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

Ryan A. Flynn et al. 2021

Presented by Manon Cahueau, Isabelle Gracien & Lucas Pradeau

# Outline

Introduction

I. Are RNAs modified with glycan structures?

II. What type of RNA are glycoRNAs?

III. What kind of glycan structures modify RNAs?

IV. Does the canonical glycan biosynthetic machineries contribute to glycoRNA production?

V. Where are localized glycoRNAs in the cell?

VI. Can Siglec receptors and anti-RNA antibodies recognize cell surface glycoRNAs?

Conclusion & perspectives

# Introduction – Cell surface membrane biomolecules

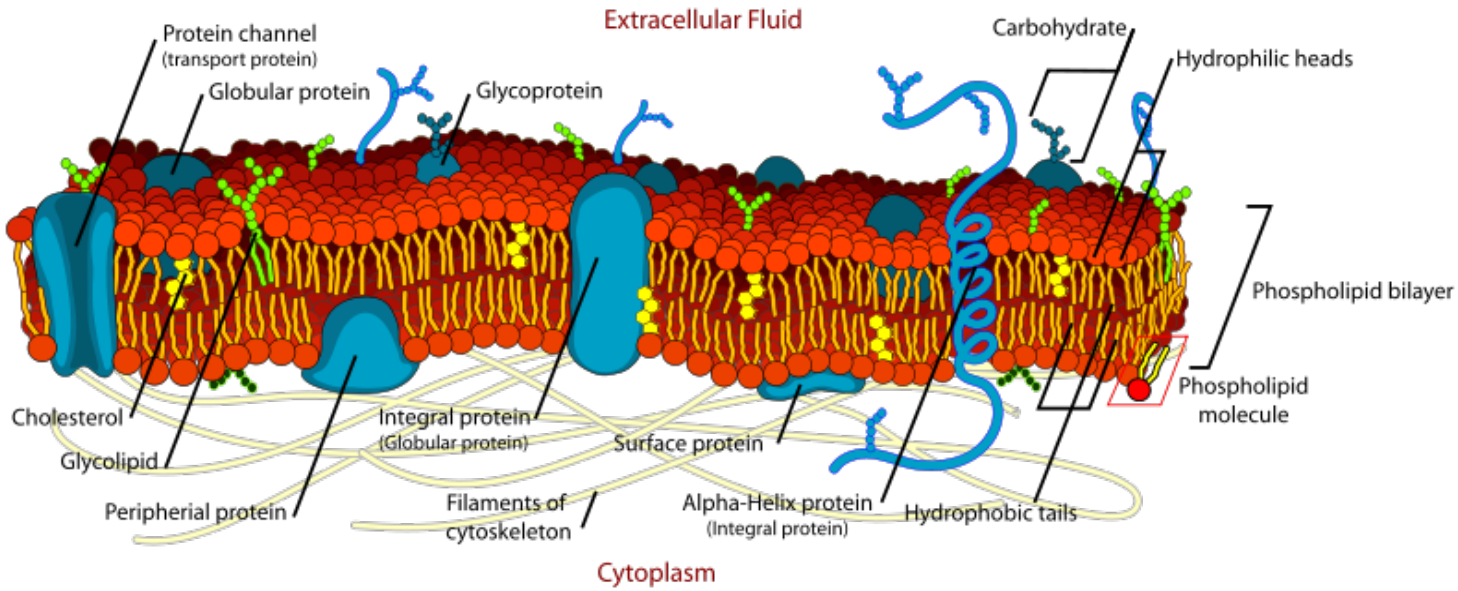



**Fig.1.** Schematic representation of the cell membrane [1]

❖ The fluid mosaic model of membranes

- Phospholipids
- Proteins
- Carbohydrates (Glycoconjugates)

❖ Different functions

- Internal / external compartmentalization
- Cell identity and integrity
- Cell interactions and communications
- Cell morphology
- Cell exchanges
- Endocytosis
- Exocytosis
- …

# Introduction – The glycoconjugate components of plasma membrane

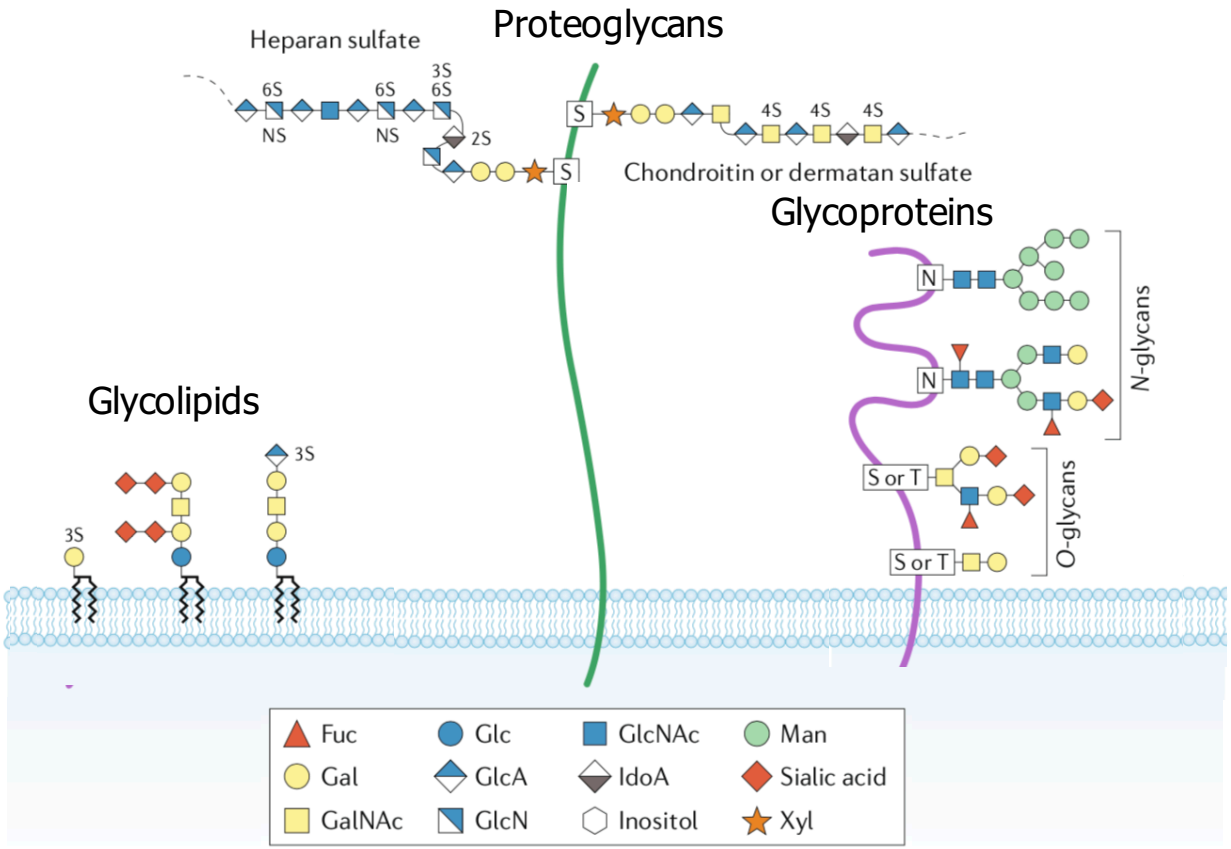
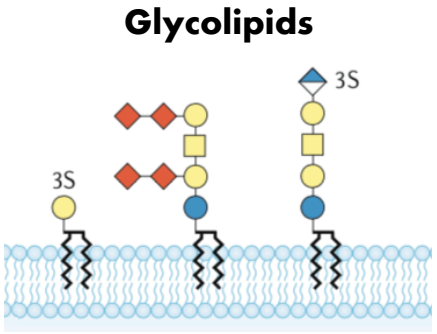



**Fig.2.** The glycoconjugate categories [2]

# Introduction – The glycoconjugate components of plasma membrane




- Glycolipids = carbohydrates + lipids
- 2 types of glycolipids:
  - Glycoglycerolipids (glycerol backbones)
  - Glyocosphingolipids (sphingosine backbones)
- Functions: Cell interactions (immune response, pathogene interactions), blood group determination

- Example: ABO blood group determinants are glycosphingolipids

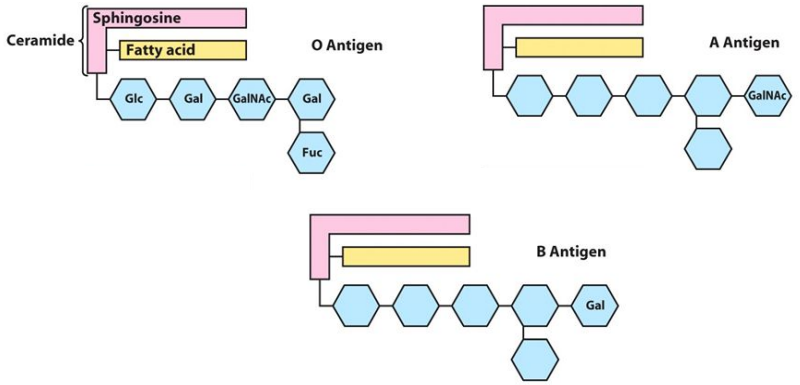
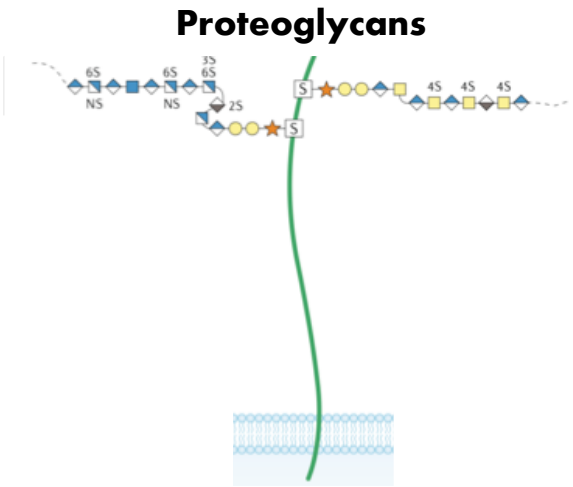


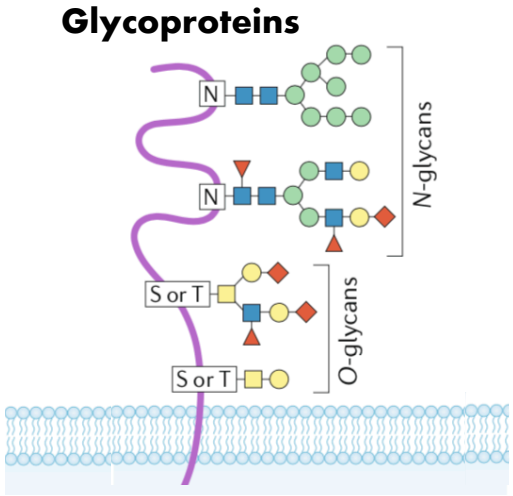

**Fig.3.** The ABO blood group glycolipid determinants [3]

# Introduction – The glycoconjugate components of plasma membrane




- Proteoglycans = glycosaminoglycans (carbohydrates) + proteins
- One of the major components of extracellular matrix
- Functions: Cell adhesion, water osmosis, molecules sequestration (growth factors)
- Example: Fibroglycans (heparan sulfate proteoglycans) interact with adhesion molecules and growth factors and support the cell shape

# Introduction – The glycoconjugate components of plasma membrane




- Glycoproteins = carbohydrates + proteins
- Functions: cell-cell recognition, cell interaction and communication, ligand binding, cell signaling, cell transport
- Example: P-glycoproteins (exportation transmembrane transporter) cause a phenomenon of multi-drug resistance

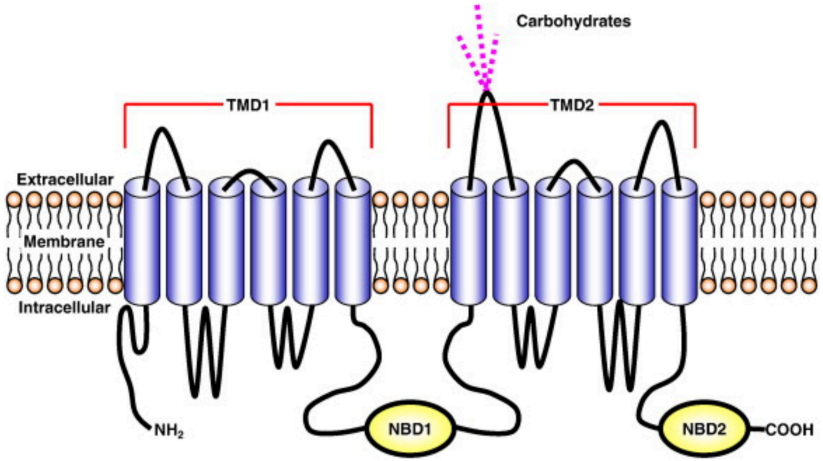
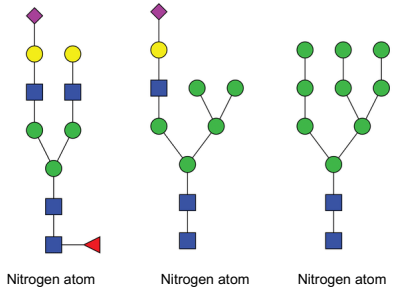
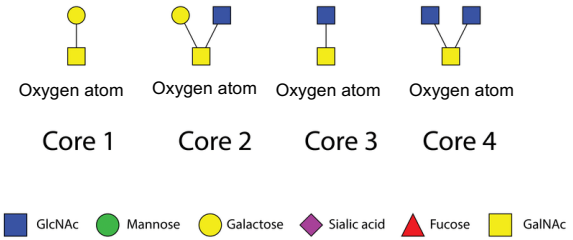



**Fig.4.** Secondary structure of the P-glycoprotein [4]

# Introduction – Two major types of glycosylation

**Glycosylation is the controlled enzymatic modification of an organic molecule (lipid or protein) by addition of a sugar molecule.**

| | N-linked Glycosylation [2, 5, 6] | O-linked Glycosylation [2, 5, 6] |
|---|---|---|
| |  |  |
| Glycosylation chemical linker | Nitrogen atom | Oxygen atom |
| Glycosylation localization | Endoplasmic reticulum and Golgi (maturation) | Golgi (only) |
| Glycosylation target | Lipids and proteins | |
| Proteins target site | Asparagine on consensus sequence<br>Asn-X-Ser/Thr (X, every AA except Pro) | Serine or Threonine<br>no known consensus sequence |
| Lipids target site | Amine group directly linked to C1 of lipids | Hydroxyl group directly linked to C1 of lipids |
| Glycosylation relevance | Folding and trafficking of proteins and lipids for secretion or membrane presentation | |
| Cell functions involving glycosylation | Cell interaction, Cell communication, Host-pathogen recognition, Immune system activation,… | |
| Occurence | Occurs mainly in Eucaryotes and Archae | Occurs in Eucaryotes, Archae and Bacteria |

# Introduction – Reminder on RNA classification

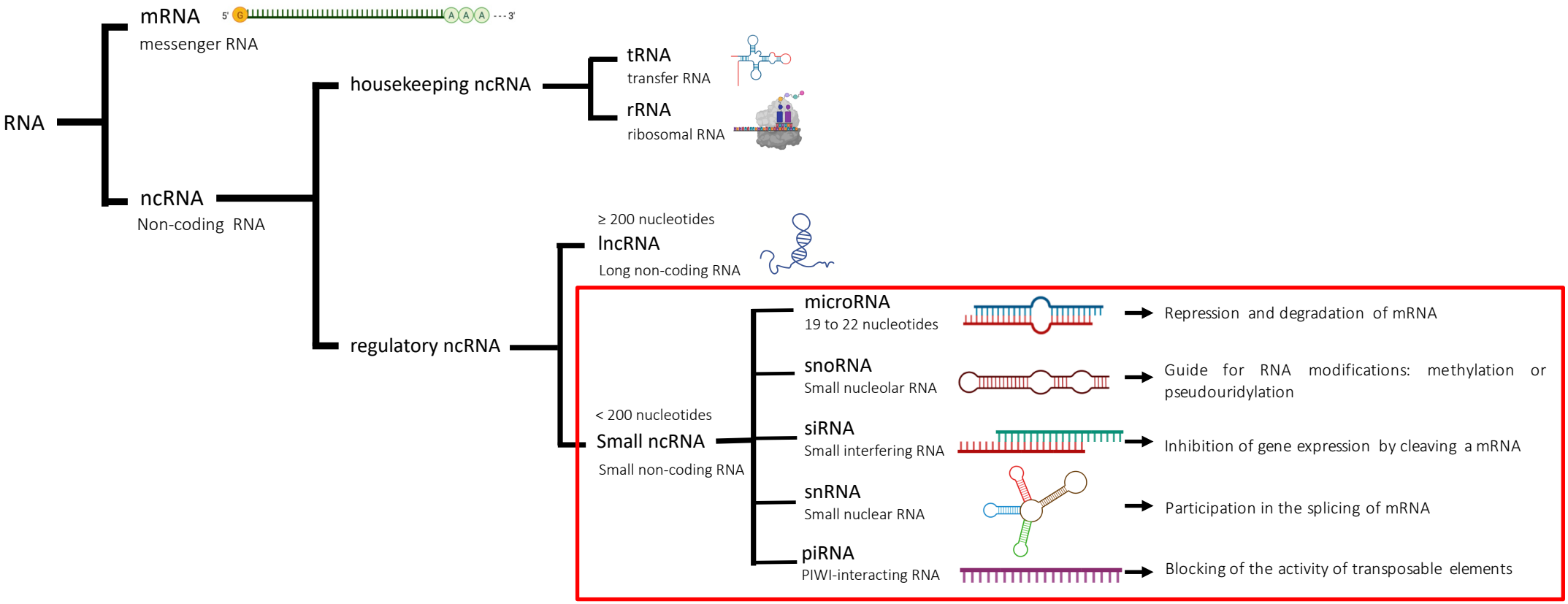



**Fig.5.** Classification of RNAs [7, 8]

# Introduction – RNA modifications

- RNA is the substrate for post-transcriptional modifications (PTMs)
- PTMs include the 5’ cap and polyA tail

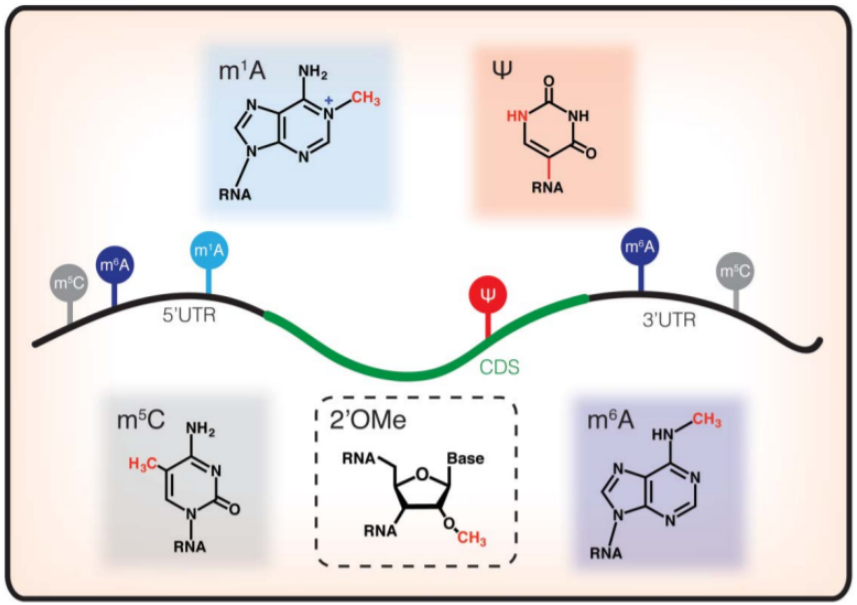



**Fig.7.** Main RNA internal modifications [10]

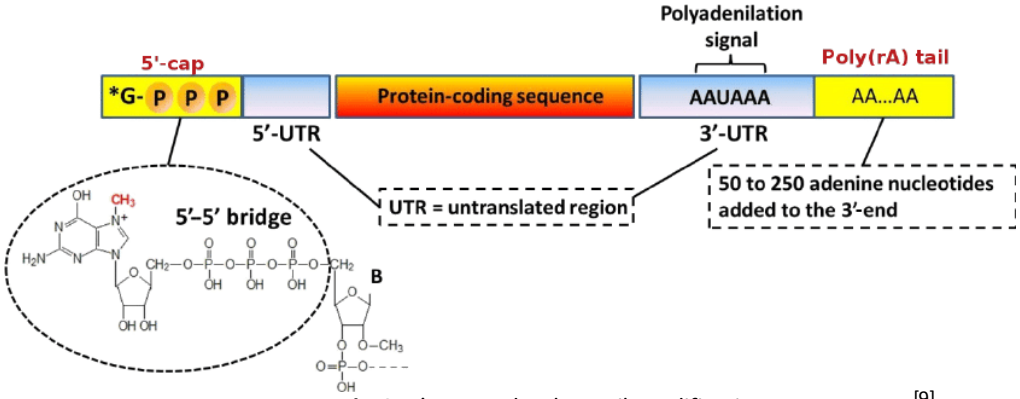
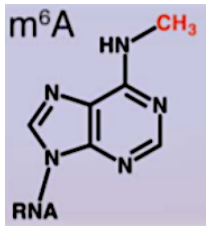
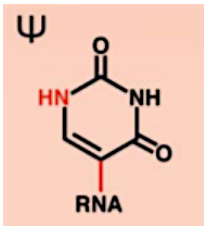
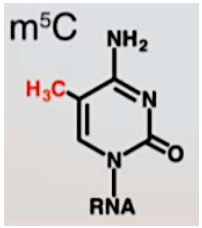
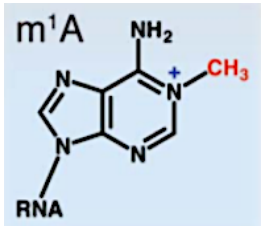
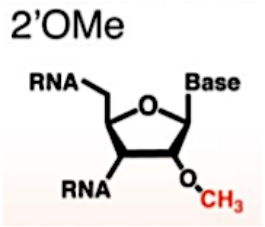



**Fig.6.** 5’ cap and polyA tail modifications on mRNAs [9]

- PTMs can also occur in the internal part of the transcripts
- Over 100 PTMs have been identified
- These RNA modifications are likely reversible and regulated

# Introduction – RNA modifications

| | $N^6$-methyladenosine | Pseudouridine | 5-methylcytosine | $N^1$-methyladenosine | 2'-O-methylation |
|---|---|---|---|---|---|
| **Structure** |   |   |   |   |   |
| **Main functions** | - Degradation of transcripts<br>- Promotion of translation initiation | - Response to stress conditions<br>- Readthrough of stop codon | - Function remains unclear<br>- Involvement in nuclear export? | - Upregulation of translation | - Roles remain to be identified |

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Article

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

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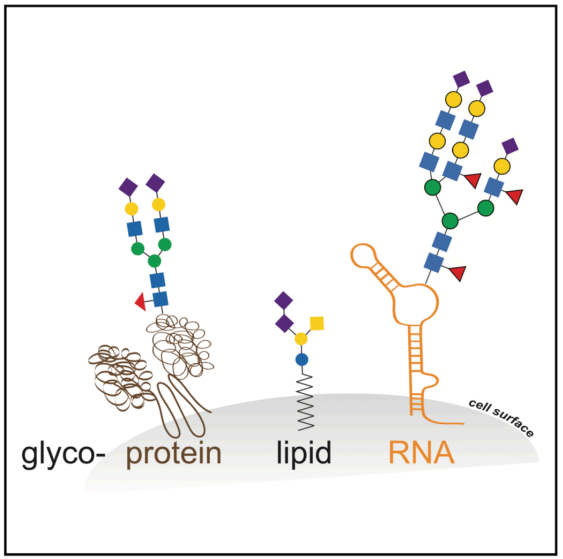
Graphical abstract



# I. Are RNAs modified with glycan structures?

**<u>Authors' hypothesis:</u> Possible existence of RNA modified with sialoglycans**

- Method: Glycoconjugates metabolic labeling and high purity RNA extraction

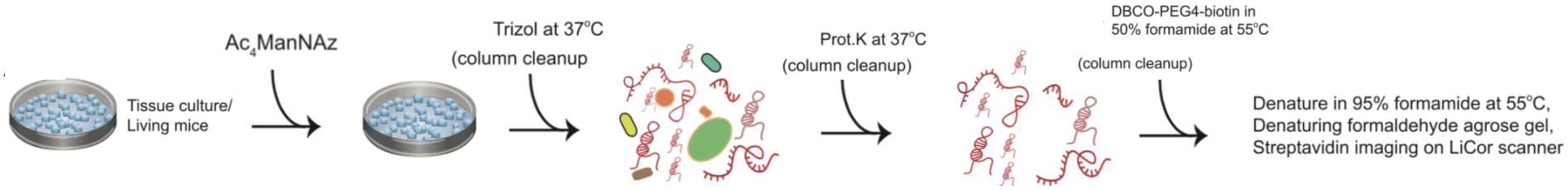



**Fig.8.** Scheme of glycan labeling and RNA extraction protocol [11]

# I. Are RNAs modified with glycan structures?

**Authors' hypothesis: Possible existence of RNA modified with sialoglycans**

- Results by RNA blotting

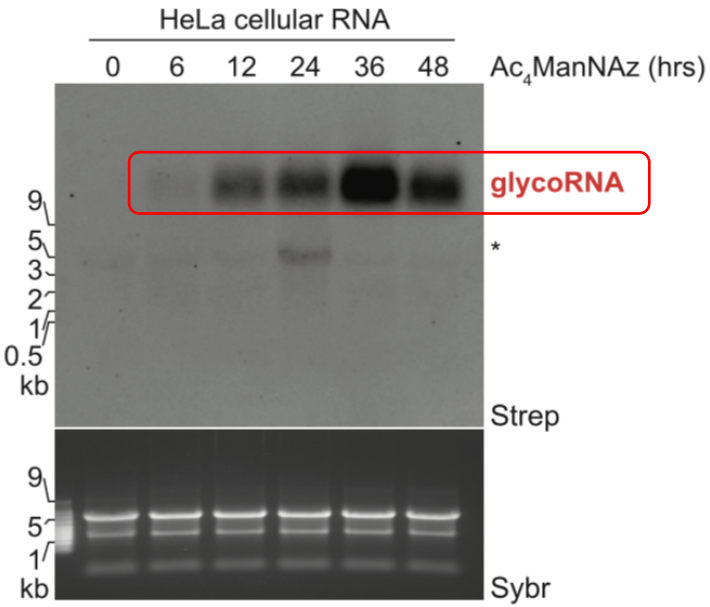



**Fig.9.** RNA blotting of RNA from HeLa cells treated with $Ac_4$ManNAz for the indicated amount of time [11]

- Glycans are revealed thanks to $Ac_4$ManZAz/DBCO-biotin conjugaison and Streptadivin visualization
- Total RNA is stained by Sybr Gold

→ **In an $Ac_4$ManNAz and time-dependent manner,** biotinylated species are identified in a high molecular weight region

# I. Are RNAs modified with glycan structures?

**Are these biotinylated species really RNA transcripts?**

- Same labeling and purification methods as previously
- Treatment with Turbo DNase, RNases and SUPERaseIn (Rnases inhibitor)
- Results by RNA blotting

❖ Treatment of RNA with DNase did not affect the glycoRNA signal

❖ Treatment of RNA with RNase cocktail digested the total RNA

❖ SUPERaseIn rescued the biotinylated glycoRNA signal

**→ Cells treated with $Ac_4$ManNAz incorporated the azide label into cellular RNA, which migrates on an agarose gel as a high MW species**

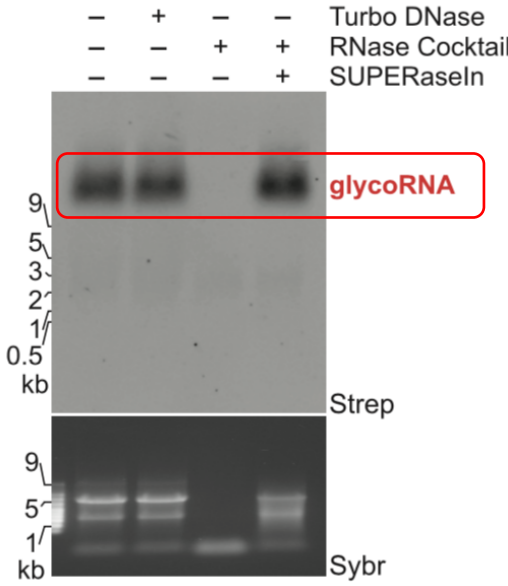



**Fig.10.** RNA blotting of $Ac_4$ManNAz labeled HeLa RNA treated *in vivo* with turbo DNase or RNase cocktail [11]

# I. Are RNAs modified with glycan structures?

**Are these glycoRNAs present in other cells types?**

- Results by RNA blotting

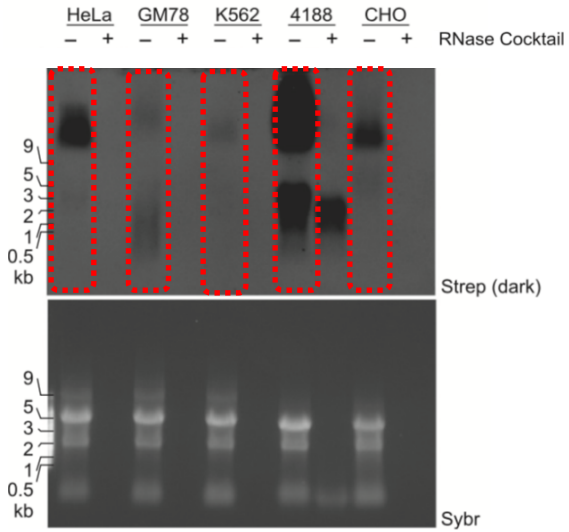



**Fig.11.** Blotting of RNA from various cell types labeled with $Ac_4$ManNAz [11]

❖ Different cell types tested:

HeLa: Human epithelial adenocarcinoma cell line

GM78 (GM12878): Human lymphoblastoid cell line

K562: Human myelogenous leukemia cell line

4188 (MYC T-ALL4188): Mouse lymphoblastic leukemia cell line

CHO: Chinese hamster ovary cell line

❖ H9 and 4188 cells showed significantly more labeling with $Ac_4$ManNAz per mass of total RNA than other cell types

**→ Evidence of the presence of glycoRNAs in different amount, in other cell types and also in another mammalian species (mouse)**

# I. Are RNAs modified with glycan structures?

**Does this labeling occur *in vivo*?**

- $Ac_4$ManNAz Intraperitoneal injection in mice
- Same labeling and purification methods as previously

**→ Dose-dependent and RNase-sensitive $AC_4$ManNAz labeling of RNAs in the same MW region as glycoRNAs from cultured cells**

- Results by RNA blotting

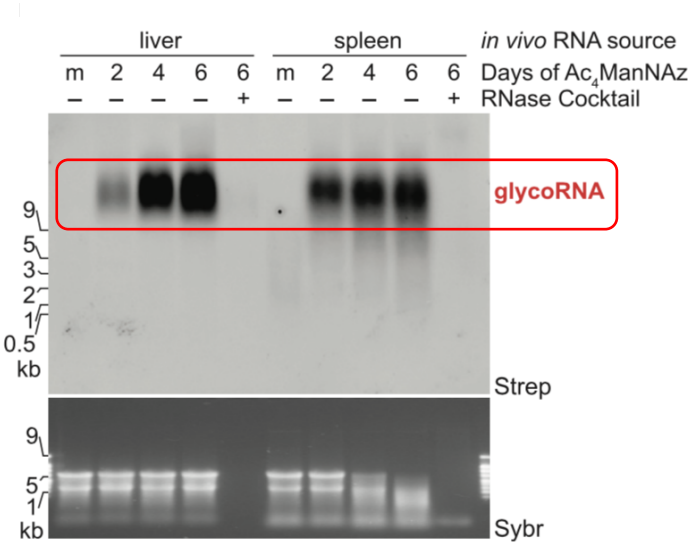



**Fig.12.** RNA blot of murine RNA after in vivo $Ac_4$ManNAz delivery via intraperitoneal injection (RNA from liver and spleen) [11]

# I. Are RNAs modified with glycan structures?

Take-home message

**This data suggests that glycoRNAs are not an artifact of tissue culture and that this modification occurs broadly: in cultured cells, *in vivo*, across multiple cell and tissue types of different mammals and at different abundances.**

# II. What type of RNA are glycoRNAs?

**<u>Authors' hypothesis:</u> If glycoRNAs migrate as a high molecular weight species, they are expected to be long length poly-adenylated RNAs**

- Method: PolyA enrichment of RNA

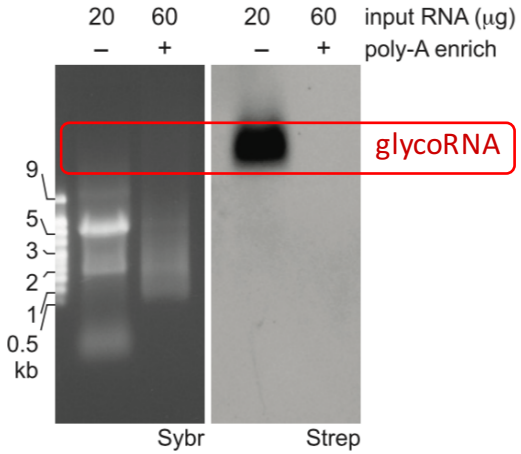



**Fig.13.** Blotting of total or poly-adenylated enriched RNA from HeLa cells treated with $Ac_4$ManNAz [11]

**→ Unable to purify glycoRNAs from extracted RNA with this method**

- Method: Fractionation method according to length

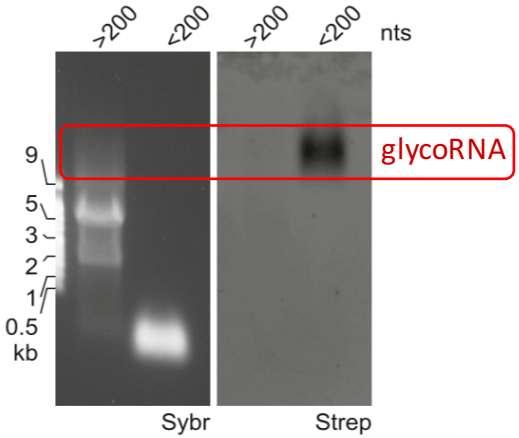



**Fig.14.** Blotting of total RNA from HeLa cells treated with $Ac_4$ManNAz after differential precipitation fractionation using silica-based columns [11]

**→ GlycoRNAs fractionated only with the small RNA population of total RNA**

# II. What type of RNA are glycoRNAs?

## Are glycoRNAs small RNAs?

- Method: RNA labeling and extraction, sucrose gradient fractionation, RNA blotting

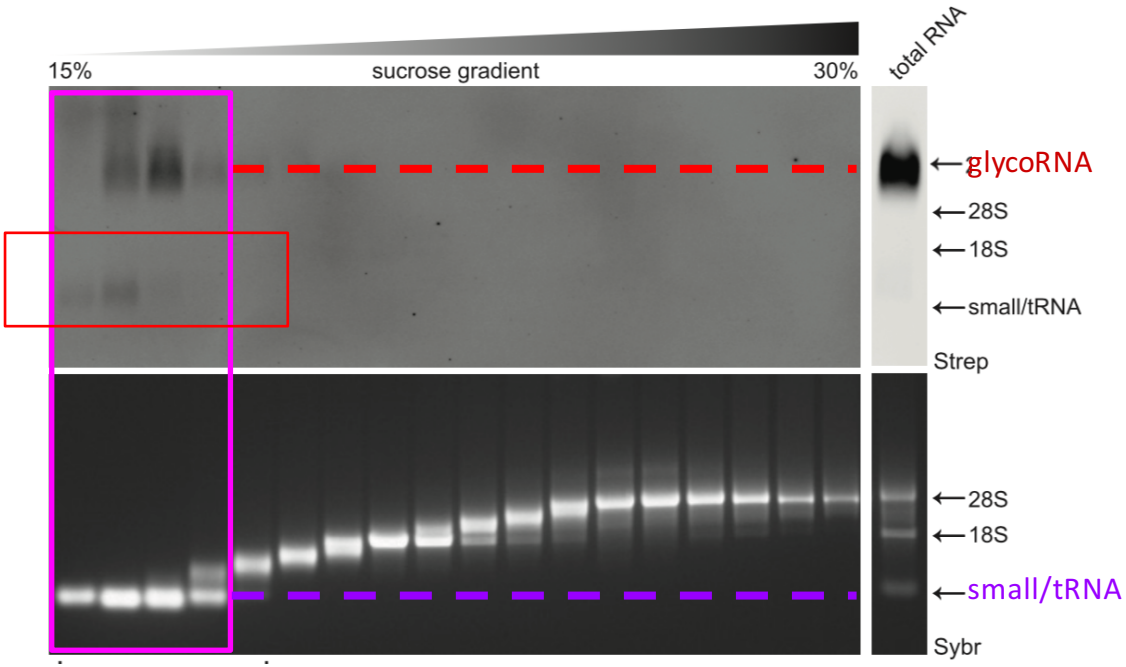



**Fig.15.** Blotting of total RNA from H9 human embryonic stem cells treated with $Ac_4$ManNAz after sucrose density gradient fractionation [11]

- The sucrose gradient separated RNAs:
  - small RNA/tRNA
  - 18S rRNA
  - 28S rRNA

**→ GlycoRNAs fractionated with small RNAs**

**→ Very slow migration of glycoRNAs can be due to their association with glycans**

# II. What type of RNA are glycoRNAs?

Take-home message

**This data shows that glycoRNAs are small non-coding RNAs (ncRNA) and tRNAs, even if they migrate slowly. This abnormal migration behavior is thought to be due to their association with glycans.**

# II. What type of RNA are glycoRNAs?

**What RNAs are selectively labeled by $Ac_4$ManNAz treatment?**

- Method:

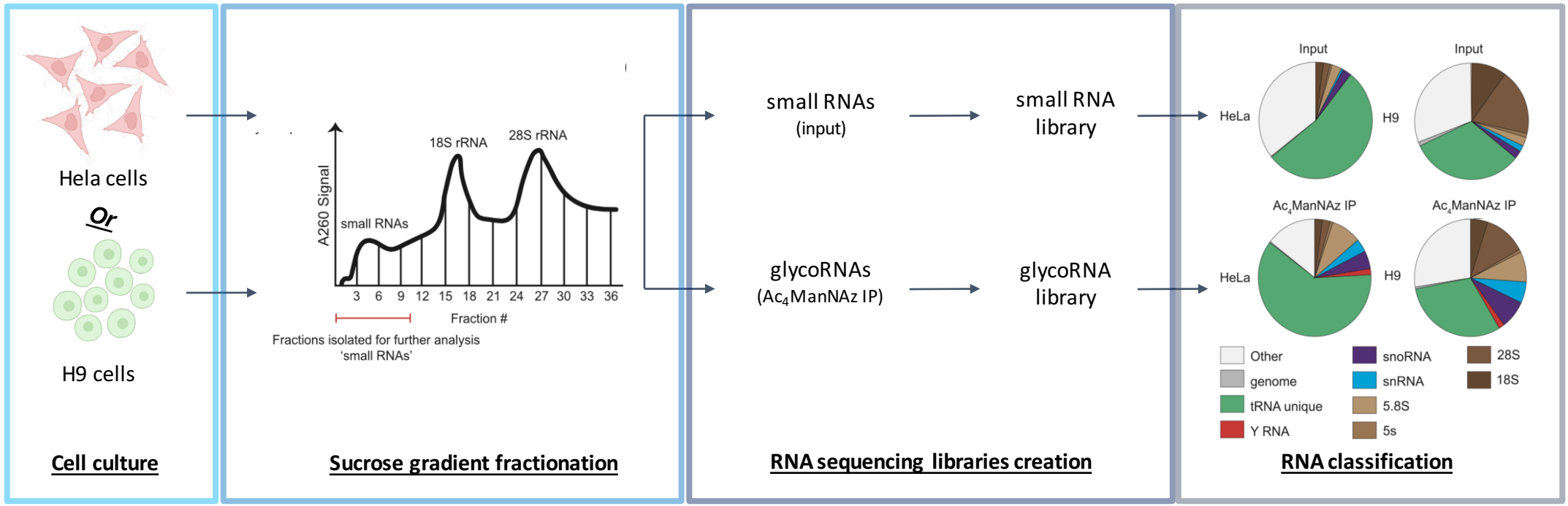



**Fig.16.** $Ac_4$ManNAz-labeled glycoRNAs identification protocol [11]

# II. What type of RNA are glycoRNAs?

**What RNAs are selectively labeled by $Ac_4$ManNAz treatment?**

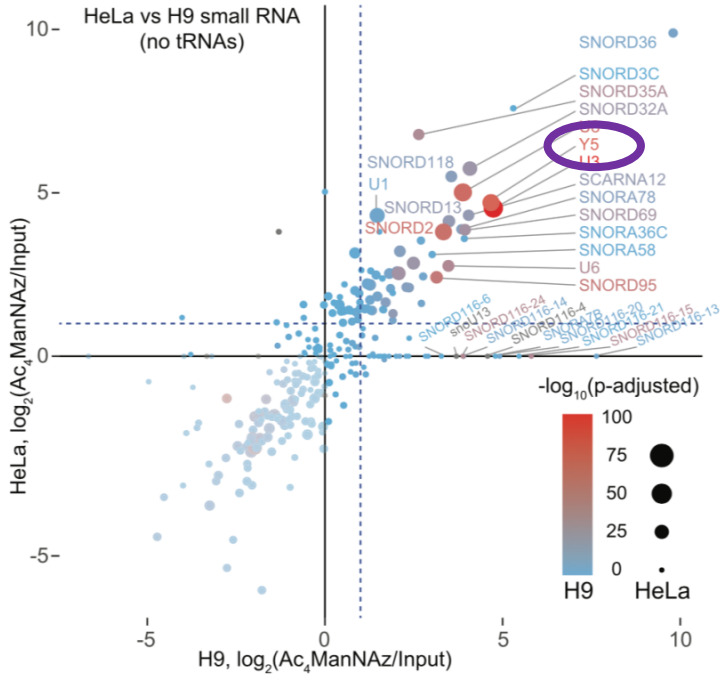



**Fig.17.** Scatterplot analysis of $Ac_4$ManNAz enriched RNAs purified from the small RNA fraction [11]

- Enrichment values are calculated as the RNA quantity after streptavidin pulldown divided by the input RNA quantity
- Y RNA, snRNA, rRNA, snoRNAs are enriched in both H9 and HeLa cells
- The enrichment values of HeLa and H9 cell glycoRNAs show a strong positive correlation

→ **Some small non-coding RNAs (Y RNA, snRNA, rRNA, snoRNAs) seem to be selectively glycosylated in both H9 and HeLa cells, despite the different lineage of these cell types**

# II. What type of RNA are glycoRNAs?

**Is Y5 really a glycoRNA? How to prove if a given RNA is a glycoRNA?**

- Y RNA family transcripts are able to bind proteins and ribonucleoproteins
- Y RNAs are highly conserved in vertebrates
- Y RNAs are known to be antigens associated with auto-immune diseases

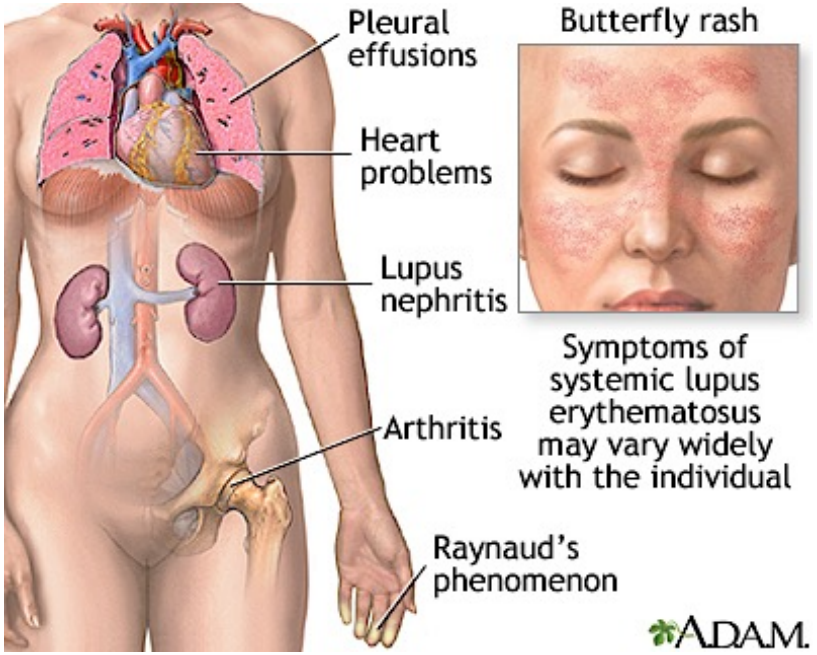



**Fig.18.** Systemic Lupus Erythematosus (SLE) [12]

# II. What type of RNA are glycoRNAs?

**Is Y5 really a glycoRNA? How to prove if a given RNA is a glycoRNA?**

- Method: Gene knock-out (KO) using CRISPR-Cas9, Northern Blot, Growth analysis

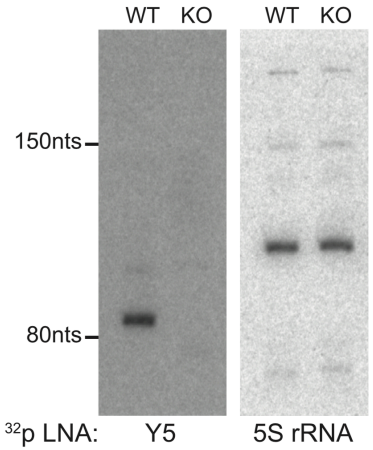



**Fig.19.** Northern blot of total RNA from WT and Y5 KO 293T cells. The KO resulted in a complete loss of the Y5 RNA, with the 5S rRNA serving as a loading control [11]

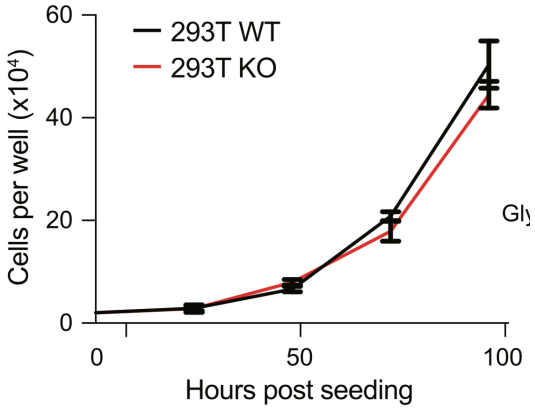



**Fig.20.** Growth rate analysis of the WT and KO clones across four days of culture. Three independent wells of cells were counted each day [11]

→ **Validation of the KO efficiency: absence of Y5 transcripts**

→ **Validation of the KO efficiency: absence of growth defects, consistent with previous reports of Y RNA silencing**

# II. What type of RNA are glycoRNAs?

**Is Y5 really a glycoRNA? How to prove if a given RNA is a glycoRNA?**

- Method: gene knock-out (KO) using CRISPR-Cas9, $Ac_4$ManNAz labeling

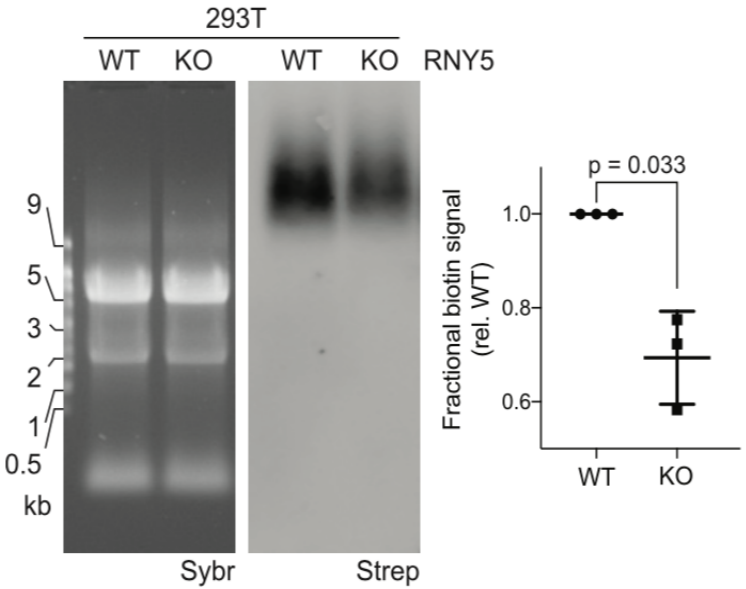



**Fig.21.** Representative blot and quantification of total RNA from WT or Y5 KO 293T cells treated with $Ac_4$ManNAz [11]

- ❖ Reduction of biotin signal compared to wild-type cells, without MW changes
- ❖ Consistent with the sequencing data, which identified Y5 as a strongly enriched RNA among a pool of other candidate glycoRNAs

**→ Y5 is one of the glycoRNAs found in Hela, H9 and HEK293T cell lines**

# II. What type of RNA are glycoRNAs?

Take-home message

**This data shows that among the glycoRNAs, we can find Y RNAs, snRNAs, rRNAs, snoRNAs, expressed in different cell types. Some of these glycoRNAs are involved in diseases, such as the Y5 RNA and SLE, an auto-immune disease. This proves the relevance of glycoRNAs studies.**

# III. What kind of glycan structures modify RNAs?

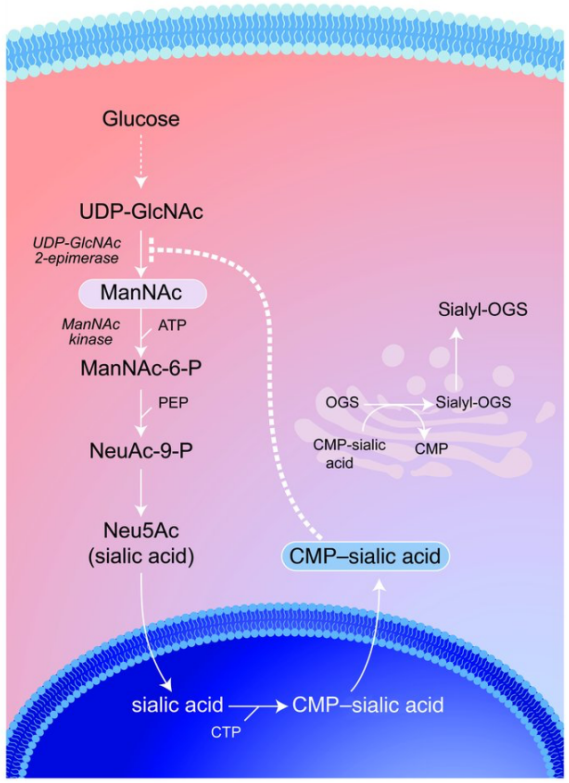



**Fig.22.** Pathway of $Ac_4$ManNAz metabolism, conversion to sialic acid and sialic acid conversion into CMP [13]

- The pathway for $Ac_4$ManNAz metabolism in human cells requires the conversion to sialic acid, then to CMP sialic acid and finally the addition to the termini of glycans

Key:

UDP-GlcNAc: Uridine diphosphate N-acetylglucosamine
ManNAc: N-Acetylmannosamine
ManNAc-6-P: N-acetyl-mannosamine 6-phosphate
NeuAc-9-P: N-acetylneuraminic acid 9-phosphate
Neu5Ac: N-acetylneuraminic acid (sialic acid)
CTP: Cytidine triphosphate
CMP: Cytidine-5'-monophosphate
OGS: Oligosaccharide

# III. What kind of glycan structures modify RNAs?

**Is $Ac_4$ManNAz shunted into unexpected metabolic pathways, thus creating artifacts?**

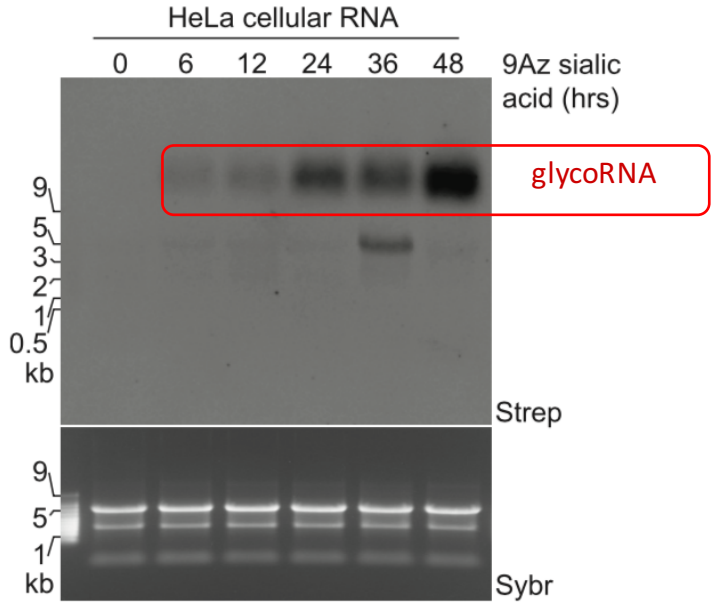



**Fig.23.** Blotting of RNA from HeLa cells treated with 9-azido sialic acid for the indicated times [11]

- Method: use of 9-Azido sialic acid that directly converts into CMP-sialic acid, instead of $Ac_4$ManNAz

❖ As previously, 9Az-sialic acid produces time-dependent labeling of slowly migrating cellular glycoRNAs

→ **This comparative labeling approach suggests that $Ac_4$ManNAz follows the canonical pathway of ManNAc, and thus, the labeling is not due of artifacts**

# III. What kind of glycan structures modify RNAs?

**Is $Ac_4$ManNAz shunted into unexpected metabolic pathways, thus creating artifacts?**

Method: *Vibrio cholerae* sialidase assay

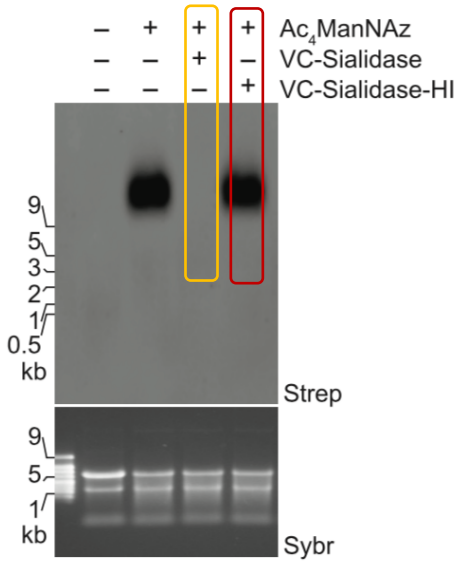



**Fig.24.** Blotting of $Ac_4$ManNAz-labeled HeLa cell RNA treated with *Vibrio cholerae* sialidase (VC-Sia) or heat-inactivated sialidase (VC-Sia-HI) [11]

- *Vibrio cholerae* sialidase (VC-Sia) is known to abolish the labeling signal obtained by the same method for glycoprotein labeling
- VC-Sia abolishes biotin signal
- Heat-inactivated (HI) VC-Sia unable to reduce the signal

**→ $Ac_4$ManNaz-dependent glycoRNA labeling signal depends on the presence of the azide-sialic group at the extremity of the glycan**

# III. What kind of glycan structures modify RNAs?

**What is the extent of the contribution of sialic acid biosynthesis enzymes?**

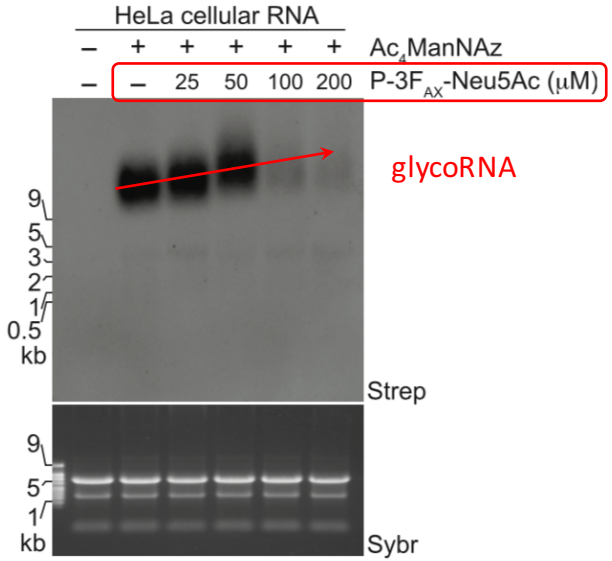



**Fig.25.** Blotting of RNA from HeLa cells treated with $Ac_4ManNAz$ and the indicated concentrations of P-$3F_{AX}$ - Neu5Ac [11]

- Method: use of P-$3F_{AX}$ - Neu5Ac, a cell permeable metabolic inhibitor of sialoside biosynthesis

❖ Treatment with P-$3F_{AX}$ - Neu5Ac resulted in a dose-dependent reduction of the total glycoRNA signal

❖ Shift to higher MW

**→ The dose-dependent reduction in total glycoRNA signal proves the involvement of canonical sialic acid biosynthesis enzymes in glycoRNA synthesis**

**→ The reduced mobility is due to the reduced amount of sialic acid and therefore the reduced negative charges per glycoRNA molecule**

# III. What kind of glycan structures modify RNAs?

**Are glycoRNAs really sialylated?**

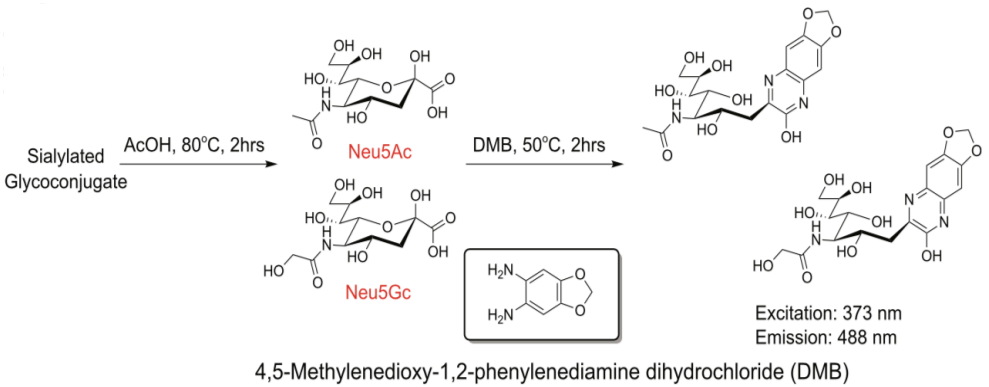



**Fig.26.** Schematic of experimental steps of the DMB assay with associated structures of the two major types of sialic acid (Neu5Ac and Neu5Gc) [11]

- Method: use of DMB (4,5-Meth- ylenedioxy-1,2-phenylenediamine dihydrochloride) probe to derivatize free sialic acids, then detect and quantify by high performance liquid chromatography (HPLC)

❖ Two forms of sialic acids in animals:
  - Neu5Ac: N-acetylneuraminic acid
  - Neu5Gc: N-glycolylneuraminic acid

# III. What kind of glycan structures modify RNAs?

## Are glycoRNAs really sialylated?

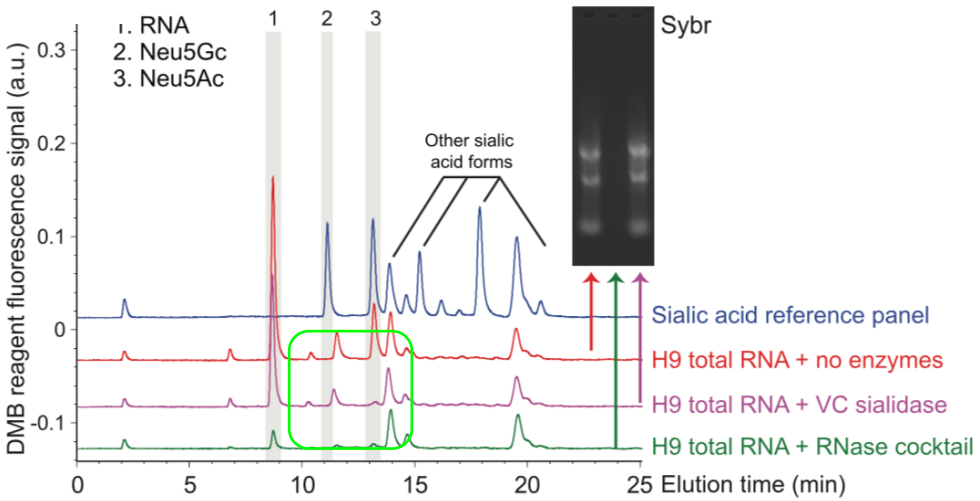



**Fig.27.** HPLC analysis of the presence and the abundance of specific sialic acid in H9 cells [11]

- Method: use of DMB (4,5-Meth- ylenedioxy-1,2-phenylenediamine dihydrochloride) probe to derivatize free sialic acids, then detect and quantify by high performance liquid chromatography (HPLC)

❖ Two forms of sialic acids in animals:
  - Neu5Ac: N-acetylneuraminic acid
  - Neu5Gc: N-glycolylneuraminic acid

❖ The peaks are reduced or gone with VC-Sia or RNase cocktail

**→ GlycoRNAs are modified with sialic acid containing glycans**

# III. What kind of glycan structures modify RNAs?

**Are other glycoforms associated with RNAs?**

- Method: mass spectrometry is used to define the composition of glycans on RNA

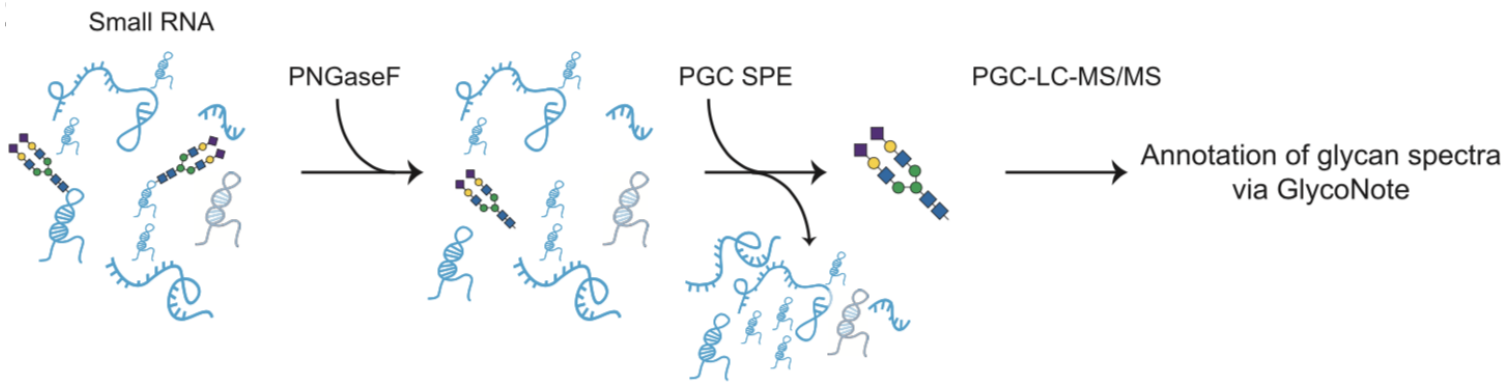



**Fig.28.** Schematic of the method used to release glycans from RNA samples and purify free glycans for mass spectrometry analysis [11]

# III. What kind of glycan structures modify RNAs?

**Are other glycoforms associated with RNAs?**

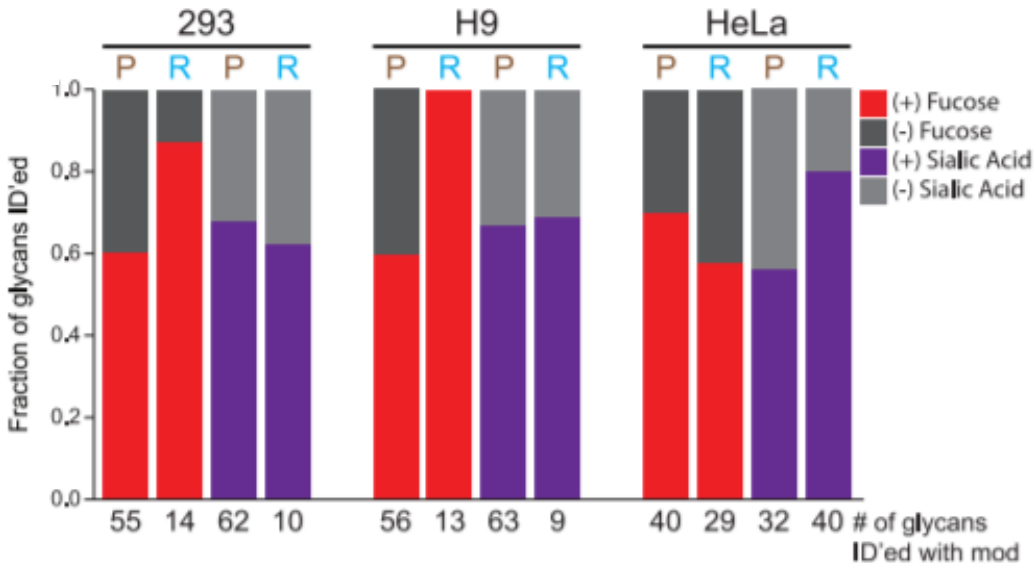



**Fig.29.** Bar plots of the fraction of glycans containing fucose (red) or sialic acid (purple) modifications that were released from proteins/peptides (P) or RNA samples (R) [11]

- Fucosylation and sialylation are the two main components of glycan structures modifying RNAs
- GlycoRNAs from 293T and H9 cells have higher fucosylation
- GlycoRNAs from HeLa cells have higher sialylation

**→ Thanks to this MS-based approach, it was revealed that the glycoforms are mainly fucosylated and sialylated**

# III. What kind of glycan structures modify RNAs?

Take-home message

**This data further confirms that glycoRNAs are modified with sialic acid containing structures, but also with fucose.**

# IV. Does the canonical glycan biosynthetic machineries contribute to glycoRNA production?

**Authors' hypothesis: similarly to glycoproteins, glycoRNAs may be related to O- and/or N-glycan biosynthetic machinery**

*1) Genetic approach*

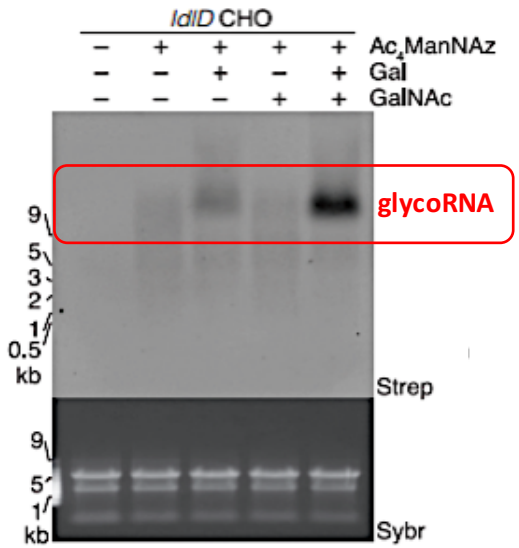



**Fig.30.** Blotting of RNA from ldlD CHO cells labeled with Ac4ManNAz, Galactose, N-acetylgalactosamine, or all [11]

- Method: use of ldlD CHO cell line, lacking GALE enzyme necessary for N-glycan elongation and initiation of O-glycosylation

❖ Culture of cells in minimal media enabled very low glycoRNA labeling

❖ Supplementation with Gal, but not with GalNac, partially restored glycoRNA labeling

❖ Supplementation with both Gal and GalNac fully restored glycoRNA labeling

**→ In CHO cells, the glycoRNA biosynthesis seems to depend on GALE activity, a N-/O- glycosylation enzyme**

# IV. Does the canonical glycan biosynthetic machineries contribute to glycoRNA production?

**Authors' hypothesis: similarly to glycoproteins, glycoRNAs may be related to O- and/or N-glycan biosynthetic machinery**

*2) Pharmacological approach*

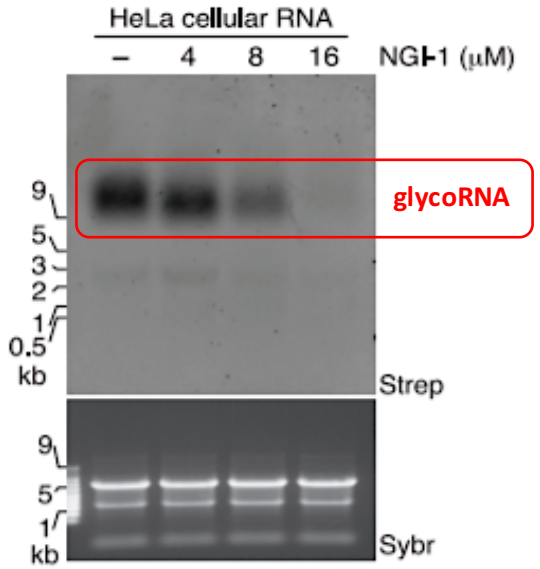



**Fig.31.** Blotting of RNA from HeLa cells treated with $Ac_4$ManNAz and indicated concentrations of NGI-1, an inhibitor of OST [11]

- Method: use of NGI-1, an OST (Oligosaccharyltransferase) inhibitor, an enzyme of the N-glycosylation pathway

❖ Treatment with NGI-1 induced a loss of glycoRNA labeling, with a dose-dependent effect

**→ N-glycosylation pathway seems to be required for glycoRNA biosynthesis**

# IV. Does the canonical glycan biosynthetic machineries contribute to glycoRNA production?

**Authors' hypothesis: similarly to glycoproteins, glycoRNAs may be related to O- and/or N-glycan biosynthetic machinery**

*3) Enzymatic approach*

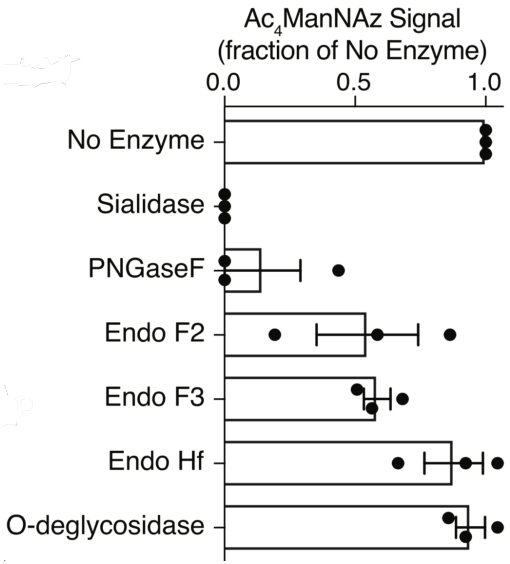



**Fig.32.** Quantification of $Ac_4$ManNAz signal after treatment of $Ac_4$ManNAz-labeled HeLa cell RNA with the indicated enzymes *in vitro* [11]

- Method: use of a panel of endoglycosidases to cleave off the glycoforms

❖ Positive control: VC-sia induces a complete loss of glycoRNA labeling

❖ Treatment with PNGaseF causes an almost complete loss of glycoRNA labeling

❖ Treatment with endo F2, F3 and Hf causes a partial loss of glycoRNA labeling

❖ Treatment with O-glycosidase has no effect

**→ GlycoRNA are likely composed of N-glycans, degraded only by N-glycan endoglycosidases**

# IV. Does the canonical glycan biosynthetic machineries contribute to glycoRNA production?

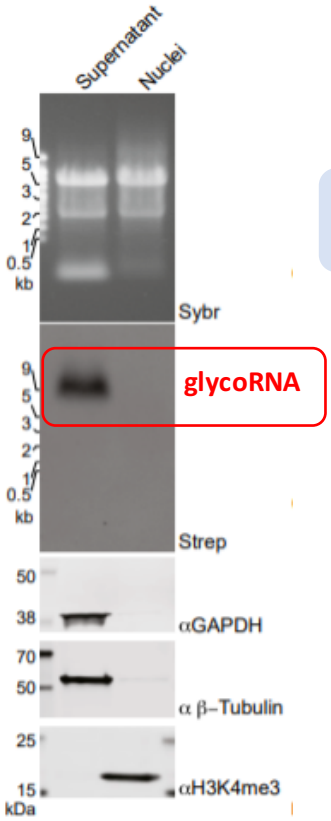
Take-home message

**This data shows that the canonical N-glycan biosynthetic machinery used in glycoprotein formation also contributes to glycoRNA production.**

# V. Where are localized glycoRNAs in the cell?

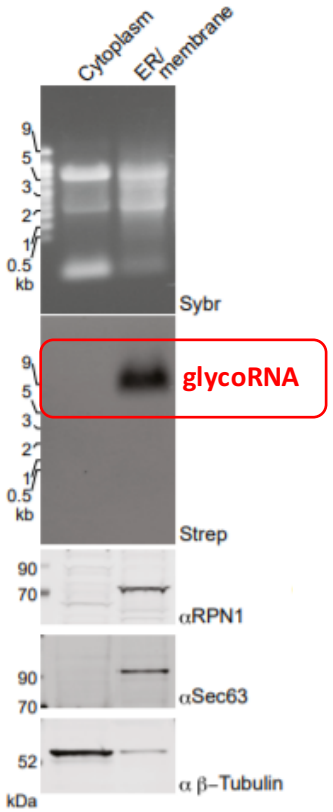
**In which cellular comparment are glycoRNAs present?**

- Method: separation of subcellular compartments and detection of glycoRNAs




→ **GlycoRNAs are not found in the nuclei fraction**

**Fig.33.** Blotting of RNA and proteins after nuclei purification. Non-nuclear proteins GAPDH and tubulin and nuclear marker H3K4me3 are visualized by western blot [11]




→ **GlycoRNAs are found exclusively in the membrane fraction**

**Fig.34.** Blotting of RNA and proteins after separatation of cytosol from membranous organelles. Membrane proteins RPN1, Sec63, and soluble tubulin are visualized by western blot [11]

# V. Where are localized glycoRNAs in the cell?

**<u>Authors' hypothesis:</u> considering the canonical trafficking and localization of glycoconjugates, glycoRNAs may be present on the extracellular surface of the plasma membrane of living cells**

- Method: VC-Sia has a specific ability to cleave sialic acids off the surface of living cells

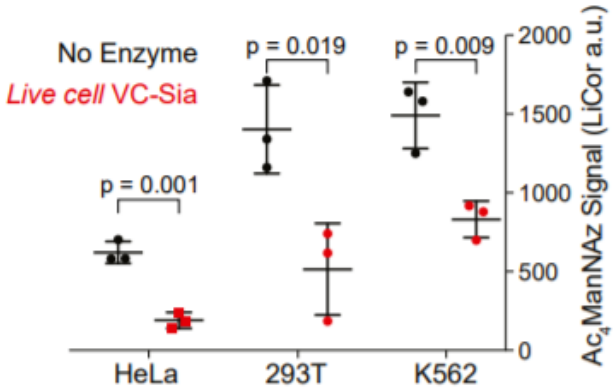



**Fig.35.** Blotting of RNA from HeLa cells labeled with $Ac_4$ManNAz and exposed or not to VC-Sia [11]

**→ VC-Sia is able to cleave glycoRNAs at the surface of living cells**

- Method: validation with an $Ac_4$ManNAz-independent labeling technique

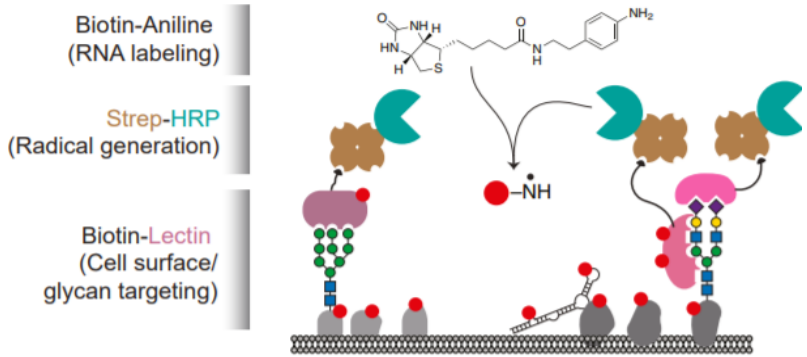



**Fig.36.** Schematic of the lectin-based proximity labeling of RNA on cell surfaces [11]

**→ This technique is able to label glycoconjugate structures on the surface of living cells**

# V. Where are localized glycoRNAs in the cell?

- Method: validation with an $Ac_4$ManNAz-independent labeling technique
- Assay on live HeLa cells

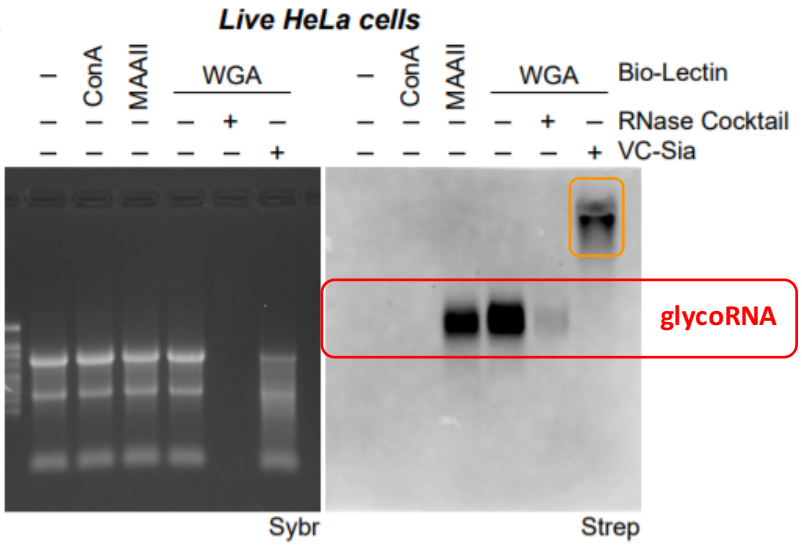



**Fig.37.** Blotting of total RNA samples after labeling. Lanes 5 and 6 were processed *in vitro* with RNase cocktail or VC-Sia [11]

- ❖ GlycoRNAs are labeled when cells are stained with MAAII and WGA but not with ConA
- ❖ Treatment of glycoRNAs with RNase strongly abrogates glycoRNA signal
- ❖ Treatment with VC-Sia induces a shift in molecular weight but does not reduce signal

**→ GlycoRNAs are found at the surface of living cells and contain sialic acid structures**

# V. Where are localized glycoRNAs in the cell?

- Method: validation with an $Ac_4$ManNAz-independent labeling technique
- Assay on HeLa cell lysate

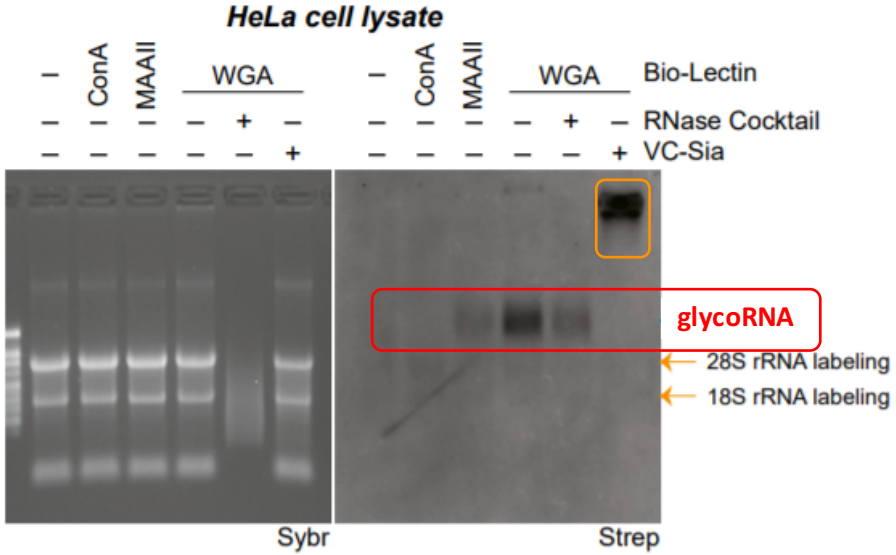



**Fig.38.** Blotting of total RNA samples from lysed cells. Lanes 5 and 6 were processed with RNase cocktail or VC-Sia [11]

- ❖ GlycoRNAs are still labeled when using MAAII and WGA even though there is no plasma membrane
- ❖ Weak but consistent labeling of internal rRNAs
- ❖ Treatment with VC-Sia induces a shift in molecular weight but does not reduce signal

**→ Most glycoRNAs are found on the cell surface**

# V. Where are localized glycoRNAs in the cell?

Take-home message

**This data suggests that the majority of glycoRNAs are exposed on the surface of living cells, on the extracellular surface of plasma membrane.**

# VI. Can anti-RNA antibodies recognize cell surface glycoRNAs?

- Method: FACS using J2 antibody recognizing dsRNA on HeLa cells

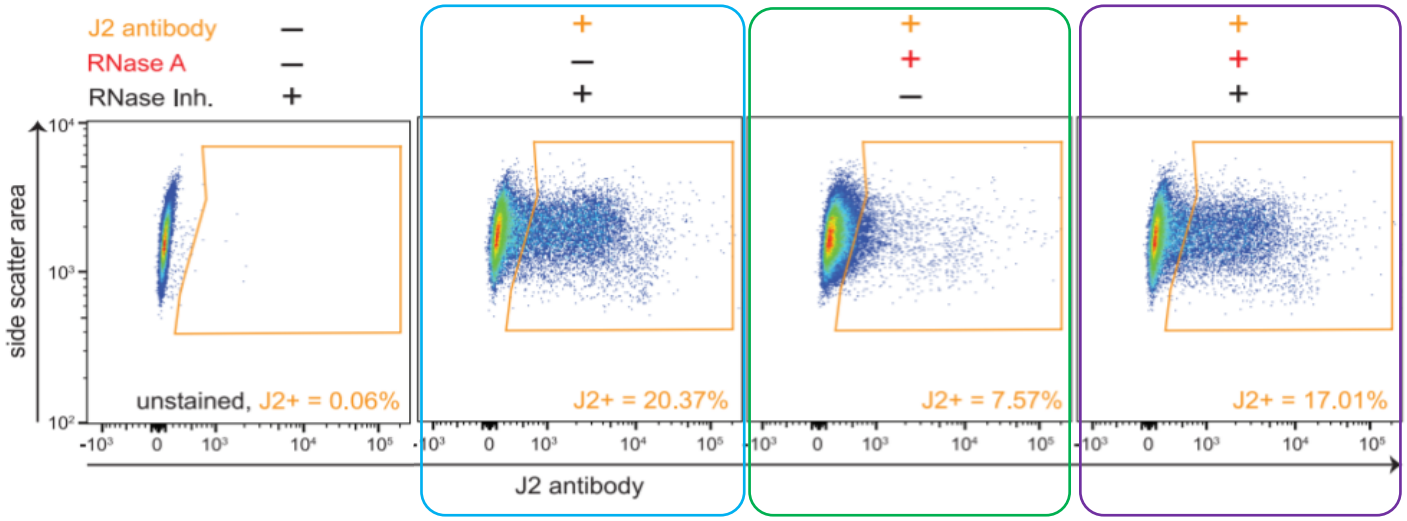



**Fig.39.** Cytometry analysis of HeLa cells pre-treated with the indicated enzymes or inhibitors and stained with J2 antibody. Gated region (orange) indicates the population shifted toward high J2 binding [11]

- 20% cells are J2-positive
- Treatment with RNase A strongly abrogates staining with J2 antibody
- Staining is rescued when a RNase inhibitor is added

**→ RNA is indeed present on the surface of live cells**

# VI. Can anti-RNA antibodies recognize cell surface glycoRNAs?

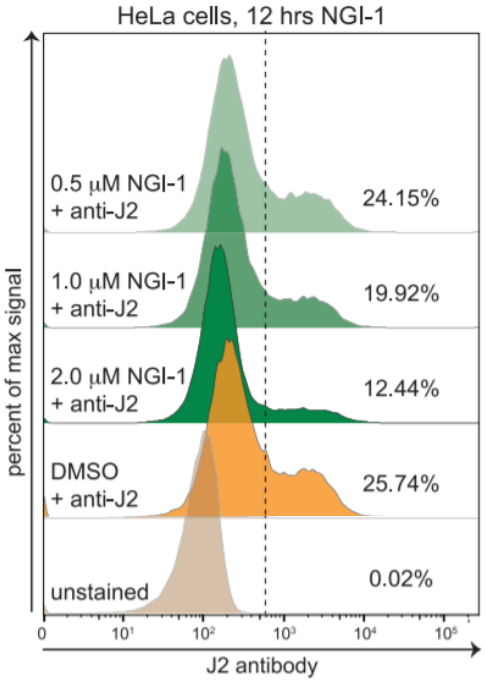



**Fig.40.** FACS analysis of single HeLa cells pre-treated with the OST inhibitor NGI-1 at the indicated concentrations. Dashed vertical line denotes a J2-high population [11]

- Method:

- FACS using J2 antibody recognizing dsRNA on HeLa cells
- Cell treatment by NGI-1, an inhibitor of the canonical glycoprotein pathway

❖ Treatment with NGI-1 induces a dose-dependent reduction of J2-positive cells

**→ The RNAs present on the cell surface revealed by J2 staining are glycoRNAs**

# VI. Can Siglec receptors recognize cell surface glycoRNAs?

**Can glycan-binding receptors interact with the glycoRNAs displayed at the cell surface?**

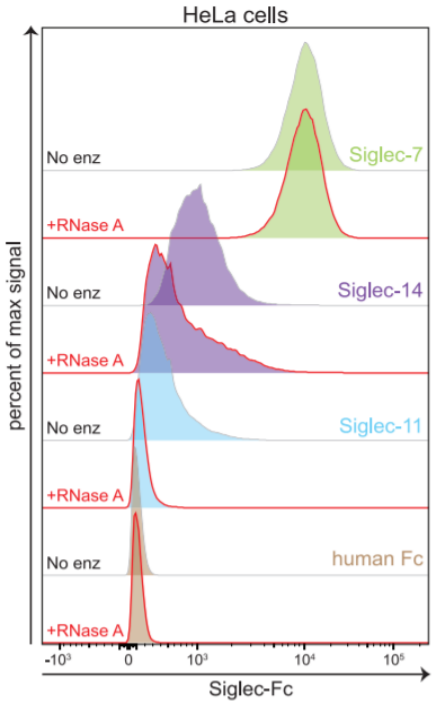



**Fig.41.** FACS analysis of single HeLa cells pre-treated or not with RNase then stained with the indicated Siglec-Fc reagents [11]

- Siglec receptors bind sialic acid structures
- 9 out of 12 Siglec-Fc reagents tested showed staining
- 2 out of the 9 (Siglec-11 and Siglec-14) were sensitive to RNase A treatment

**→ GlycoRNAs could be ligands of Siglec receptors**

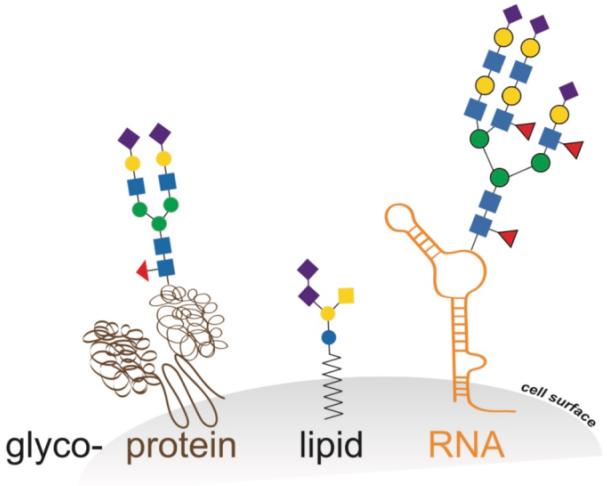
# VI. Can Siglec receptors and anti-RNA antibodies recognize cell surface glycoRNAs?

Take-home message

**This data suggests that glycoRNAs found at the surface of live cells may play a role in molecular interactions at cell junctions, notably with receptors of the Siglec family, which have a role in immune modulation.**

# Conclusion and perspectives

- In mammals, proteins and lipids are not the only biomolecules to be glycosylated in cells
- In mammalian species, RNA was found to be modified with glycan structures rich in sialic acid and fucose
- These glycoRNAs are small non-coding RNAs and are conserved across species and cell types
- GlycoRNAs are present on the surface of living cells and are ligands for Siglec receptors




→ Perspectives

- Defining in more details all glycoforms that can possibly modify RNAs
- Defining the chemical linkage between the RNA transcript and the glycoform
- Pathology implication (auto-immune diseases)

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# Thank you for your attention!
# Any questions?

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