Poster #67
Thursday
3-5 pm

Hyperactive GlcNAc-1-Phosphotransferase (S1S3 PTase) Dramatically Alters Glycosylation of Lysosomal Enzymes Leading to Enhanced Phosphorylation for Improved CI-MPR Binding

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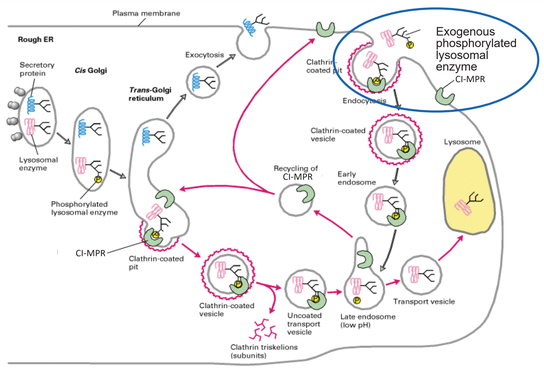
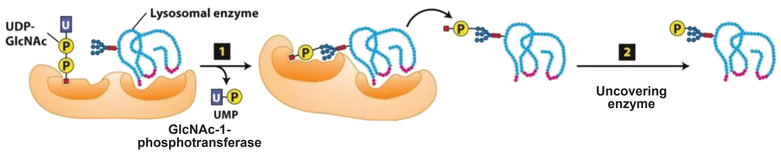
20th ANNUAL WORLDSymposium February 4-9 2024

Natural M6P Receptor Pathway Enables Phosphorylated Exogenous Lysosomal Enzymes Cellular Uptake for Treatment of Lysosomal Storage



Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 17.7

Phosphorylation of Lysosomal Enzymes is Mediated by GlcNAc-1-Phosphotransferase That is Inherently Inefficient in Cells



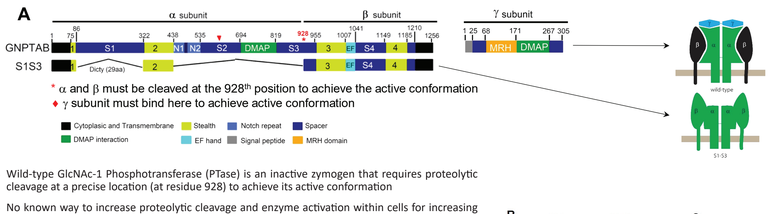
GlcNAc-1-phosphotransferase catalyzes the transfer of GlcNAc-1-phosphate from sugar donor UDP-GlcNAc onto certain terminal mannose residues of the N-linked oligosaccharides in cis-Golgi

NAGPA (N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase), also known as uncovering enzyme, removes covering GlcNAc group to reveal mannose 6-phosphate (M6P)

Section 17.7 Protein Glycosylation in the ER and Golgi Complex, Molecular Cell Biology, 8th edition, 2016

- GlcNAc-1-Phosphotransferase is a large and complex protein that is responsible for adding phosphate groups to mannose residues on N-linked glycans of newly synthesized lysosomal enzymes in Golgi to create mannose 6-phosphate (M6P)
- Phosphorylation of lysosomal enzymes is an inherently inefficient process in nature, it is further compromised when overexpressing lysosomal enzyme
- Increasing GlcNAc-1-Phosphotransferase activity within cells for producing therapeutic lysosomal enzymes with higher M6P content has not been achievable thus far with traditional ERT and gene therapy approache

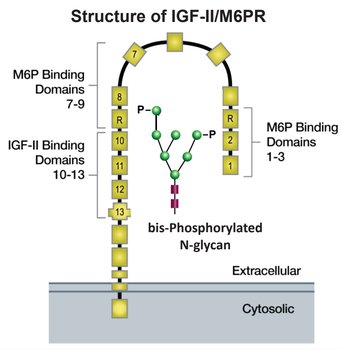
S1S3 Variant Has Key Attributes That Enable Its Use for Development of Best-In-Class Recombinant Enzymes and Gene Therapies



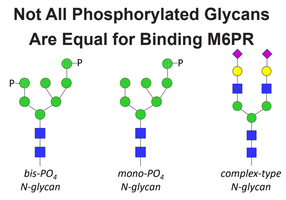
- Wild-type GlcNAc-1 Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at a precise location (at residue 928) to achieve its active conformation
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity for improving phosphorylation
- S1S3 PTase is a truncated, pre-activated variant with significant advantages:
  - Has 20-fold higher specific activity
  - Enables efficient phosphorylation of soluble lysosomal enzymes
  - S1S3 Ptase is a transmembrane protein; confined to the cis-golgi
  - Has proper conformation without need for γ-subunit
  - Small gene size enables its incorporation into expression constructs for S1S3 PTase co-expression with therapeutic enzymes within cells for ERT and gene therapy applications

Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65.

Structure of IGF-II/Cation-Independent M6P Receptor (IGF-II/CI-MPR) and Measured Binding Affinities of Carbohydrate Ligands

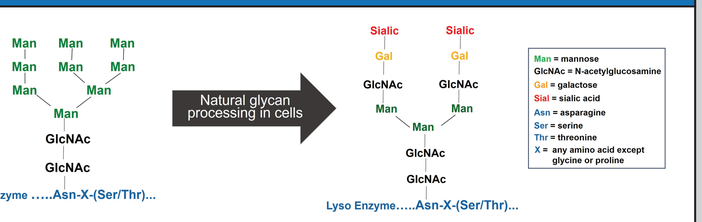


Not All Phosphorylated Glycans Are Equal for Binding M6PR



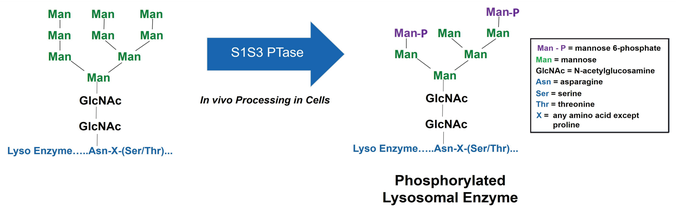
Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

The Problem: Most N-glycans on Lysosomal Enzymes are Not Phosphorylated and Processed to Complex-Type Structures During Over-Expression



- Identical Man9 N-glycan structure is added to glycoproteins in endoplasmic reticulum during synthesis; N-glycan processing primarily occurs in Golgi
- Phosphorylation of recombinant lysosomal enzymes is highly inefficient in cells leading to mostly complex-type structures
- Complex-type N-glycans are typical of plasma proteins and do not enable cellular uptake or delivery of exogenous lysosomal enzymes to lysosomes

The Solution: S1S3 PTase Co-Expression Platform Ensures Production of Therapeutic Lysosomal Enzymes with High Levels of M6P



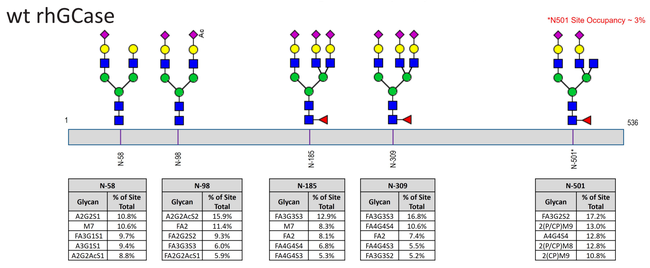
- Co-expression of S1S3 PTase with therapeutic protein produces lysosomal enzyme with high levels of M6P to enable much improved drug targeting
- Cation-independent M6P receptor (CI-MPR) is present on nearly all cells for cellular uptake of exogenous M6P-bearing lysosomal enzymes

M011: GCase^{M6P} (GCase Co-Expressed with S1S3 PTase) is an ERT Therapy with Increased Phosphorylation, Superior Receptor Binding and Higher CI-MPR Affinity

- β-Glucocerebrosidase (GCase) is a lysosomal enzyme that cleaves by hydrolysis the β-glycosidic linkage of glucocerebroside
- It is an ~60 kDa glycoprotein with 5-6 N-linked glycosylation sites (6th site engineered into M011 for added stability)
- Gaucher Disease is caused by reduced or absent GCase activity resulting in glucosylceramide accumulation in the liver, spleen, and macrophage lineage cells.

Funadamental Change in GCase Glycosylation
(LC-MS/MS Site Specific Glycosylation)

wt rhGCase



N-58	
Glycan	% of Site Total
A2G2S1	10.8%
M7	10.6%
FA3G1S1	9.7%
A3G1S1	9.4%
A2G2Ac51	8.8%

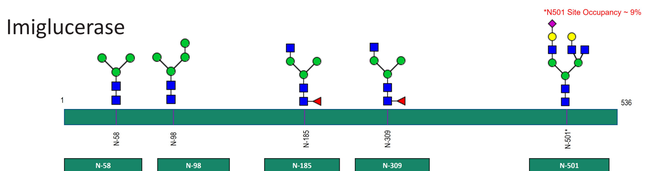
N-98	
Glycan	% of Site Total
A2G2Ac52	15.9%
FA2	11.4%
FA2G2S2	9.3%
FA3G3S3	6.0%
FA2G2Ac51	5.9%

N-185	
Glycan	% of Site Total
FA3G3S3	12.9%
M7	8.3%
FA2	8.1%
FA4G4S4	6.8%
FA4G4S3	5.3%

N-309	
Glycan	% of Site Total
FA3G3S3	16.8%
FA4G4S4	10.6%
FA2	7.4%
FA4G4S3	5.5%
FA3G3S2	5.2%

N-501	
Glycan	% of Site Total
FA3G2S2	17.2%
2(P/CP)M9	13.0%
A4G4S4	12.8%
2(P/CP)M8	12.8%
2(CP)M9	10.8%

Imiglucerase



N-58	
Glycan	% of Site Total
M3	61.9%
PM6	11.1%
M5	5.4%
A1	4.5%
2(P)M7	3.4%

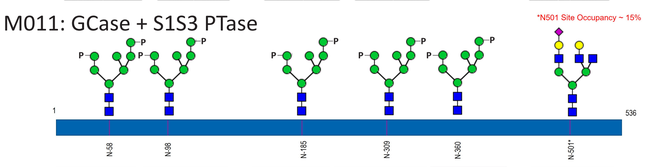
N-98	
Glycan	% of Site Total
M4	24.9%
M5	19.5%
A1	15.8%
FA1	14.6%
PM6	10.8%

N-185	
Glycan	% of Site Total
FA1	45.3%
FA2	24.0%
FA2G1	4.5%
PM5	4.4%
PM6	3.7%

N-309	
Glycan	% of Site Total
FA1	31.7%
PM6	30.5%
FA2	9.2%
A1PM6	7.6%
PM5	7.6%

N-501	
Glycan	% of Site Total
A3G2S1	44.9%
A3G1S1	23.8%
FA3G1S1	9.2%
FA3G2S1	8.5%
A3G2Sg1	5.3%

M011: GCase + S1S3 PTase



N-58	
Glycan	% of Site Total
2(P)M7	62.1%
PM6	9.7%
A1PM6	6.9%
2(P/CP)M7	6.6%
2(P)M6	3.7%

N-98	
Glycan	% of Site Total
2(P)M7	59.5%
2(P)M6	10.8%
2(P/CP)M6	5.6%
2(P/CP)M7	3.9%
PM6	3.3%

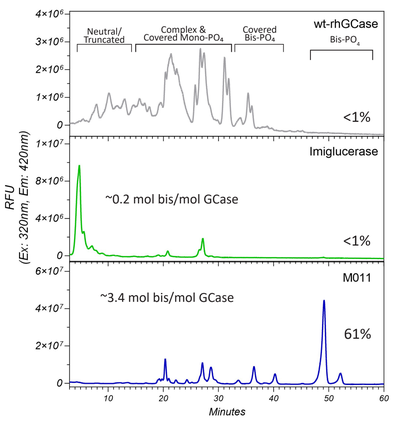
N-185	
Glycan	% of Site Total
2(P)M7	39.8%
PM6	12.4%
A1PM6	10.0%
PM5	10.0%
2(P/CP)M6	8.3%

N-309	
Glycan	% of Site Total
2(P)M7	33.2%
2(P)M6	13.5%
A1PM6	6.4%
2(P/CP)M7	5.8%
FA1G1S1PM6	5.8%

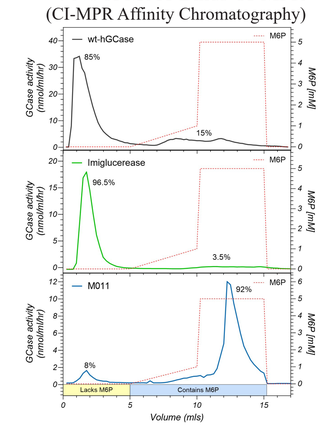
N-360	
Glycan	% of Site Total
2(P)M7	37.8%
A1PM6	12.2%
PM6	8.1%
A2G2S1	6.3%
FA2G2S1	4.1%

N-501	
Glycan	% of Site Total
A3G2S1	28.8%
FA4G3S2	21.0%
A3G1S1	20.3%
FA2G2S2	15.6%
A3G2Sg1	3.5%

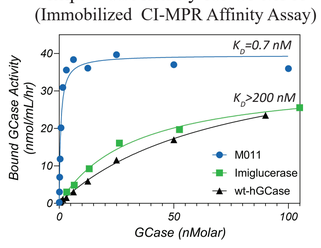
Improved Bis-PO_4 Glycan Content
(2AB Oligosccharide Chromaotography)



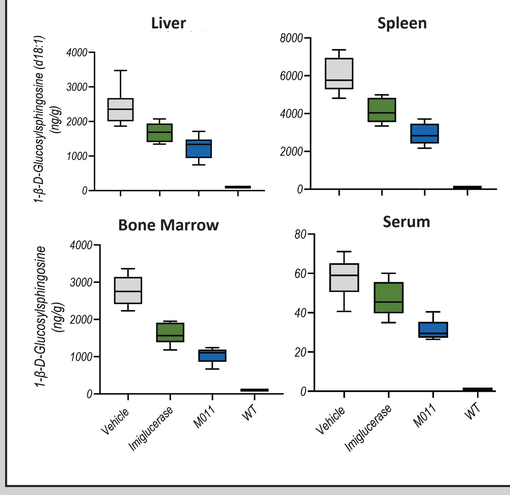
>90% of M011 Binds CI-MPR
(CI-MPR Affinity Chromatography)



Improved Affinity for CI-MPR
(Immobilized CI-MPR Affinity Assay)

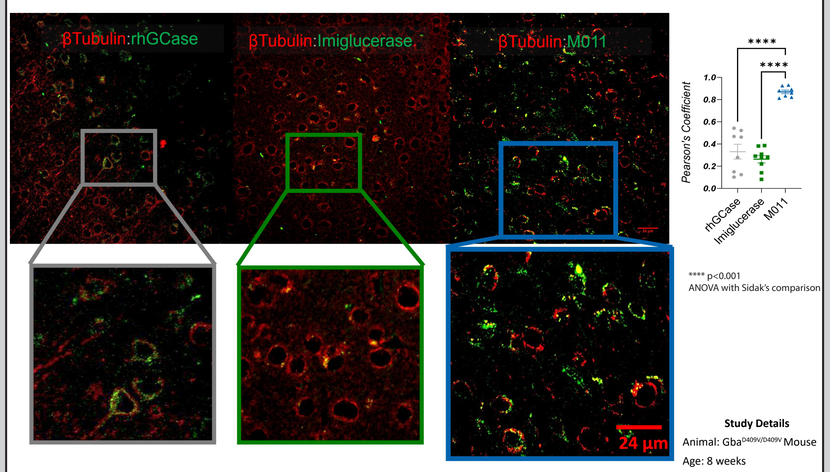


M011 is Significantly Better than SOC for Reducing Accumulated Substrate Under Identical Experimental Conditions



Study Details
Animals:
Strain: D409V/null Gaucher Mouse
Gender: Mixed
Age: 8-14 weeks
N: 8 per group – 4 male/4 female
Treatment:
Weekly dosing for 4 weeks
Imiglucerase @ 1.5 mg/kg
M011: GCase^{M6P} @ 1.5 mg/kg
EOL: 1 week following last dose
Analysis: GC content in serum and tissues

Phosphorylation Enables Significantly Better Cellular Uptake of M011 in Neurons than Imiglucerase After Identical ICM Injection



**** p<0.001
ANOVA with Sidak's comparison

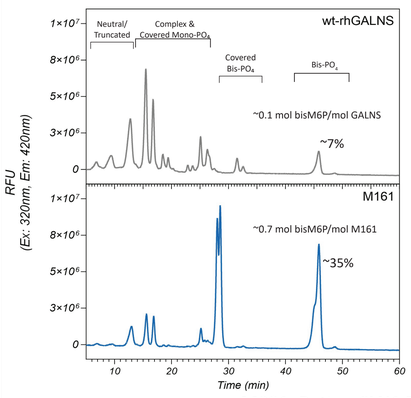
Study Details
Animal: Gba$^{D409V/D409V}$ Mouse
Age: 8 weeks
Dose: 140 µg
Dose route: ICM
Dose Volume: 10 µL
EOL: 24 hrs post dosage

Same S1S3 PTase Approach Can be Utilized for Increasing M6P Levels on Vast Majority of Soluble Lysosomal Enzymes

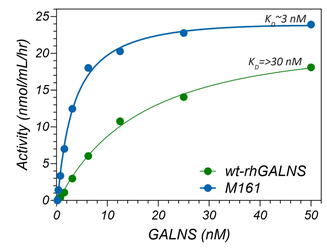
M161: MucoPolySaccharidosis Type IV A ERT Development

- Morquio Syndrome A, also known as MucoPolySaccharidosis Type IV A (MPS IV A), is a rare metabolic disorder in which the body cannot process certain types of sugar molecules called glycosaminoglycans
- N-acetylgalactoseamine 6-sulfatase (GALNS) is a lysosomal enzyme required to breakdown glycosylaminoglycans (GAGs)
- GALNS deficiency causes the accumulation of keratan sulfate and chondroitin sulfate
- Active GALNS is a ~55 kDa glycoprotein with 2 N-linked glycosylation sites

5x Improvement for Bis-PO_4 Glycan Content
(2AB Oligosccharide Chromaotography)



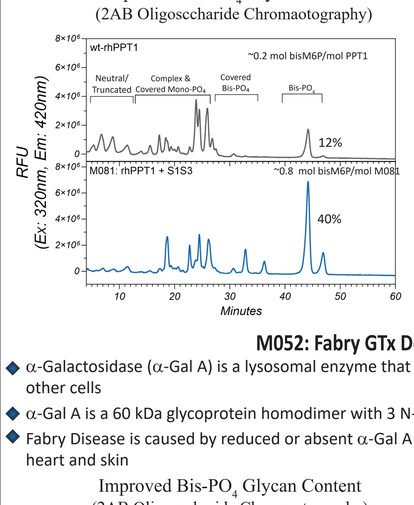
Improved Affinity for CI-MPR
(Immobilized CI-MPR Affinity Assay)



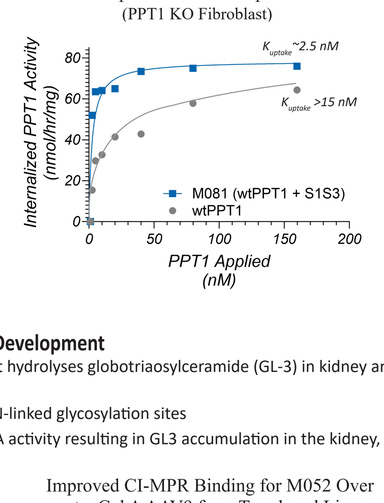
M081: Batten CLN1 ERT Development

- Palmitoyl-protein thioesterase 1 (PPT1) is a lysosomal enzyme that removes thioester-linked fatty acyl groups during catabolism of lipid-modified proteins
- It is a 32.7 kDa glycoprotein with 2 N-linked glycosylation sites
- Batten CLN1 is caused by diminished or missing PPT1 Activity in the CNS

Improved Bis-PO_4 Glycan Content
(2AB Oligosccharide Chromaotography)



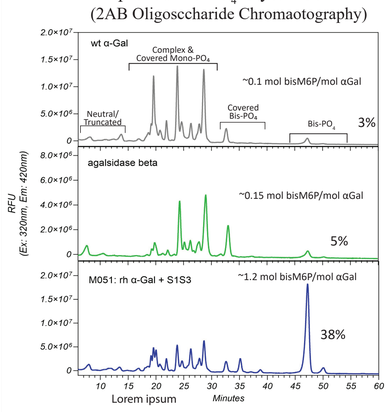
Improved Cellular Uptake
(PPT1 KO Fibroblast)



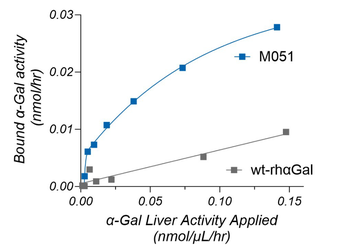
M052: Fabry GTx Development

- α-Galactosidase (α-Gal A) is a lysosomal enzyme that hydrolyses globotriaosylceramide (GL-3) in kidney and other cells
- α-Gal A is a 60 kDa glycoprotein homodimer with 3 N-linked glycosylation sites
- Fabry Disease is caused by reduced or absent α-Gal A activity resulting in GL3 accumulation in the kidney, heart and skin

Improved Bis-PO_4 Glycan Content
(2AB Oligosccharide Chromaotography)

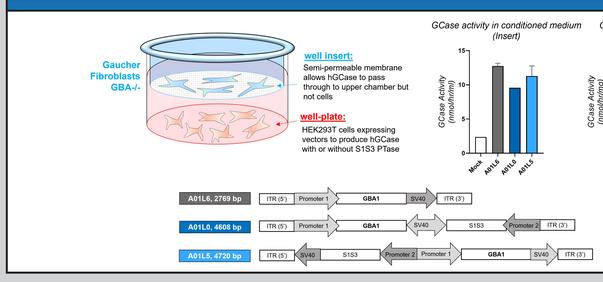


Improved CI-MPR Binding for M052 Over wt-αGal A AAV9 from Tranduced Liver

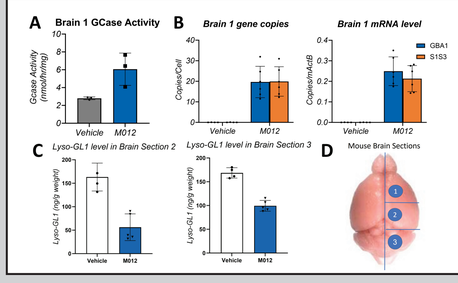


Following AAV9 Tranduction with either wt-aGal M052 (wt-αGal +S1S3 PTase co-expression) CI-MPR binding was evaluted for liver tissue following transduction via AAV9 capsid

M6P Enables Efficient Cellular Uptake of hGCase for Cross-Correction of Gaucher Fibroblasts



GBA^{D409V} mice treated with M012 AAV9 Enhances GCase Activity, Gene Expression and Substrate Reduction in Brain Tissue



AAV9 M012 treated Gaucher^{D409V} mice display enhanced GCase Activity (A), DNA copy and mRNA copy (B), and reduced Lyso-GL1 levels (C). (D) shows brain sections that were analyzed.

(More Details Avaialble at Poster #137 Thursday 3-5)

Conclusions

- CI-MPR is a receptor pathway that enables cellular uptake of exogenous lysosomal enzymes in nearly all cells and tissues including CNS neurons
- Phosphorylation is inherently inefficient for many recombinant lysosomal enzymes which limits in vivo efficacy for both ERTs and GTs
- Increasing GT dosage does not fix the lysosome targeting problem since protein overexpression exacerbates poor phosphorylation
- S1S3 PTase, a truncated highly active GlcNAc-1-phosphotransferase, is a major scientific breakthrough that overcomes this problem
- Co-expression of S1S3 PTase ensures that lysosomal enzymes are produced with high levels of bis-M6P resulting in more potent ERTs and GTs
- Highly phosphorylated lysosomal enzymes have superior CI-MPR binding and cellular uptake leading to better substrate clearance

Acknowledgements

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