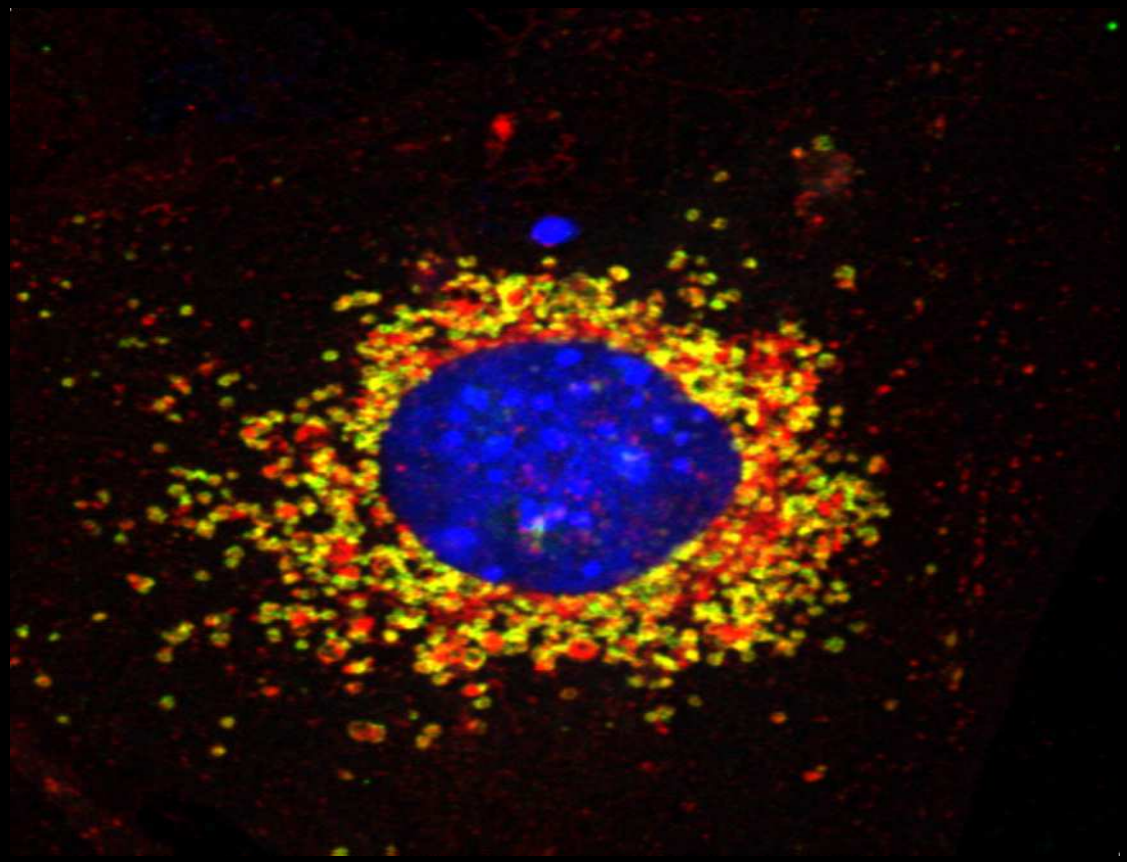
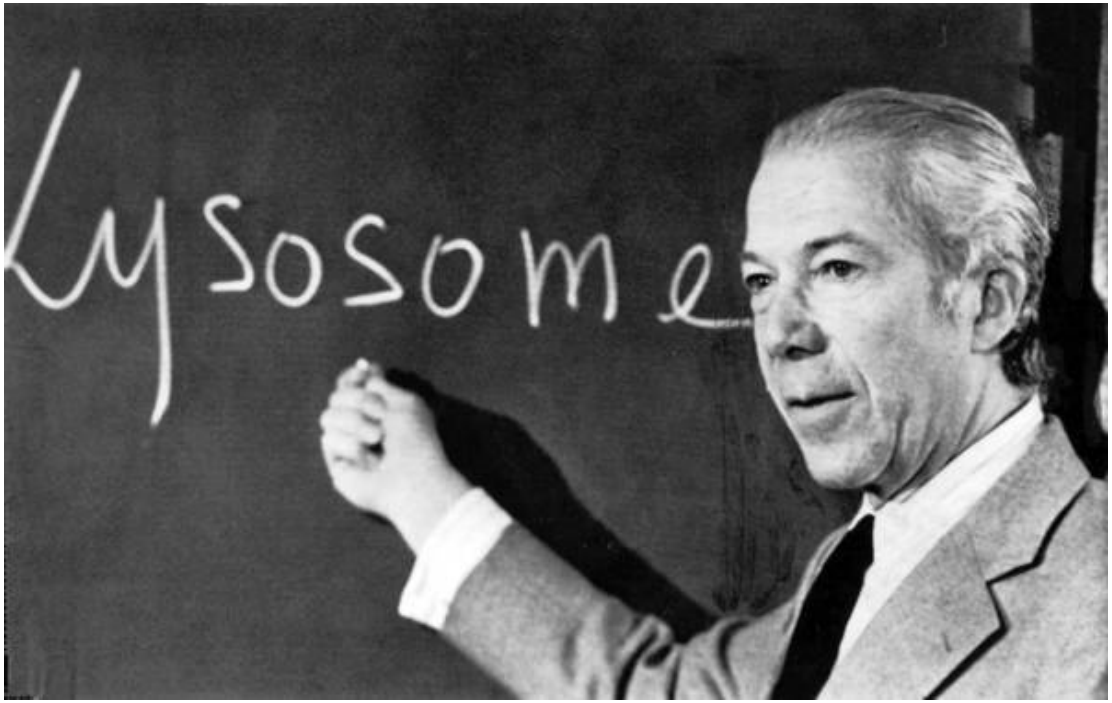
Biosynthesis of lysosomal enzymes



Thomas Braulke

Children‘s Hospital-Biochemistry

University Medical Center Hamburg-Eppendorf



'blue skies' research

1949: **Christian de Duve**, University of Louvain

Insulin action on liver cells:

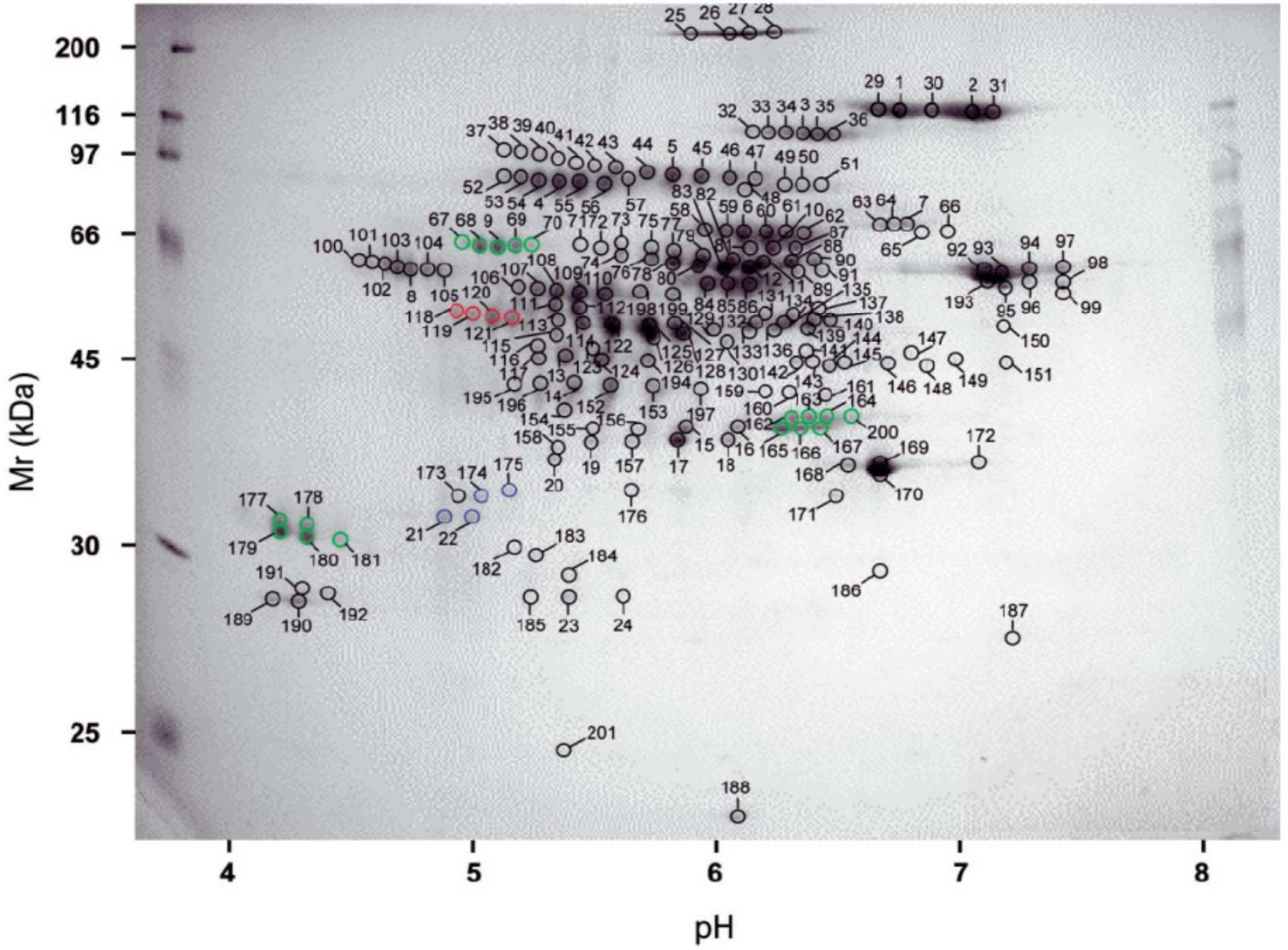
Localization of glucose 6-phosphatase /acid phosphatase

Differential centrifugation

1955: hydrolases in saclike structures- lysosomes

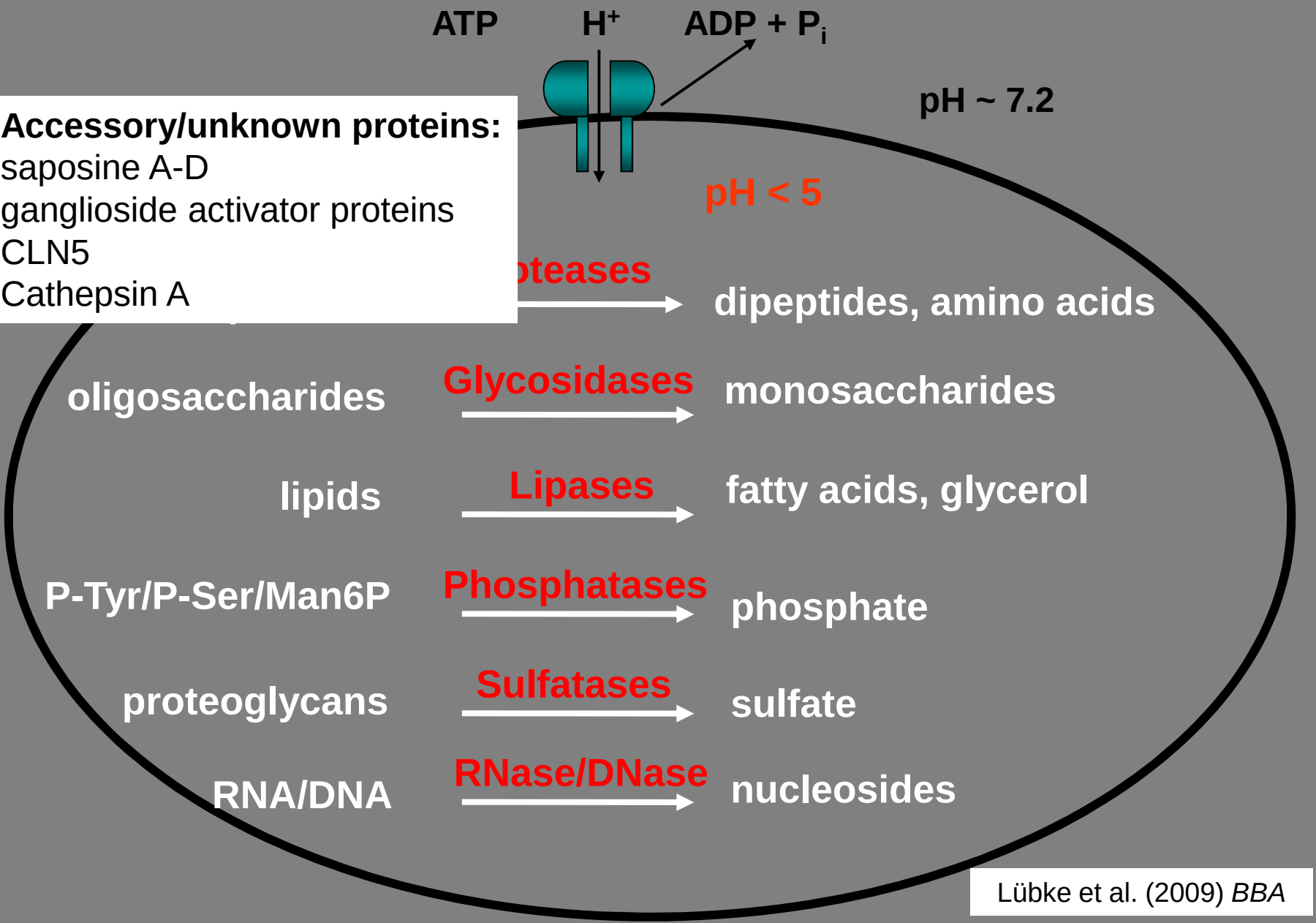
1974: Nobel prize

~70 known soluble lysosomal proteins/enzymes



+ 40 proteins not currently thought to be lysosomal

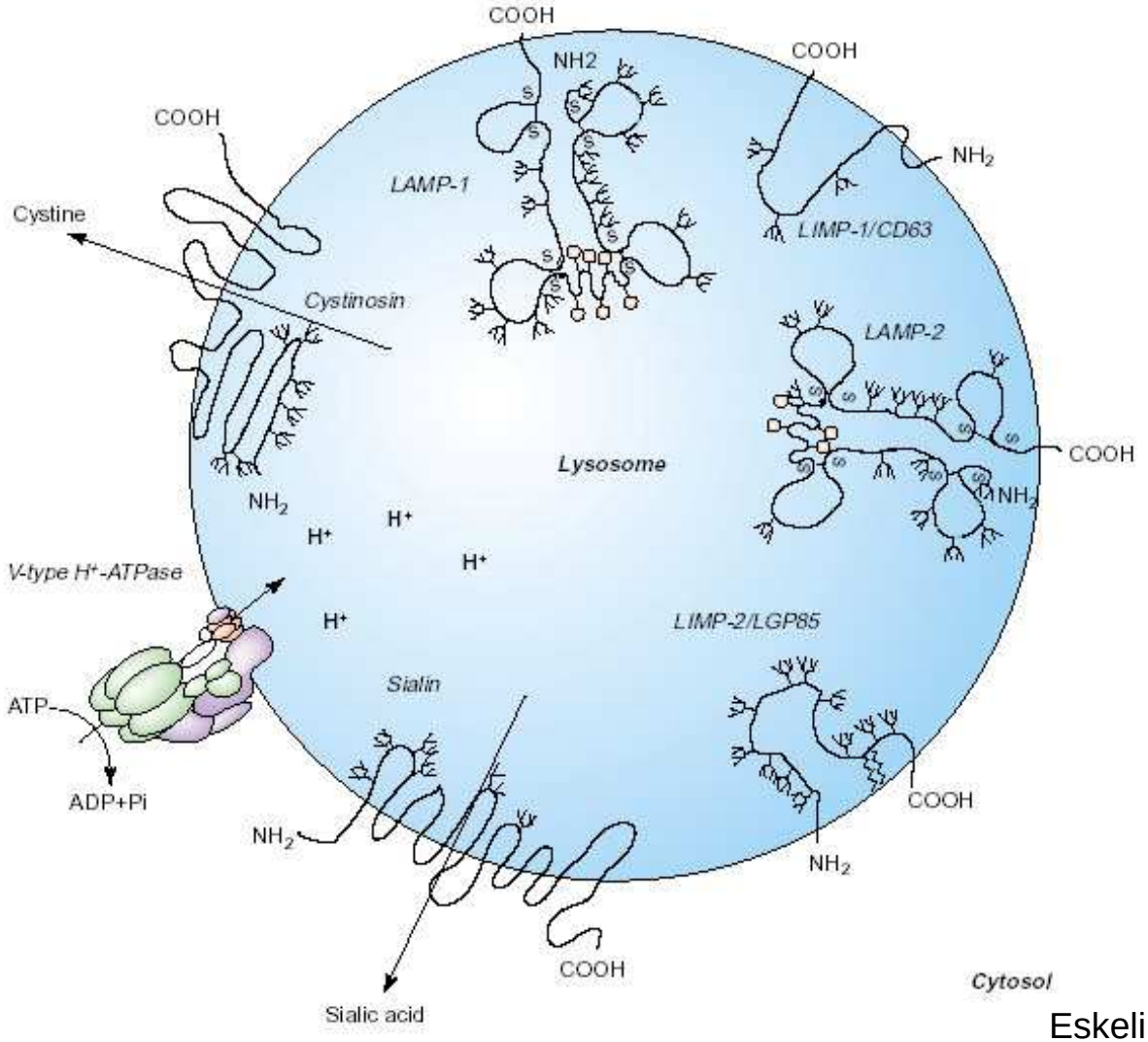
Degradative functions of lysosomes



Accessory/unknown proteins:
saposine A-D
ganglioside activator proteins
CLN5
Cathepsin A

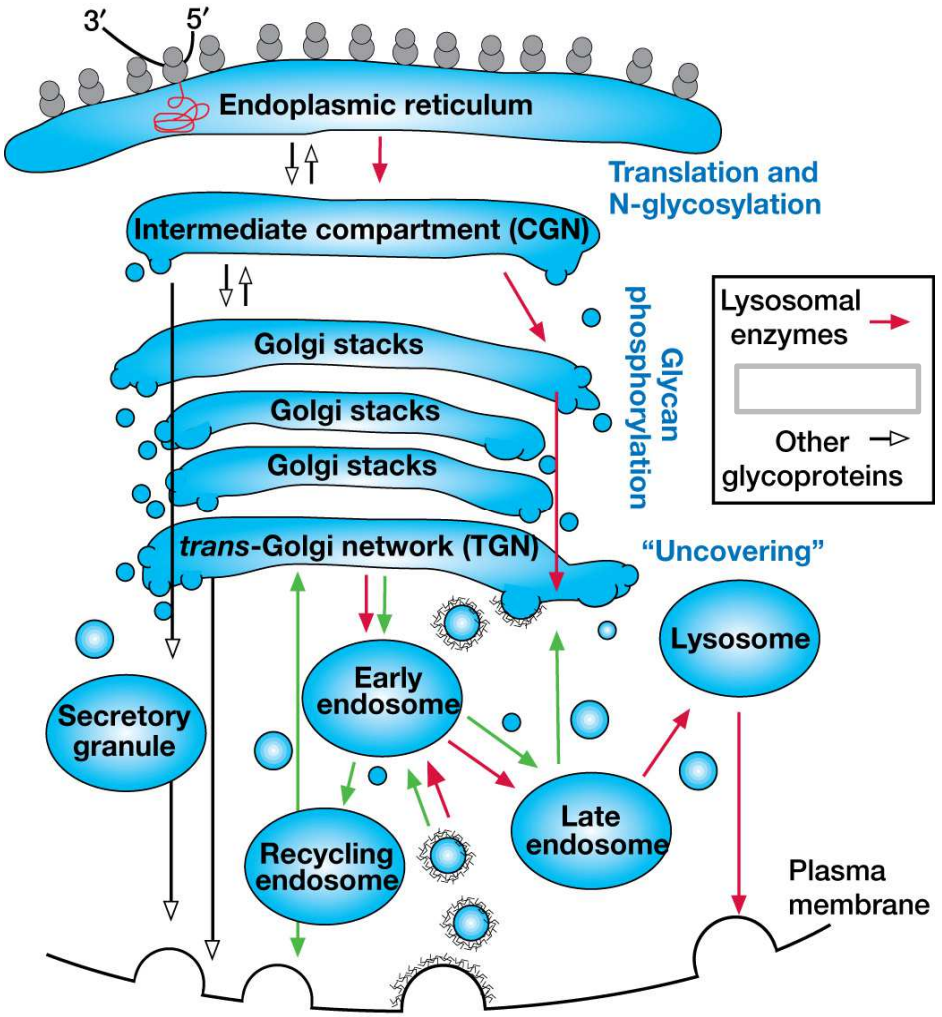
Lübke et al. (2009) *BBA*

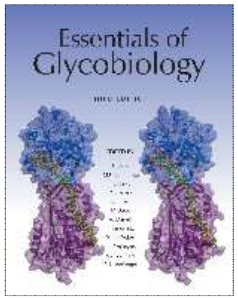
~140-300 lysosomal membrane proteins



Eskelinen et al. (2003)
Trends Cell Biol

Subcellular Trafficking Pathways of Glycoproteins and Lysosomal Enzymes



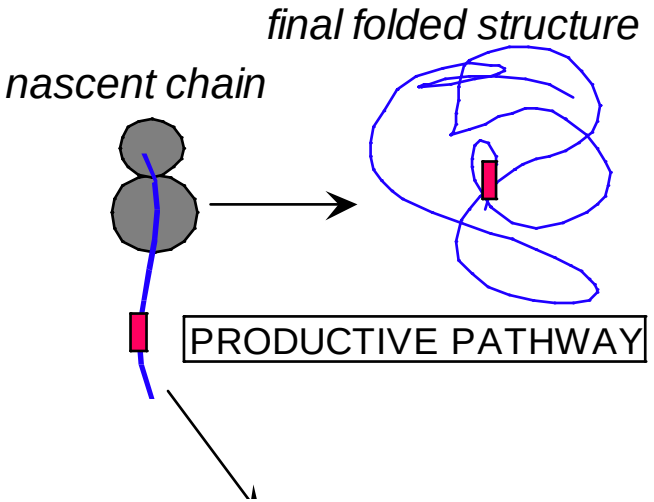


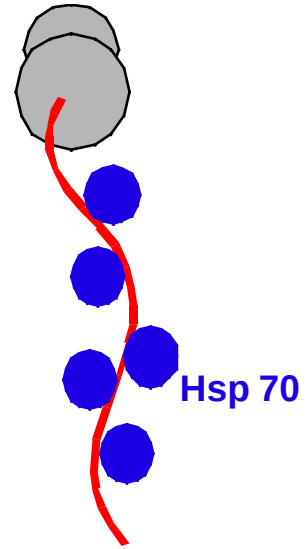
Varki & Kornfeld (2017)

Folding

Most polar residues face the surface whereas hydrophobic residues face the interior of the protein (interaction with solvent or with each other, respectively)

Exposure of hydrophobic regions during synthesis causes aggregation





Hsp 70 stabilizes the nascent chain

- Recogition of unfolded proteins
  - Recognizes exposed hydrophobic patches
  - No binding to specific sequences
  - Bind permanently to misfolded protein
  - Regulated by ATP hydrolysis

Glycosylation

A. Glycoproteins

B. Glycolipids

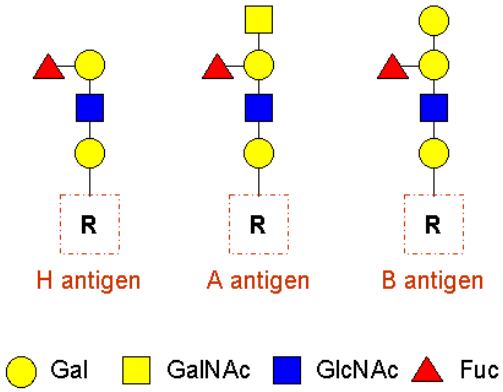
C. Proteoglycans

> 100 glycosyltransferases, glycosidases, transporter

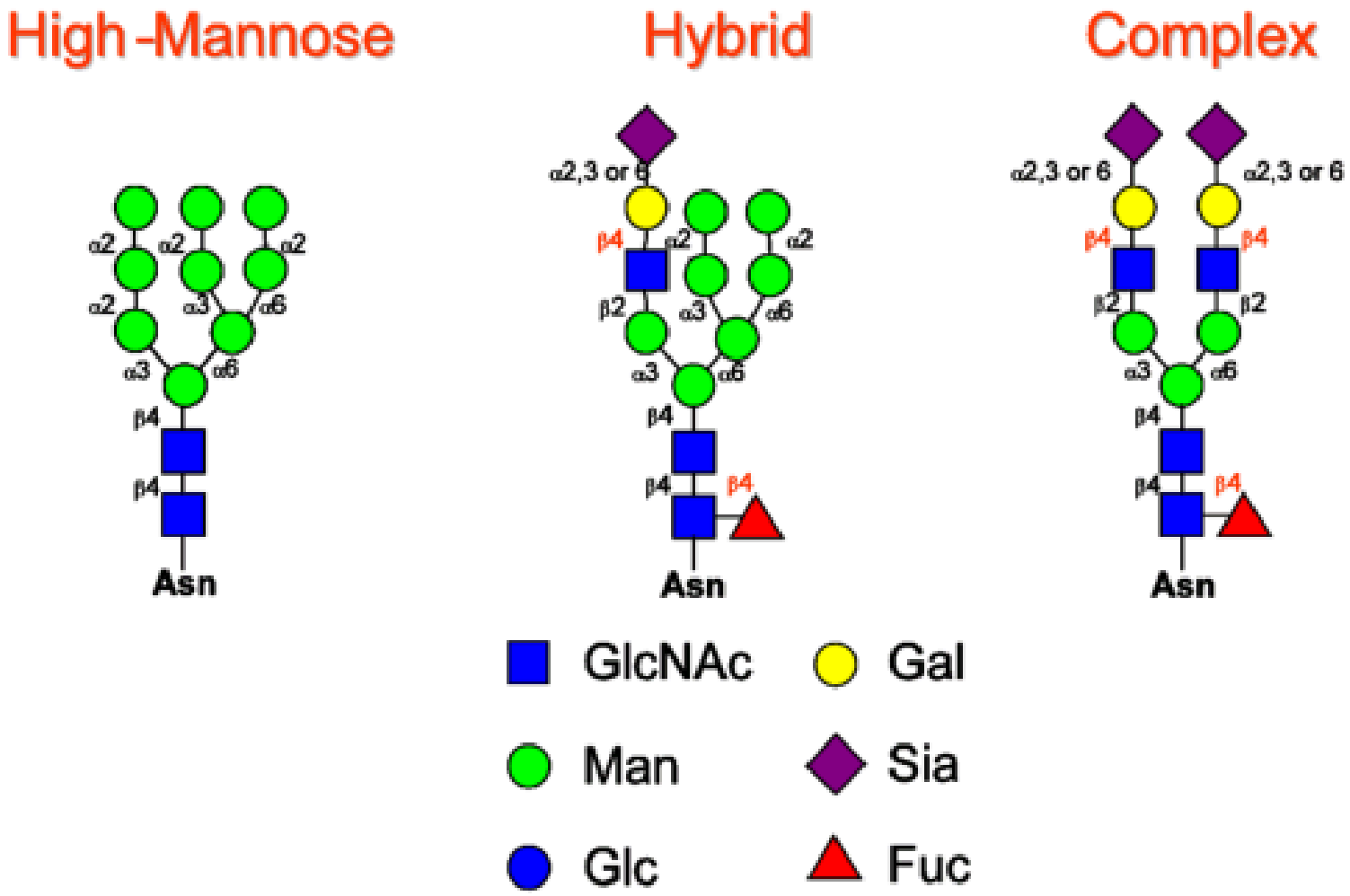
***N*-linked oligosaccharides (Asn)**

glucose, galactose, mannose, fucose, GalNAc, GlcNAc and neuraminic acid

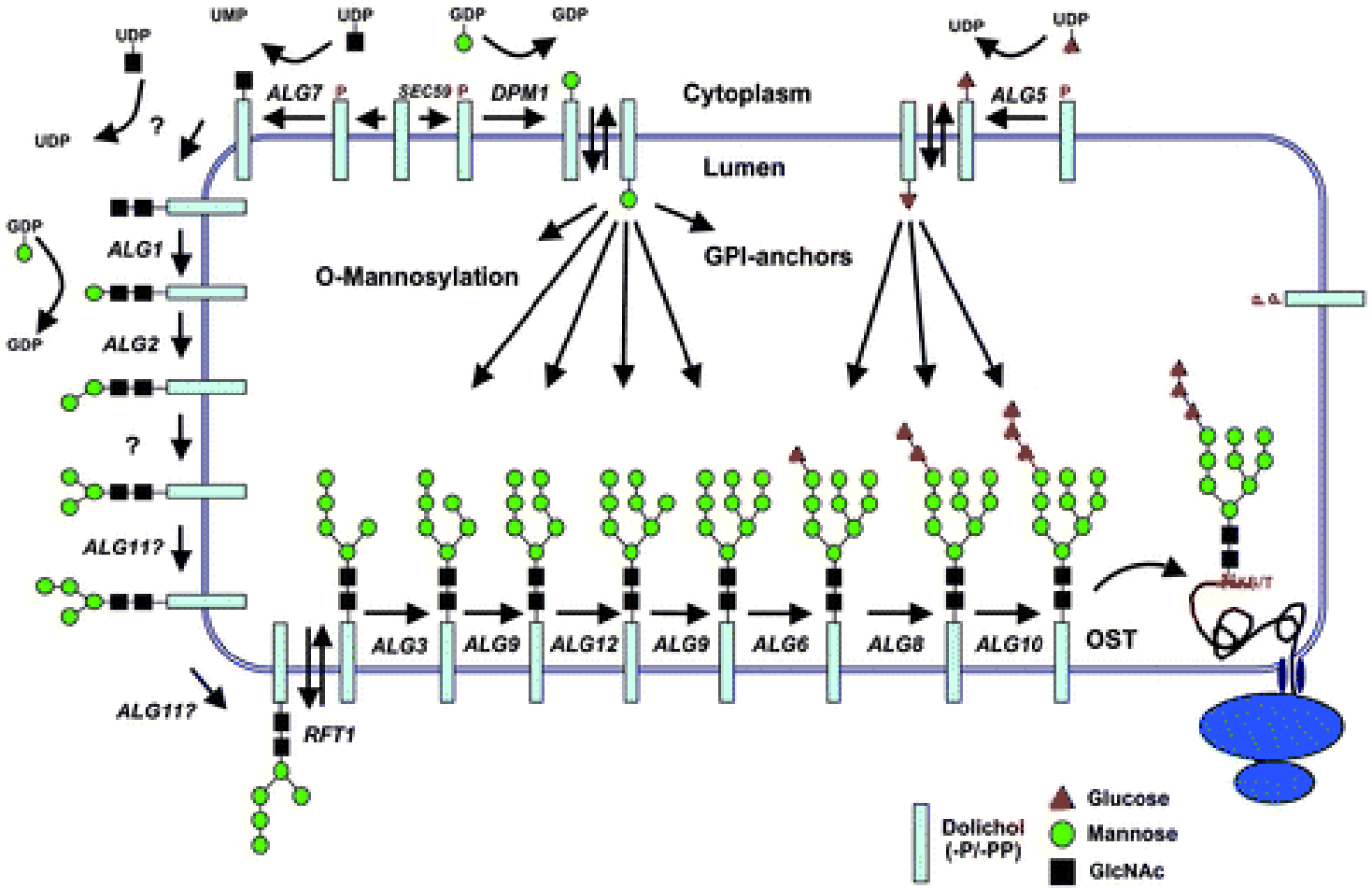
***O*-linked oligosaccharides (Ser/Thr)**



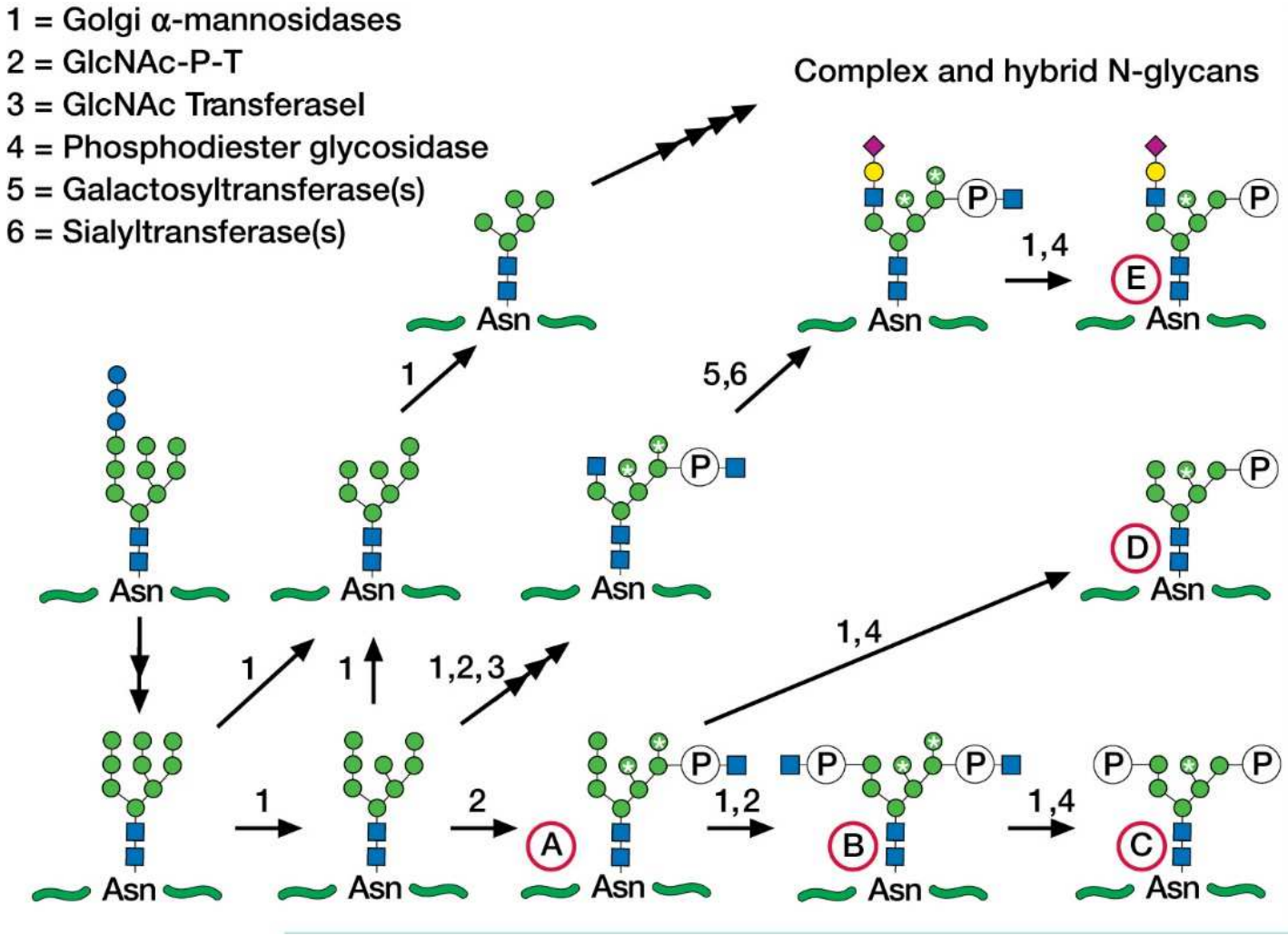
Structures of *N*-linked Oligosaccharides



N-glycosylation (NXT/S)

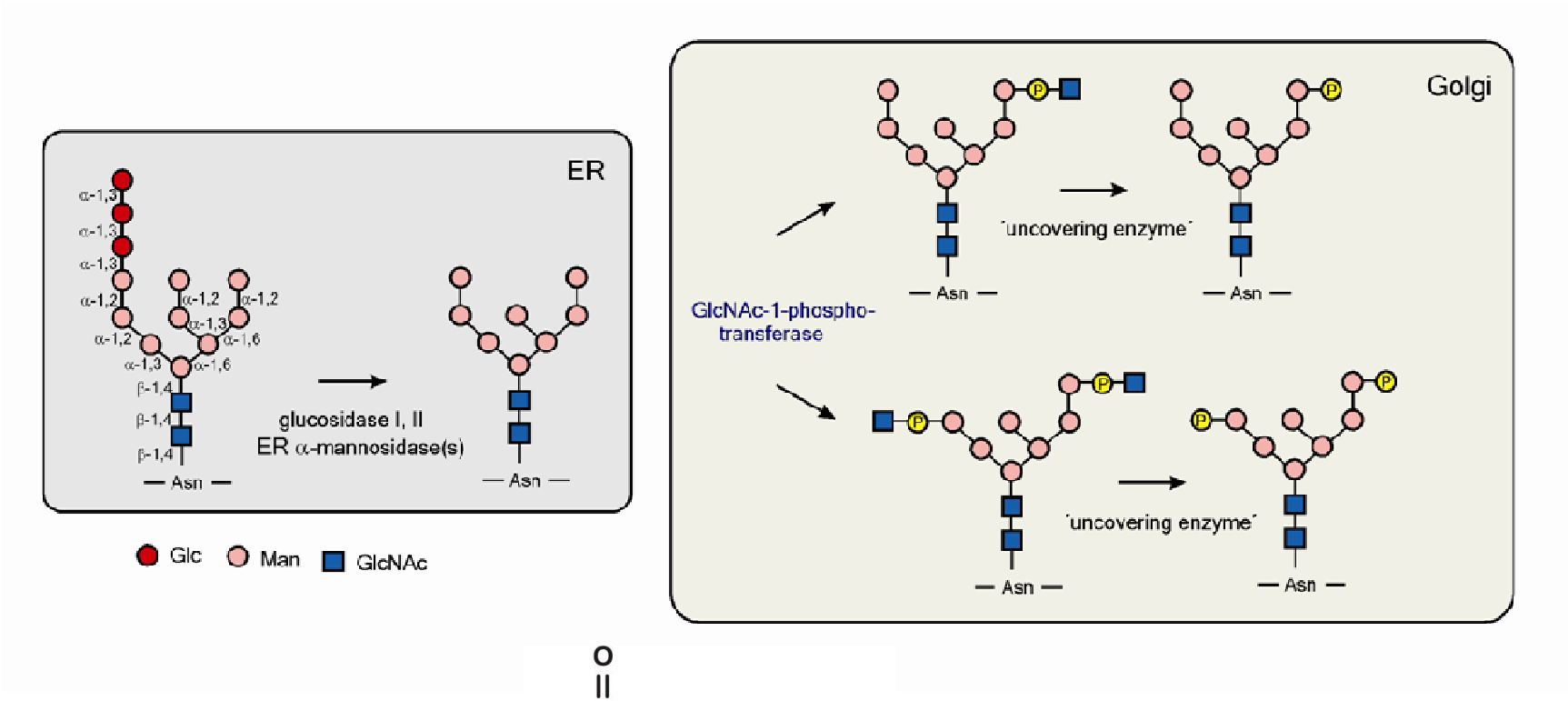


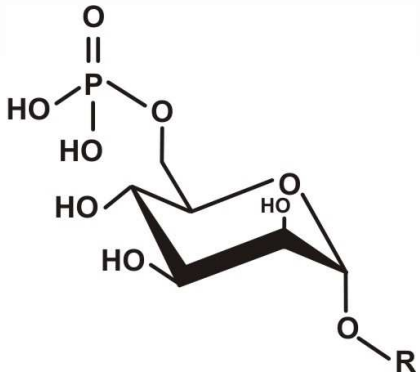
Pathways for Biosynthesis of N-Glycans Bearing the Mannose 6-Phosphate (M6P) Recognition Marker



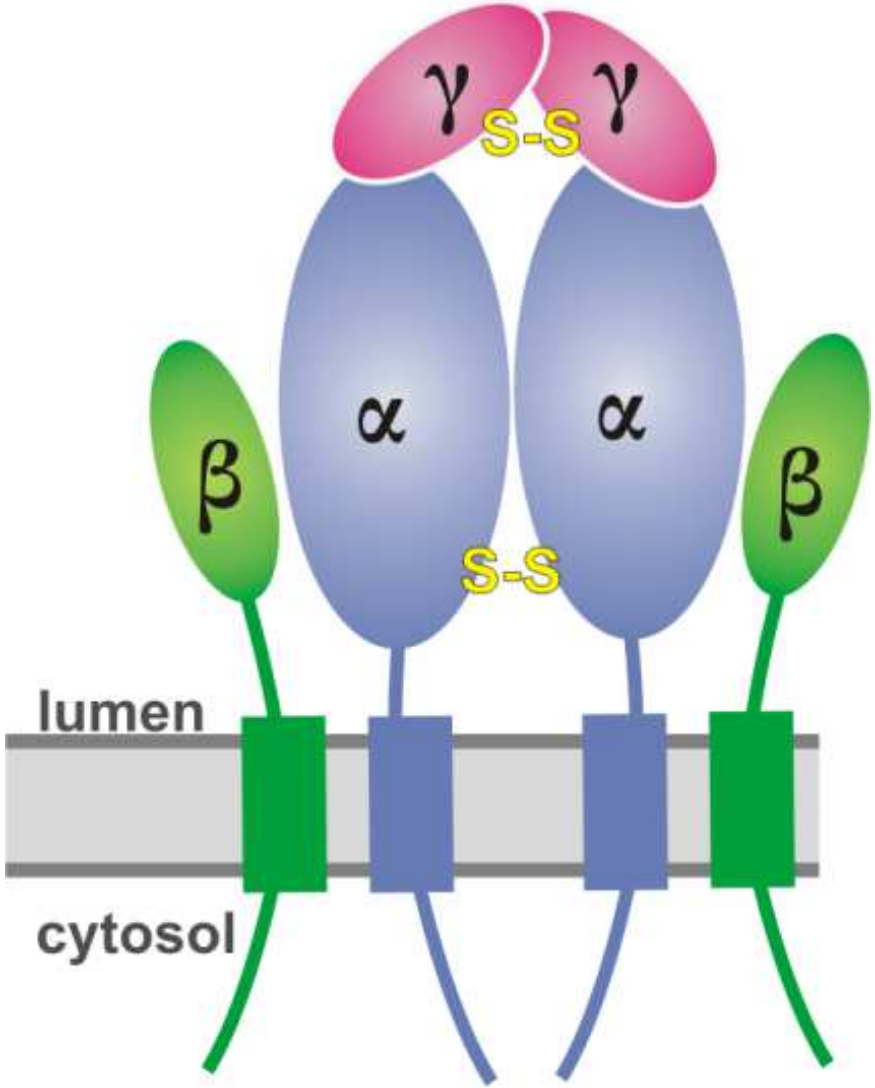
Varki & Kornfeld (2017)

Generation of M6P residues in the Golgi apparatus



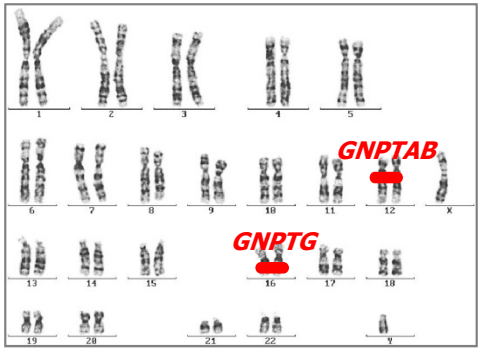


GlcNAc-1-phosphotransferase subunit encoding genes



GNPTG

GNPTAB



Bao et al. 1996; Raas-Rothschild et al. 2000; Tiede et al. 2005; De Pace et al. (2015)

Mucolipidosis II alpha/beta (I-cell disease)

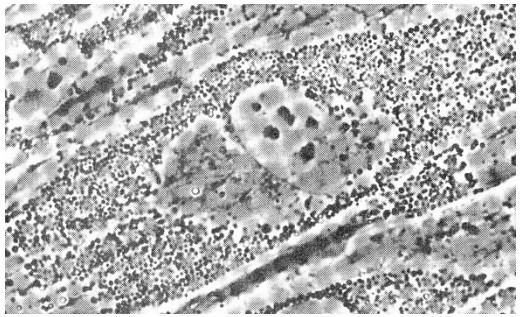
Severe psychomotoric retardation

Clinical symptoms and radiological alterations resemble M. Hurler (MPS I)

Mucopolysaccharidurie is seldom

Fast progressive course

Death between 5-8 yrs



Coarse facies

Growth retardation

restricted joint mobility

Hepatosplenomegalie

Affected heart and lung functions

Mucolipidosis III gamma

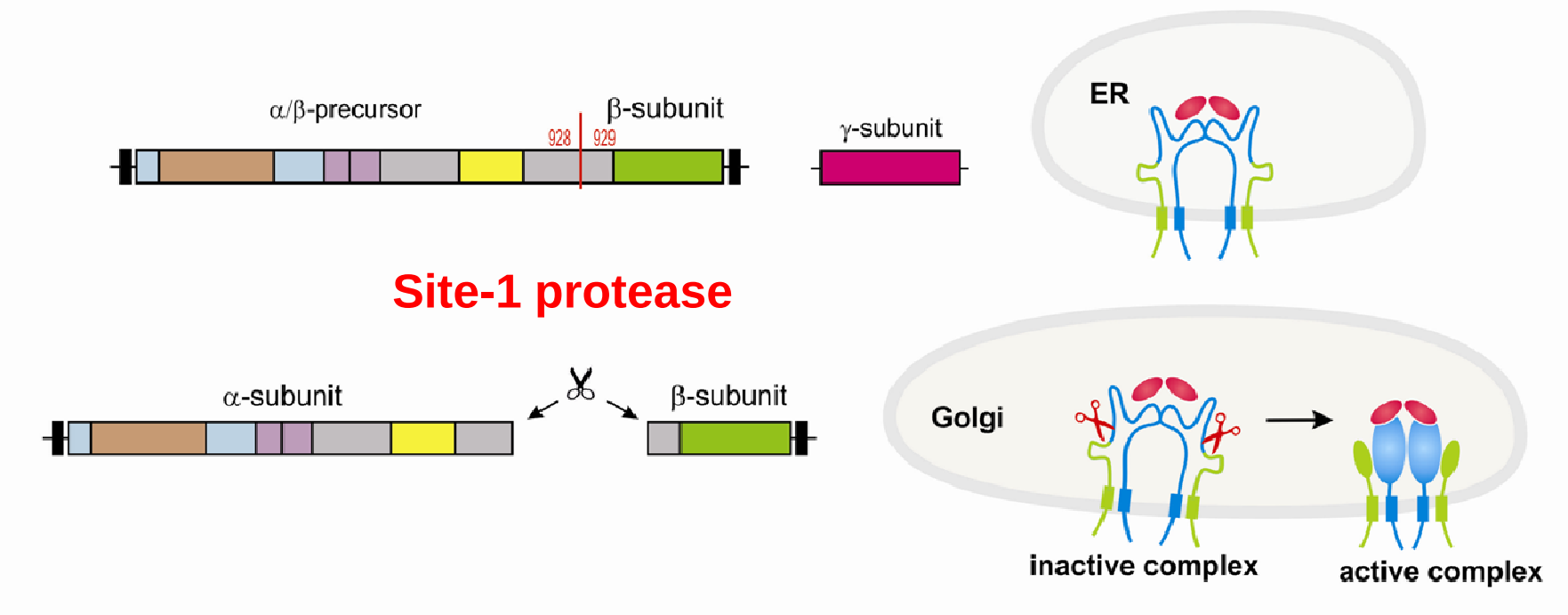
Milder course, later onset (2-4 yrs)

Stiffness of the hands and shoulders

Reduced capabilities to learn in 50 %

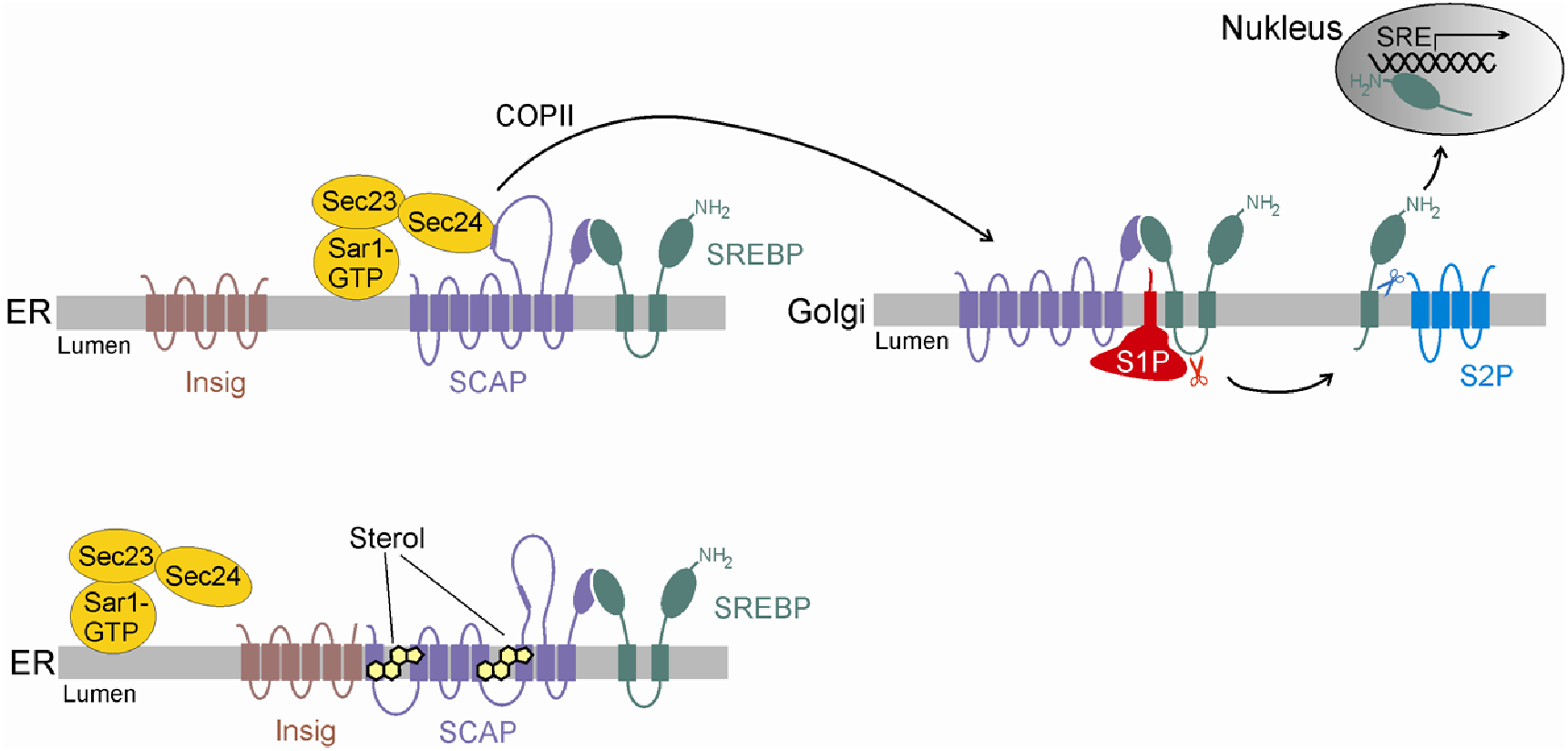
age > 30 yrs (80 yrs)

Assembly and proteolytic activation of GlcNAc-1-phosphotransferase complex

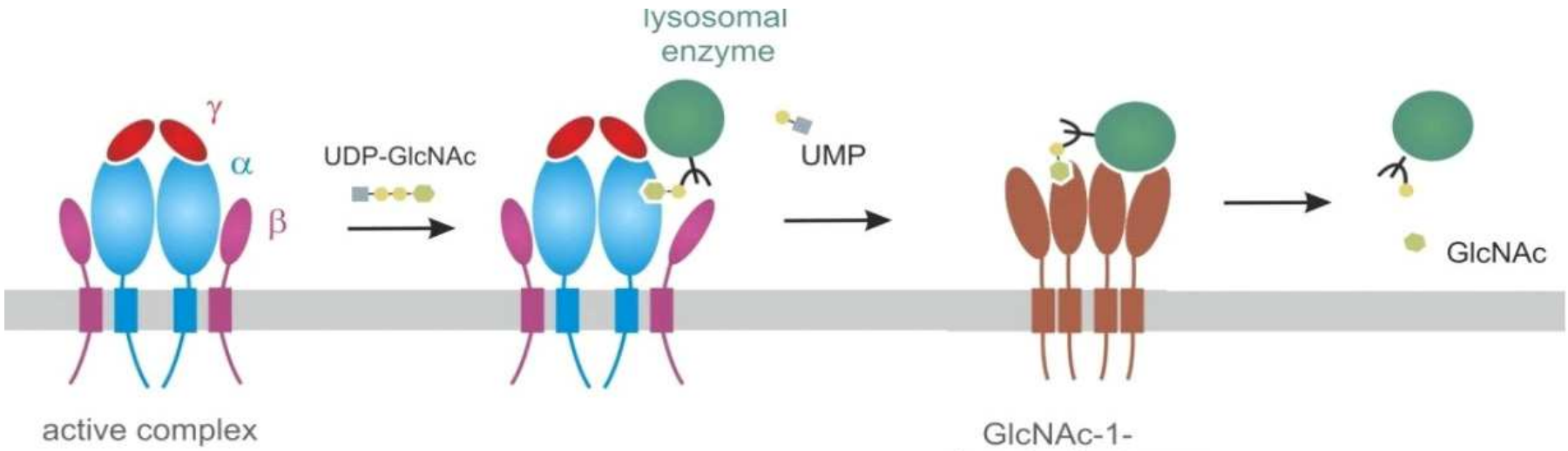


Encarnação et al. (2011) J Biol Chem
Marschner et al. (2011) Science

Site-1 protease: regulation of cholesterol biosynthesis

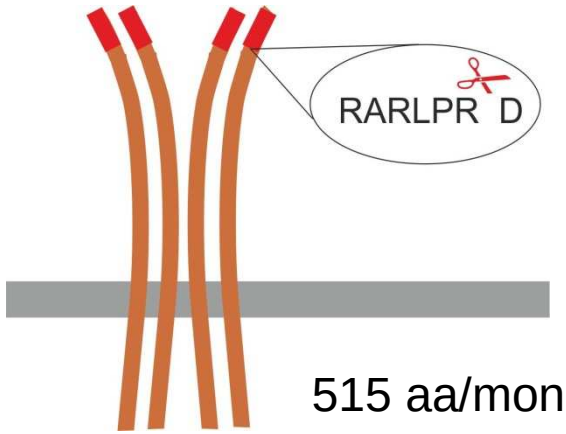


GlcNac-1-phosphodiester-α*N*-acetylglucosaminidase (UCE)



Proteolytic activation by furin

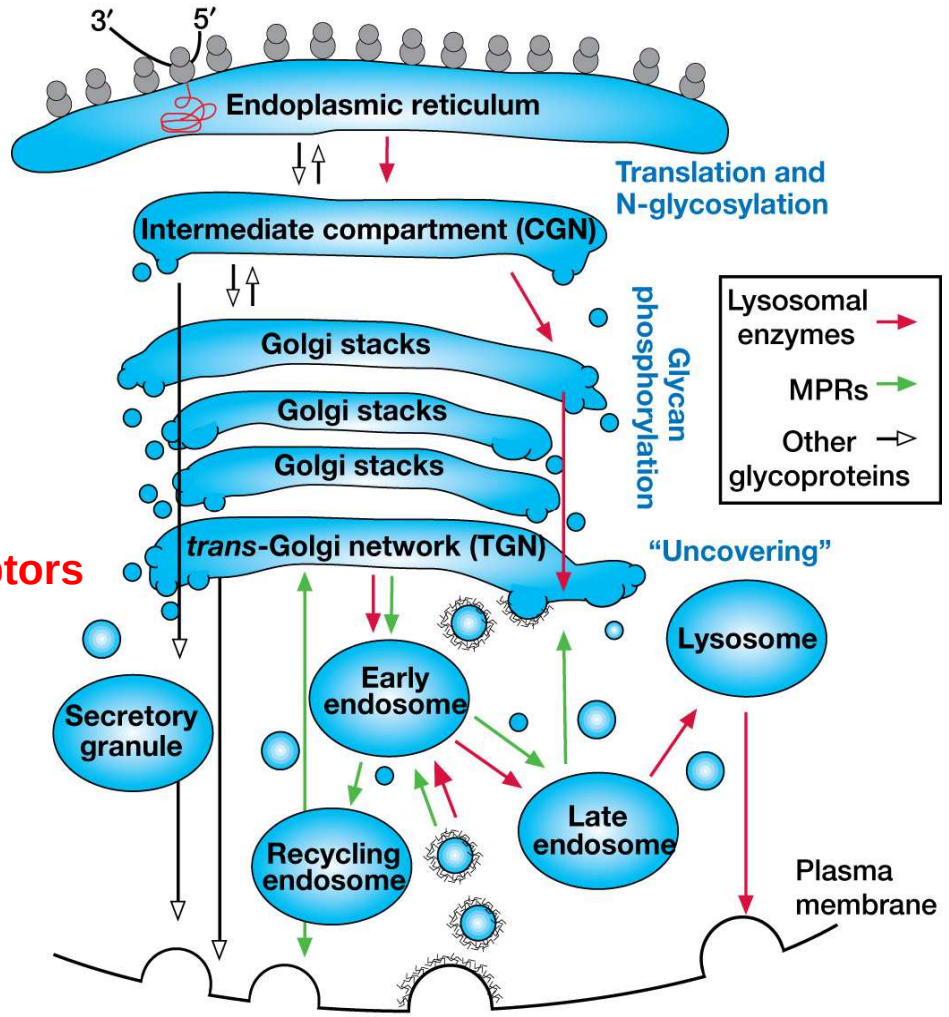
(Ca²⁺-dependent subtilisin-like serine endopeptidase)

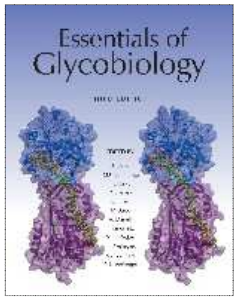


515 aa/monomer

Subcellular Trafficking Pathways of Glycoproteins, Lysosomal Enzymes, and M6P Receptors

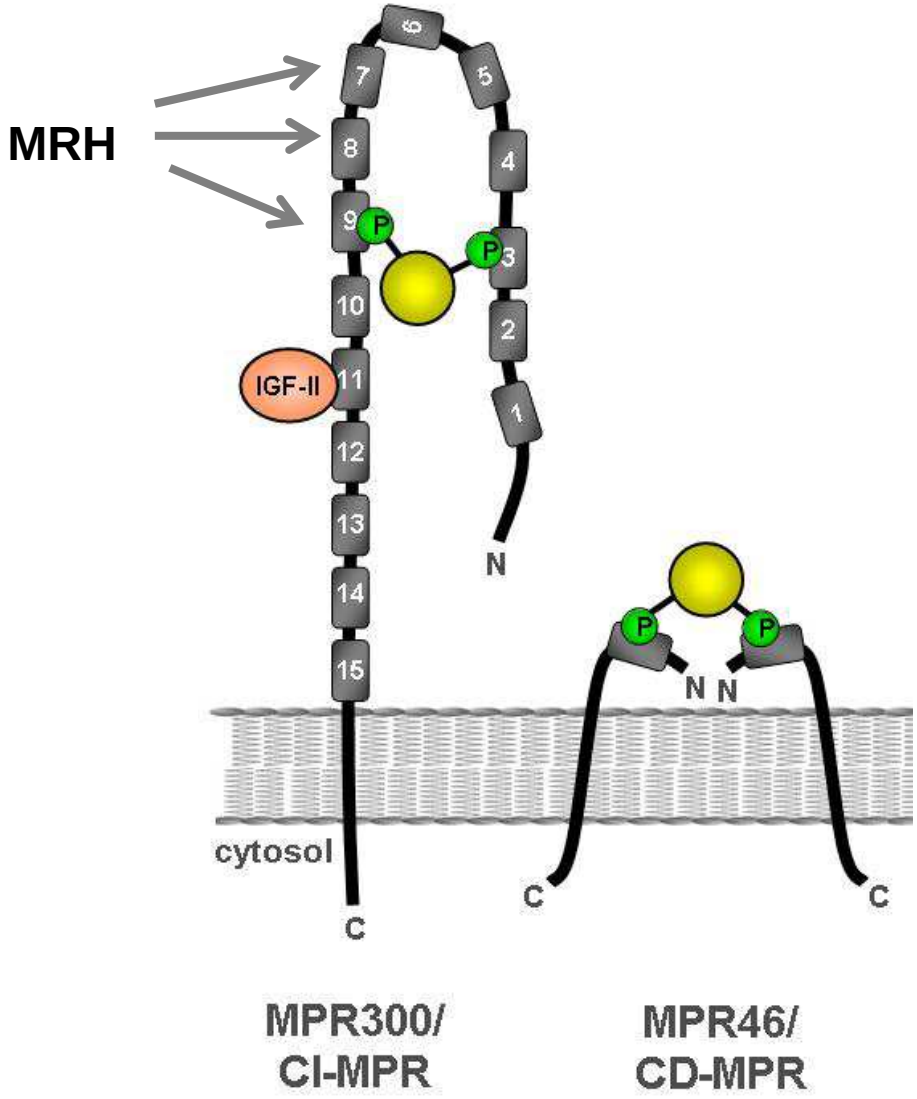
Binding to M6P receptors





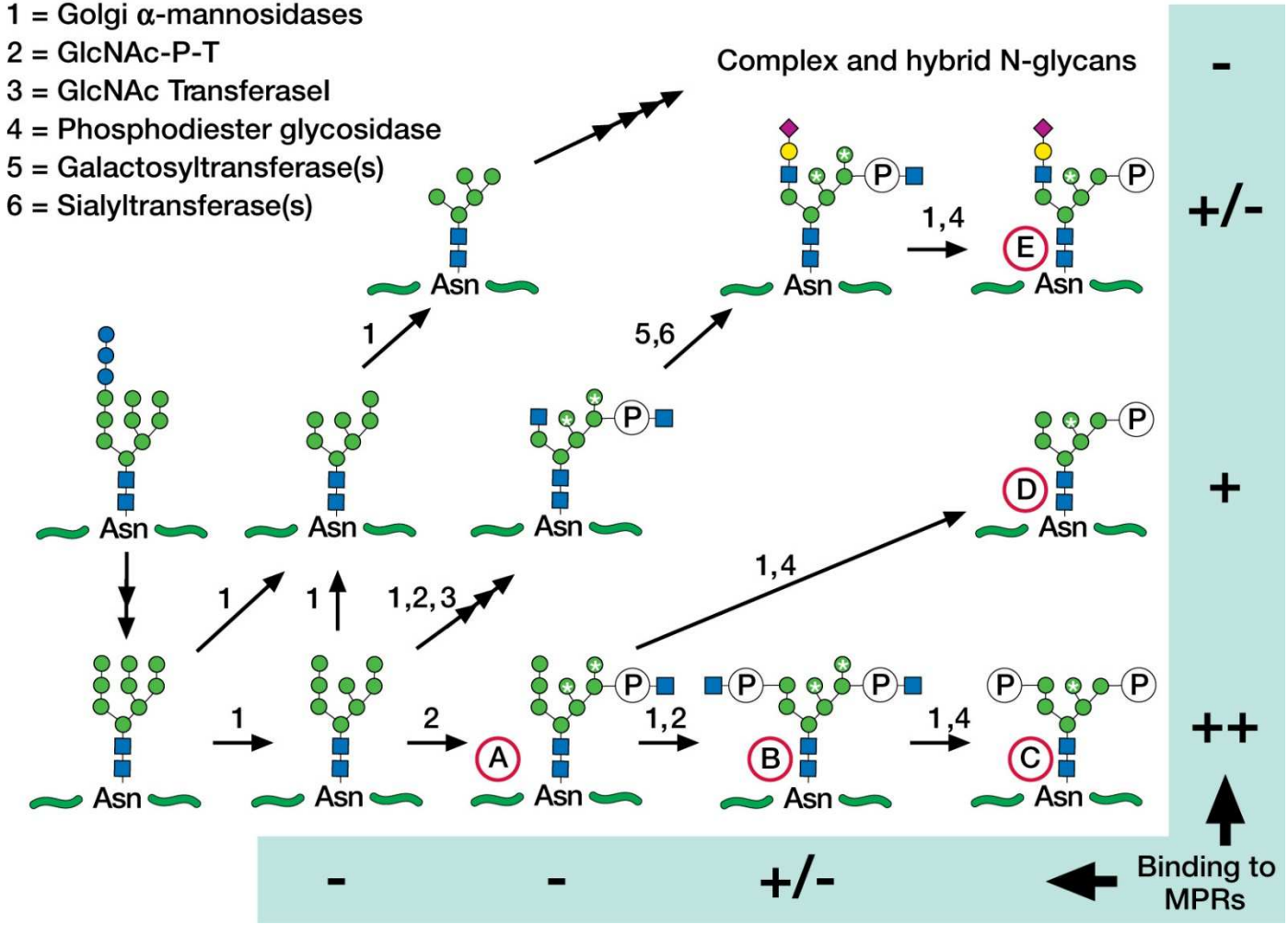
Varki & Kornfeld (2017)

Mannose 6-phosphate receptors



Braulke & Bonifacino
(2009) *BBA*

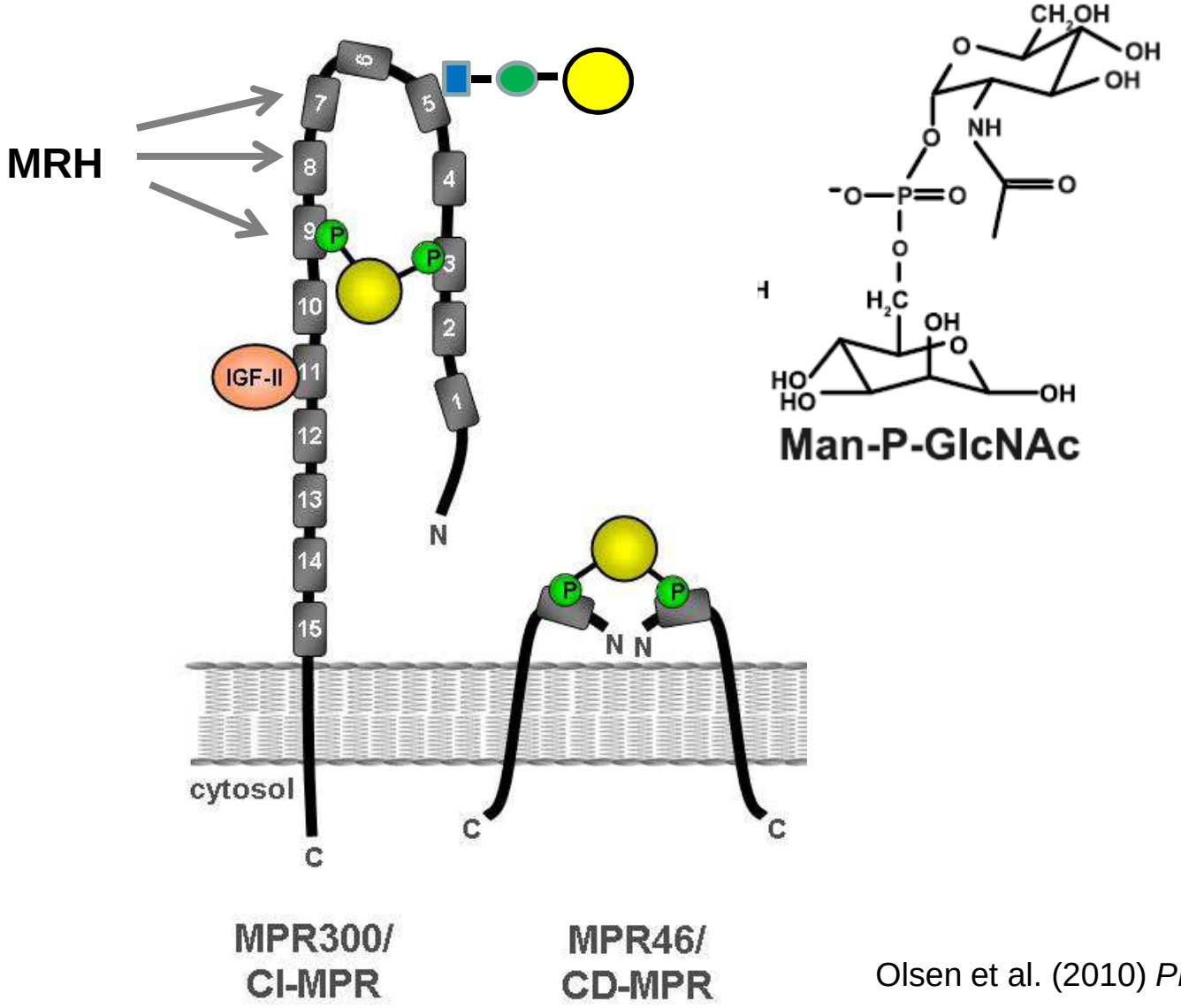
Binding affinity of M6P-containing lysosomal proteins



++, strong; +, moderate; +/−, weak; −, no binding.

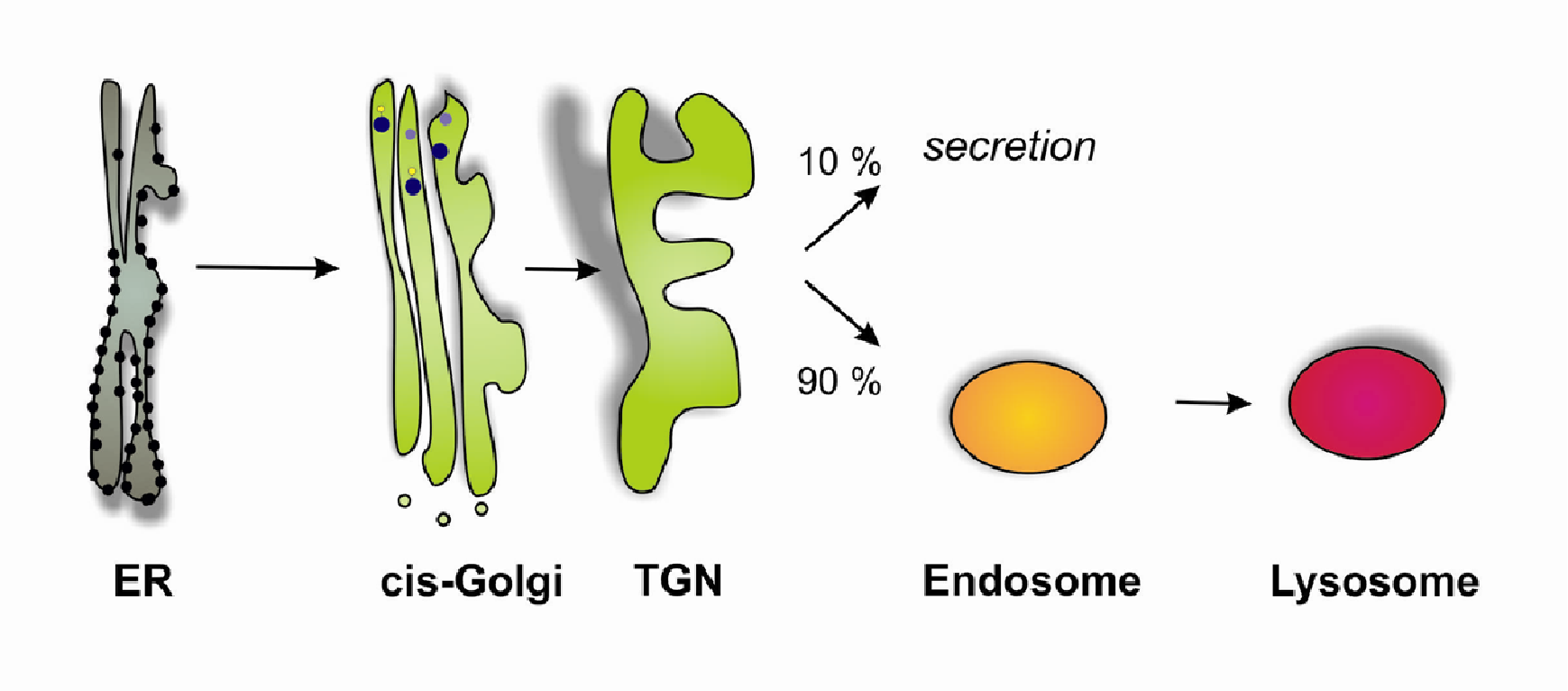
Varki & Kornfeld (2017)

Binding of phosphorylated lysosomal proteins to MPRs



Olsen et al. (2010) *PNAS*

Mannose 6-phosphate-dependent transport of lysosomal hydrolases



Chimeric-enzyme-replacement therapy

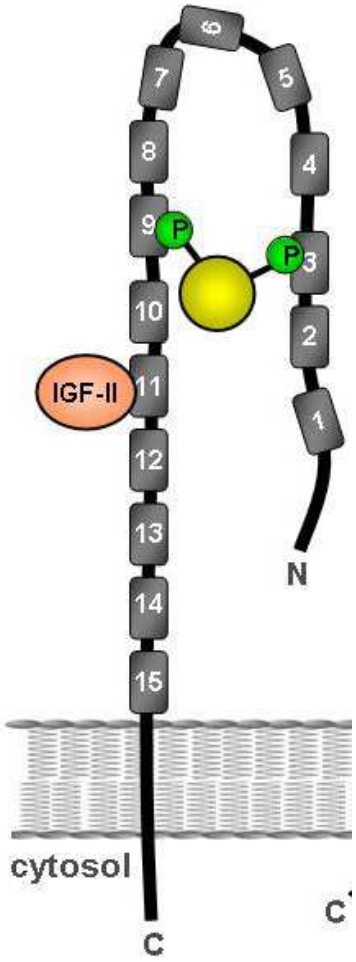
β-glucuronidase IGF-II

Ultragenyx

α-N-ac-glucosaminidase IGF-II

α-glucosidase IGF-II

Biomarin



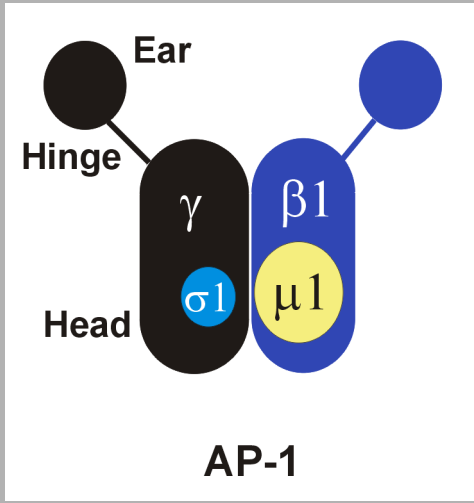
MPR300/ CI-MPR

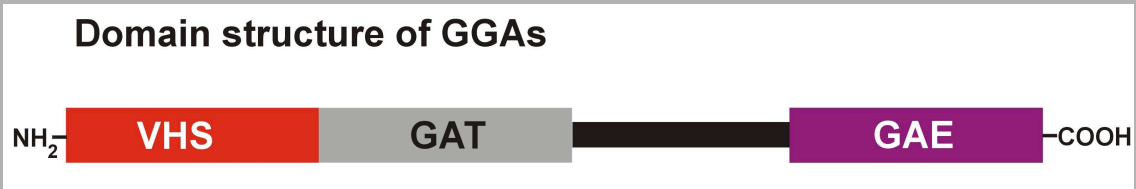
Sorting signals in the cytoplasmic domains of MPR/lysosomal membrane proteins

- **Tyrosine- based motifs** — **YXXΦ, FXNPXY**
- **Di-Leucine- based motifs** — **LL, LI, LM, LV**
- **Di-hydrophobic- based motifs** — **FF, FW**
- **Cluster of acidic residues + CK2 recognition site**

DSEDEVL, FHDDSDED, GEESEERDD

Transport proteins associated with sorting signals

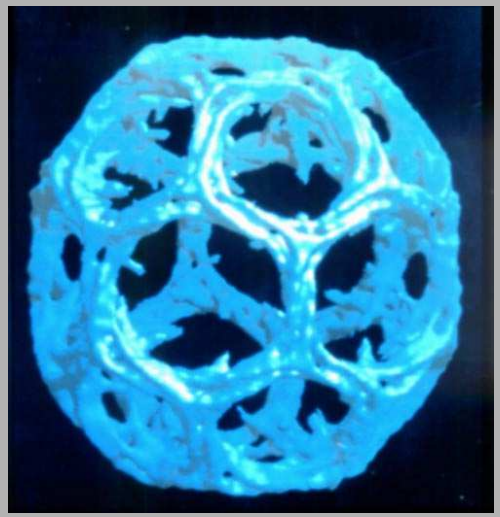




Golgi-localized, γ-ear containing ARF binding protein (GGAs)

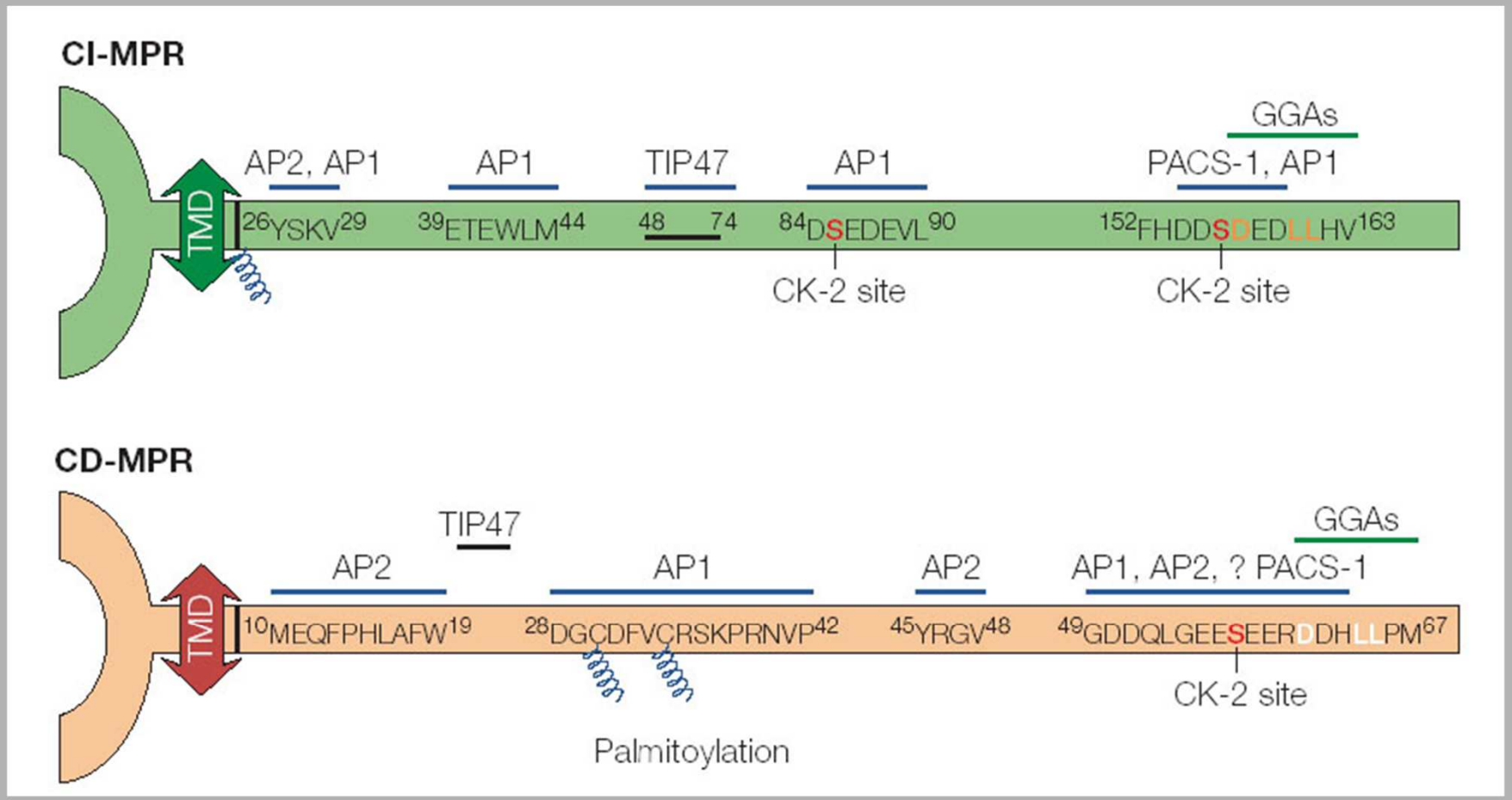
Adaptor protein (AP) 1 -5

Clathrin coat



- TIP 47
- PACS-1
- other proteins linking MPR to actin cytoskeleton

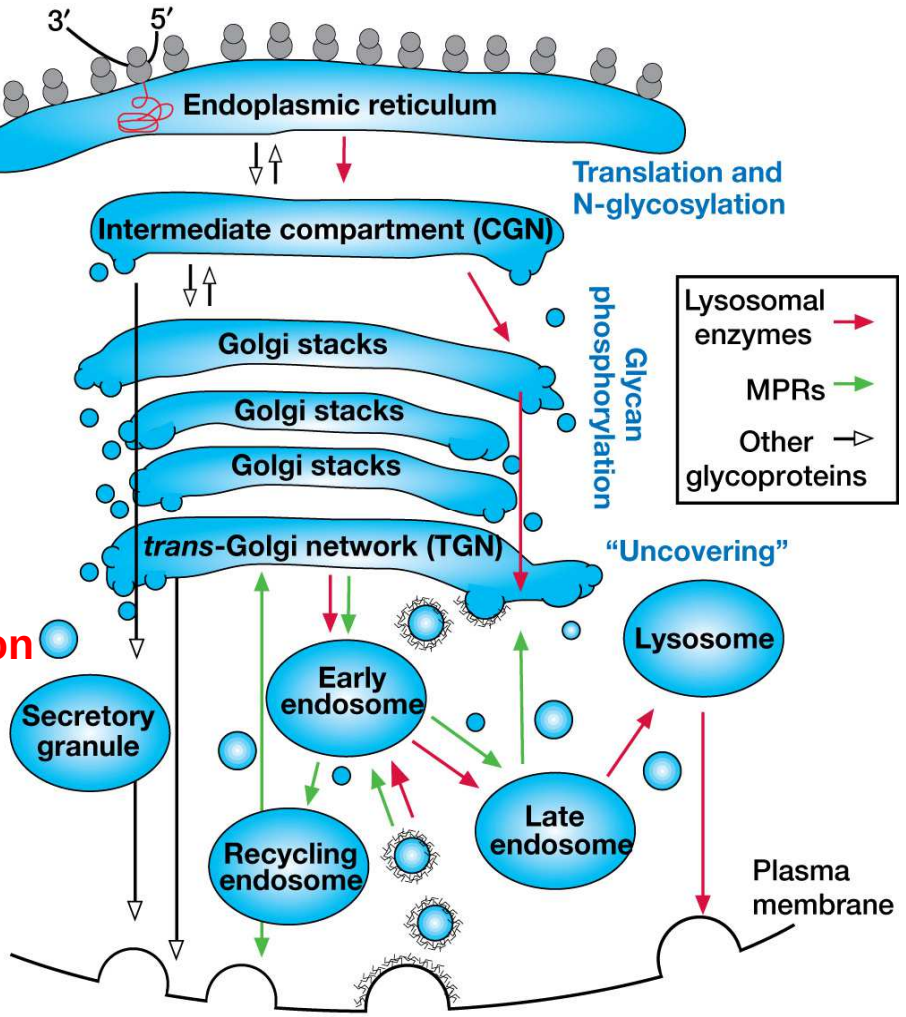
Signal-dependent interaction between MPR tails and cytoplasmic adaptors



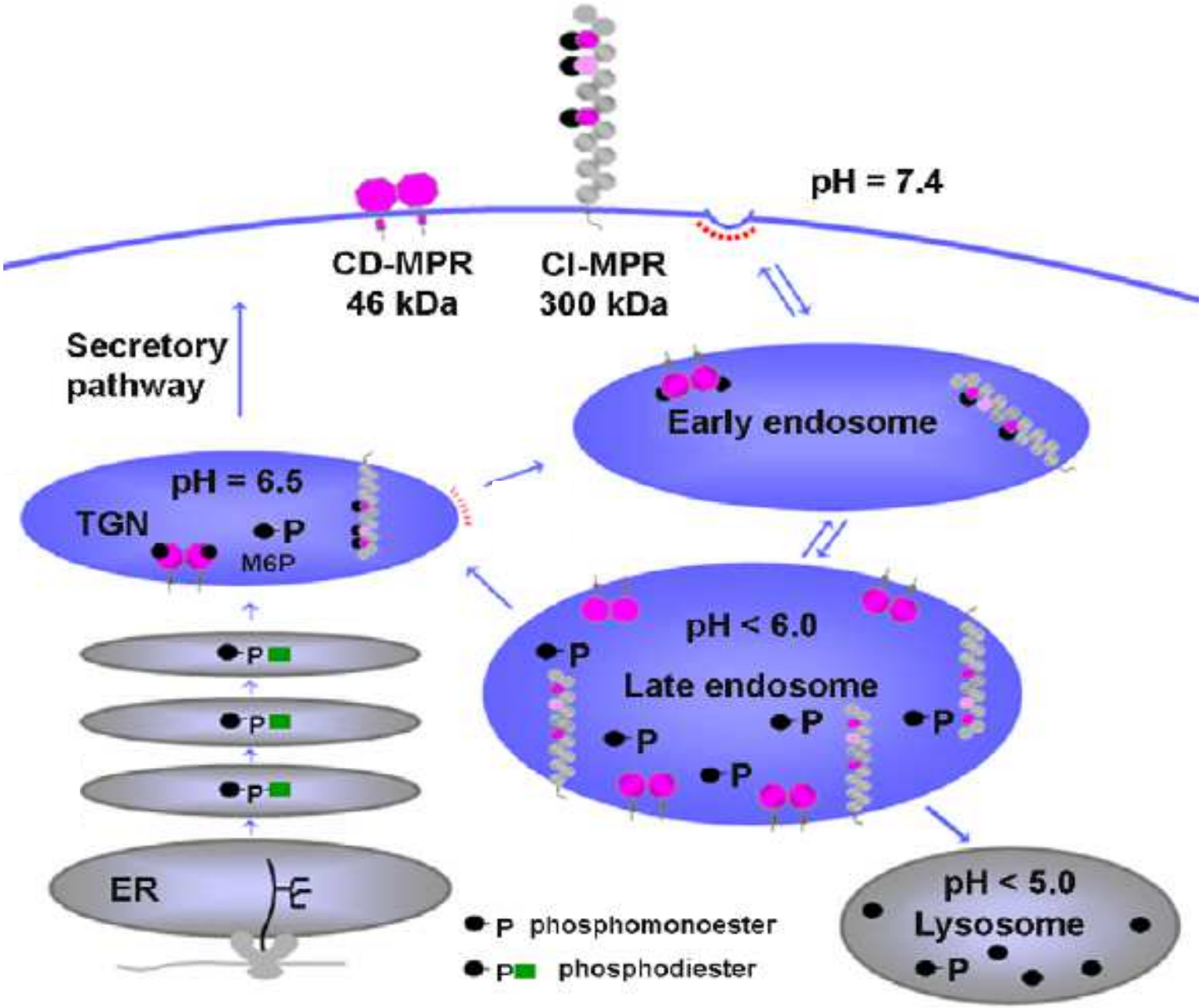
Gosh et al. (2003) Nature Rev.

Subcellular Trafficking Pathways of Glycoproteins, Lysosomal Enzymes, and M6P Receptors

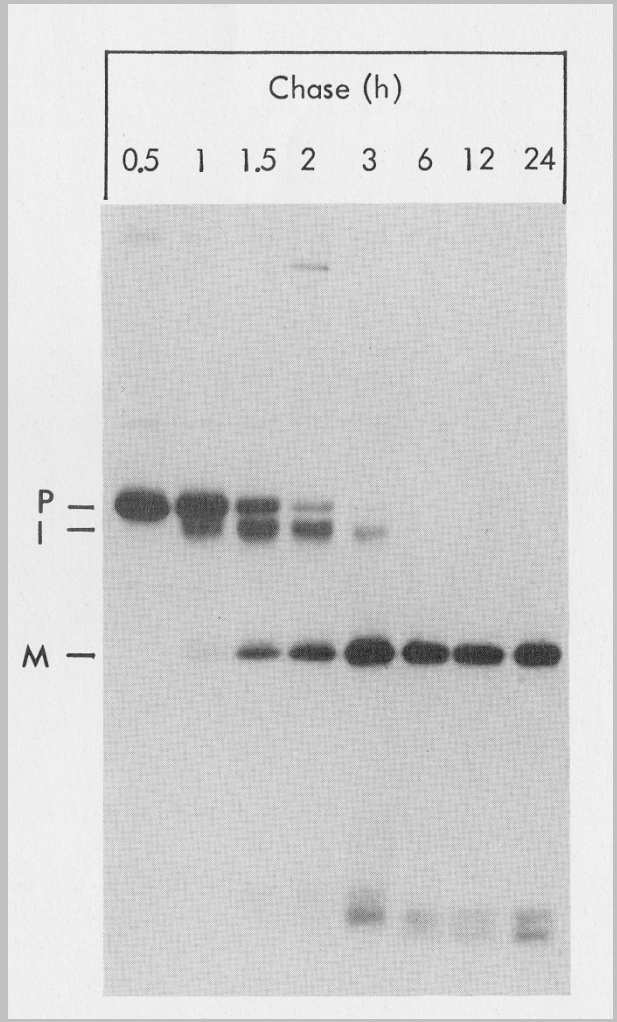
low pH-dependent dissociation



Varki & Kornfeld (2017)

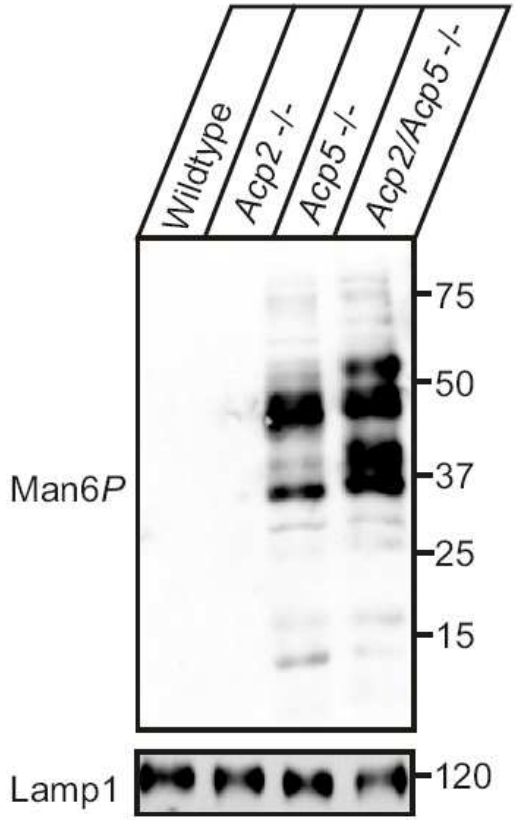

pH = 7.4
CD-MPR
46 kDa
CI-MPR
300 kDa
Secretory
pathway
Early endosome
pH = 6.5
TGN
P
M6P
pH < 6.0
Late endosome
P
ER
pH < 5.0
Lysosome
P phosphomonoester
P phosphodiester

Proteolytic processing and activation of lysosomal enzymes during the transport to the lysosome



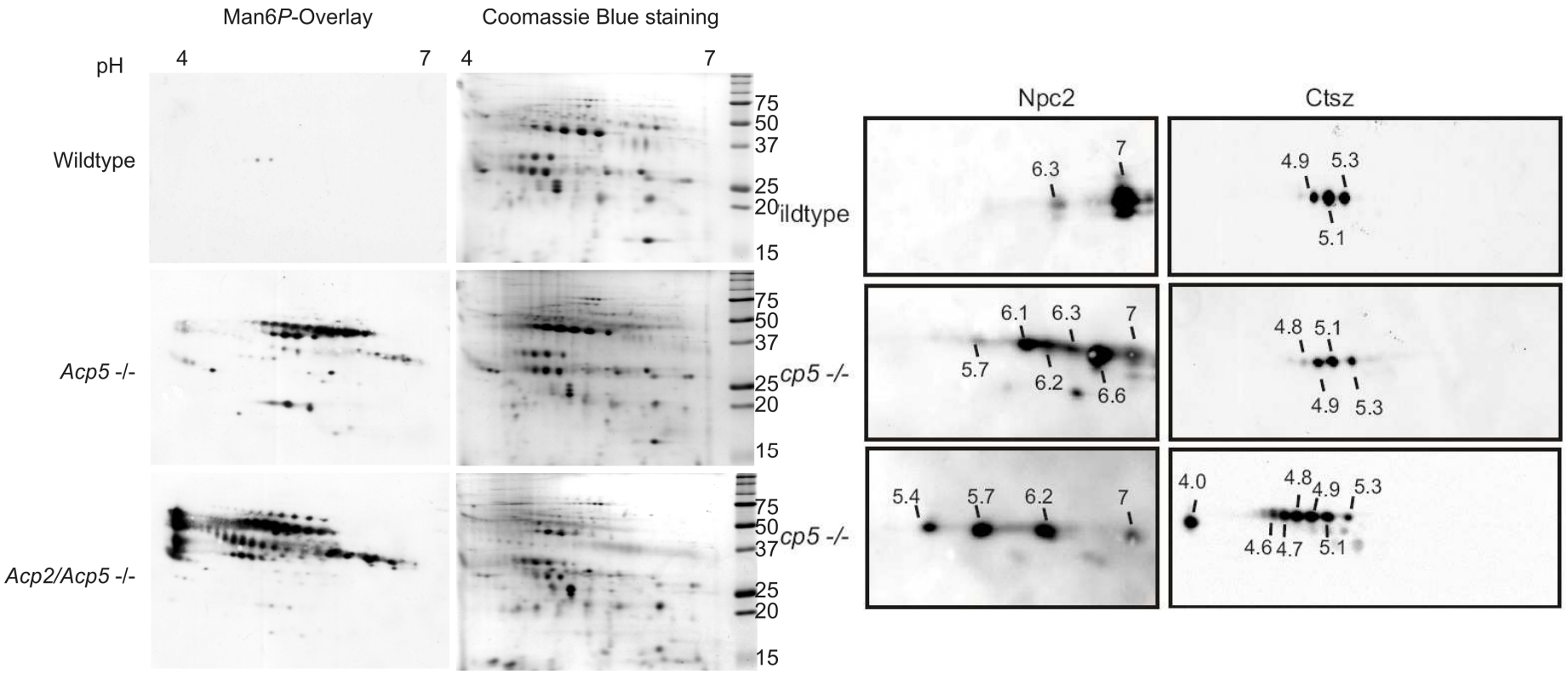
Cathepsin D

Dephosphorylation of lysosomal proteins upon arrival in lysosomes by Acp2 (LAP) and Acp5 (TRAP)

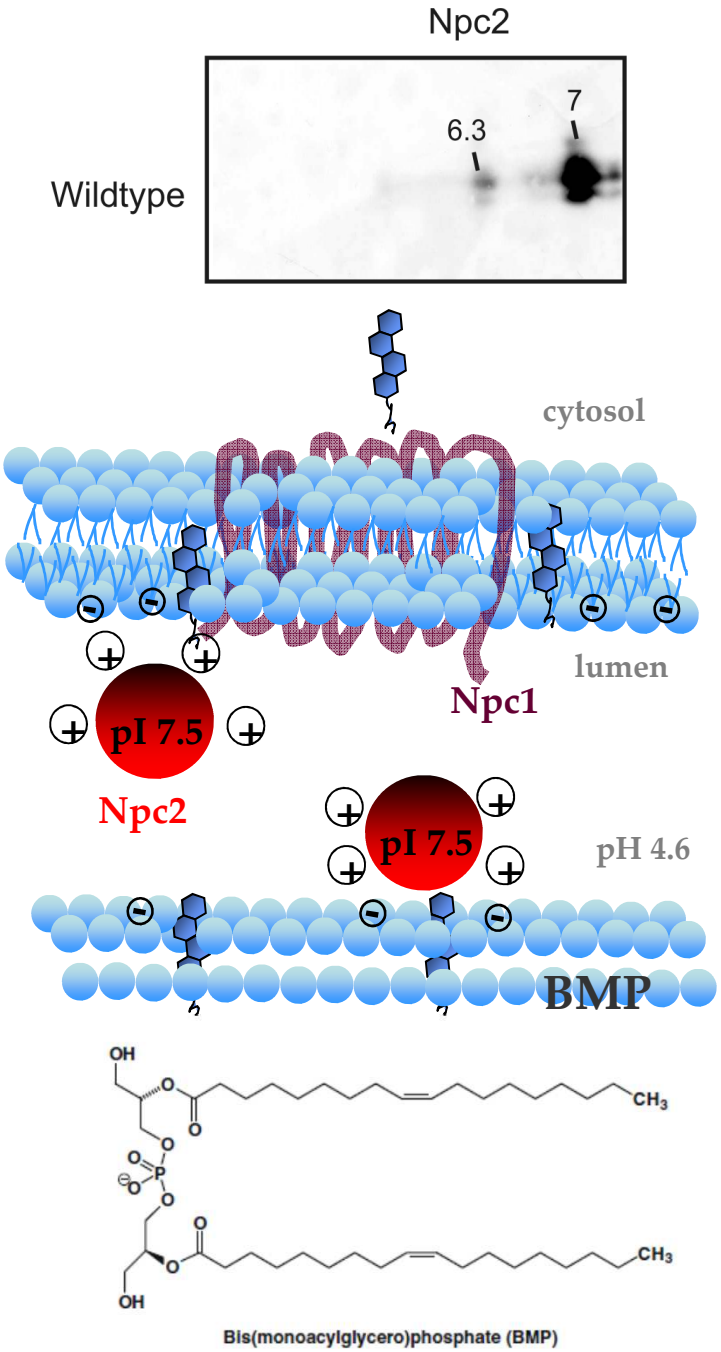


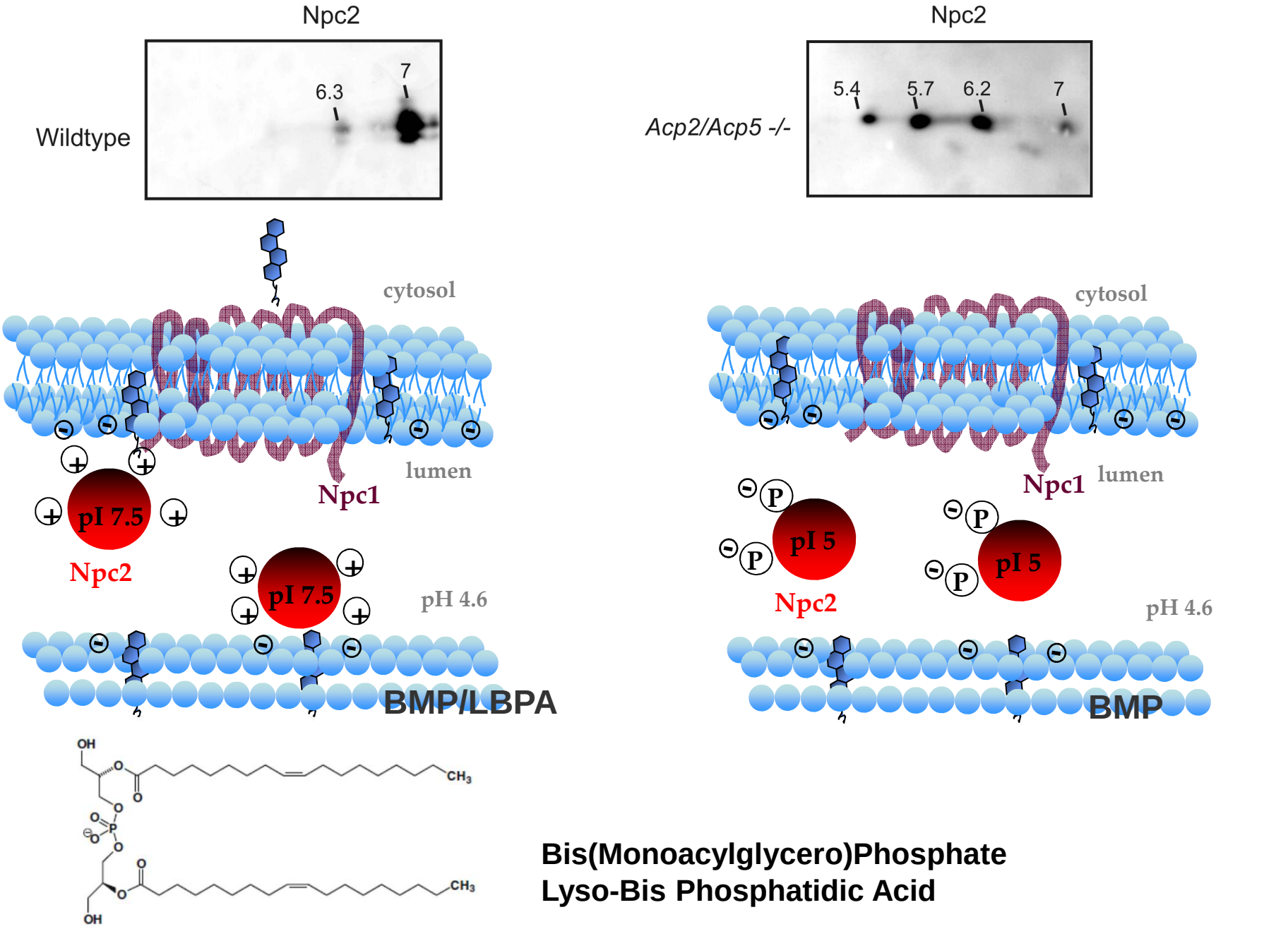
Sun et al., *PNAS* 2008; Makrypidi et al., *Mol Cell Biol* 2012

Dephosphorylation of Man6P residues affects the pI and the interaction of lysosomal proteins with negatively charged lysosomal membranes



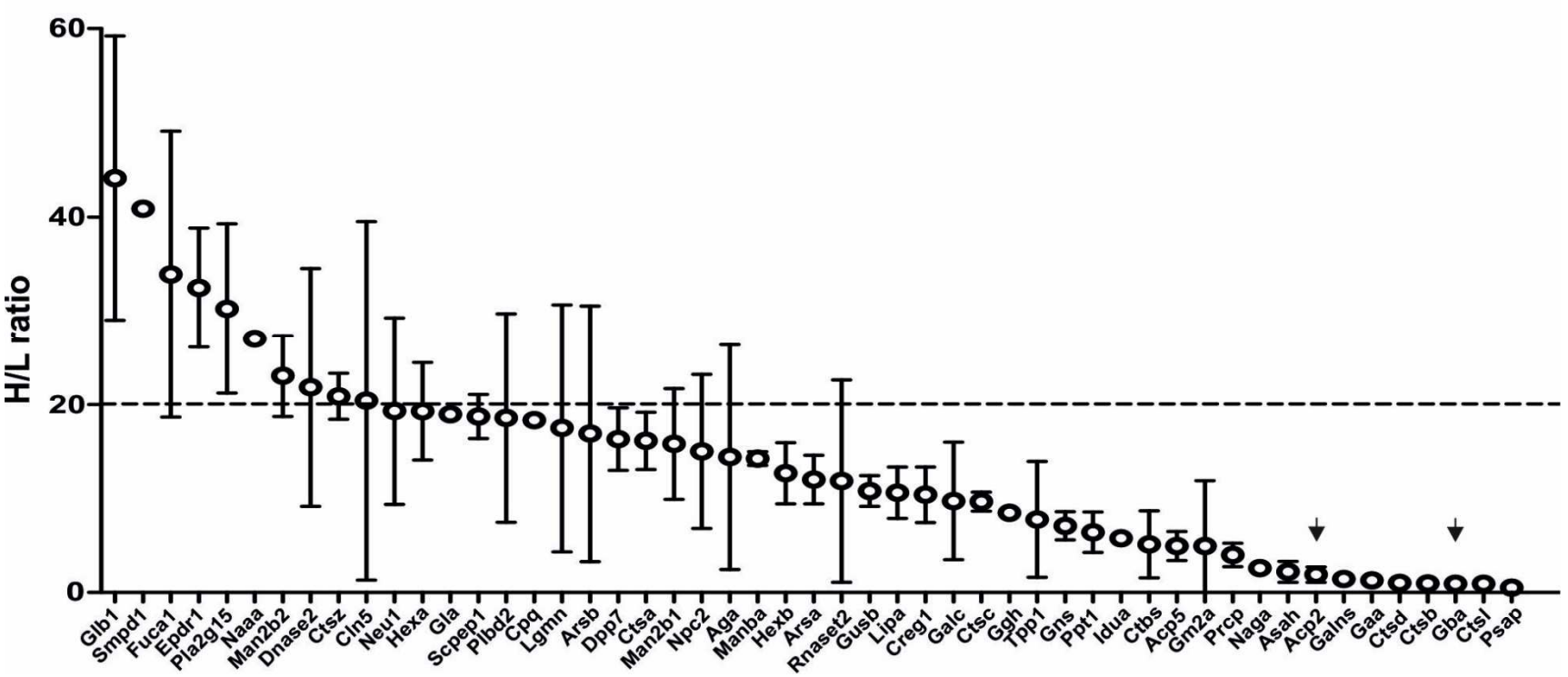
Makrypidi et al, *Mol Cell Biol* 2012


Npc2
Wildtype
6.3
7
cytosol
lumen
Npc1
pI 7.5
Npc2
pI 7.5
pH 4.6
BMP
OH
CH3
CH3
OH
Bis(monoacylglycero)phosphate (BMP)



Bis(Monoacylglycero)Phosphate
Lyso-Bis Phosphatidic Acid

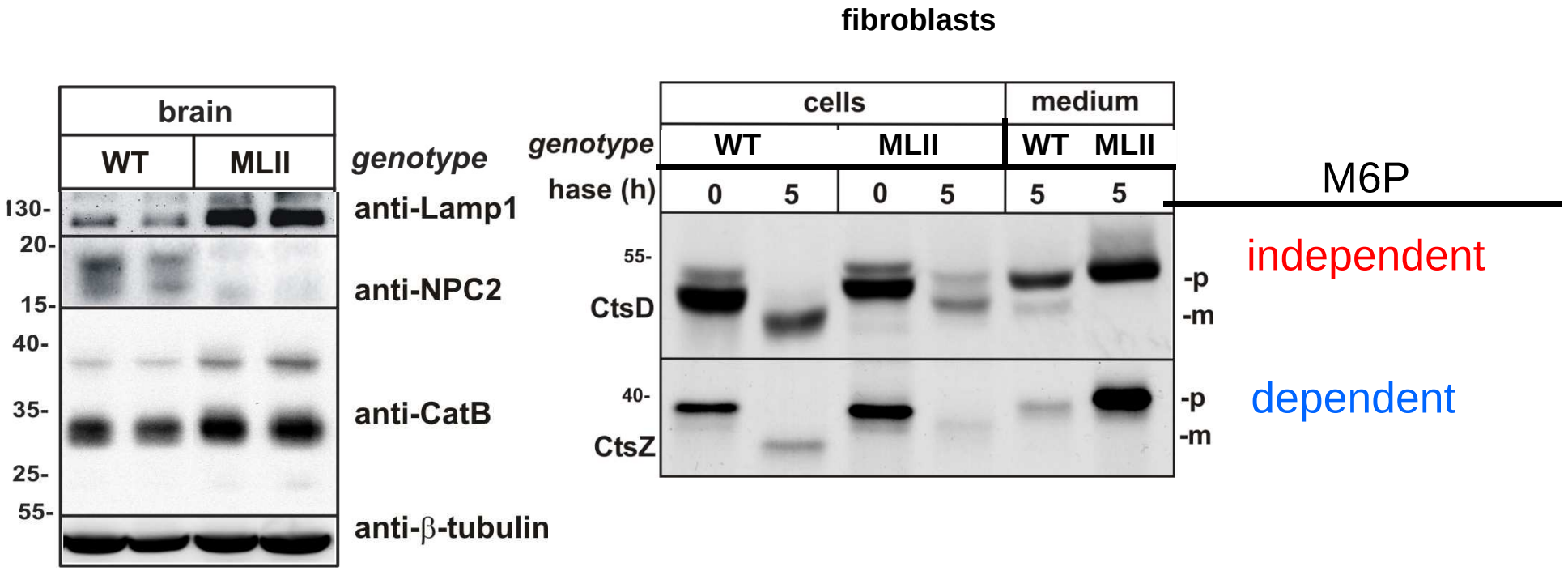
Lysosomal enzyme targeting in the absence of M6P



β-galactosidase (**Glb1**)
sphingomyelin phosphodiesterase (**Smpd1**)
α-L-fucosidase (**Fuca1**),
mamalian ependymin-related protein MERP-1 (**Epdr1**)
phospholipase A2 Group XV (**Pla2g15**)
N-acylethanolamine acid amidase (**Naaa**)
epididymis α-mannosidase (**Man2b2**)
deoxyribonuclease-2-α (**Dnase2**)
cathepsin Z (**Ctsz**)
Cln5

Markmann et al., *Traffic* 2015

Enzyme-dependent missorting of lysosomal proteins



Kollmann et al, *Brain* 2012

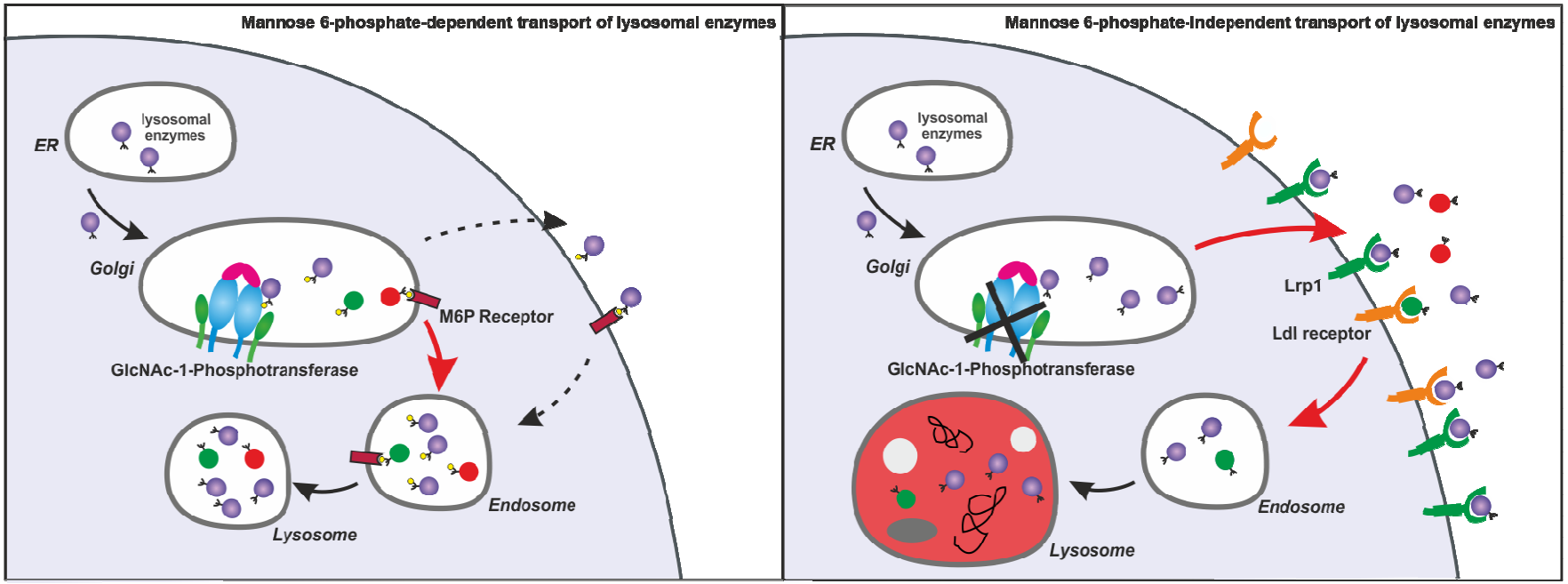
M6P-independent lysosomal targeting

Candidates

- sortilin
- LDL receptor-related protein (LRP), LDLR
- mannose and galactose-specific receptor
- LIMP-2 (β-glucocerebrosidase)

Secretion-recapture in the absence of M6P

About 15% of all lysosomal enzymes require M6P residues for lysosomal targeting in *fibroblasts* (limiting for lysosomal function)



The majority of lysosomal enzymes reach lysosomes by alternative receptors in a cell- and enzyme-dependent manner (sufficient for normal function)

Impact for enzyme replacement therapy (ERT)

Inter-organelle connections

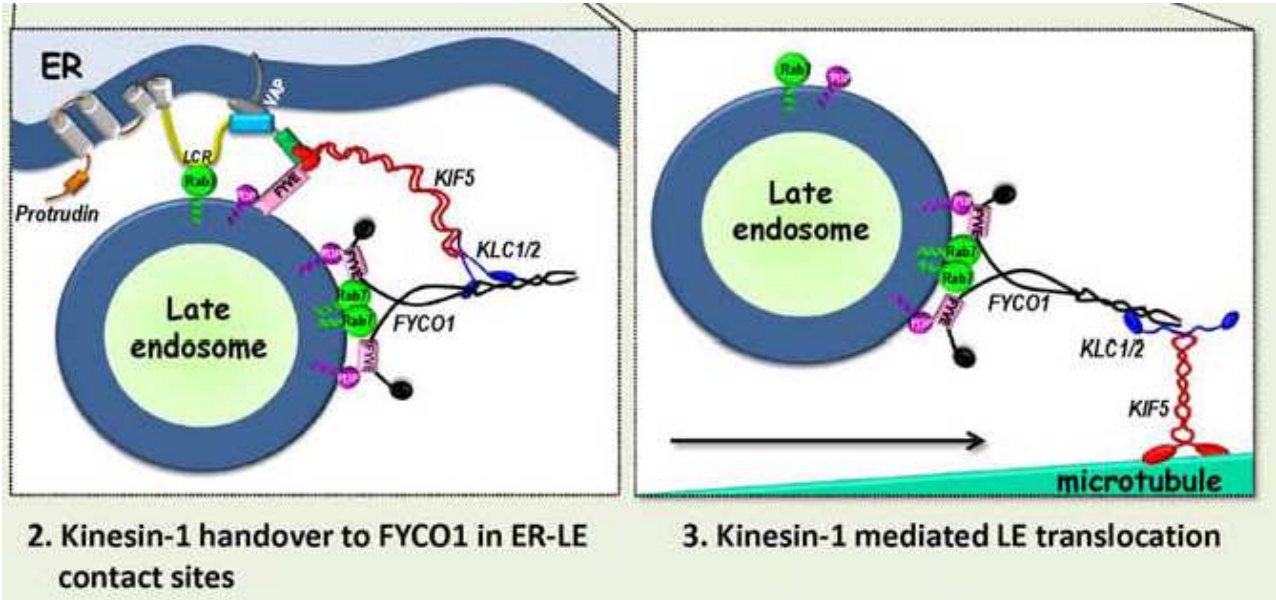
ER-mitochondria (Friedman et al. 2011 Science; Ah Young et al 2015 PNAS): mitochondrial division, **PC**

ER-endosomes (Rowland et al. 2014 Cell; Raiborg et al. 2015 Nature)

ER-vacuoles/lysosomes (Henne et al.2015 JCB; Murlay et al. 2015 JCB): **SL, sterol**

ER- plasma membrane (Idevall-Hagren et al. 2015 EMBO J; Kim et al. 2015 Dev Cell) **PI-PA**

Mitochondria- vacuole (Elbaz-Alon et al. 2014 Dev Cell; Hönscher et al. 2014 Dev Cell) : **PL**



BORC
Pu et al. 2015 Dev Cell)

Lysosomes/Golgi
(Nobel Prize for Medicine - 1974)
Christian de Duve/George E Palade

SIGNAL HYPOTHESIS (ER)
(Nobel Prize for Medicine - 1999)
Günter Blobel

Vesicular Transport Machinery
(Nobel Prize for Medicine - 2013)
Jim Rothman, Randy Schekman, Thomas Südhof

Autophagy
(Nobel Prize for Medicine - 2016)
Yoshinori Ohsumi