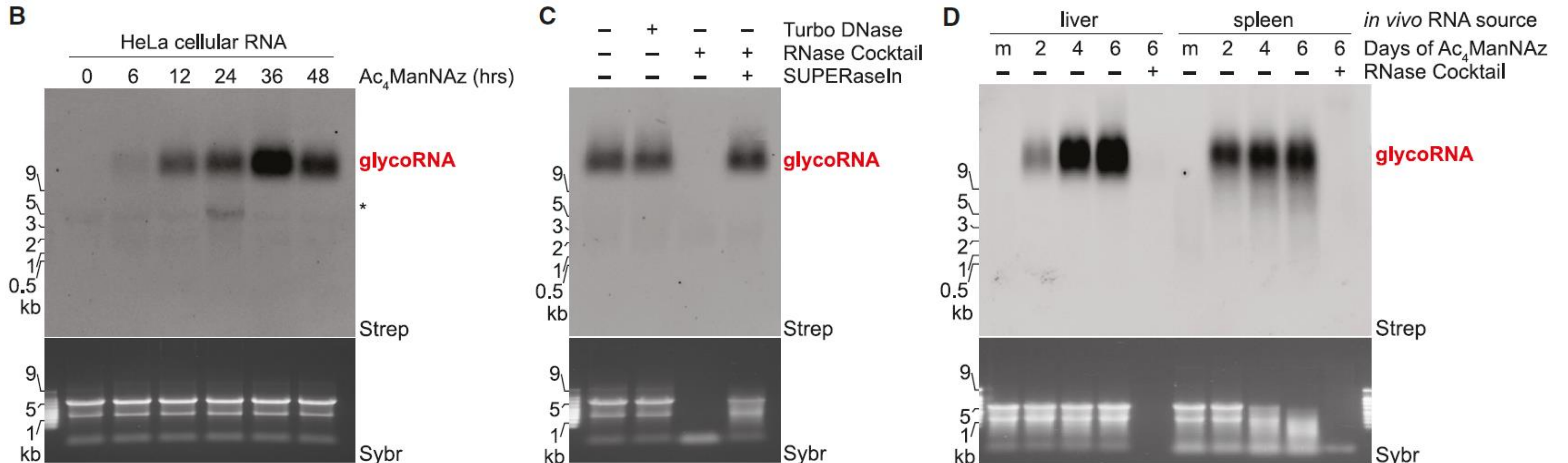


- Next, assessed this labeling in vivo.
- To this end, performed intraperitoneal injections of Ac4ManNAz into mice for 2, 4, or 6 days.
- In the liver and spleen, the organs that yielded enough total RNA for analysis, we observed dose-dependent and RNase-sensitive Ac4ManNAz labeling of RNAs in the same MW region as glycoRNAs from cultured cells
- These data suggest that glycoRNA is not an artifact of tissue culture and occurs broadly across multiple cell and tissue types and at various abundances.

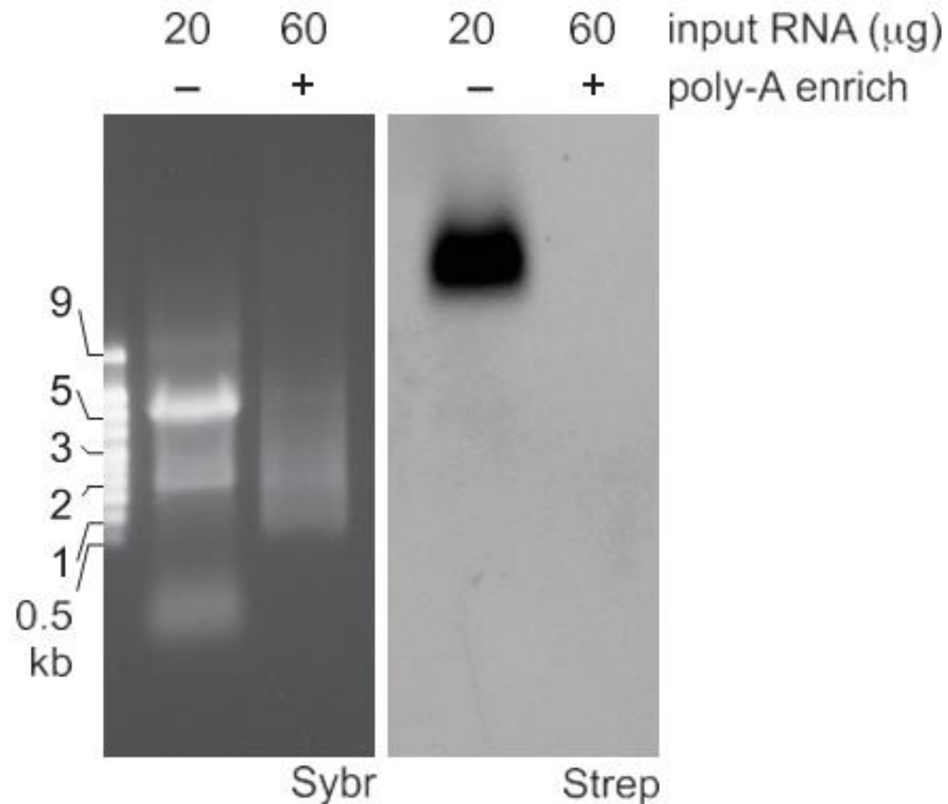


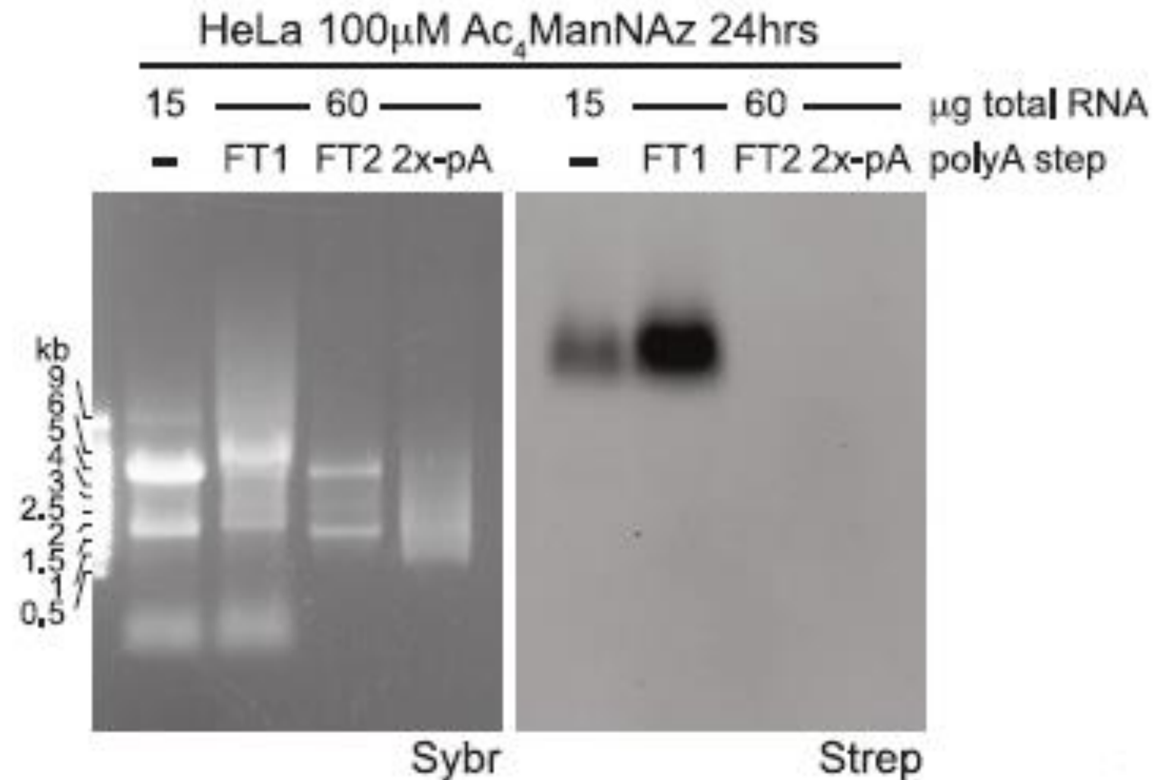
# glycoRNAs are small noncoding RNAs

- ✓ Across all cell types and organs tested, glycoRNA was found to migrate very slowly by denaturing agarose gel electrophoresis.

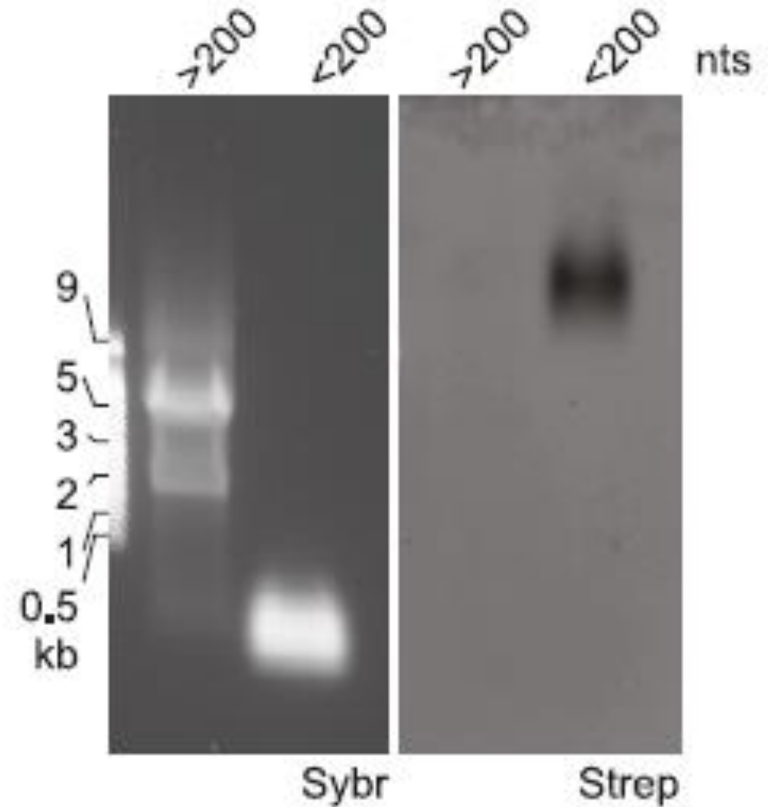


- ✓ We hypothesized that if glycoRNA's are indeed large RNAs, they would likely be polyadenylated (poly-A). However, we were consistently unable to purify glycoRNA from extracted RNA via poly-A enrichment.
- ✓ This was not due to cleavage or degradation of the glycoRNA during the poly-A enrichment procedure.

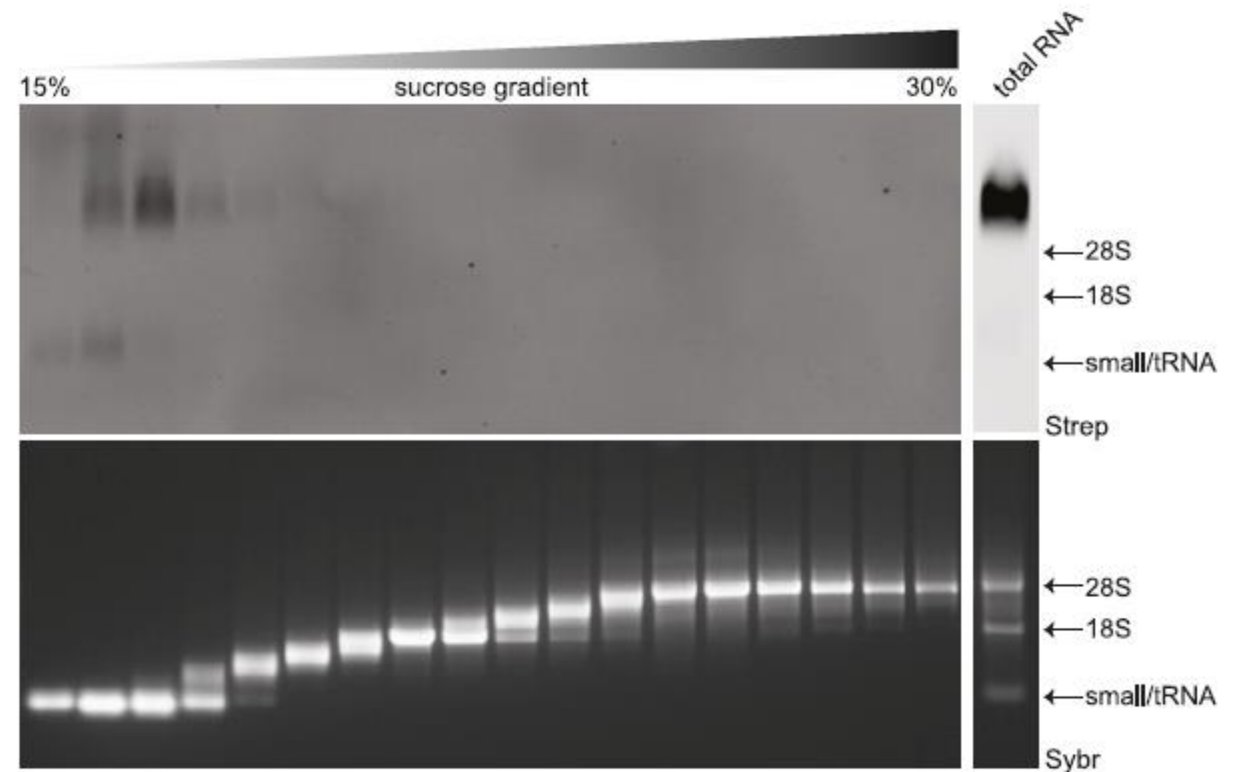


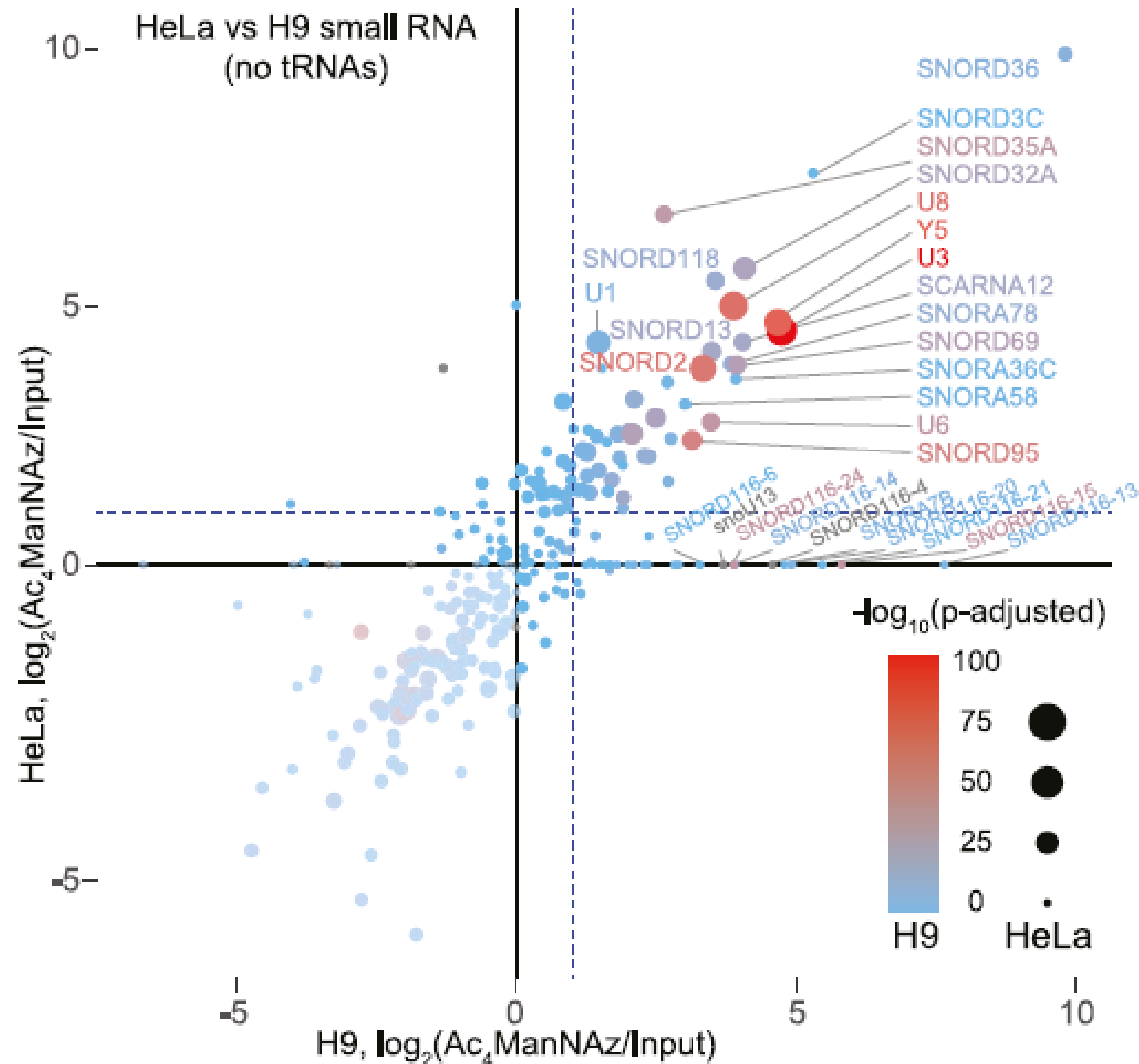
- ✓ As an alternative enrichment strategy, we used a commercial fractionation method that leverages length-dependent RNA precipitation and binding to silica columns to separate out ‘‘large’’ (>200 nt) from ‘‘small’’ (<200 nt) transcripts (STAR Methods). To our surprise, the glycoRNA fractionated exclusively with the small RNA population of total RNA



- ✓ The sucrose gradient robustly separated the major visible RNAs such as small RNAs/tRNA, 18S rRNA, and 28S rRNA
- ✓ glycoRNA's anomalous migratory behavior is caused by its associated glycans



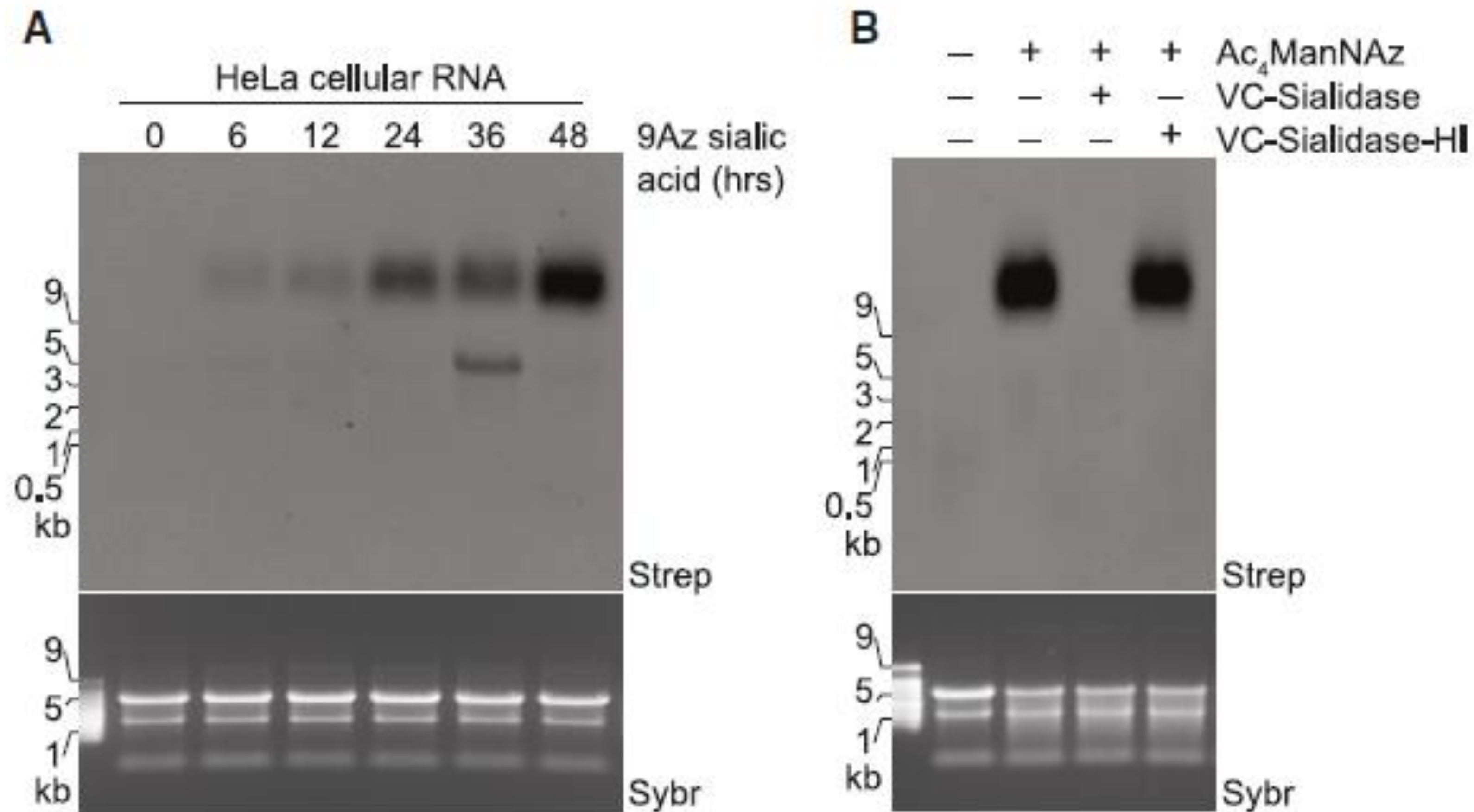
# A common set of transcripts are glycosylated across diverse cell types



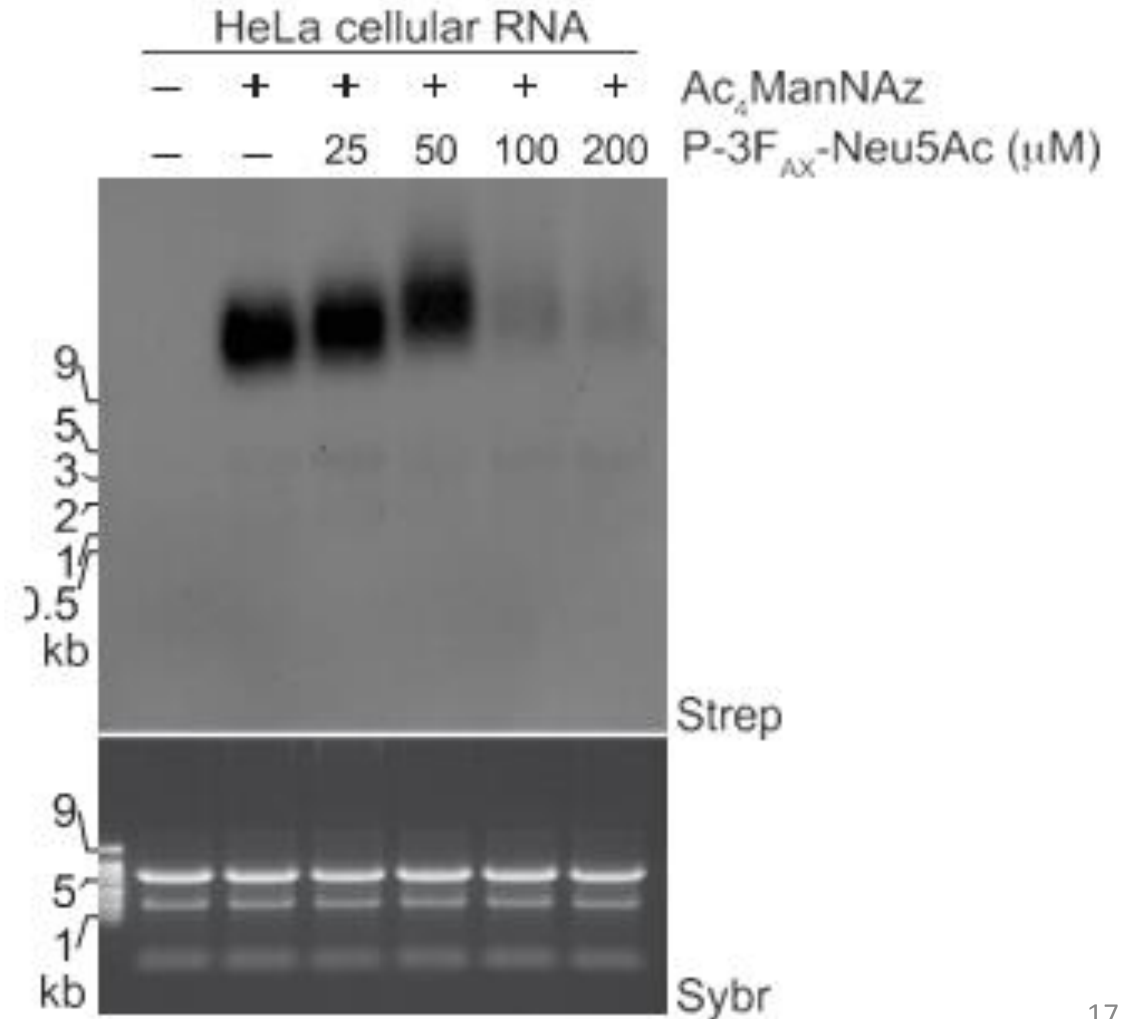
# Label and label-free detection of sialic acid in glycoRNA

- ✓ **Next step is determination the glycan structures on glycoRNAs**
- ✓ **Use 2 different method: metabolic and non-metabolic**

- ✓ To exclude the possibility that Ac4ManNAz is shunted into unexpected metabolic pathways, used 9-azido sialic acid (9Azsialic acid), which is directly converted into CMP-sialic as a metabolic label.



- ✓ assessed the contribution of canonical sialic acid biosynthesis enzymes through the use of P-3$F_{AX}$-Neu5Ac, a cell-permeable metabolic inhibitor of sialoside biosynthesis



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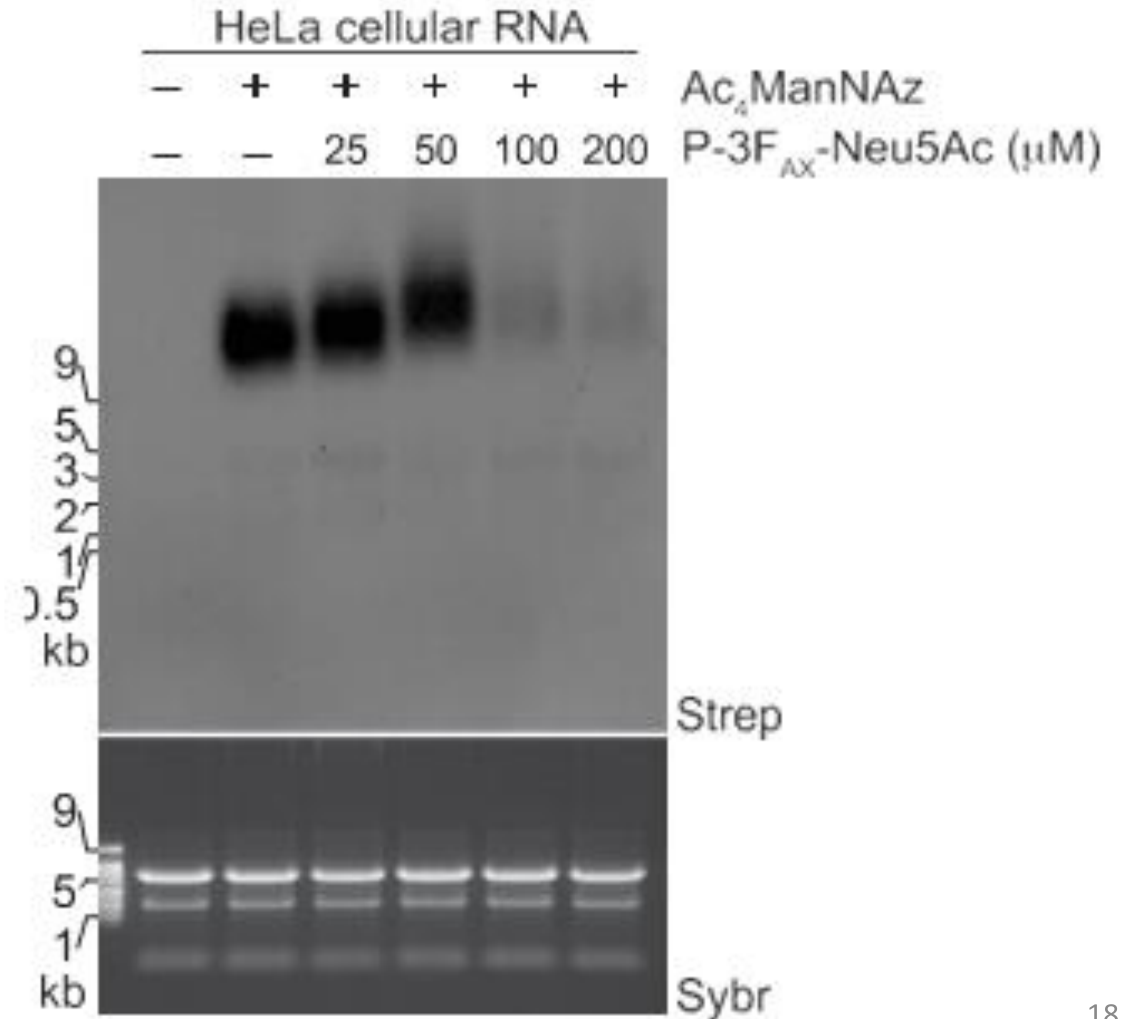
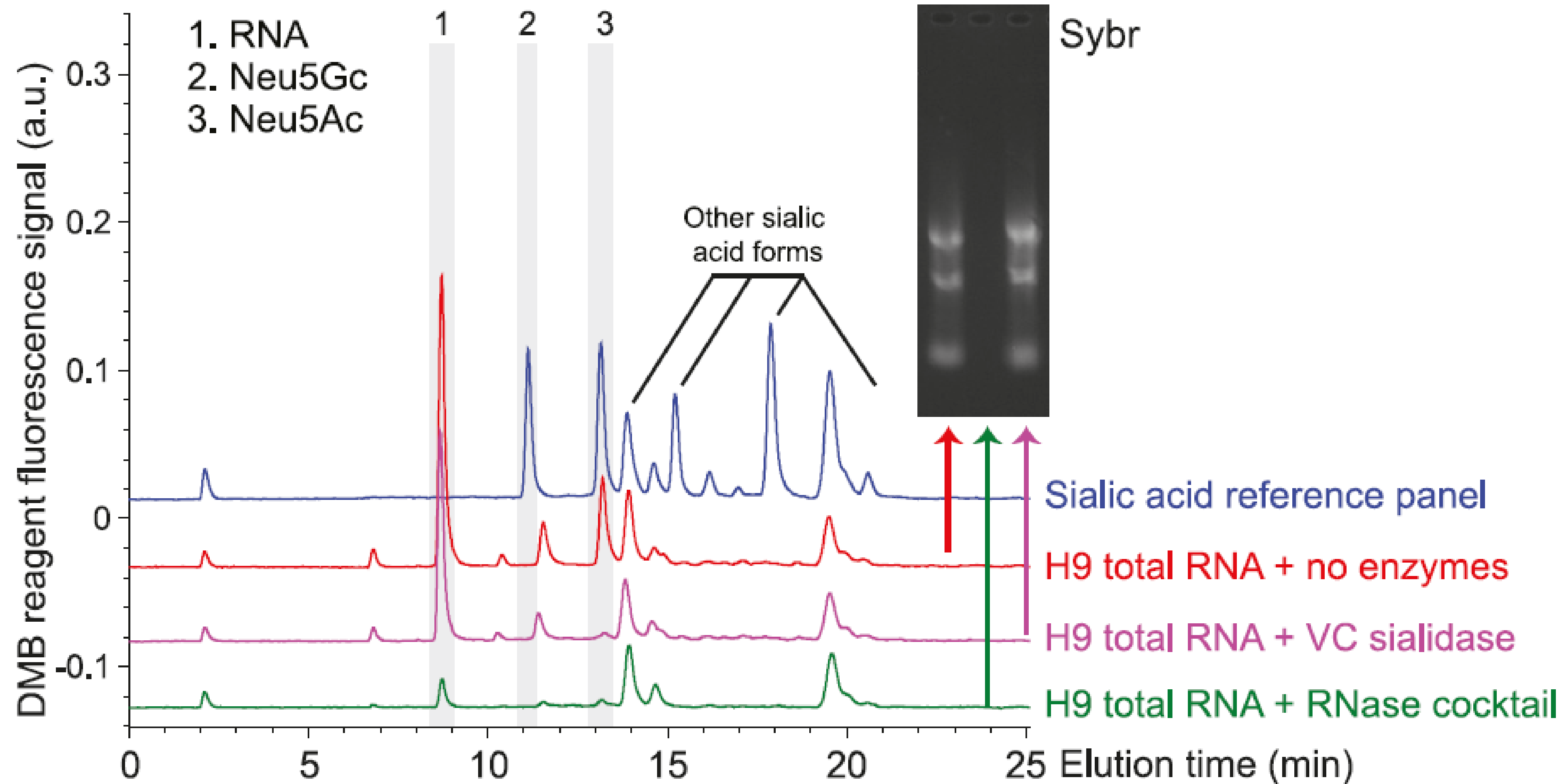
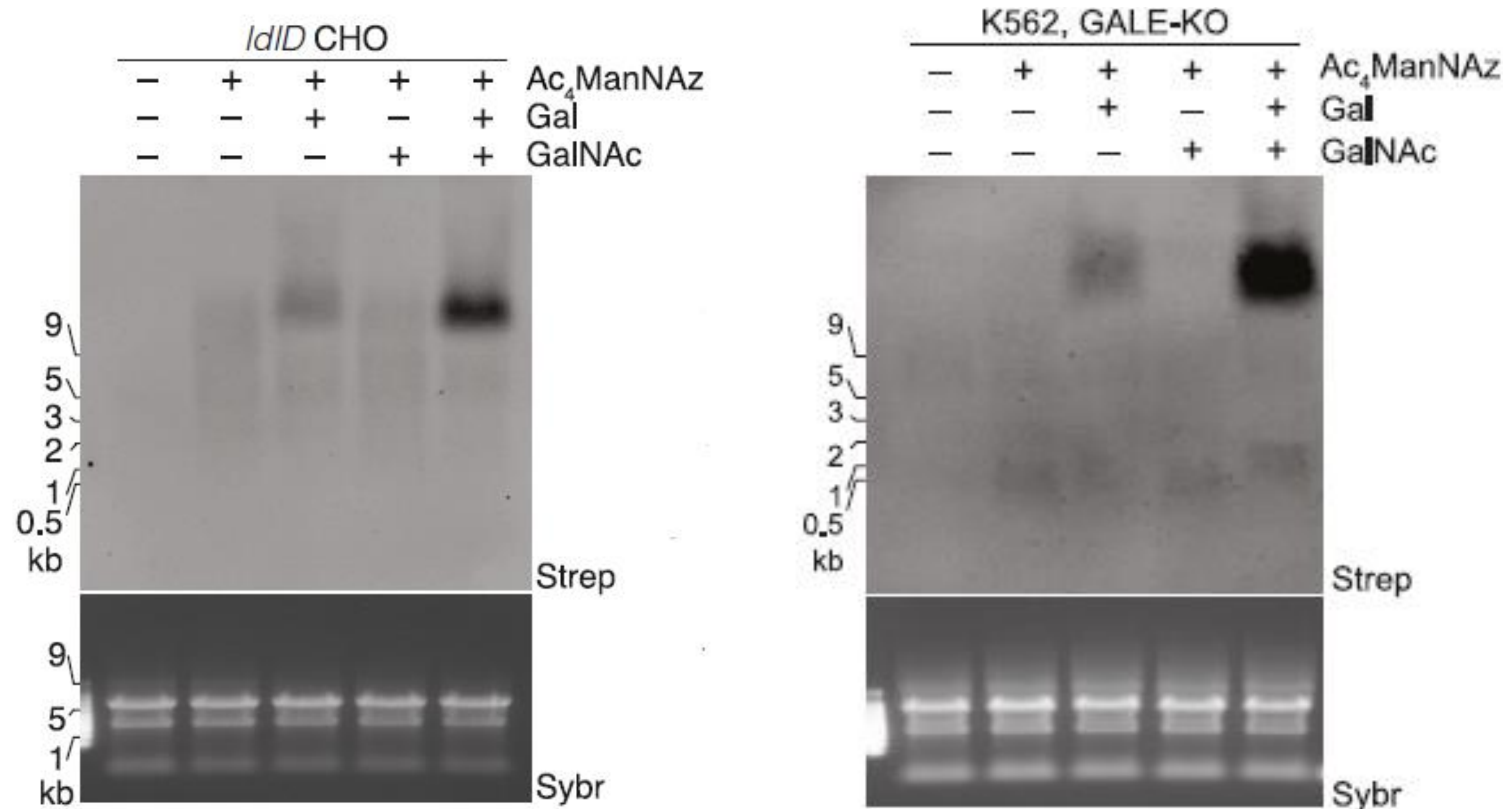


- ✓ To confirm that glycoRNAs are sialylated, used an independent method not relying on metabolic reporters
- ✓ The fluorogenic 1,2-diamino-4,5-methylenedioxybenzene (DMB) probe is used to derivatize free sialic acids for detection and quantitation by high-performance liquid chromatography (HPLC)-fluorescence

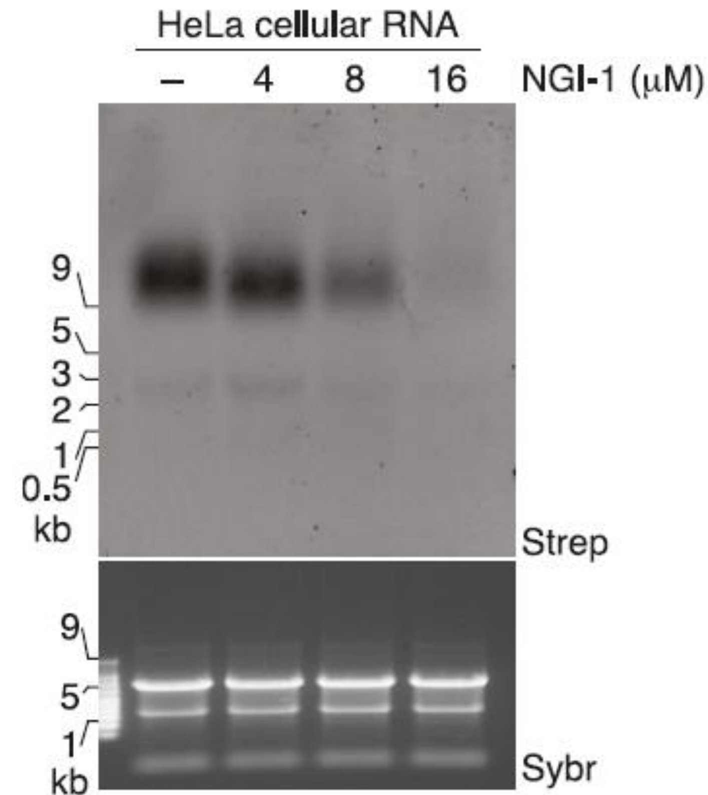


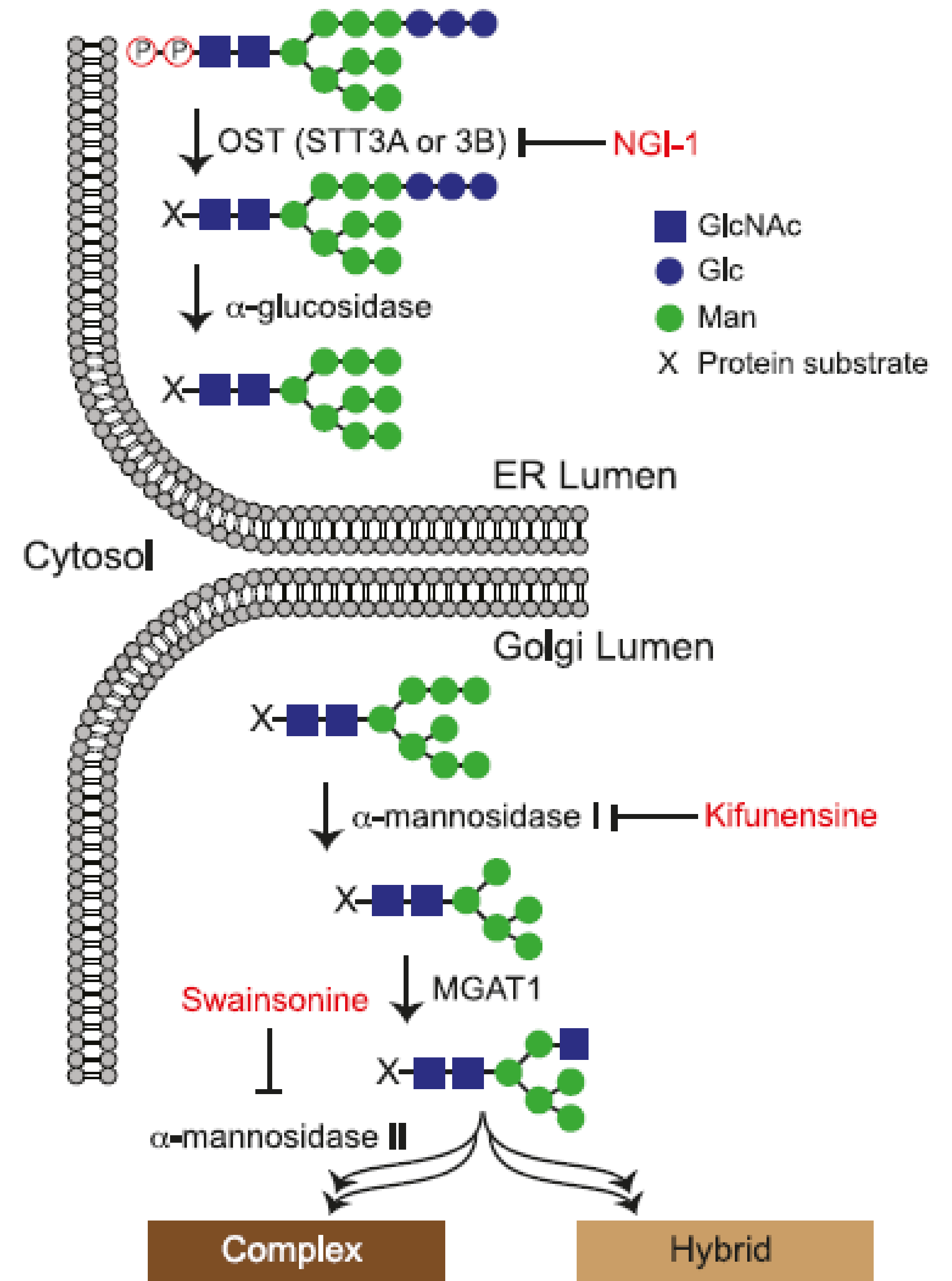
# Canonical N-glycan biosynthetic machinery contributes to glycoRNA production

- ✓ There are two main classes of glycans on proteins, N- and O-glycans, and both can be sialylated
- ✓ The ldlD mutant CHO cell line lacks the ability to interconvert GlcNAc into GalNAc
- ✓ human K562 cell line with a CRISPR-Cas9 targeted KO of UDP-galactose-4-epimerase (GALE), which mimics the phenotype of the ldlD CHO cell line



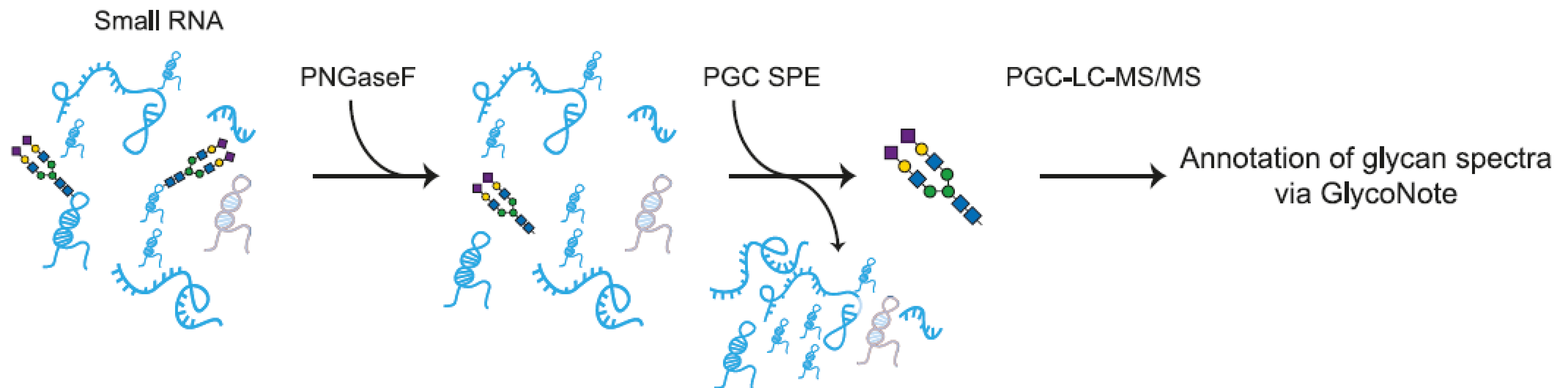
- ✓ Oligosaccharyltransferase (OST) mediates protein N-glycosylation by transferring a 14-sugar glycan to asparagine residues on nascent polypeptides during their translocation through the Sec/translocon
- ✓ tested the effect of NGI-1, a specific and potent small molecule inhibitor of OST , on glycoRNA production



# Mass spectrometry defines distinct compositions of glycans on RNA

- ✓ To develop a more precise view of the glycoforms associated with RNA, optimized a workflow based on PNGaseF-mediated release of glycans from pools of small RNAs, followed by analysis of those glycans by a porous graphitized carbon-based liquid chromatography MS strategy
- ✓ because the MS- based approach does not require sialic acid for enrichment or visualization, we were able to reveal an expanded set of glycan compositions that are often fucosylated and sometimes asialylated



# glycoRNAs are associated with cellular membrane

- ✓ The localization of Y RNAs has been reported to be mainly cytoplasmic with a minor fraction in the nucleus
- ✓ Other major classes of glycoRNA transcripts such as tRNAs and sn/snoRNAs are classically localized to the soluble cytosol and nucleus, respectively.

- ✓ To determine where glycoRNAs are distributed inside cells, used two biochemical strategies:
  1. Isolates nuclei away from membranous organelles and the cytosol
  2. Separates the soluble cytosolic compartment away from membranous organelles

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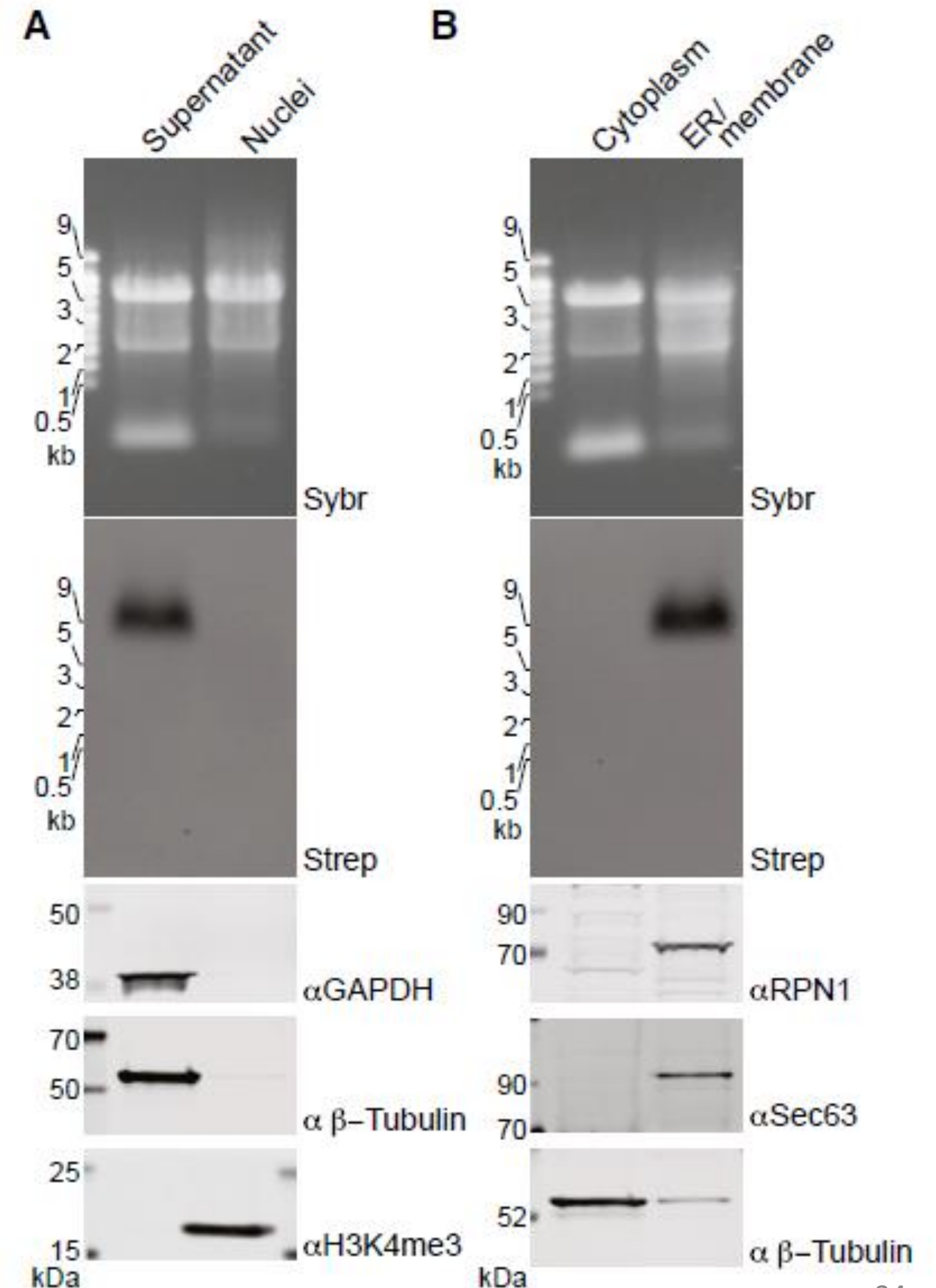
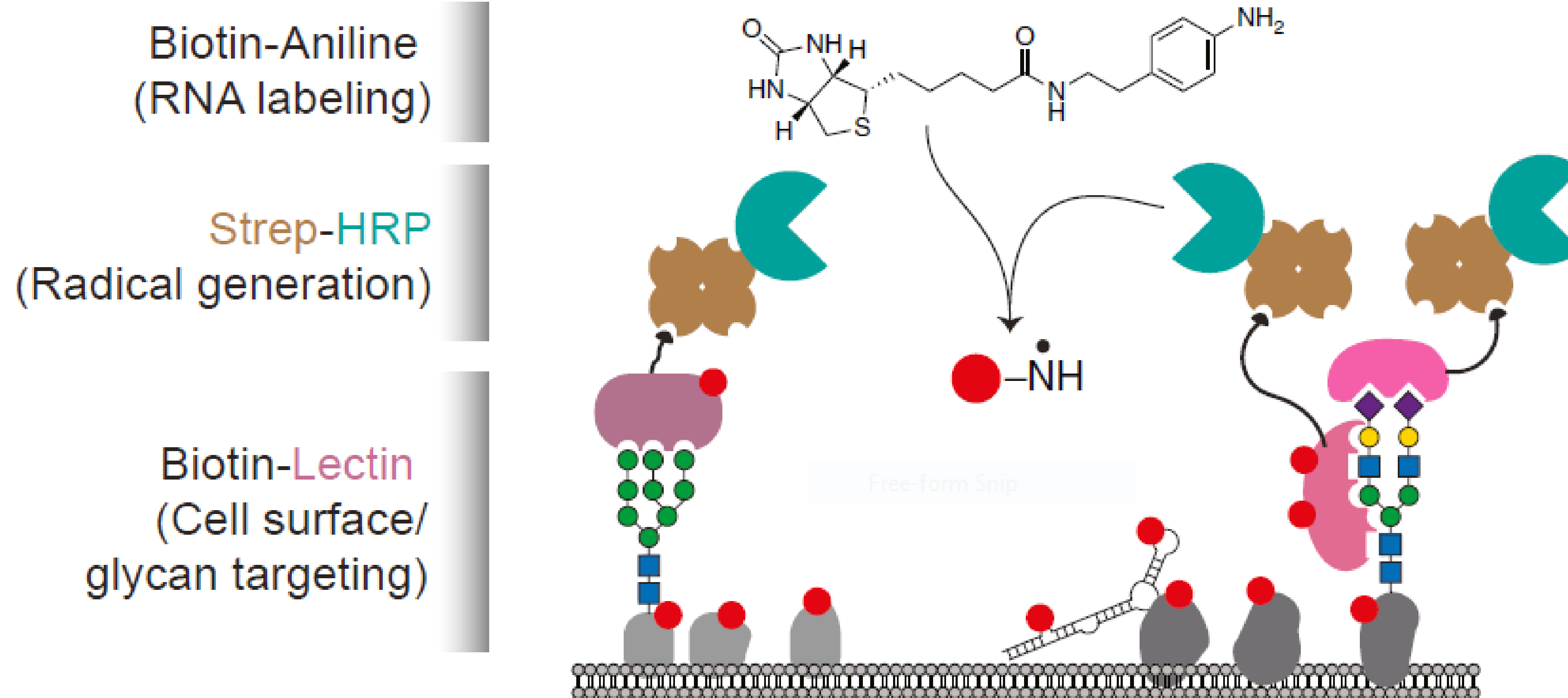


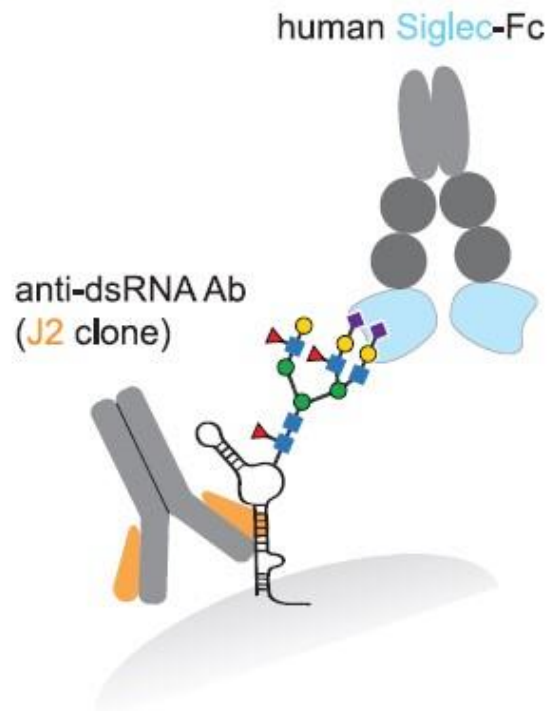
# glycoRNAs gain access to the surface of living cells

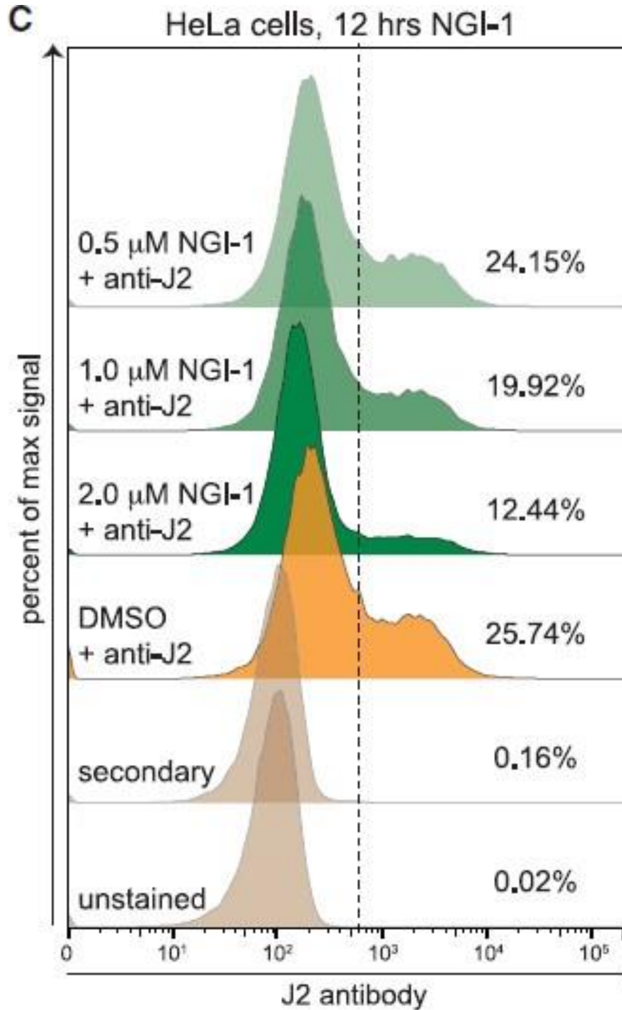
- ✓ done strictly at 4oC to reduce or eliminate vesicular trafficking

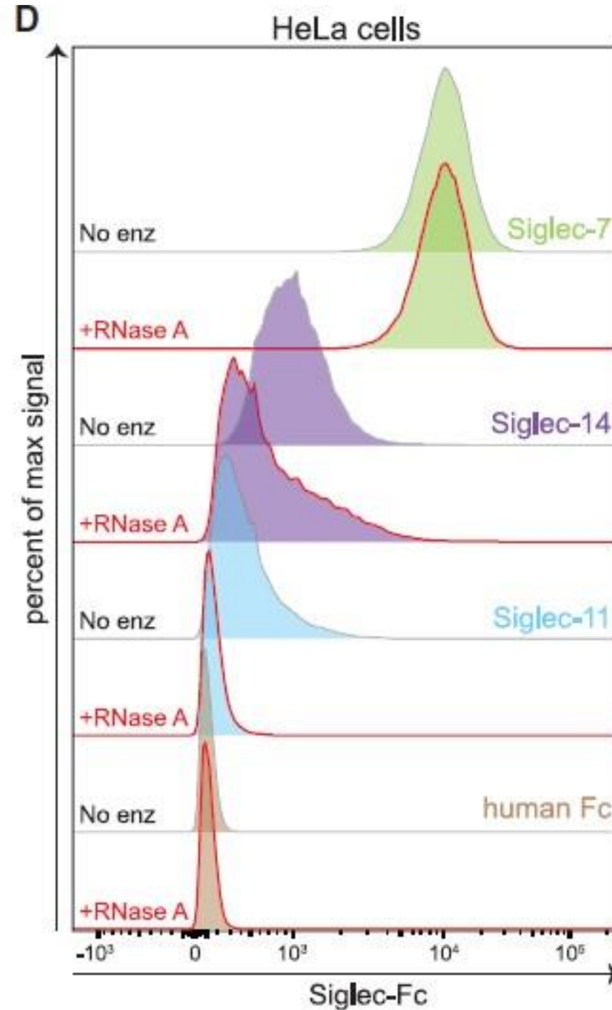


# Siglec receptors and anti-RNA antibodies recognize cell surface glycoRNAs

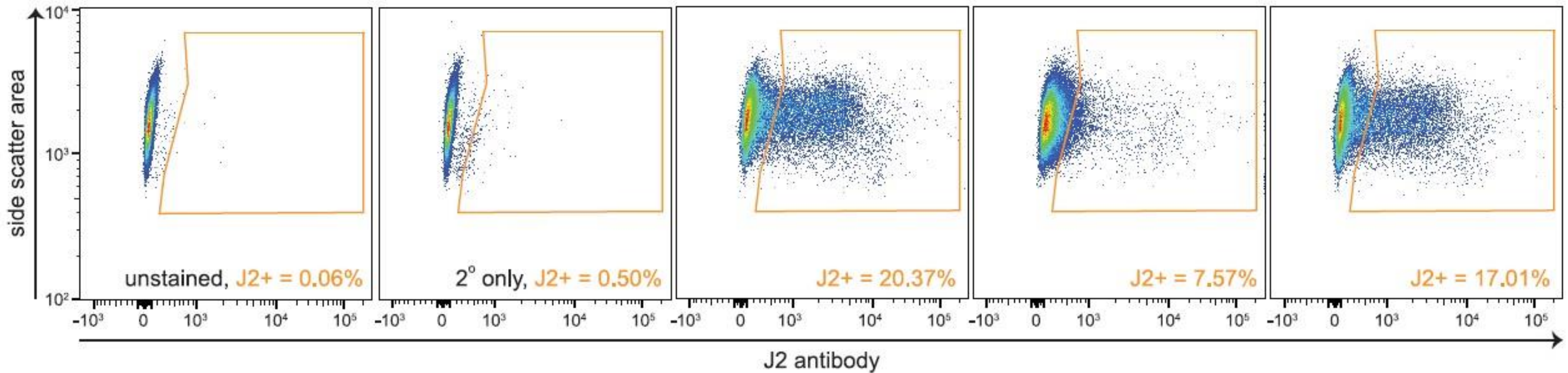
- ✓ Use antibodies that targeting RNA which have been associated with systemic lupus erythematosus (SLE)



- ✓ Approximately 20% of a population of cultured HeLa cells showed positivity with J2 staining



# Chemical linkage of RNA to glycan

- ✓ Although the precise nature of the glycan-RNA linkage has not yet been determined, we speculate that direct glycosylation of native RNA bases is unlikely.
- ✓ The observed sensitivity to PNGase F, which cleaves the glycosidic linkage between asparagine and the proximal GlcNAc of N-glycans, implies an amide bond-containing linker that native nucleobases lack.
- ✓ It is possible that a precursor guanosine modification is necessary to establish an asparagine-like functionality capable of modification by OST (Oligosaccharyltransferase), or that a preassembled N-glycan carrier moiety is attached to nu- cleobases by some other chemistry.
- ✓ These possibilities are consistent with sedimentation of glycoRNAs in the sucrose gradient, which suggests a linker with a relatively small molecular weight.

# Limitations of study

- ✓ A major focus of the work presented leverages selective metabolic labeling of sialic acid with Ac4ManNAz.

- ✓ Because not all glycans contain sialic acid, it is possible that glycoform's beyond those reported here may also be conjugated to RNAs

- ✓ The precise linkage between the RNA template and carbohydrate remains unknown