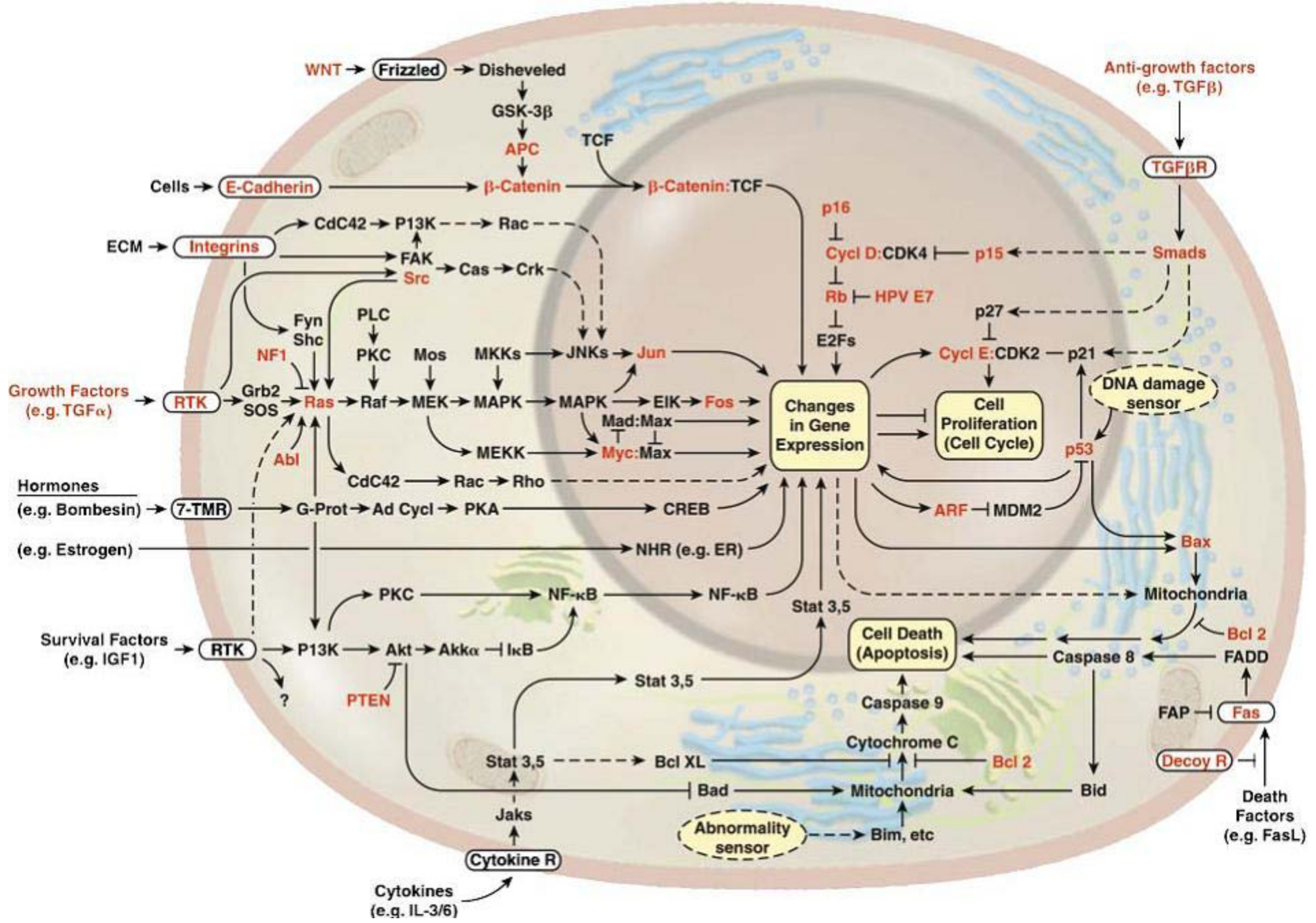
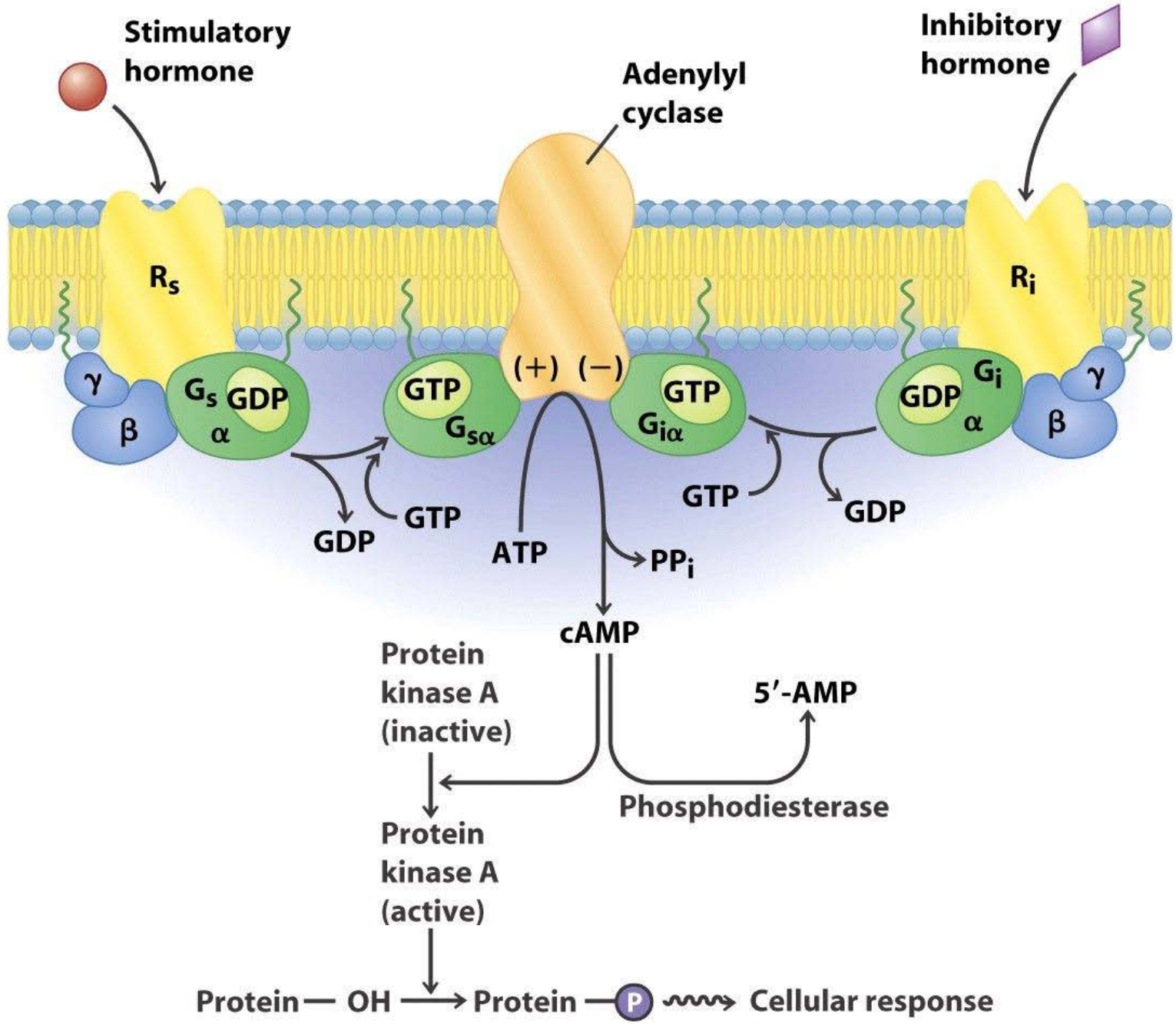
Reversible protein glycosylation in health and disease

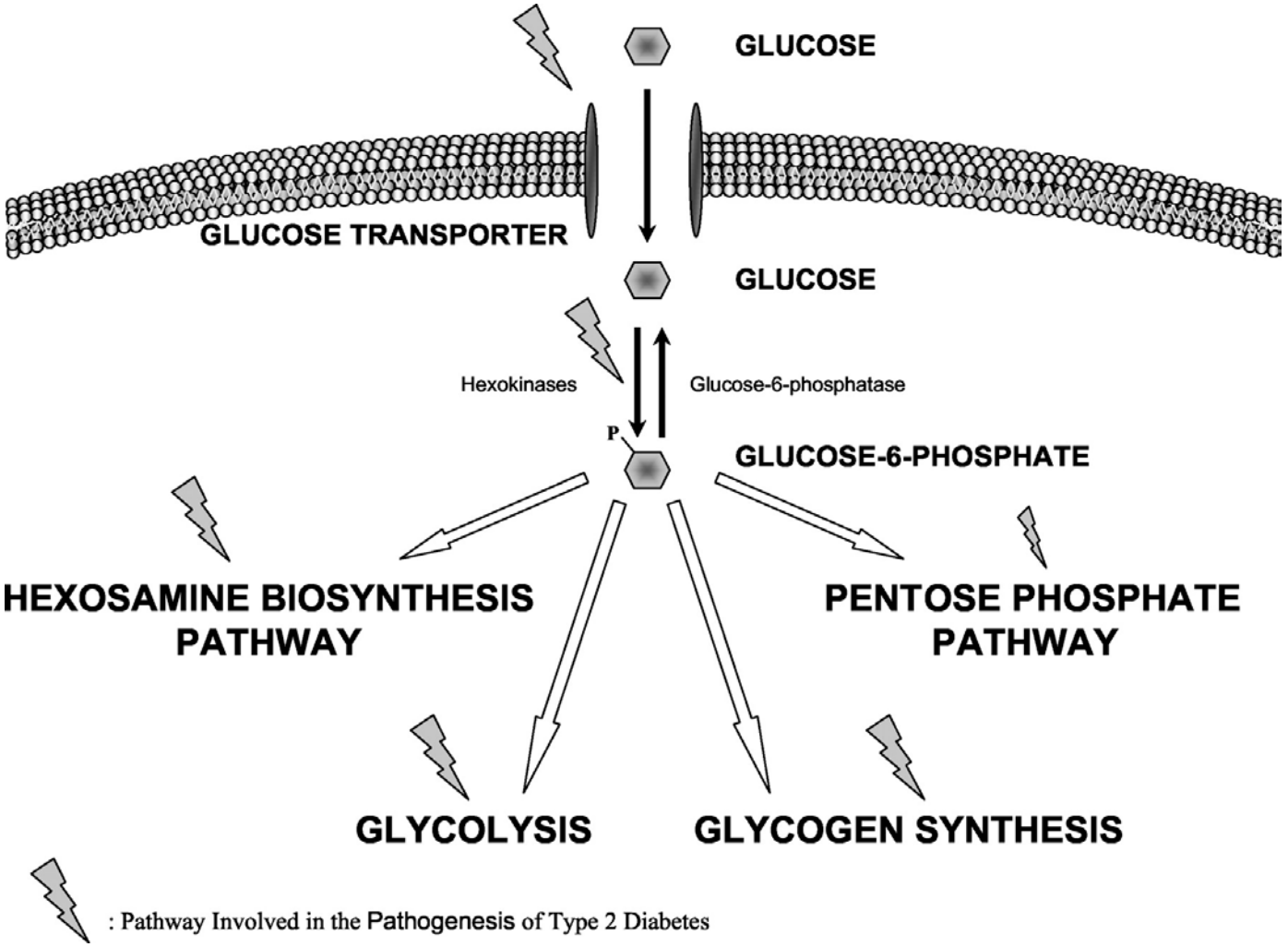
The *O*-GlcNAc modification

Line M. Grønning-Wang
Department of Nutrition
MBV9100-31.10.11


WNT
Frizzled
Disheveled
GSK-3β
APC
TCF
β-Catenin
β-Catenin:TCF
Cells
E-Cadherin
Anti-growth factors
(e.g. TGFβ)
TGFβR
Smads
p16
Cycl D:CDK4
p15
Rb
HPV E7
p27
E2Fs
Cycl E:CDK2
p21
ECM
Integrins
CdC42
P13K
Rac
FAK
Src
Cas
Crk
Fyn
Shc
PLC
PKC
NF1
Mos
MKKs
JNKs
Jun
Growth Factors
(e.g. TGFα)
RTK
Grb2
SOS
Ras
Raf
MEK
MAPK
MAPK
ElK
Fos
Mad:Max
Myc:Max
MEKK
Changes
in Gene
Expression
Cell
Proliferation
(Cell Cycle)
DNA damage
sensor
p53
Abl
Hormones
(e.g. Bombesin)
7-TMR
G-Prot
Ad Cycl
PKA
CREB
CdC42
Rac
Rho
ARF
MDM2
(e.g. Estrogen)
NHR (e.g. ER)
Bax
PKC
NF-κB
NF-κB
Stat 3,5
Mitochondria
Survival Factors
(e.g. IGF1)
RTK
P13K
Akt
Akkα
IκB
?
PTEN
Cell Death
(Apoptosis)
Caspase 8
Bcl 2
FADD
Stat 3,5
Caspase 9
FAP
Fas
Cytochrome C
Stat 3,5
Bcl XL
Bcl 2
Decoy R
Bad
Mitochondria
Bid
Jaks
Abnormality
sensor
Bim, etc
Death
Factors
(e.g. FasL)
Cytokine R
Cytokines
(e.g. IL-3/6)



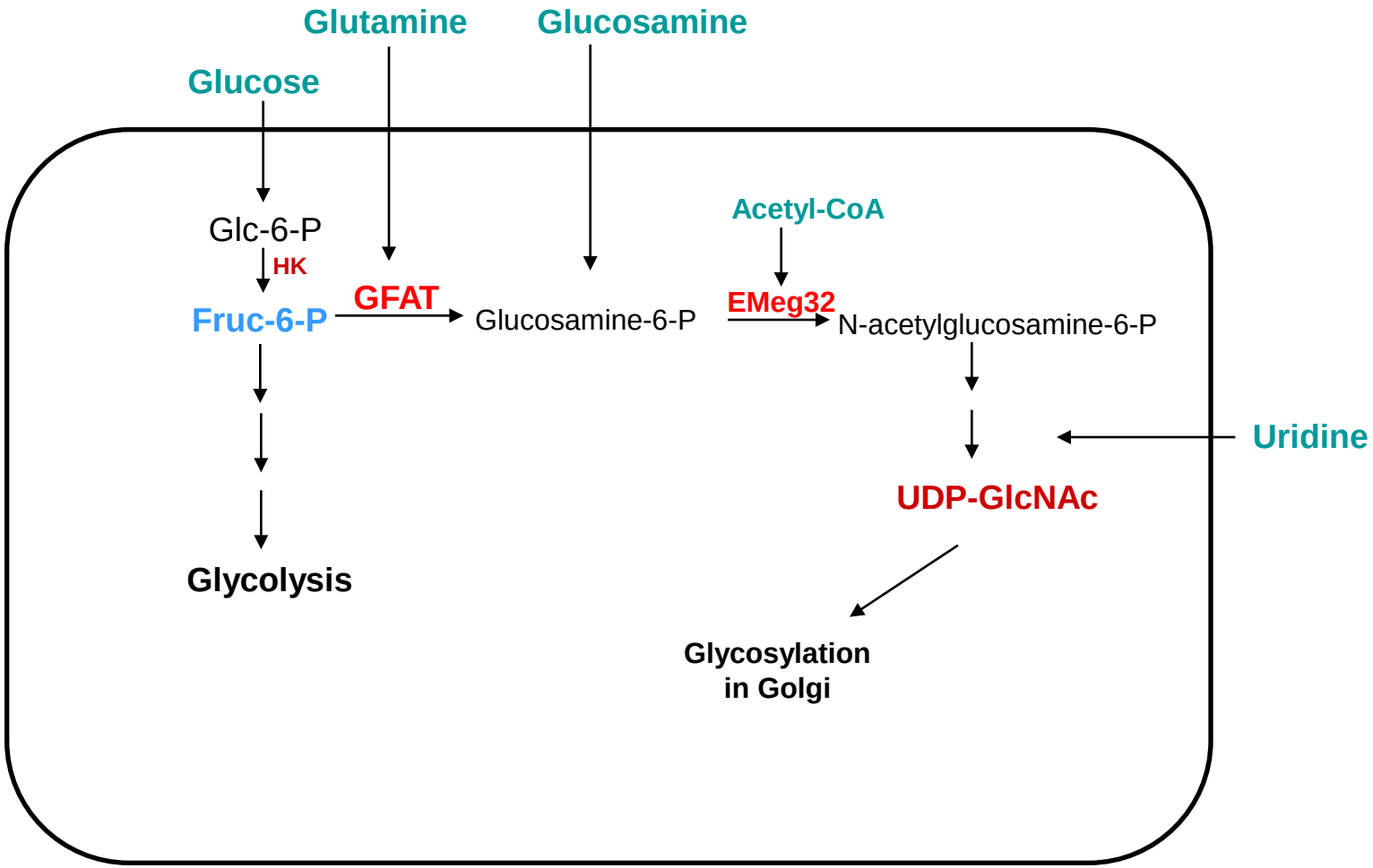
What happens to glucose when it enters into cells?



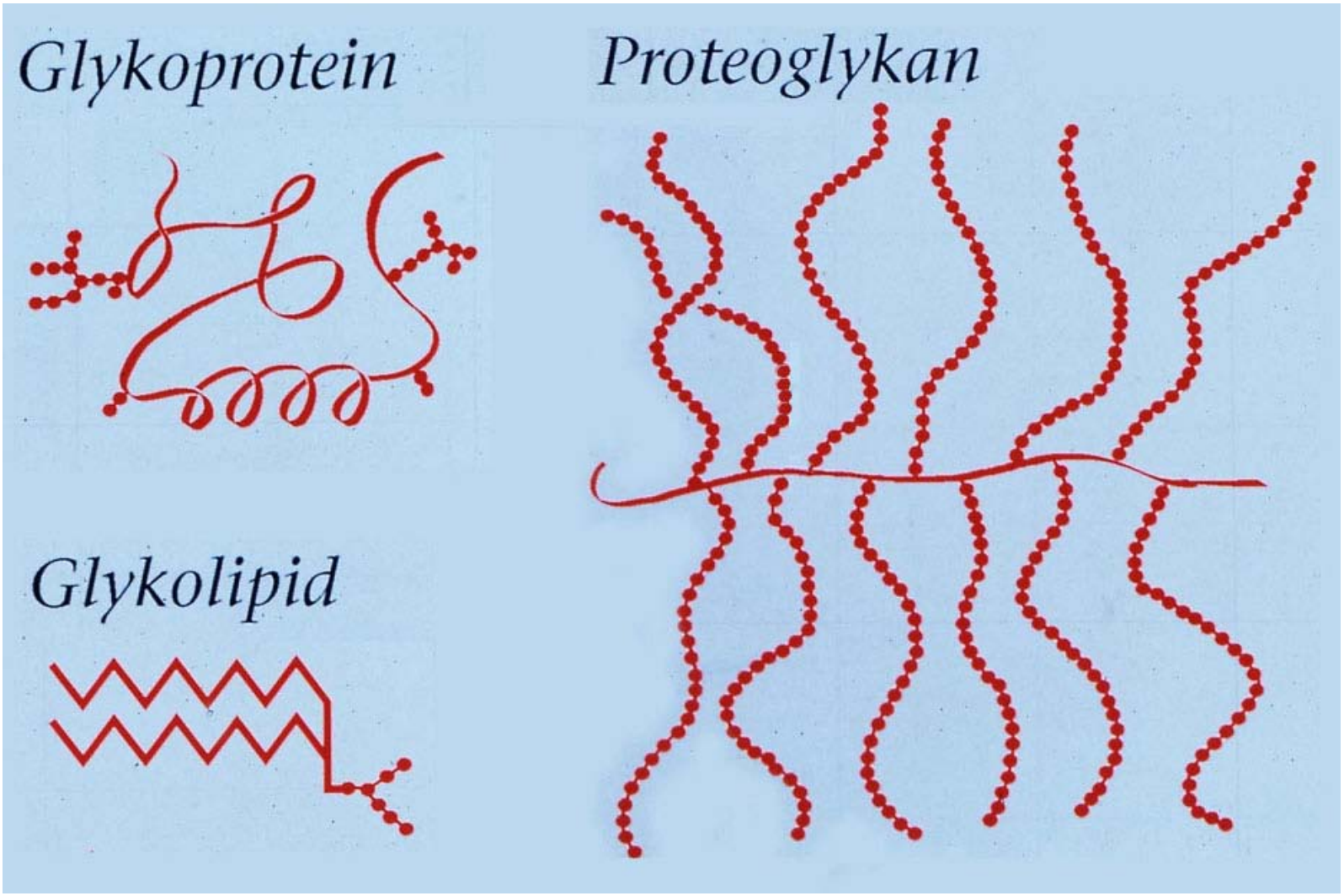
Bouche, C. et al. Endocr Rev 2004;25:807-830

ENDOCRINE REVIEWS

The hexosamine pathway-a nutrient responsive pathway

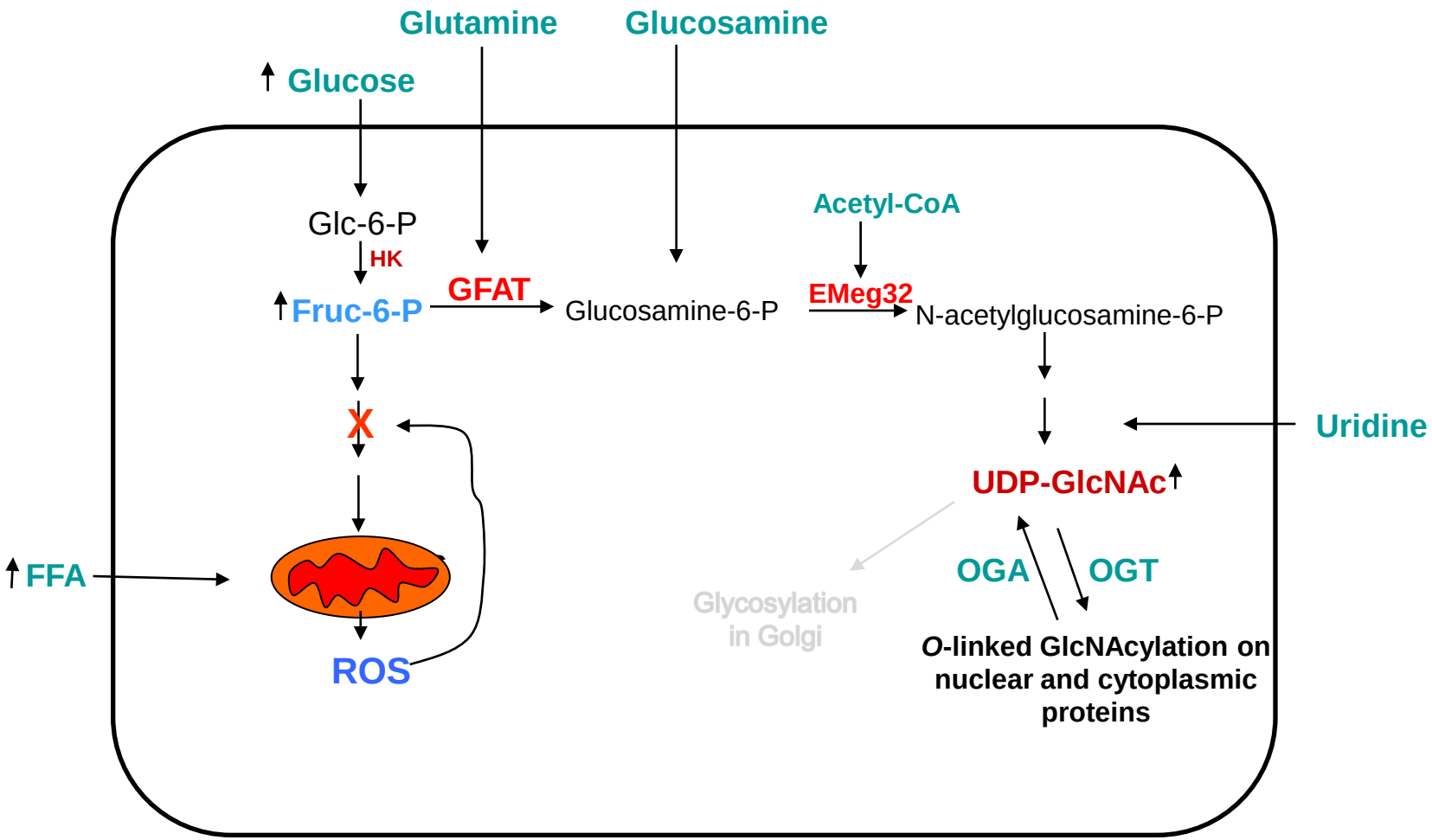


Proteoglycans and other glycoconjugates

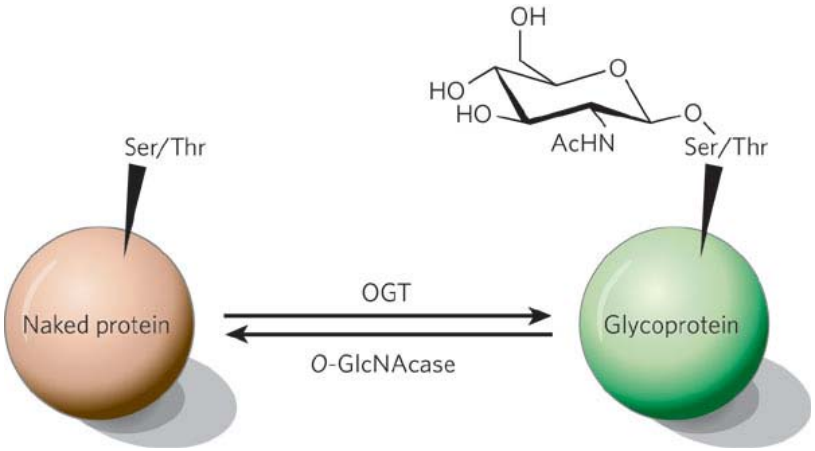


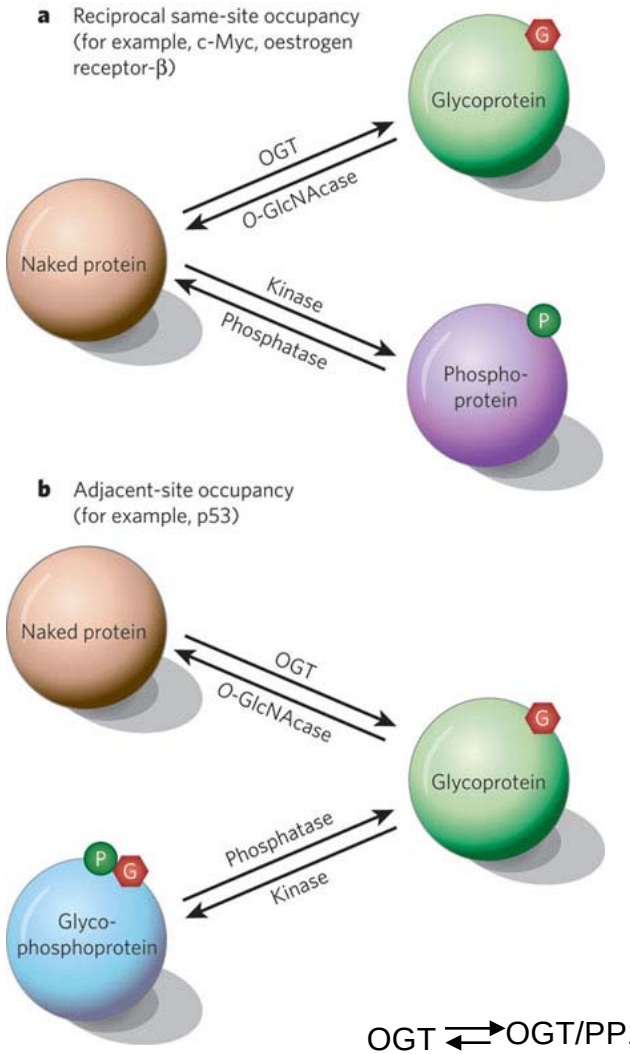
Marion Kusche-Gullberg

The hexosamine pathway-a nutrient responsive pathway



O-linked β-N-acetylglucosamine (O-GlcNAc)- a dynamic posttranslational Modification (PTM) analogous to phosphorylation

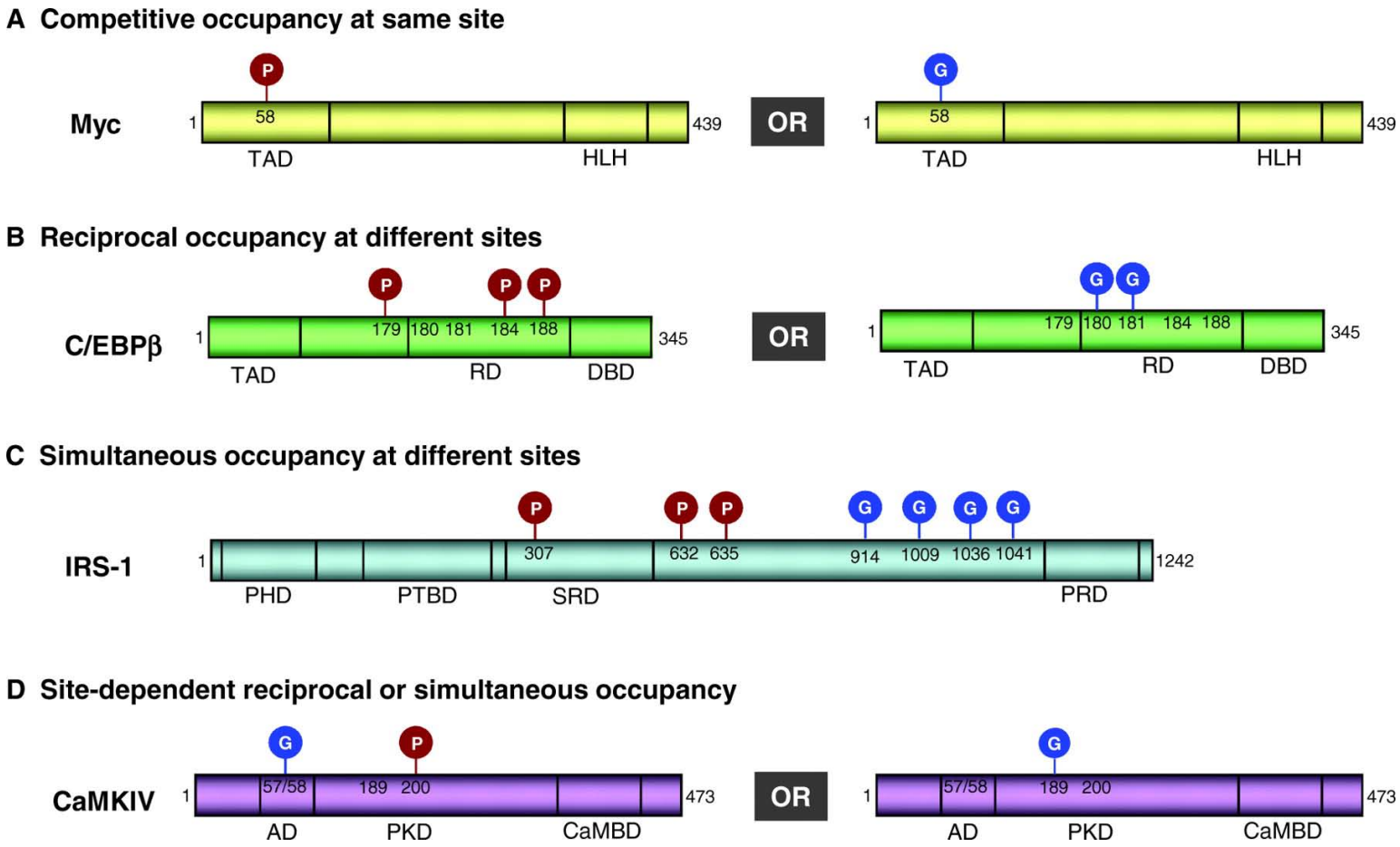




OGT ⇄ OGT/PP1c

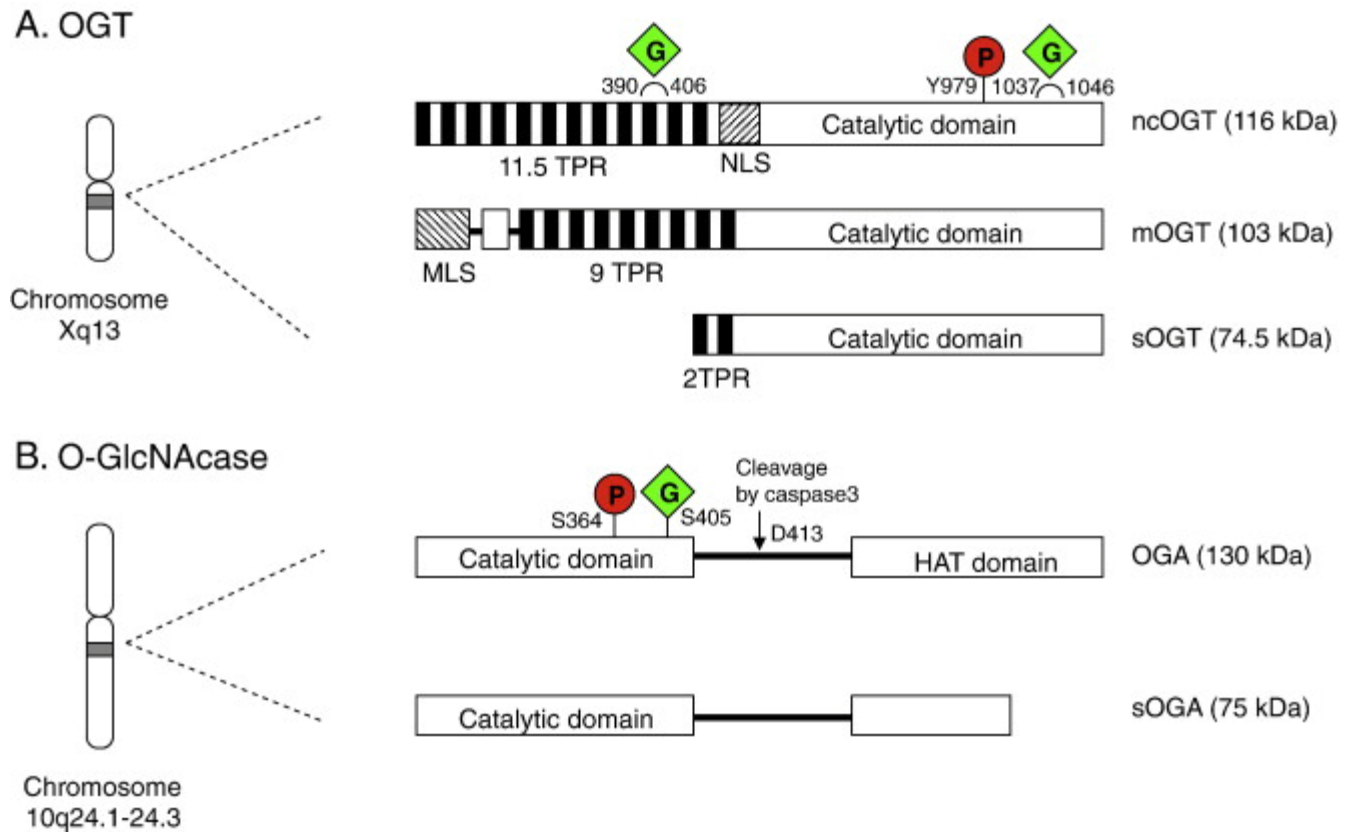
Hart GW et al, NATURE vol 446, 2007

Four different types of O-GlcNAc-phosphate crosstalk on protein substrates

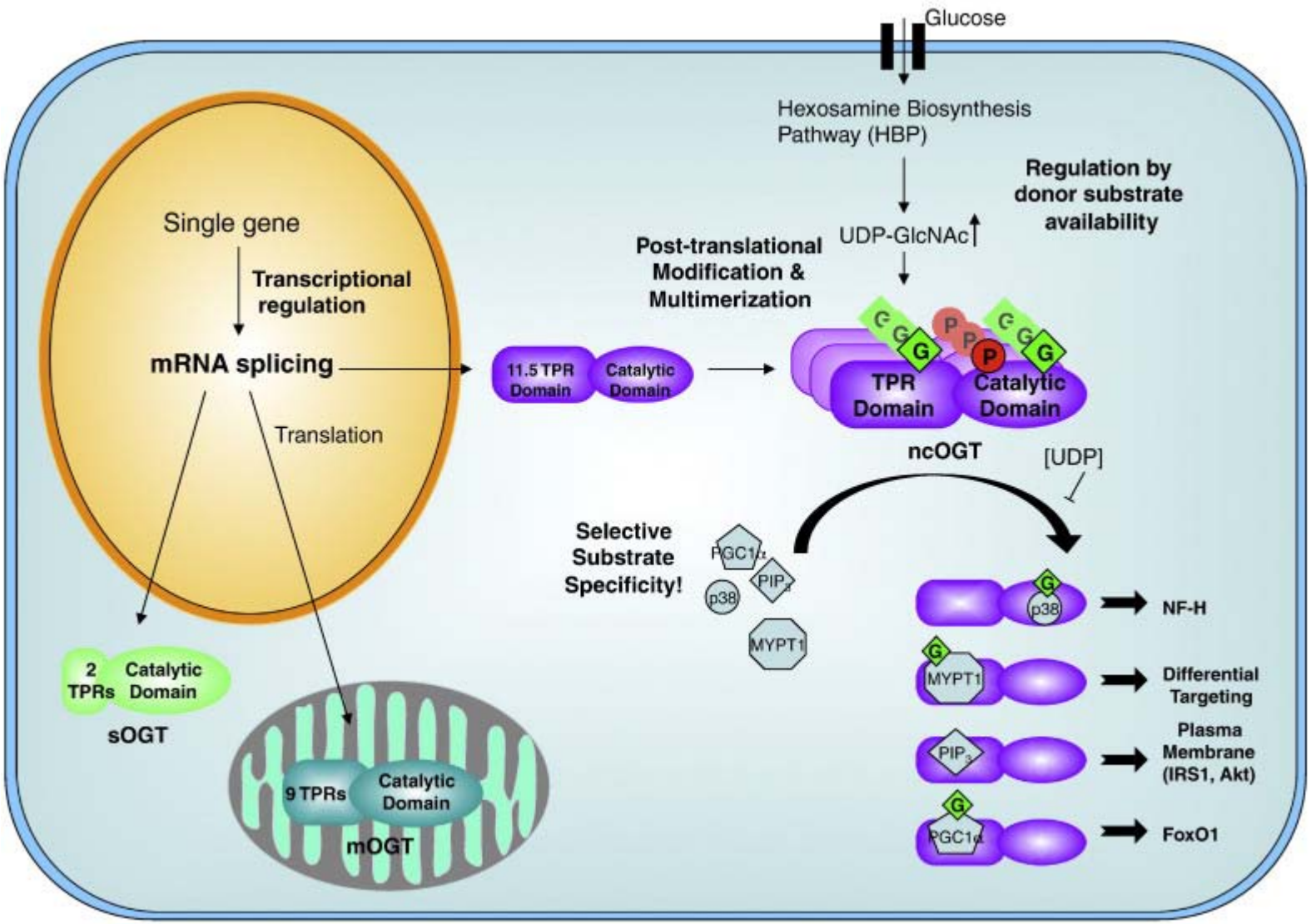


Zeidan Q and Hart GW, Journal of Cell Science, 2010

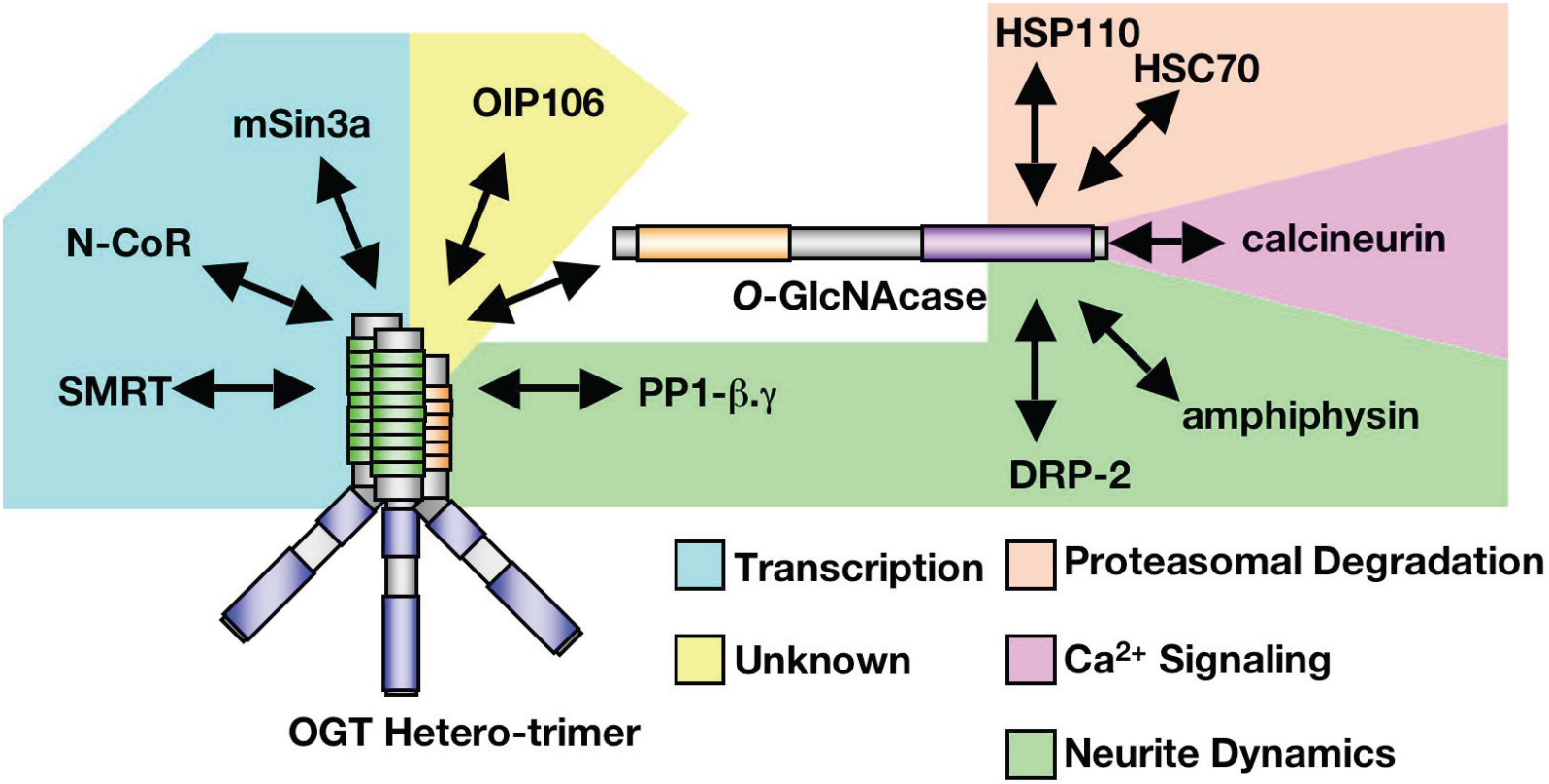
Three different forms of human OGT are produced by alternative splicing from a single gene



Butkinaree C et al, Biochim et Biophys Acta, 2010

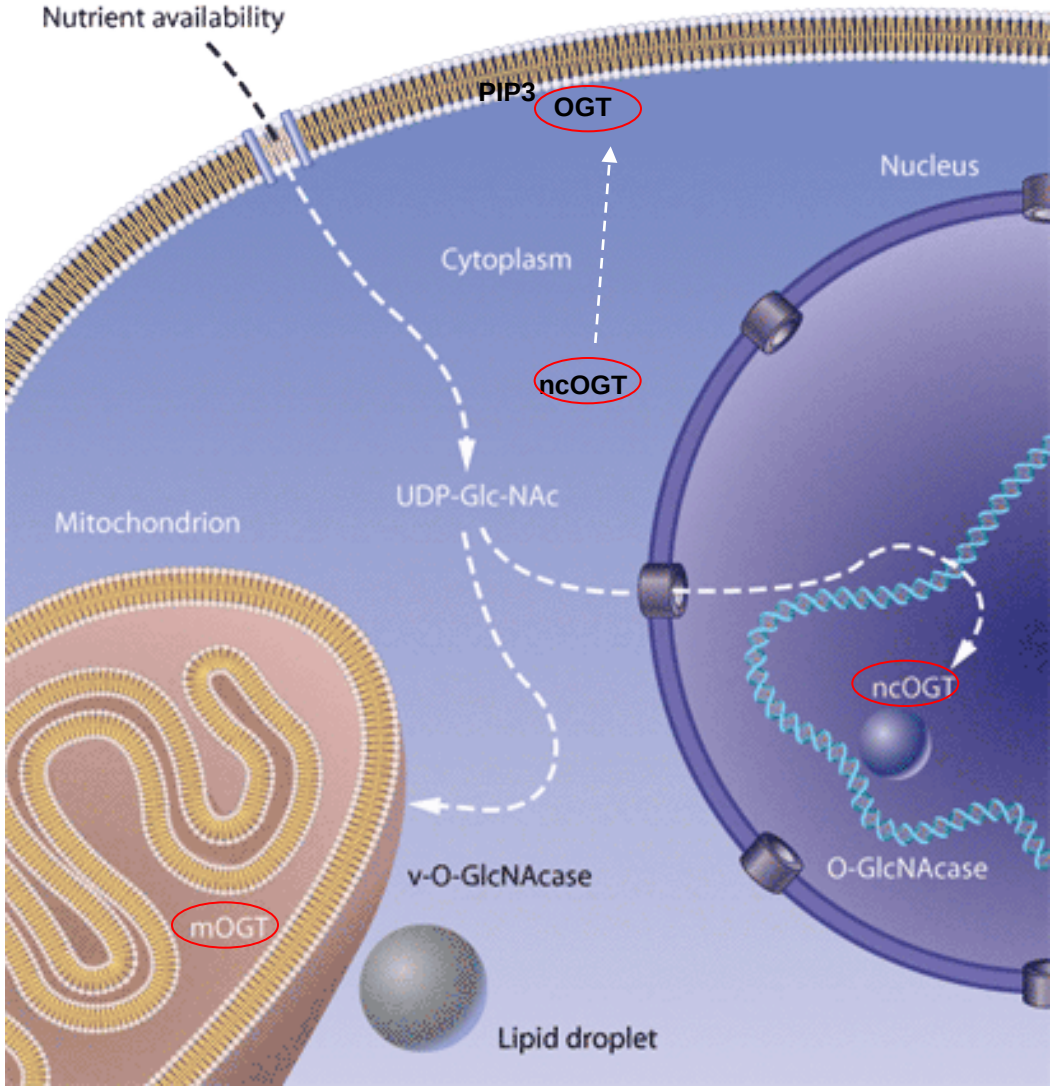


Butkinaree C et al, Biochim et Biophys Acta, 2010



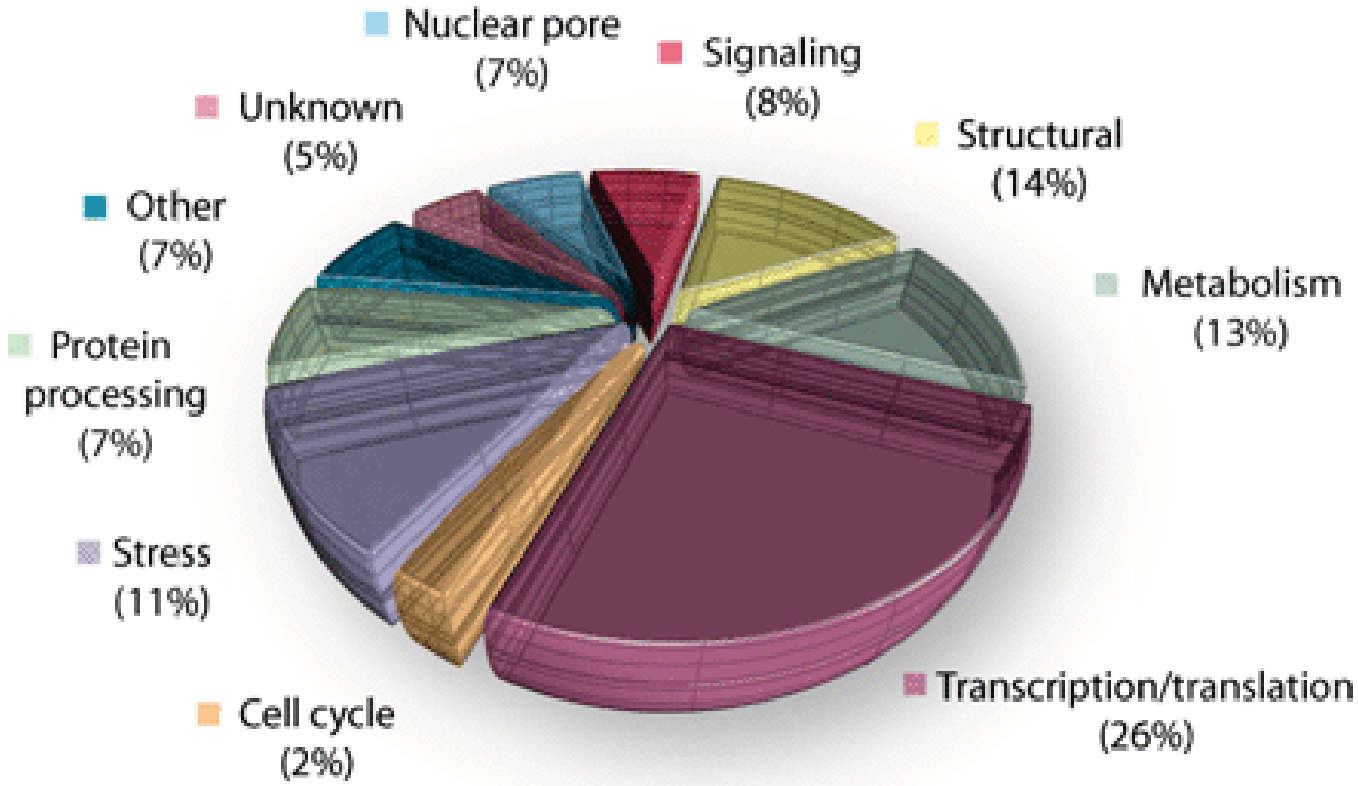
http://etd.caltech.edu/etd/available/etd-05292007-183523/unrestricted/Chapter2.pdf

Specificity in O-GlcNAc signaling: OGT localization



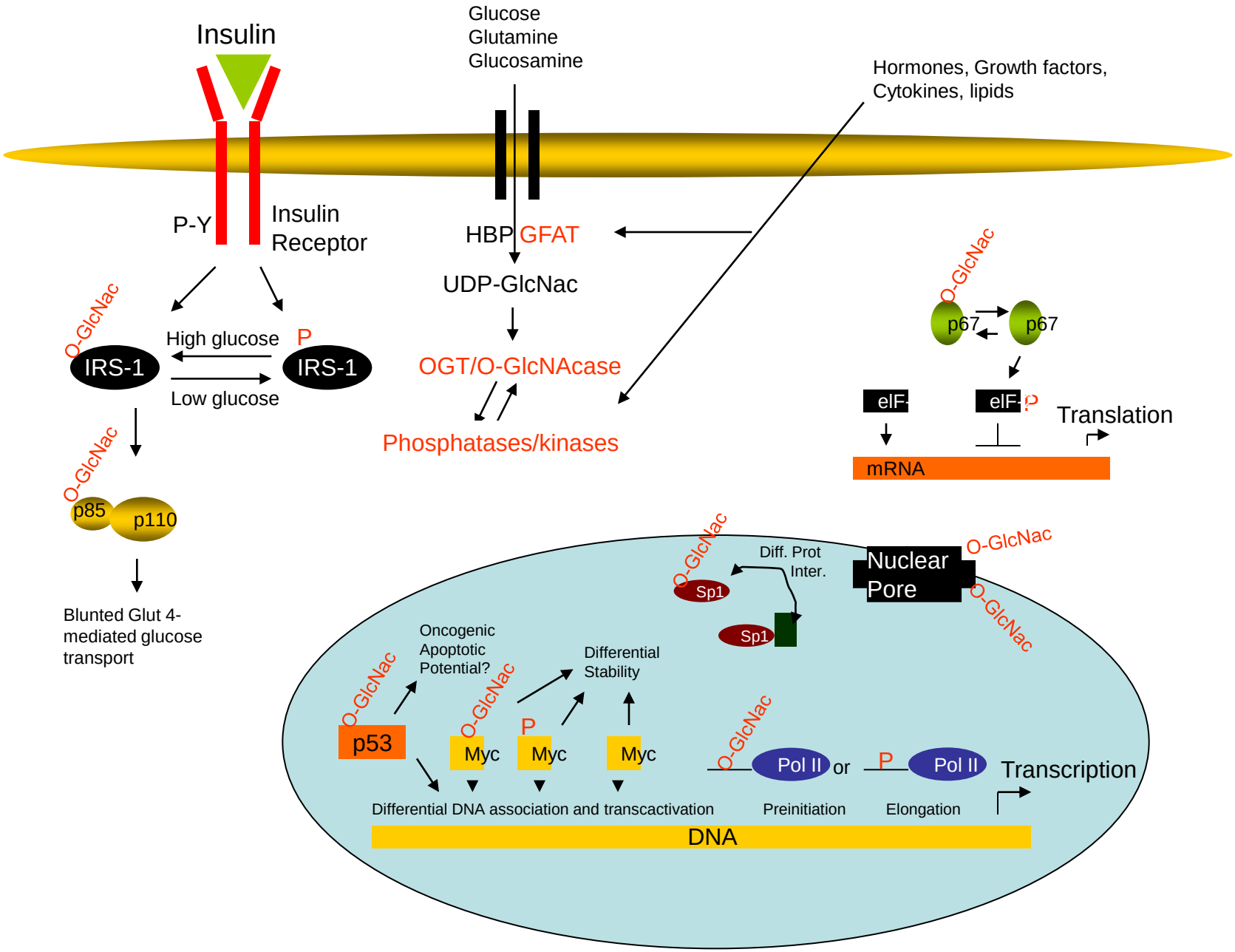
Love DC and Hanover A., Science's STKE. 2005

Known O-GlcNAc substrates.



Love DC and Hanover A., Science's STKE. 2005

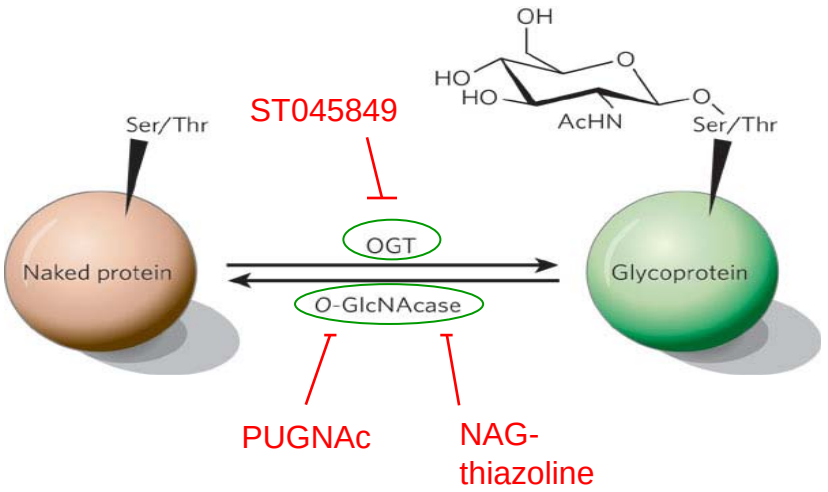
CELLULAR TRANSFORMATION CAUSED BY ACTIVATION OF THE HEXOSAMINE PATHWAY



Tools for the detection of O-GlcNAcylated proteins

Glucosamine
Treat cells with glucosamine
to increase levels of UDP-GlcNAc

PUGNAc/NAG-thiazoline
Inhibitors of O-GlcNAcase (OGA);
inhibit O-GlcNAc removal
from proteins



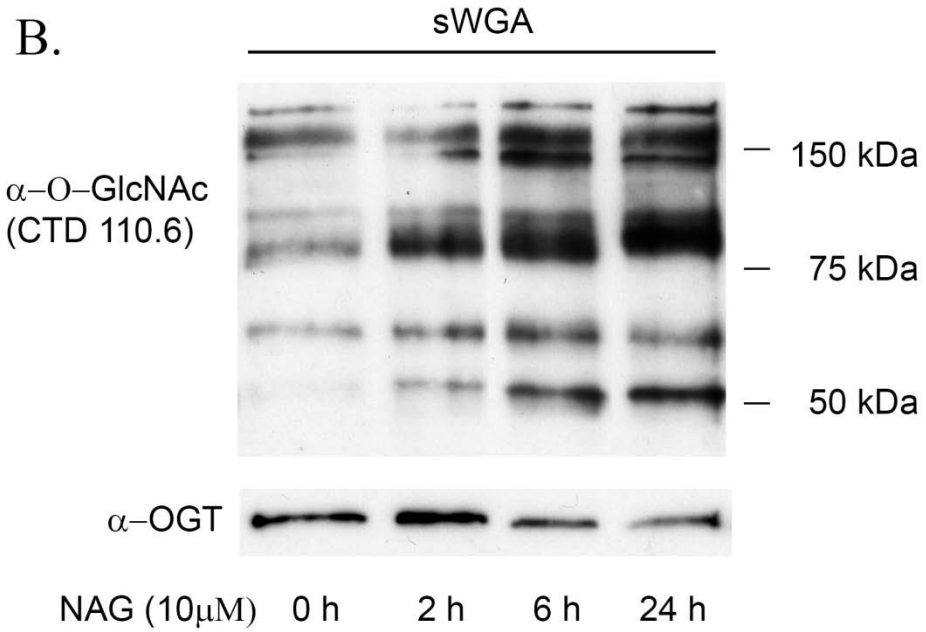
sWGA agarose
Succinylated wheat germ agglutinin (lectin) binds to terminal GlcNAc residues on proteins. sWGA agarose is used to precipitate N- and O-linked GlcNAcylated proteins from cytosolic, nuclear or whole cell lysates.

Monoclonal anti-*O*-GlcNAc antibodies CTD 110.6 and RL2
Monoclonal antibodies specificially recognizing O-GlcNAcylated Proteins. Used in immunoprecipitation and Western blotting.

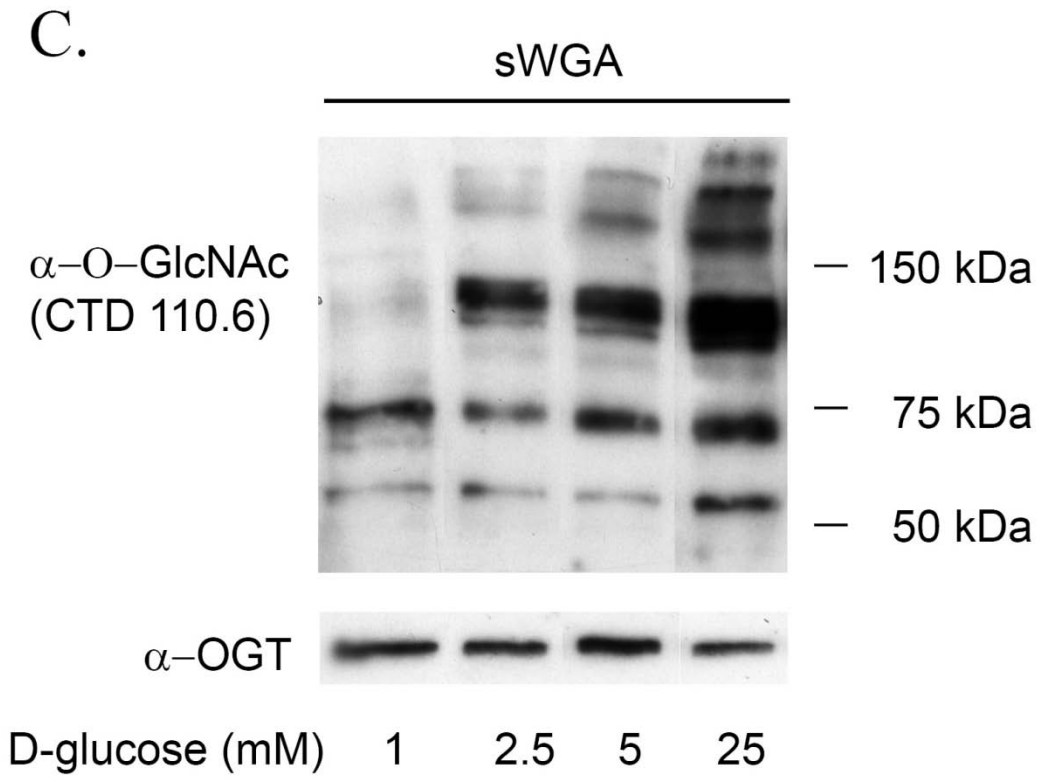
ST045849
Inhibitor of OGT

Predictions of O-ß-GlcNAc attachment sites in eukaryotic protein sequences
New: dpOGAP: http://cbsb.lombardi.georgetown.edu/OGAP.html
YinOYang: http://www.cbs.dtu.dk/services/YinOYang/

Treatment with NAG-thiazoline increases protein *O*-GlcNAcylation



Effects of glucose on protein O-GlcNAcylation



Essential roles of protein O-GlcNAcylation

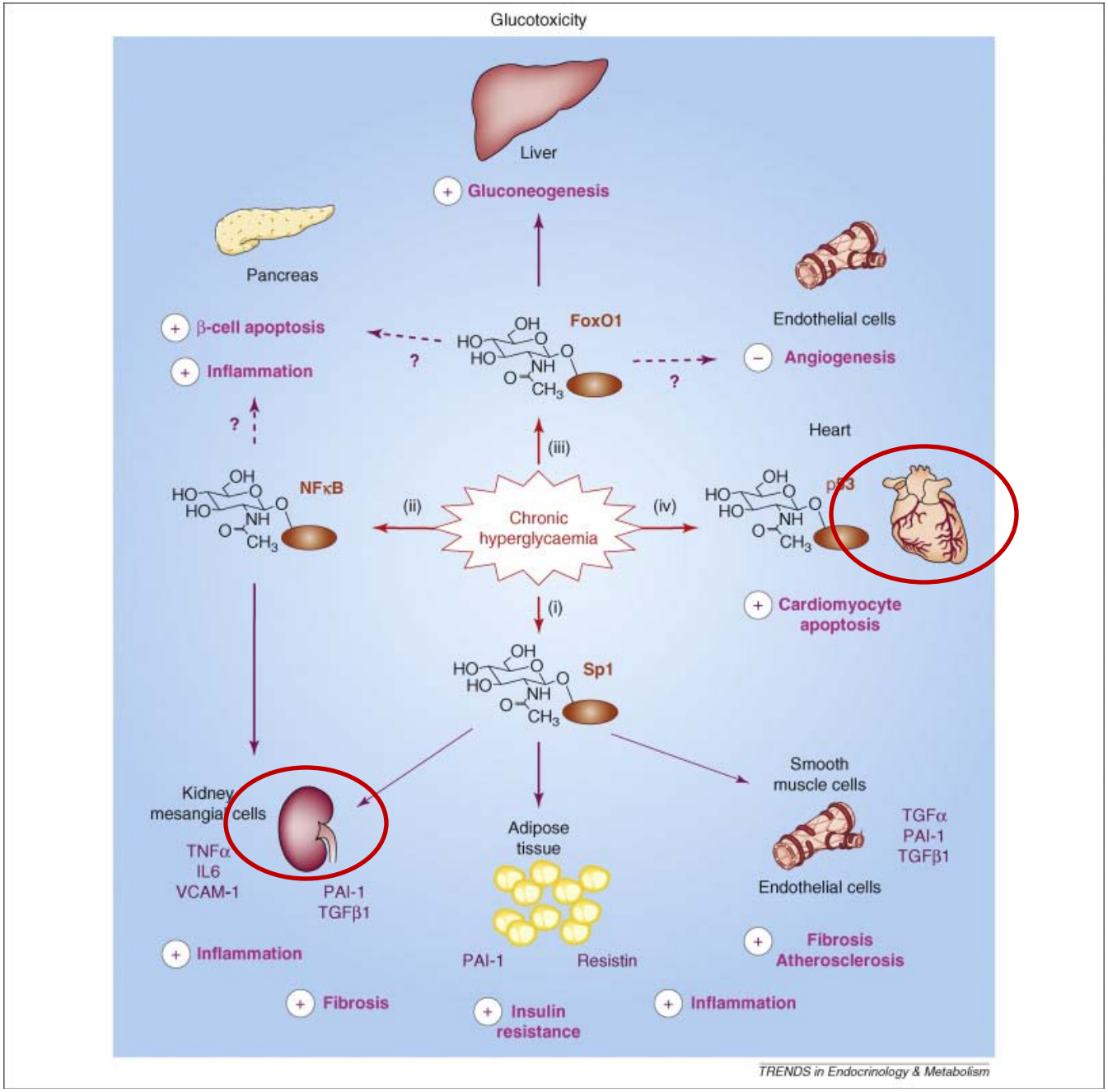
Cell cycle: **Important for G2/M phase entry, controls level of phosphorylation and expression of cyclins** **(Slawson et al, JBC, 2005, Dehennaut et al, JBC, 2007).**

Increased tolerance to stress: **Rapid and transient elevation in O-GlcNAcylation involving up-regulation of Heat Shock Proteins and Chaperones, decrease in calcium influx inhibiting calpain-mediated proteolysis** **(Chatham et al, Shock, 2008, Zachara and Hart, Biochim et Biophys Acta, 2006).**

Pathologies associated with aberrant O-GlcNAcylation

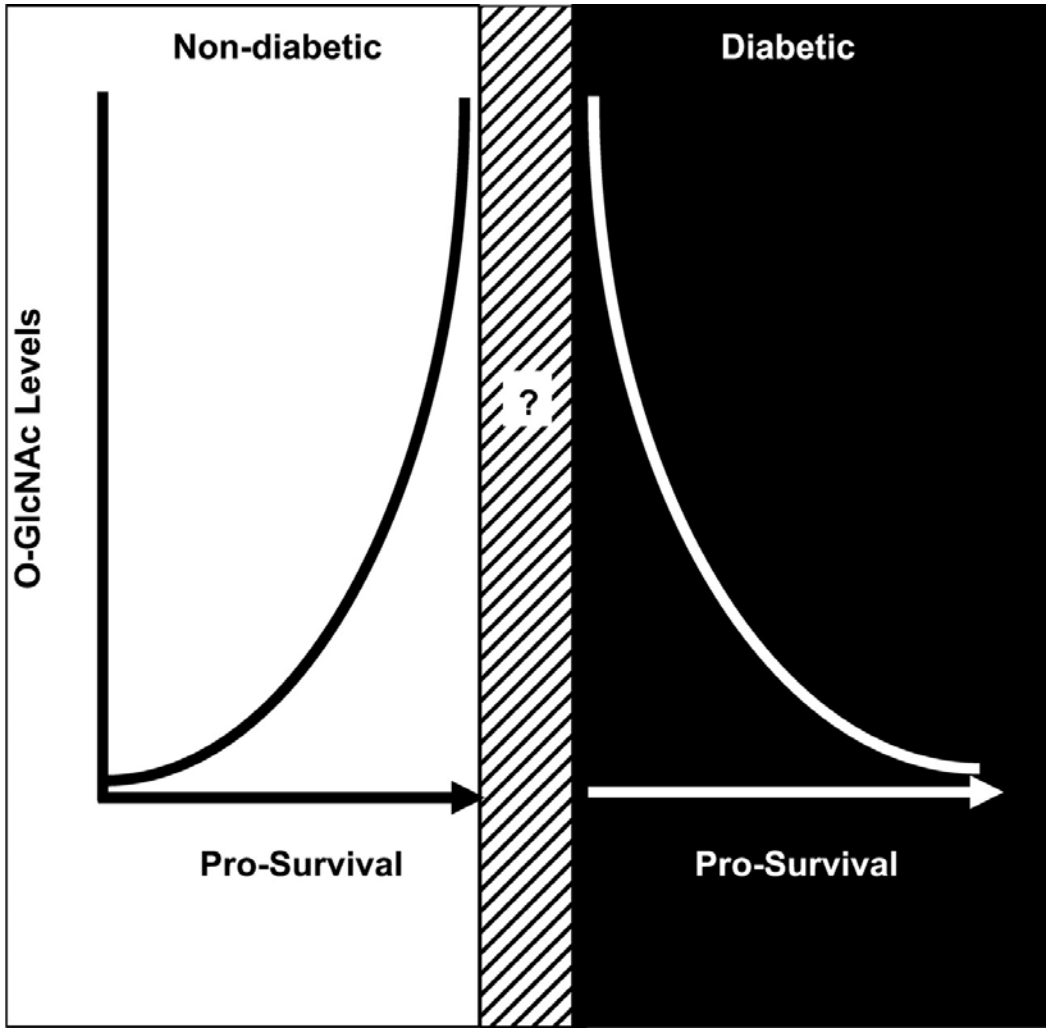
O-GlcNAc	Target	Pathology
↑	IRS, p85/PI3-kinase, PKB/Akt Glut4 vesicle associated proteins	Insulin resistance; decreased insulin signaling and Glut4 translocation.
↑	eNOS	Endothelial dysfunction; reduced NO production.
↑	nuclear proteins	Diabetic Cardiomyopathy; prolonged calcium transient decays and reduced SERCA2 expression (Clark et al. JBC, 2003).
↓	microtubule-associated protein, tau	Alzheimer's; hyperphosphorylation of tau
↓	Increased O-GlcNAcase activity	Breast carcinoma tumor growth

* Slawson C and Hart GW, Nature Reviews Cancer, 2011



Issad T and Kuo M, Trends Endocrinol. Metab, 2008

In both cell culture and animal models that are not diabetic, O-GlcNAc levels are rapidly elevated in response to stress



Zachara, N. E. Am J Physiol Heart Circ Physiol 293: H1324-H1326 2007;
doi:10.1152/ajpheart.00697.2007

AJP - Heart and Circulatory Physiology

Conclusions:

O-GlcNAc is a major regulatory PTM in eukaryotes

O-GlcNAc is required for life. Deletion of OGT is lethal

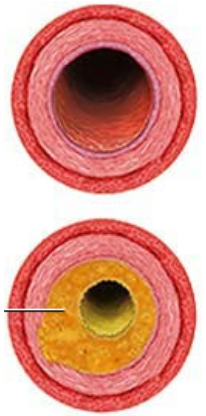
O-GlcNAc is as abundant and phosphorylation and often competes with it

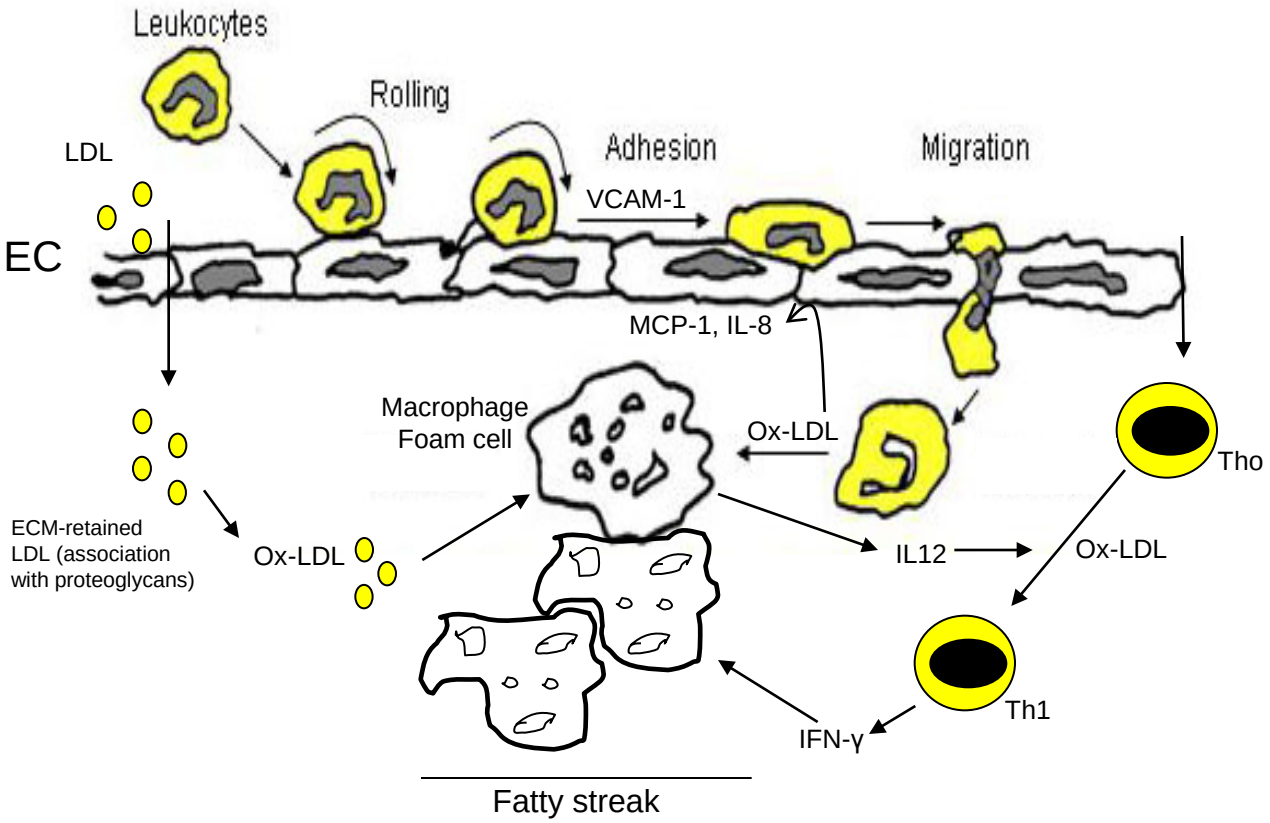
O-GlcNAc is a metabolic and stress sensor that modulate signaling and transcription in response to cellular status

Many toxic effects of Hyperglycemia likely result from dysregulation of the balance between *O*-GlcNAc and *O*-phosphate

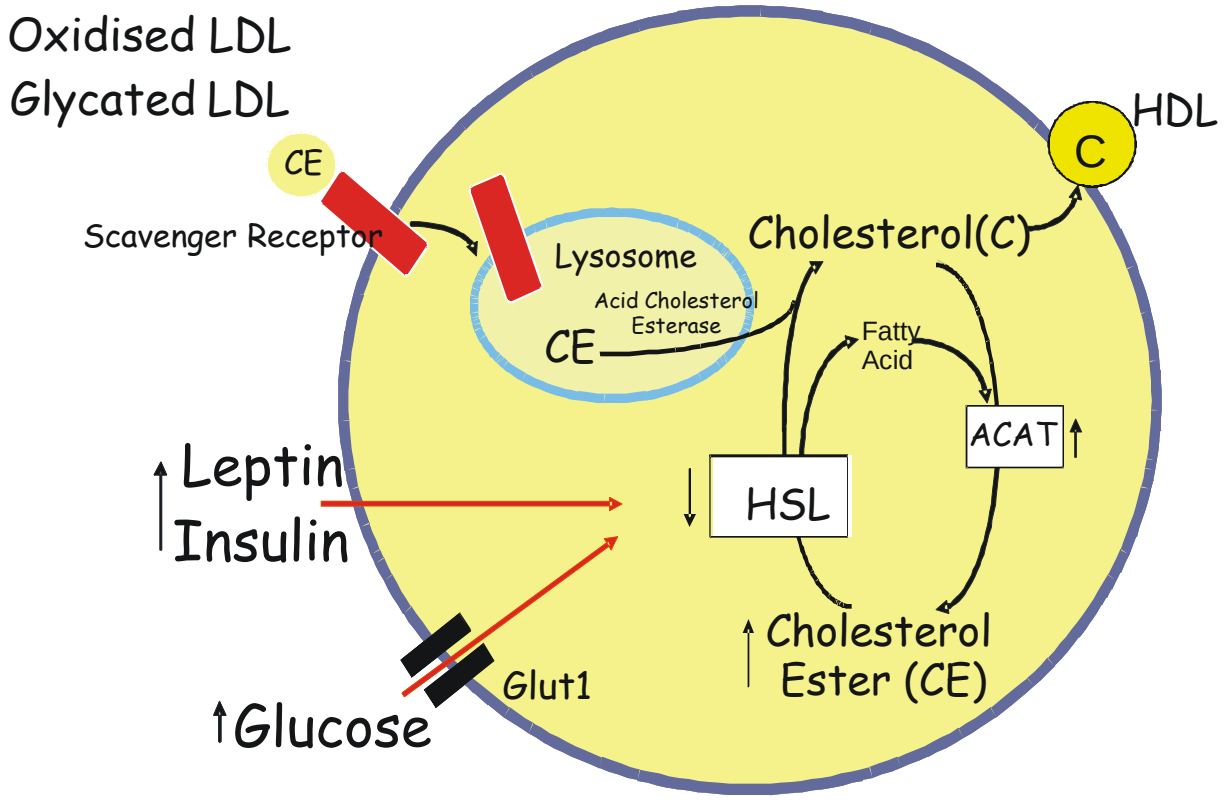
Contradiction between beneficial and adverse effects of O-GlcNAc can be explained by acute and chronic increases in *O*-GlcNAc on proteins by the concept of allostasis; activation of processes/signaling pathways that are protective and improve survival in the short term may lead to patophysiology in the long term

Development of Atherosclerosis





Mechanisms regulating macrophage cholesterol ester deposition in diabetics?



O'Rourke L, Grønning LM, Yeaman SJ, Shepherd PR, JBC, 2002

Reciprocal regulation of inflammation and lipid metabolism by liver X receptors

SEAN B. JOSEPH[1], ANTONIO CASTRILLO[1], BRYAN A. LAFFITTE[1], DAVID J. MANGELSDORF[2] & PETER TONTONOZ[1]

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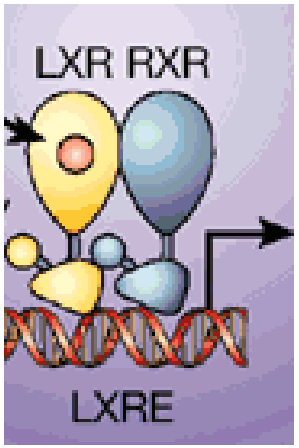
[2]*Howard Hughes Medical Institute, Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas, USA*
S.B.J. and A.C. contributed equally to this work.
Correspondence should be addressed to P.T.; email: ptontonoz@mednet.ucla.edu

Published online 13 January 2003; doi:10.1038/nm820

Macrophages have important roles in both lipid metabolism and inflammation and are central to the pathogenesis of atherosclerosis. The liver X receptors (LXRs) are established mediators of lipid-inducible gene expression, but their role in inflammation and immunity is unknown. We demonstrate here that LXRs and their ligands are negative regulators of macrophage inflammatory gene expression. Transcriptional profiling of lipopolysaccharide (LPS)-induced macrophages reveals reciprocal LXR-dependent regulation of genes involved in lipid metabolism and the innate immune response. *In vitro*, LXR ligands inhibit the expression of inflammatory mediators such as inducible nitric oxide synthase, cyclooxygenase (COX)-2 and interleukin-6 (IL-6) in response to bacterial infection or LPS stimulation. *In vivo*, LXR agonists reduce inflammation in a model of contact dermatitis and inhibit inflammatory gene expression in the aortas of atherosclerotic mice. These findings identify LXRs as lipid-dependent regulators of inflammatory gene expression that may serve to link lipid metabolism and immune functions in macrophages.

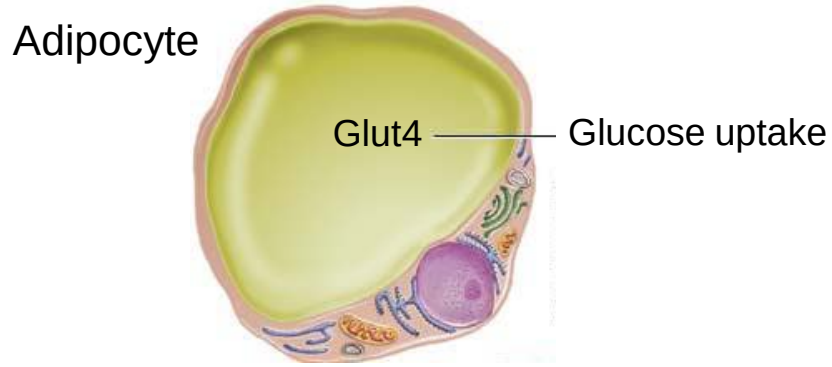
Nature Medicine, 2003

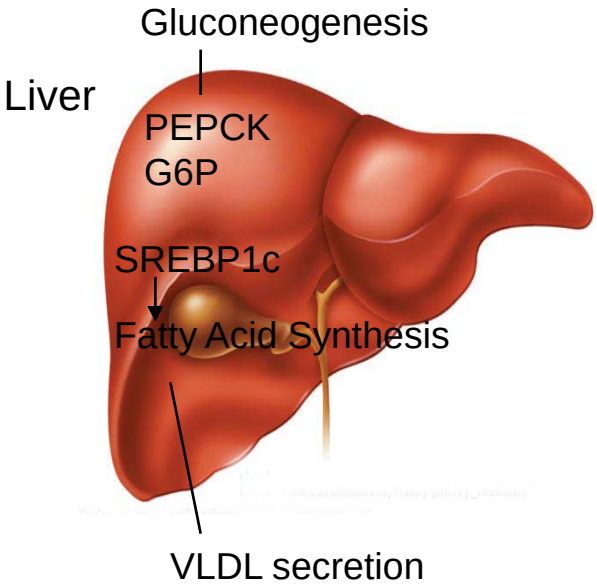
The Liver X Receptor

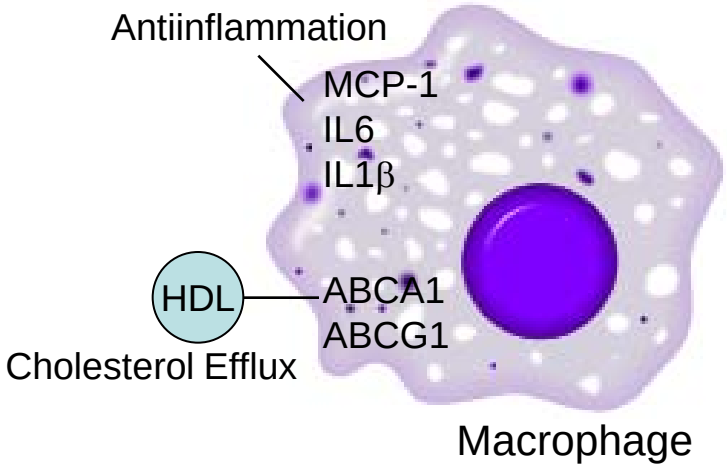


- Two forms; LXRα and LXRβ
- Member of the nuclear hormone receptor family (incl. PPARs, RXR, GR, ER,..)
- Work as heterodimer with the retinoid X receptor (RXR)
- LXRα is expressed in macrophages, liver, adipose tissue, intestine and spleen
- LXRβ is expressed ubiquitously
- Control gene expression linked to cholesterol and fatty acid homeostasis in response to oxygenated cholesterol metabolites (oxysterols).

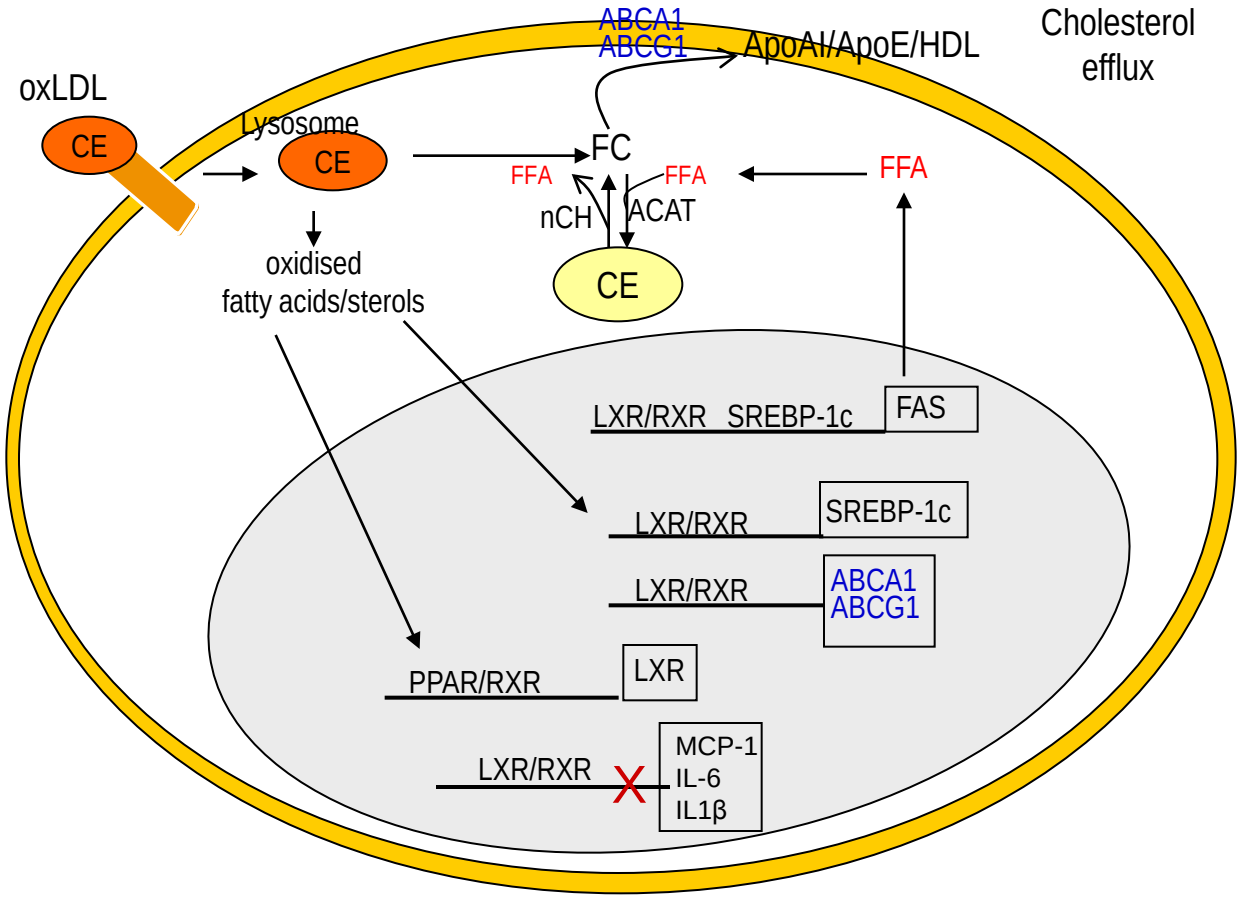
LXR:Functions

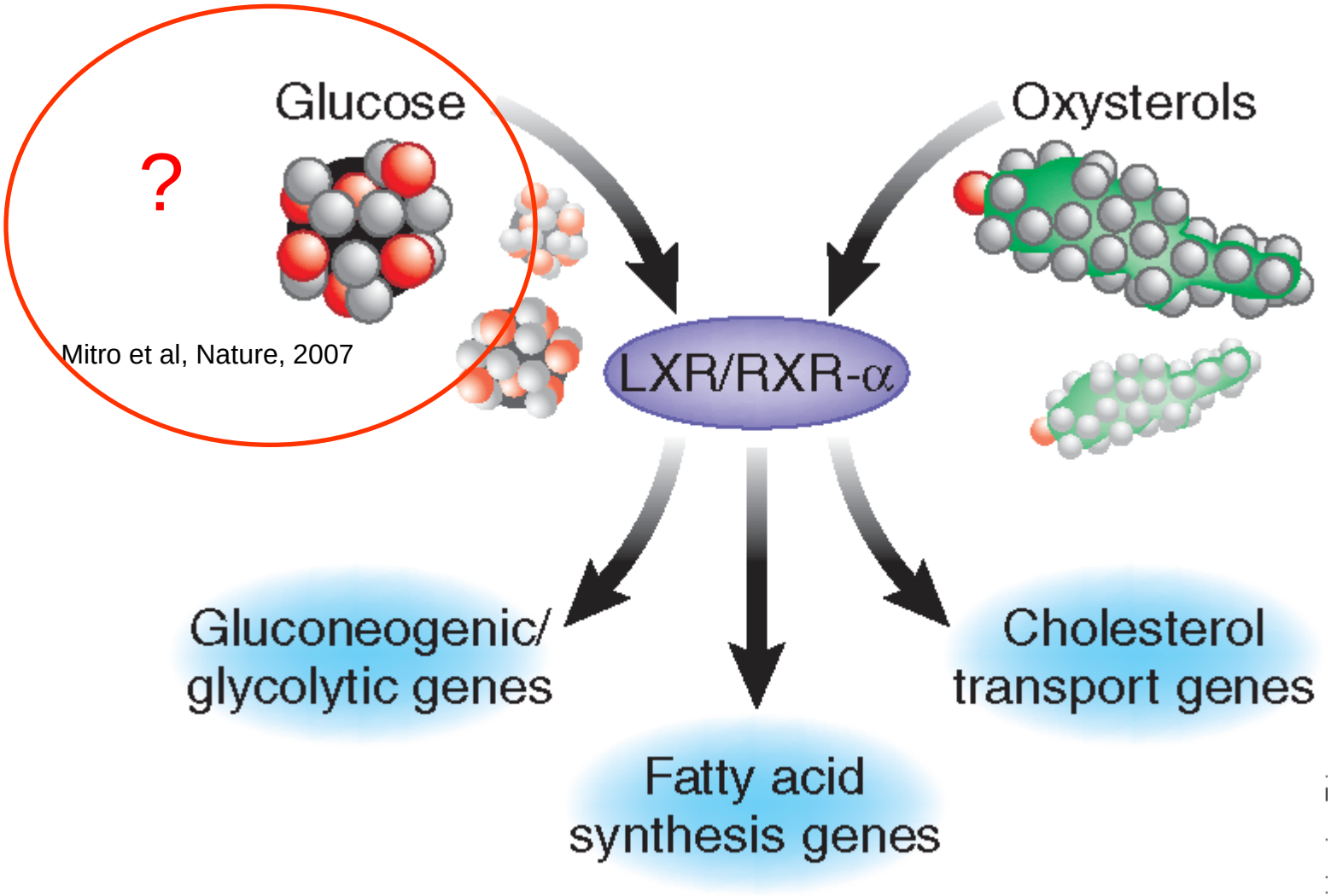






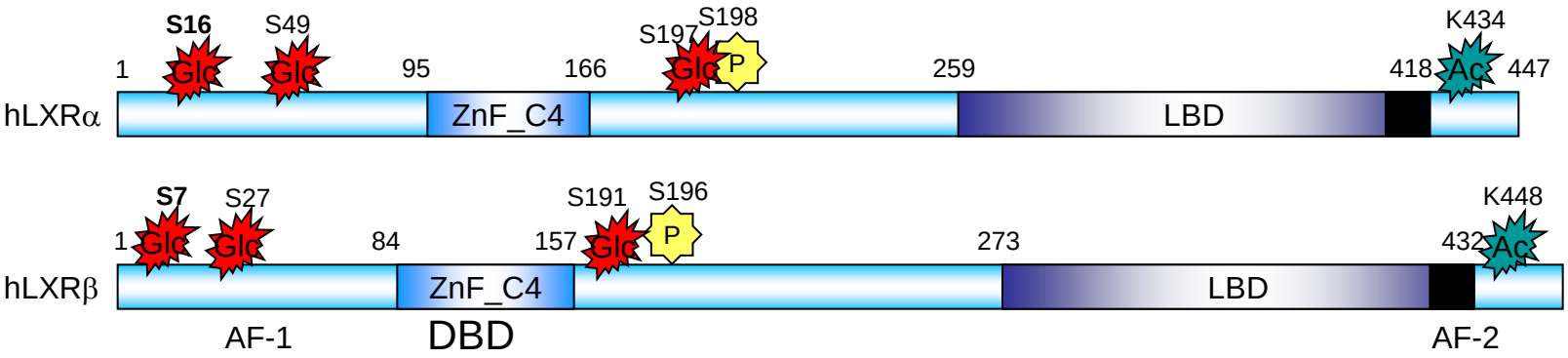
Role of LXR in macrophage lipid metabolism and inflammation





Rodgers JT and Puigserver P, Nature Medicine 13, 2007

Post-translational modifications of LXR with putative O-GlcNAcylation sites

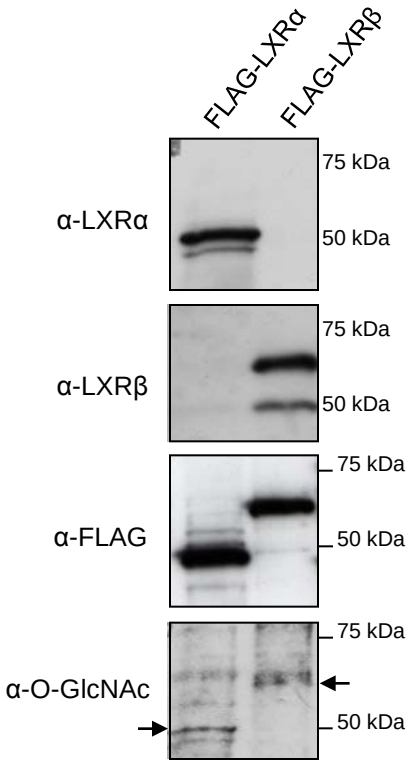


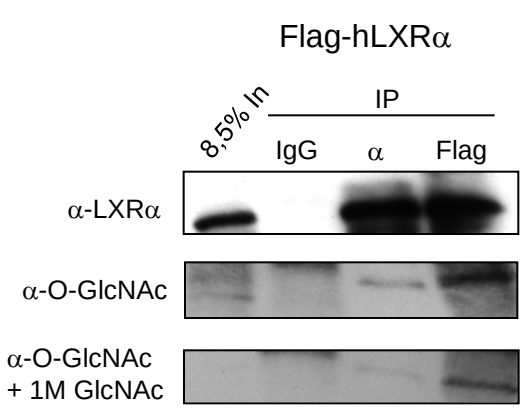
Glc

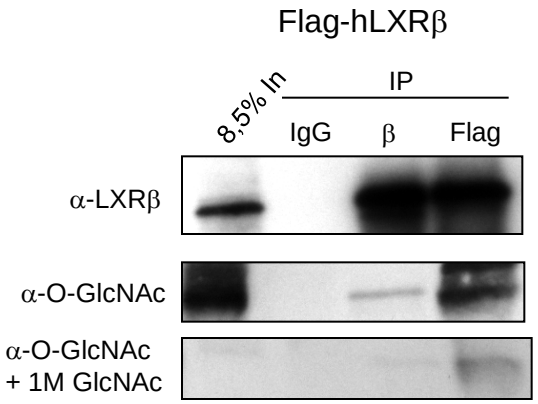
http://cbsb.lombardi.georgetown.edu/OGAP.html

S/T relative to proline at -3/-2

LXRα and LXRβ are modified by O-GlcNAc in Huh7 cells

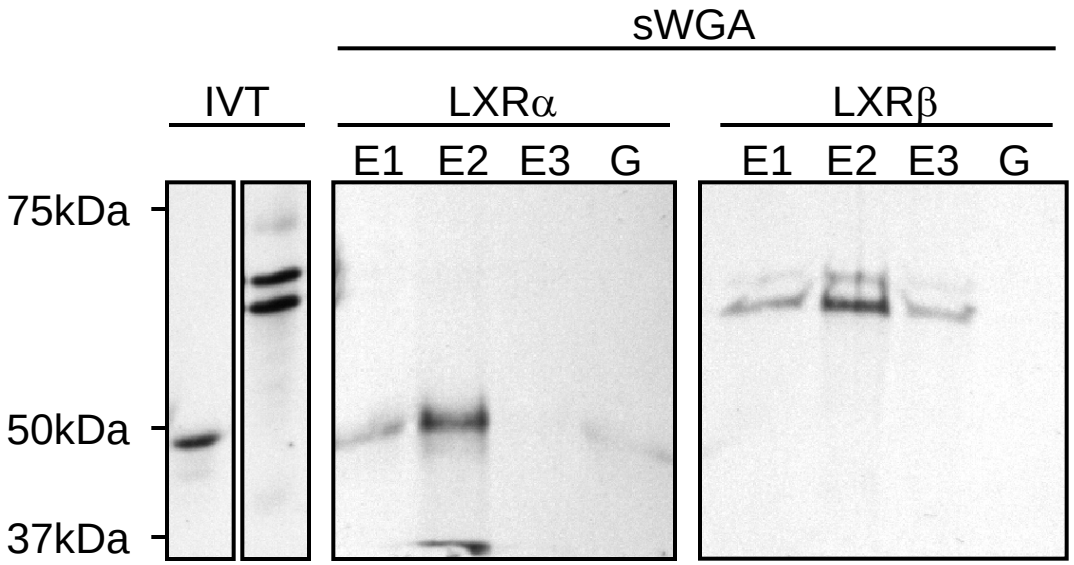






Anthonisen EH et al, JBC, 2010

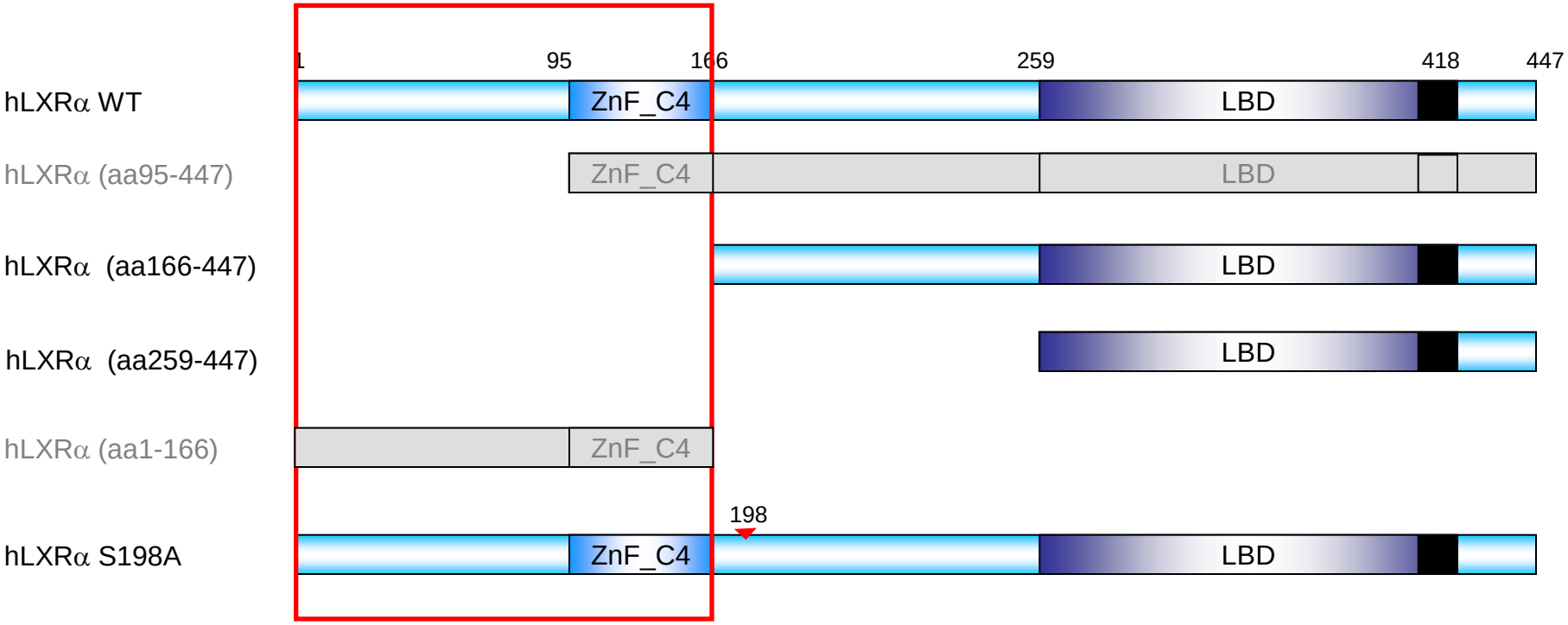
LXRα and LXRβ are Modified by *O*-GlcNAc *In Vitro*

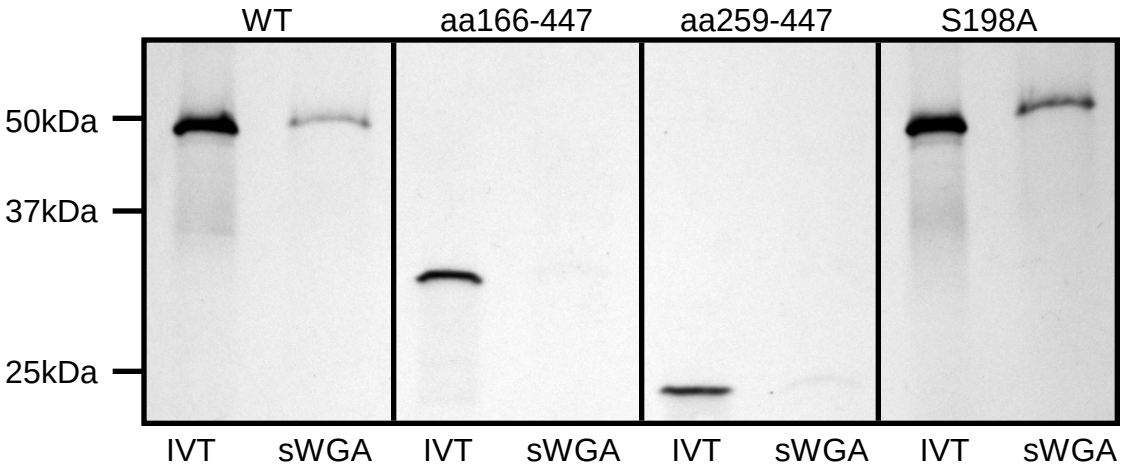


G – 0.5M Galactose
E – 0.5M GlcNAc

Anthonisen EH et al, JBC, 2010

In Vitro Glycosylation-Assay, LXRα

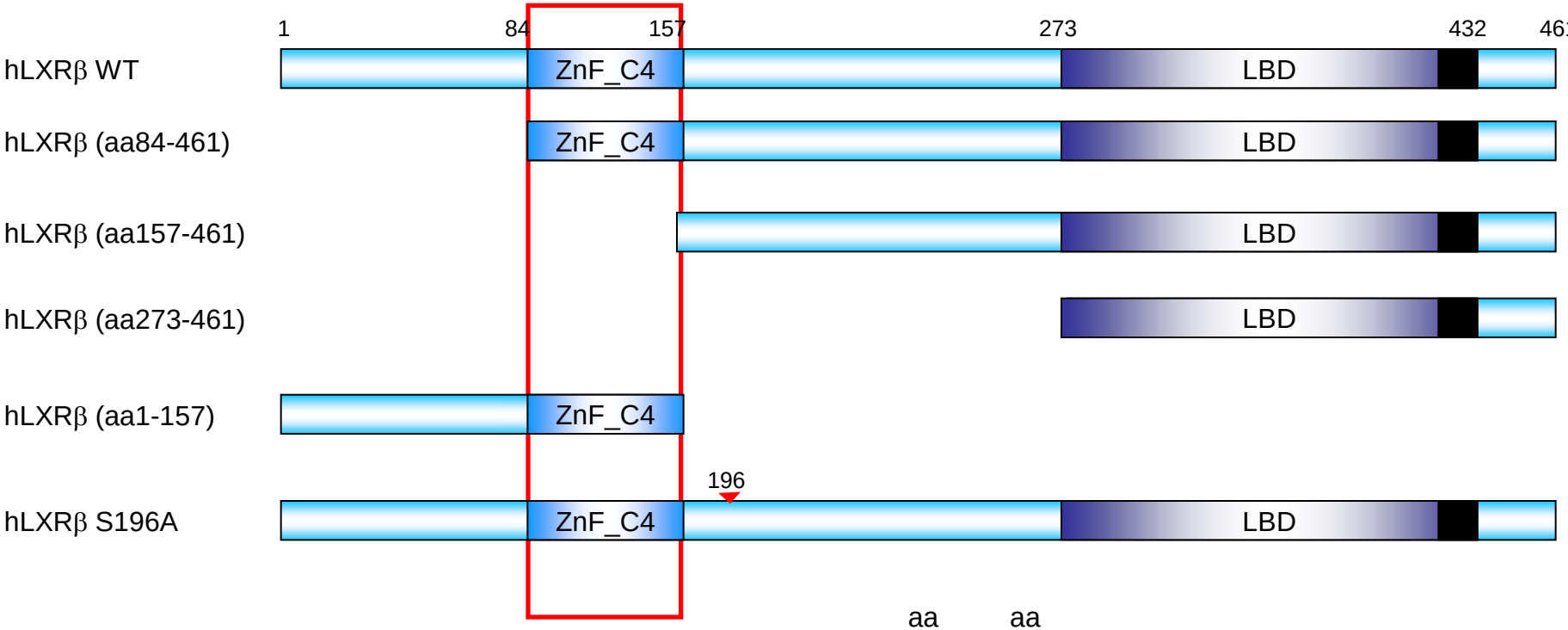


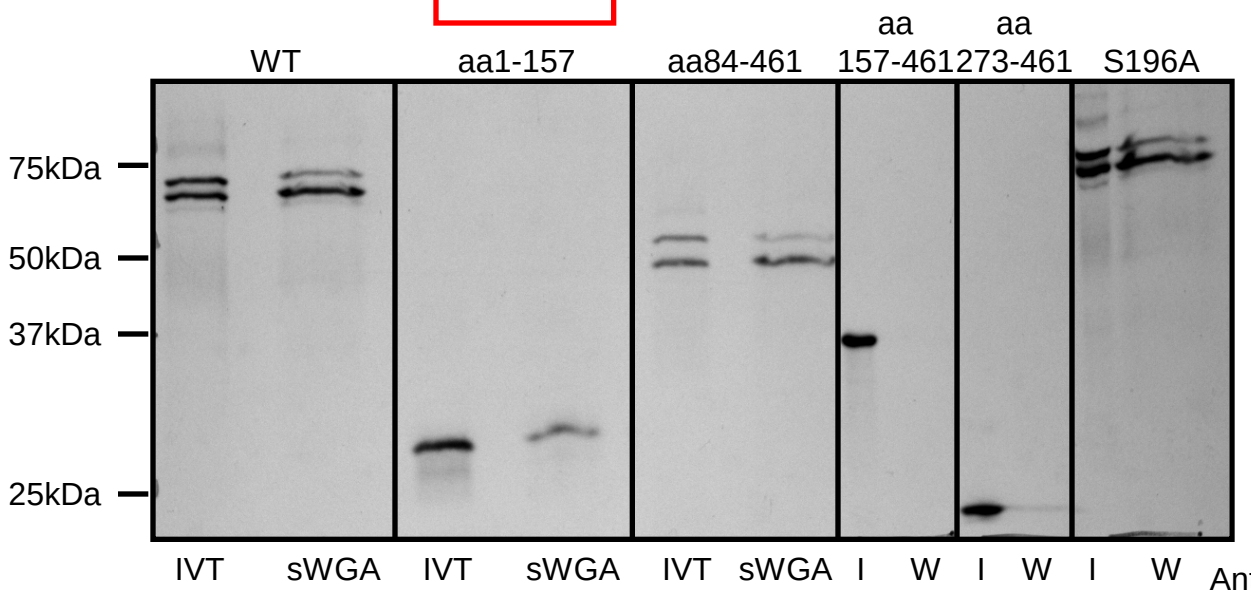


Not tested

Anthonisen EH et al, JBC, 2010

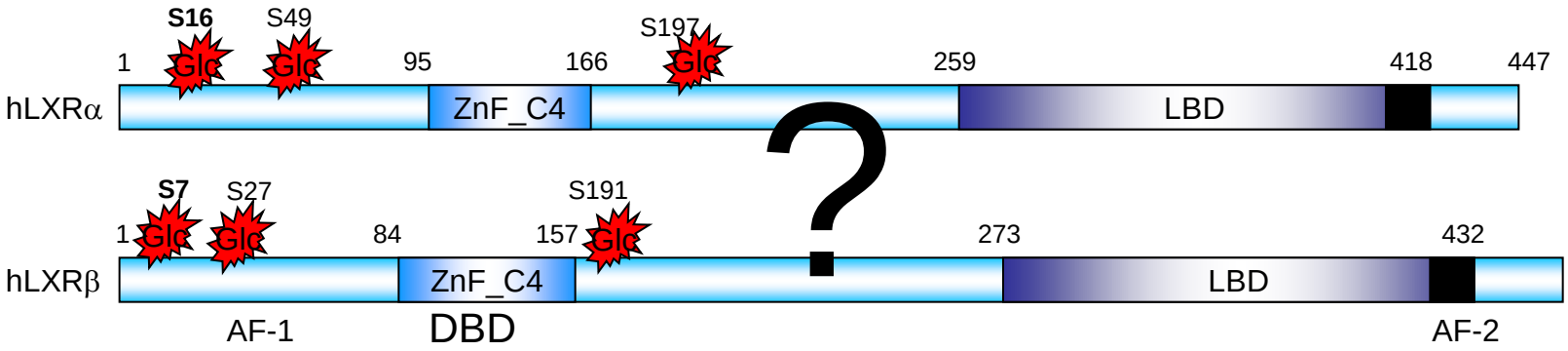
In Vitro Glycosylation-Assay, LXRβ





Anthonisen EH et al, JBC, 2010

Mapping of LXR O-GlcNAcylation sites by MS



Glc

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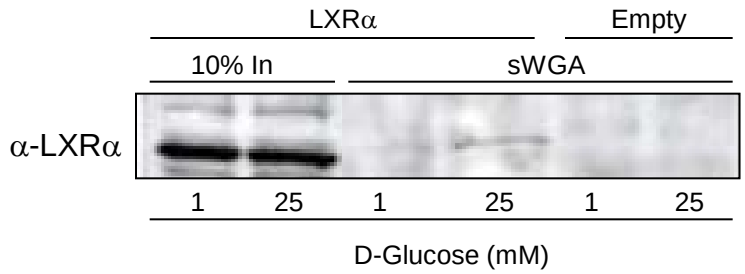
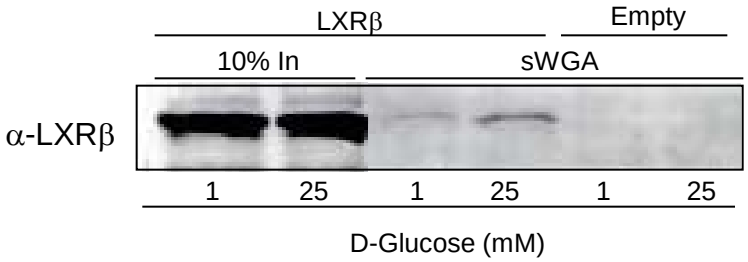
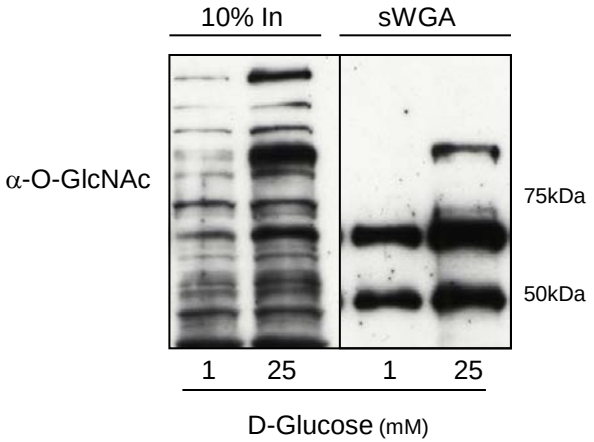
S/T relative to proline at -3/-2

O-GlcNAc modification of LXR is regulated by glucose in Huh7 cells

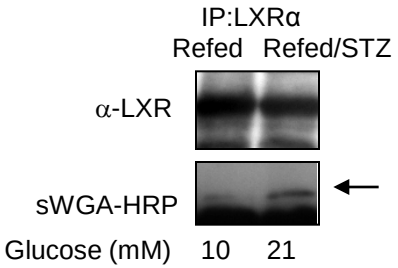


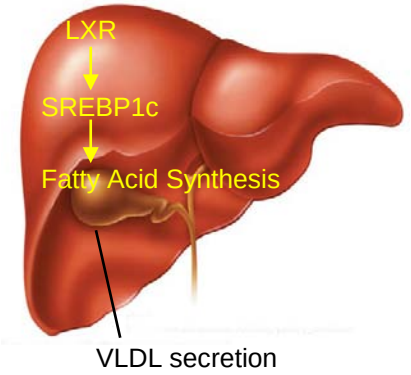


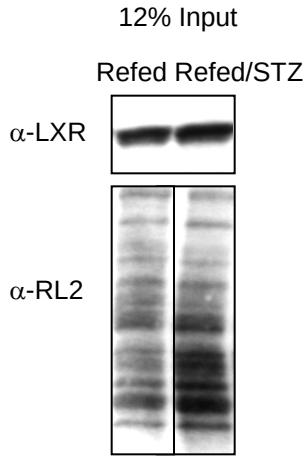


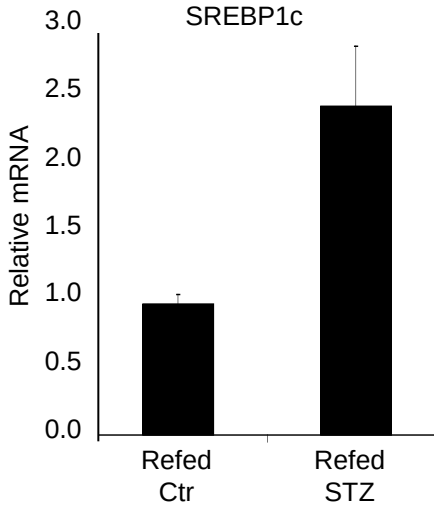
Anthonisen EH et al, JBC, 2010

In vivo O-GlcNAc modification of mLXRα is increased in STZ treated diabetic mice



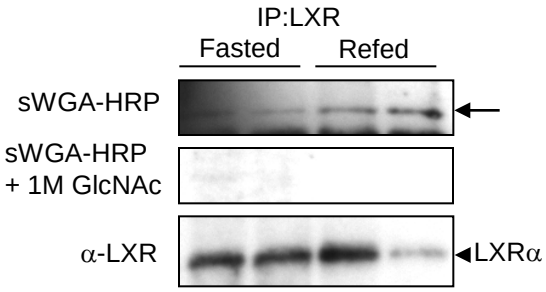




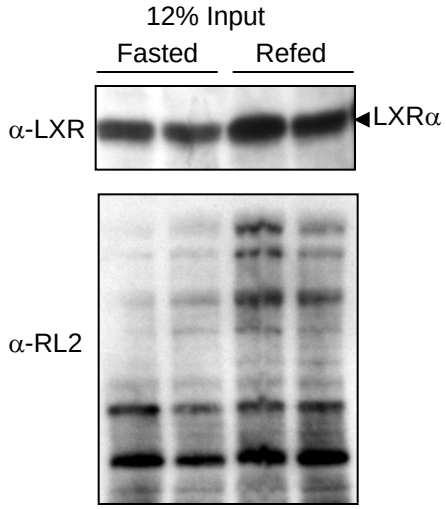


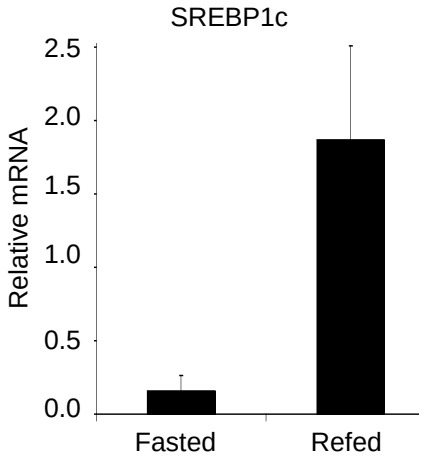
Anthonisen EH et al, JBC, 2010

In vivo O-GlcNAc modification of mLXRα is induced during fasting – refeeding



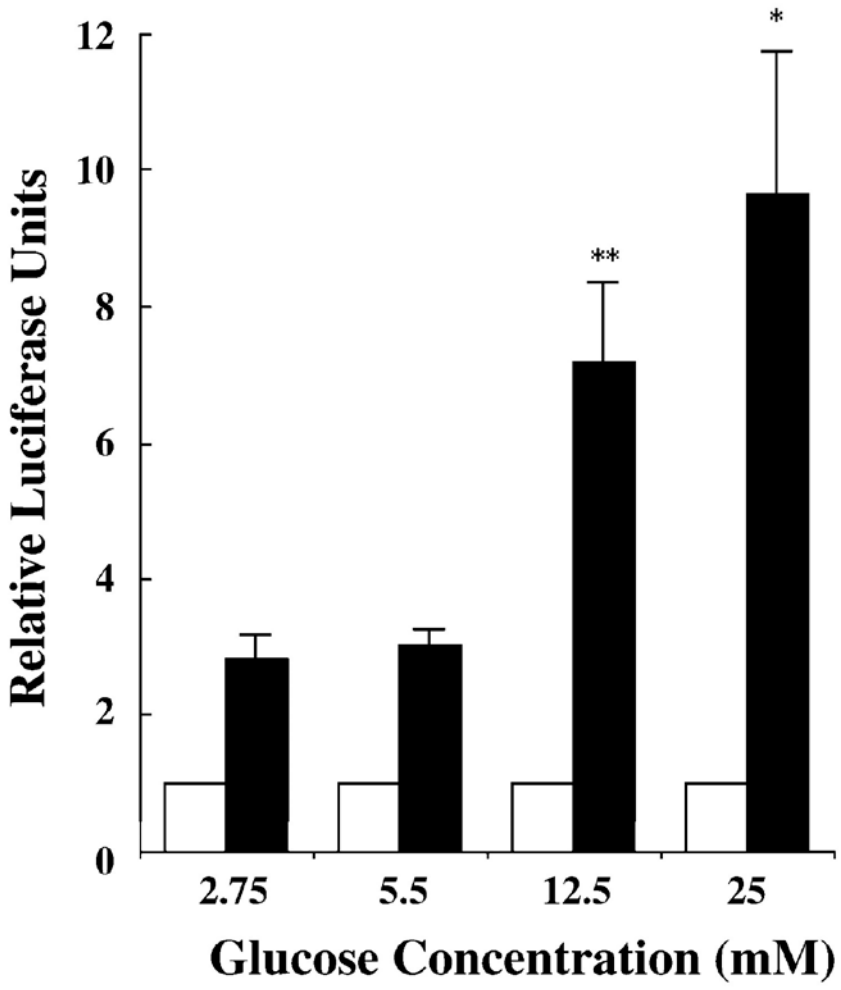
	Glucose (mM)	
Fasted	4,5	4,8
Refed	9,9	11,2

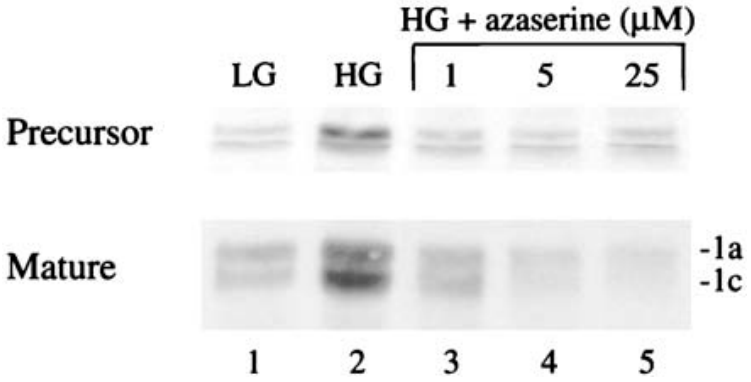


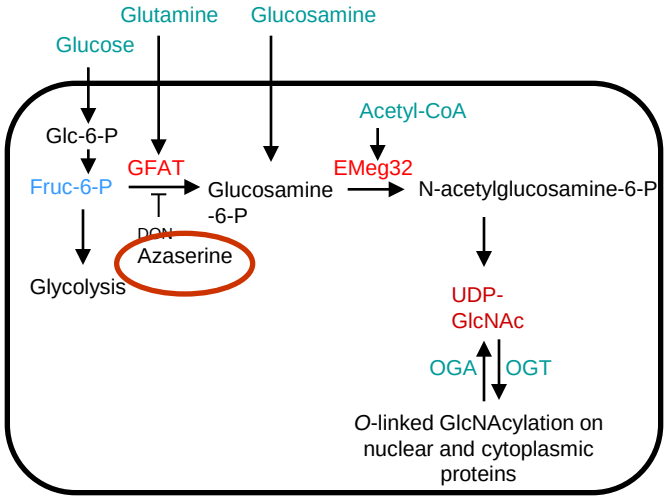


Anthonisen EH et al, JBC, 2010

Glucose effects on SREBP-1c expression

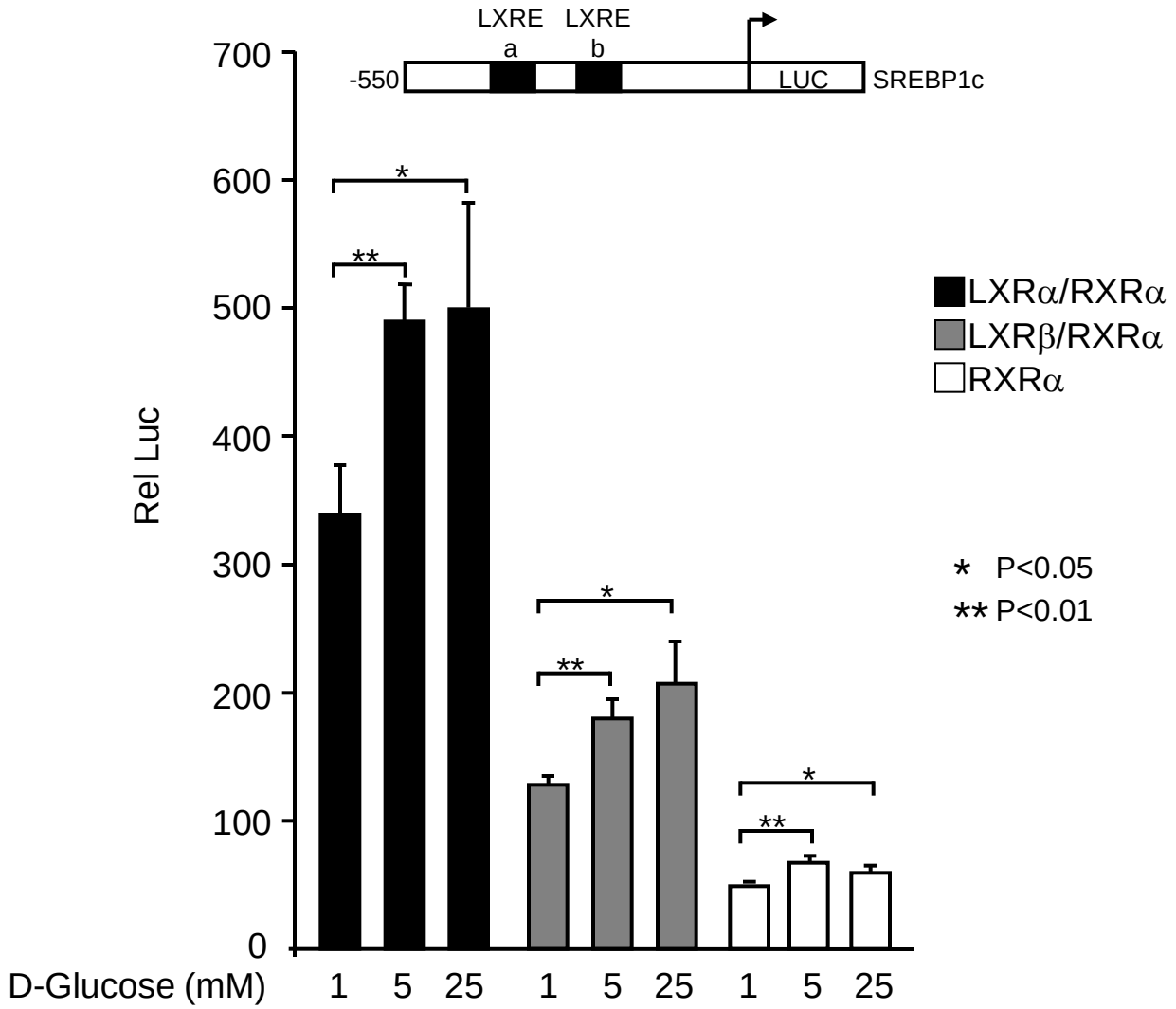






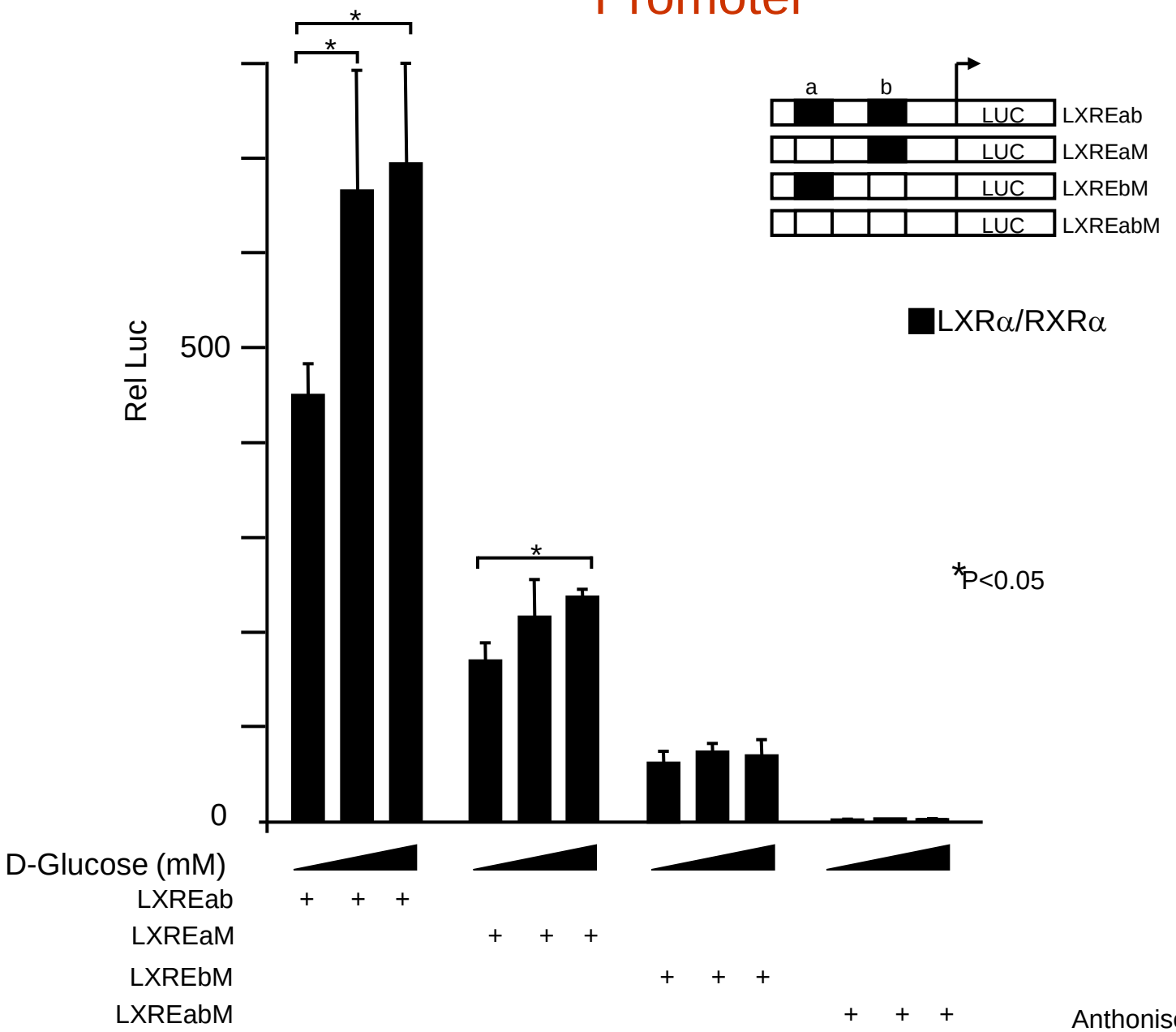
Hasty, A. H. et al. J. Biol. Chem. 2000;275:31069-31077

O-GlcNAc Regulates LXR Transactivation of the SREBP-1c Promoter



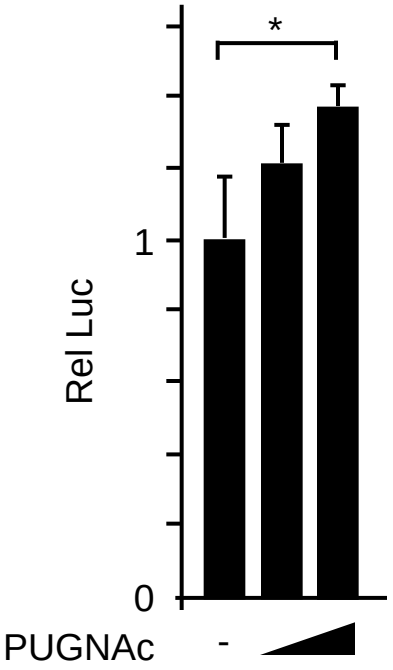
Anthonisen EH et al, JBC, 2010

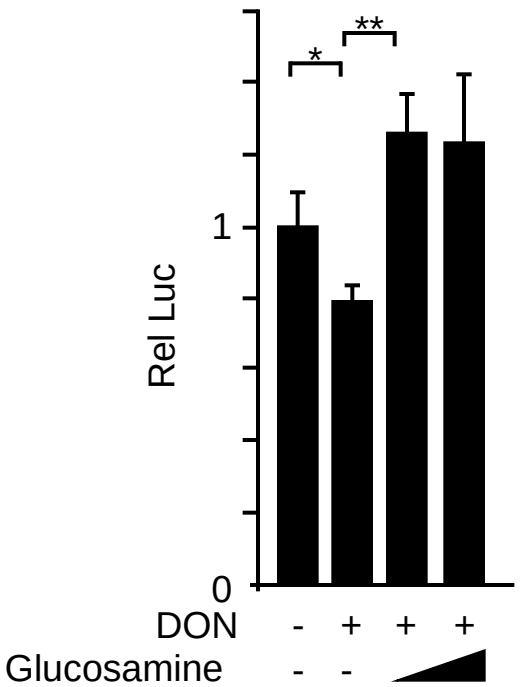
O-GlcNAc Regulates LXRα Transactivation of the SREBP-1c Promoter

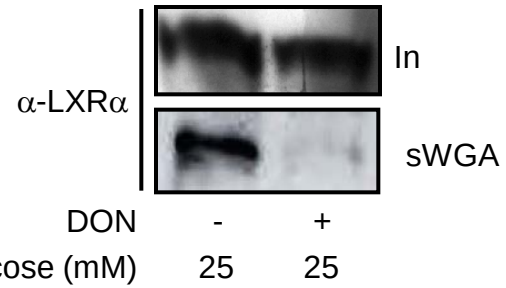


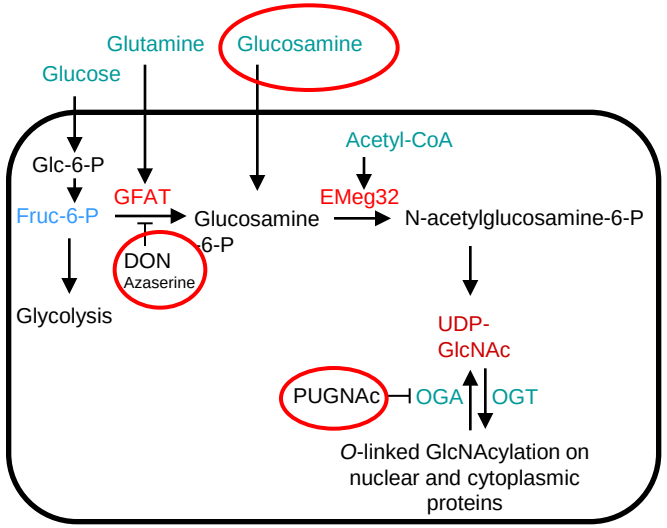
Anthonisen EH et al, JBC, 2010

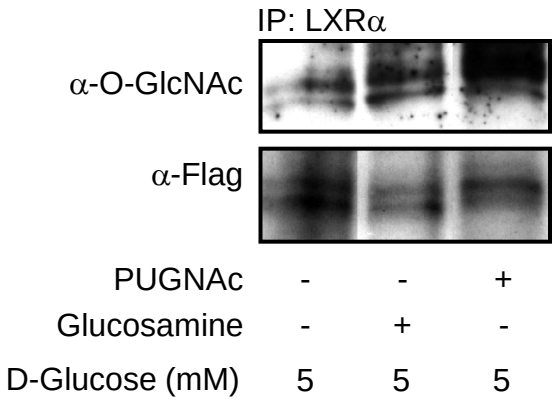
O-GlcNAc Regulates LXRα Transactivation of the SREBP-1c Promoter









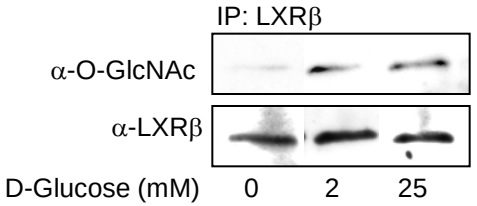


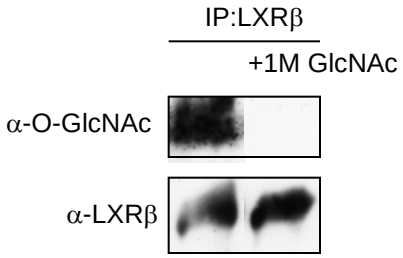
* P<0.05

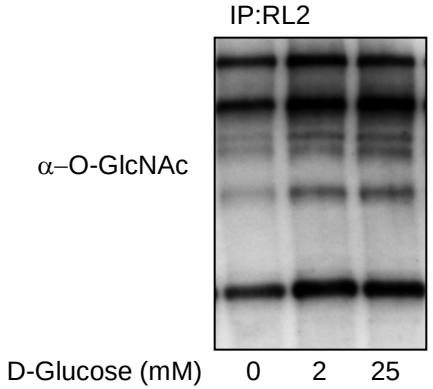
** P<0.01

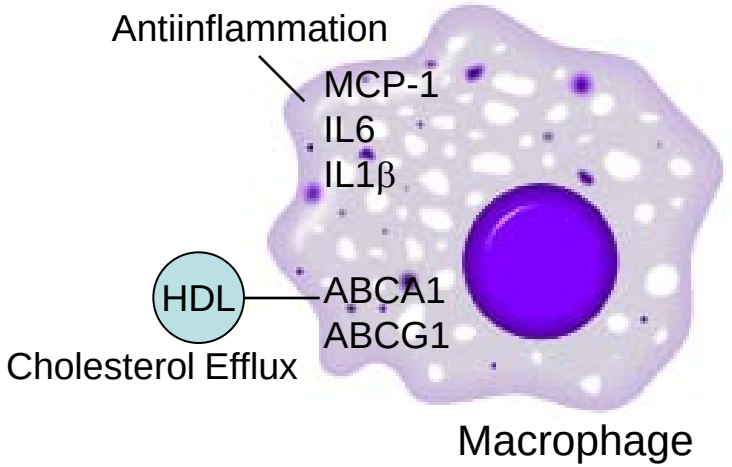
Anthonisen EH et al, JBC, 2010

O-GlcNAc modification of LXRβ is regulated by glucose in human THP-1 macrophages

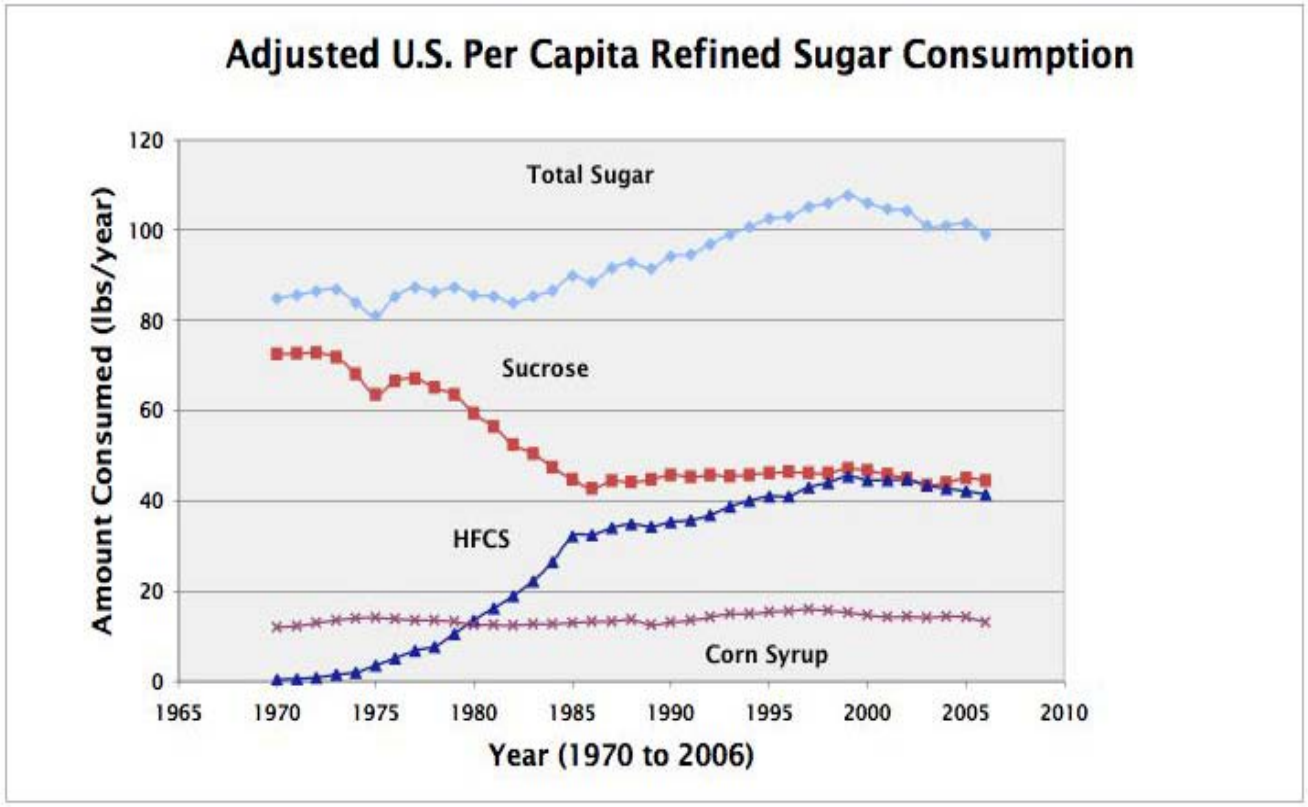




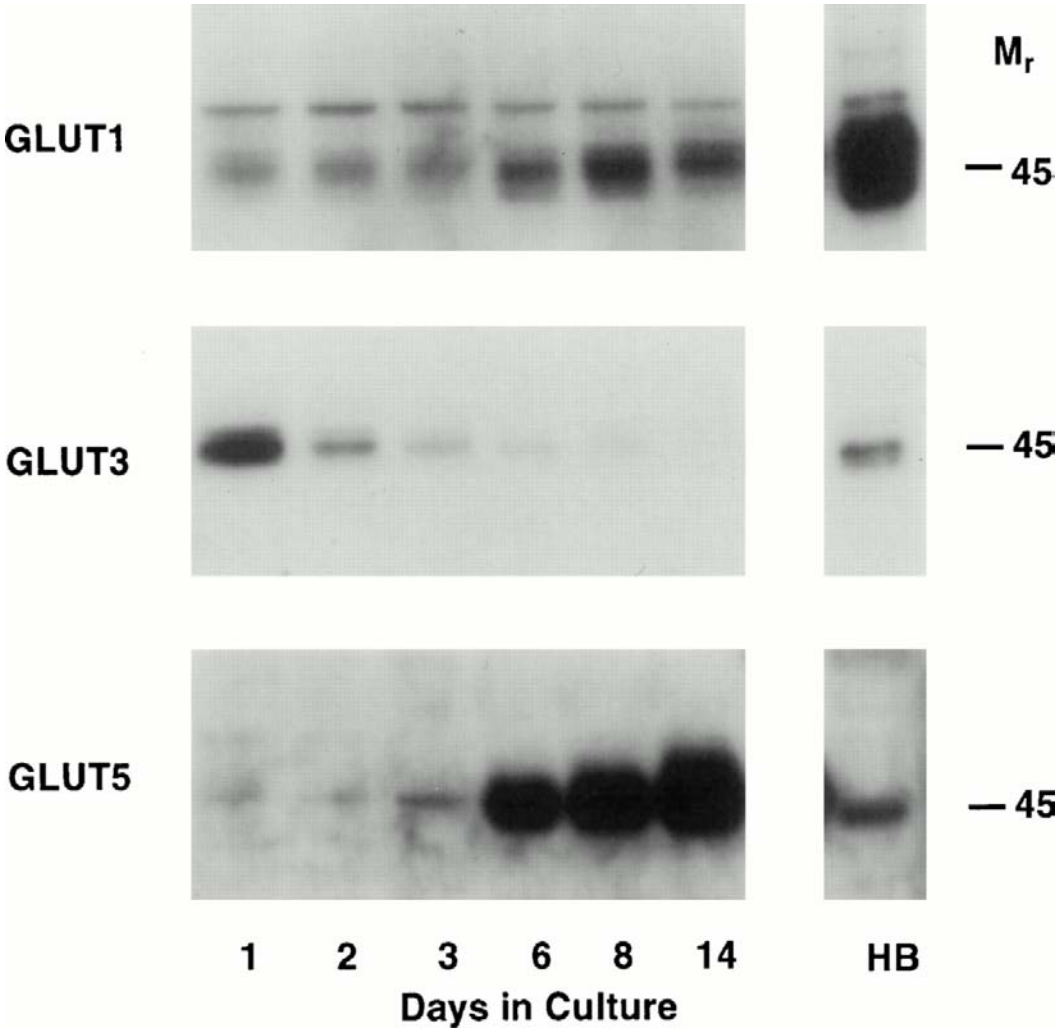




FRUCTOSE CONSUMPTION

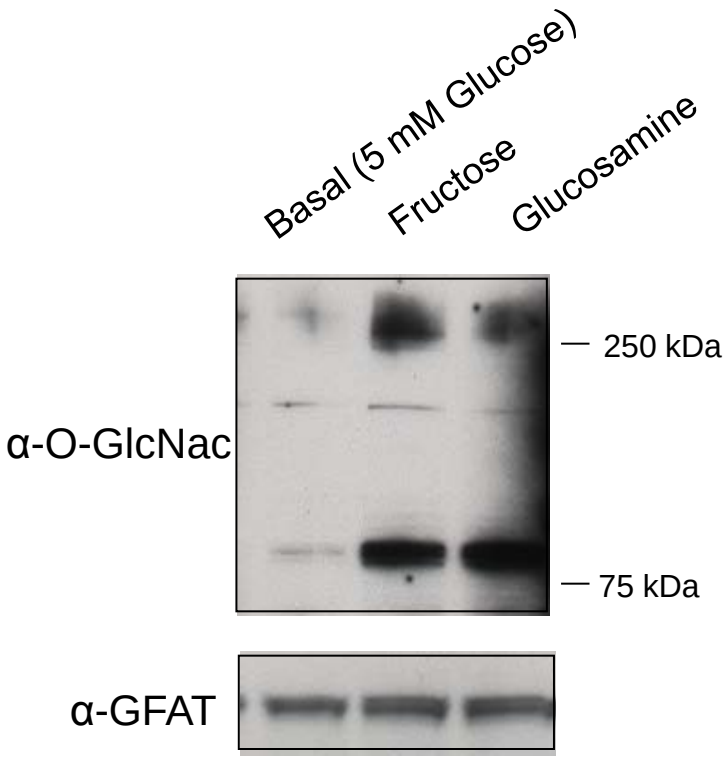


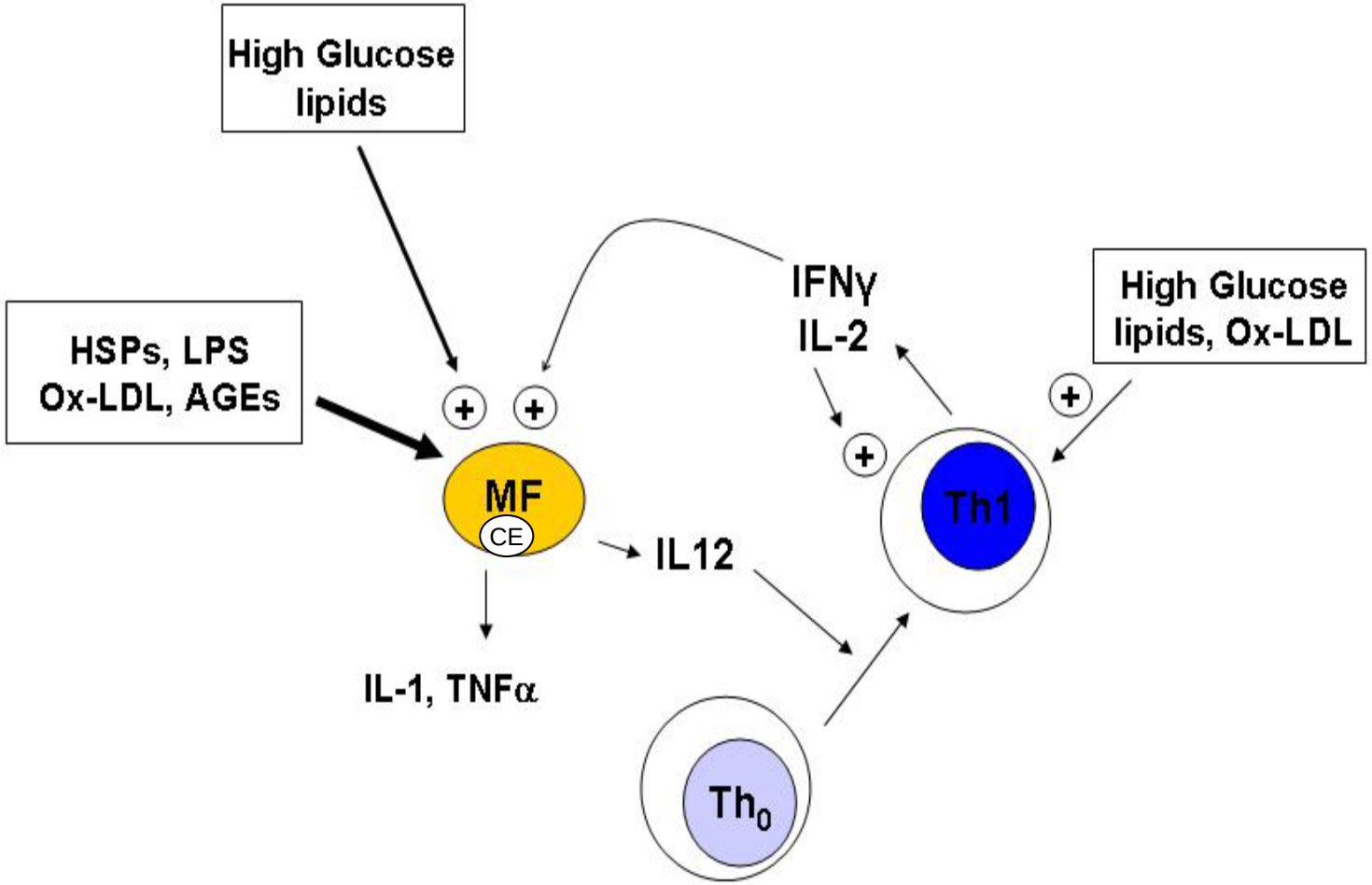
Human macrophages, but not circulating monocytes, express Fructose transporter, Glut5



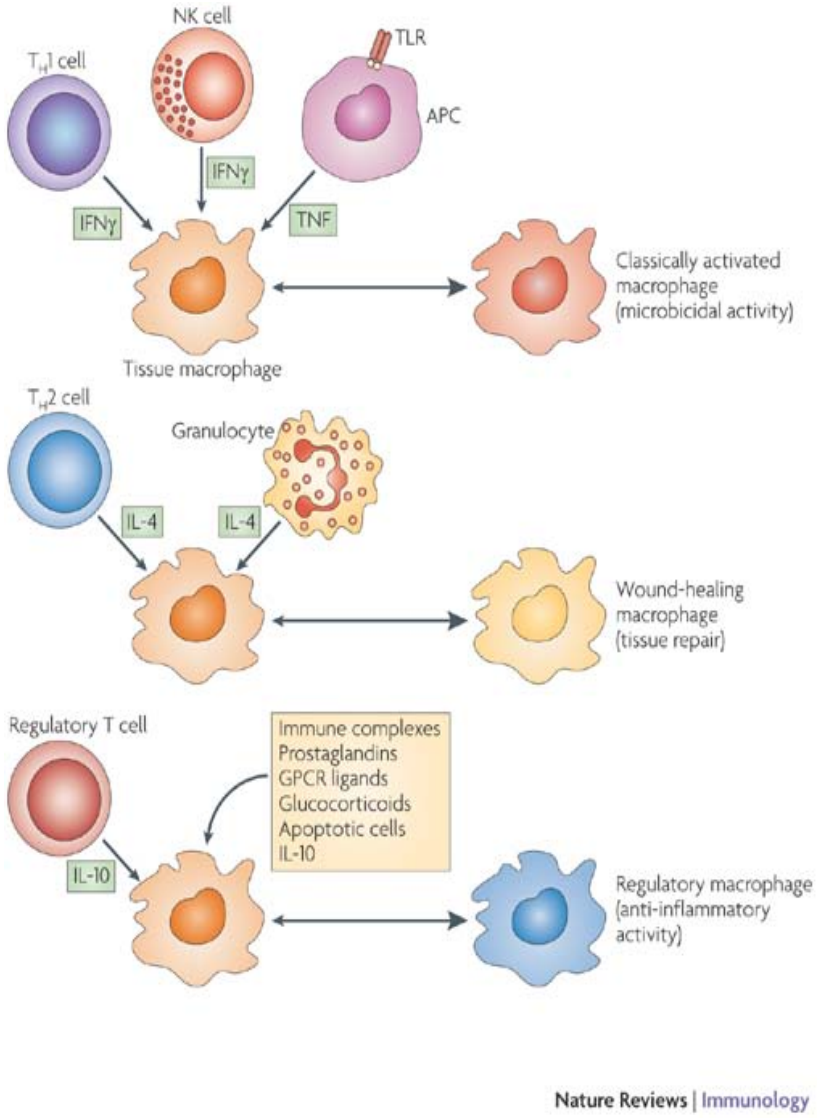
Malide, D. et al. Am J Physiol Endocrin Metab 274, 1998

Fructose increases protein O-GlcNAcylation in primary human blood-derived macrophages




High Glucose
lipids
HSPs, LPS
Ox-LDL, AGEs
IFNγ
IL-2
High Glucose
lipids, Ox-LDL
MF
CE
Th1
IL12
IL-1, TNFα
Th0

Differentiation and polarization of human monocyte-derived macrophages



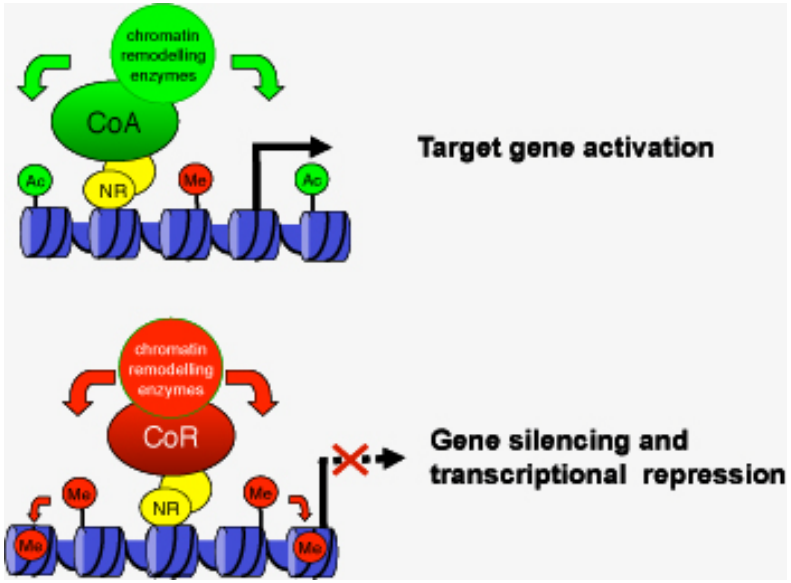
Mosser DM and Edwards JP, Nature Reviews 2008

AIM OF phD PROJECT (MLS)

Elucidate the role of the hexosamine signalling pathway and O-linked glycosylation in macrophages (resting and activated) in relation to sugar (glucose/fructose)-induced macrophage foam cell formation and pro-inflammatory cytokine production in atherosclerosis.

Specific aims:

A1. Elucidate the role of O-GlcNAcylated LXR in sugar-induced lipid metabolism (storage and transport) and pro-inflammatory cytokine production



A2. Identification of novel, sugar-induced O-GlcNAc modified proteins by MS analysis

Further Reading

- **Butkinaree C et al, Biochim et Biophys Acta, 2010:**

O-linked beta-N-acetylglucosamine (O-GlcNAc): Extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress

- **Anthonisen EH et al, JBC, 2010:**

Nuclear receptor liver X receptor is O-GlcNAc-modified in response to glucose