

**Identification and Characterization of the Plasticity-Relevant
Fucose- α (1-2)Galactose Glycoproteome from Mouse Brain**

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...for my family and friends who have helped me over the years...

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Abstract

Fuc α (1-2)Gal carbohydrates have been implicated in cognitive processes such as learning and memory. However, a molecular level understanding of their functions has been lacking. This thesis describes multiple chemical and biological approaches that we have undertaken to elucidate the molecular mechanisms by which fucosyl sugars mediate neuronal communication. We demonstrate that Fuc α (1-2)Gal carbohydrates play an important role in the regulation of synaptic proteins and neuronal morphology. We identify synapsins Ia and Ib as prominent Fuc α (1-2)Gal glycoproteins in rat hippocampus, and fucosylation protects synapsin I from proteolytic degradation by the calcium-activated protease calpain. Synapsin fucosylation has important consequences on neuronal growth and morphology, with defucosylation leading to stunted neurites and delayed synapse formation. In addition, we identify the Fuc α (1-2)Gal proteome from mouse olfactory bulb using lectin affinity chromatography. We discover four major classes of Fuc α (1-2)Gal glycoproteins, including the immunoglobulin superfamily of cell adhesion molecules, ion channels and solute carriers/transporters, ATP-binding proteins, and synaptic vesicle-associated proteins. Protein fucosylation is regulated by FUT1 in mouse olfactory bulb, and olfactory bulb development is impaired in FUT1-deficient mice. In particular, FUT1 KO animals exhibit defects in the olfactory nerve and glomerular layers of olfactory sensory neurons expressing the fucosylated cell adhesion molecules NCAM and OCAM. Lastly, we explore the molecular mechanisms of protein fucosylation by metabolic labeling with alkynyl- and azido-fucose derivatives. We demonstrate that fucosylated glycoconjugates are present along both axons and dendrites

of developing neuronal cultures, as well as in the Golgi body. We identify the fucosylated proteome from cultured cortical neurons, and demonstrate that proteins such as NCAM, the MARCKS family of proteins, and the inositol 1,4,5 triphosphate receptor are fucosylated. In addition, we can label fucosylated glycans *in vivo*, which will have important consequences for studies on the dynamics of protein fucosylation in living animals. Cumulatively, our studies suggest important functional roles for fucosyl-carbohydrates in the nervous system, and implicate an extended role for fucose in the molecular mechanisms that may underlie synaptic plasticity and neuronal development.

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List of Abbreviations

1D	one-dimensional
2D	two-dimensional
2-dGal	2-deoxy-D-galactose
2-fucosyllactose	L-fucose α (1-2)galactose β (1-4)glucose
3-dGal	3-deoxy-D-galactose
4-dGal	4-deoxy-D-galactose
6-dGal	6-deoxy-D-galactose
2-dGlc	2-deoxy-D-glucose
AAA	<i>Anguilla anguilla</i> agglutinin
Ab	antibody
Ac	acetyl, acetate
AgNO ₃	silver nitrate
AOB	accessory olfactory bulb
aq	aqueous
ATP	adenosine triphosphate
Baf. A1	bafilomycin A1
BCA	bicinchoninic acid
β -Gal	β -galactosidase

BSA	bovine serum albumin
°C	degree Celsius
CaCl ₂	calcium chloride
Cacna2d1	alpha 2/delta subunit of the dihydropyridine-sensitive channel
cAMP	cyclic adenosine monophosphate
CAMs	cell adhesion molecules
CDG	congenital disorder of glycosylation
CH ₃ CN	acetonitrile
CHCl ₃	chloroform
CHO	Chinese hamster ovary
CMF-HBSS	Calcium and Magnesium Free Hank's Balanced Salt Solution
CNS	central nervous system
CO ₂	carbon dioxide
CRMP-2	collapsin response mediator protein
CV	column volume
ddH ₂ O	double distilled water
D-Gal	D-galactose
DIV	days in vitro
DMEM	Dulbecco's Minimal Eagle's medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E18	embryonic day 18
EBI-IPi	European Bioinformatics Institute-International Protein Index
ECL	enhance chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
Endo H	endoglycosidase H
ER	endoplasmic reticulum
FCS	fetal calf serum
Fuc	L-fucose
Fuc α (1-2)Gal	fucose α (1-2) galactose
Fuc α (1-3)Gal	fucose α (1-2) galactose
Fuc α (1-4)Gal	fucose α (1-4) galactose
Fuc α (1-6)GlcNAc	fucose α (1-6) <i>N</i> -acetylglucosamine
FUT1	α (1-2) fucosyltransferase 1
FUT2	α (1-2) fucosyltransferase 2
FUT3	α (1-3,4) fucosyltransferase 3
FUT4	α (1-3,4) fucosyltransferase 4
FUT5	α (1-3) fucosyltransferase 5
FUT6	α (1-3) fucosyltransferase 6
FUT7	α (1-3) fucosyltransferase 7
FUT8	α (1-6) fucosyltransferase 8
FUT9	α (1-6) fucosyltransferase 9
FUT10	putative α (1-3) fucosyltransferase 10

FUT11	putative $\alpha(1-3)$ fucosyltransferase 11
g	gram, gravitational force
Gal	galactose
GalNAc	<i>N</i> -acetylgalactosamine
GDP-fucose	guanosine diphosphatyl-fucose
Glc	glucose
GlcA	D-glucuronic acid
GlcN	D-glucosamine
GlcNAc	<i>N</i> -acetylglucosamine
GluR1	glutamate receptor 1
GTP	guanosine triphosphate
h	hour
H	homogenate
HIO ₄	periodate
hnRNP	heterogeneous ribonucleoprotein
H ₂ O	water
HOAc	acetic acid
HRP	horse-radish peroxidase
Hsc/Hsp 70	heat shock chaperonin/heat shock protein 70
IACUC	institute of animal care and use committee
IgSF	immunoglobulin superfamily
IgG	immunoglobulin
IP	immunoprecipitated
K ⁺	potassium ion
K _{assoc}	association constant
KCl	potassium chloride
kDa	kilodalton
K ₃ Fe(CN) ₆	potassium ferricyanide
KO	knockout
L	liter
LAC	lectin affinity chromatography
LAD II	leukocyte adhesion deficiency type II
LC/MS ⁿ	liquid-chromatography mass spectrometry
LTL	<i>Lotus tetragonolobus</i> lectin
LTP	long-term potentiation
LS1	soluble fraction of synaptosome lysis
LS2	crude synaptosol
LP1	insoluble fraction of synaptosome lysis
LP2	crude synaptic vesicle preparation
M	molar
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
Man	mannose
MAP2	microtubule associated protein 2
MEM	Minimal Eagle's Medium
MeOH	methanol
μ g	microgram

MG132	proteasome inhibitor
MgCl ₂	magnesium chloride
min	minutes
m	milli or meter
μ	micro
μCi	micro-Curie
MOB	main olfactory bulb
mol	mole
MS	mass spectrometry
Munc18	syntaxin-binding protein
MWCO	molecular weight cut-off
n	nano
N	normal
Na ⁺	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
Na ₂ CO ₃	sodium bicarbonate
NaN ₃	sodium azide
Na ₂ S ₂ O ₃	sodium thiosulfate
NCAM	neural cell adhesion molecule
NCBI	national center for biotechnology information
NETFD	SDS-neutralization lysis buffer
Neu5Ac	sialic acid
NH ₄ HCO ₃	ammonium bicarbonate
NIH	national institute of health
NP-40	nonidet P-40 detergent
NPI	neuronal pentraxin I
NSF	<i>N</i> -ethylmaleimide sensitive factor
NTCB	2-nitro-5-thiocyanobenzoic acid
OCAM	olfactory cell adhesion molecule
OEt	<i>O</i> -ethyl
ONL	olfactory nerve layer
OSN	olfactory sensory neuron
P0	post-natal day 0 mouse or rat pup
P1	insoluble fraction 1
P2	insoluble fraction 2
P2'	insoluble fraction 2'; crude synaptosomes
P3	post-natal day 3 mouse or rat pup
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PEPCase	phosphoenolpyruvate carboxylase
PNGase F	<i>N</i> -glycosidase F
POFUT1	<i>O</i> -fucosyltransferase 1
POFUT2	<i>O</i> -fucosyltransferase 2
PSA	polysialic acid
PSD-95	post synaptic density protein 95

PTM	post-translation modification
PVDF	polyvinylidene difluoride
Rab-GDI	Rab guanine-dissociation inhibitor
RNA	ribonucleic acid
rpm	revolutions per minute
rt	room temperature
S1	soluble fraction 1
S2	soluble fraction 2
S2'	soluble fraction 2'
SDS	sodium dodecyl sulfate
Slc12a2	solute carrier family member 2
Sec1	noncatalytic $\alpha(1-2)$ fucosyltransferase
SEM	standard error of the mean
SG	sucrose-gradient purified synaptic vesicles
SNAP-25	synaptosomal-associated protein of 25 kDa
SynI	synapsin I
Syn KO	synapsin knockout
TBST	tris buffered saline with Tween-20
TCEP	tris(2-carboxyethyl)phosphine
TEAA	triethylammonium acetate
Tris-Cl	tris chloride
TRPV5	transient receptor potential cation channel
UEAI	<i>Ulex europeaus</i> agglutinin I
UDP	uridyl-diphosphate
UV	ultraviolet
VDAC1	voltage-dependent anion channel 1
vol	volume
w/v	weight per volume
WGA	wheat germ agglutinin
WT	wild type
Xyl	xylose