FEBS National Lecture

Galectin-3: from molecule to biomarker and back

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30th Turkish Biochemical Society Congress & 27th Balkan Clinical Laboratory Federation Meeting, Antalya, Turkey, October 27-31, 2019

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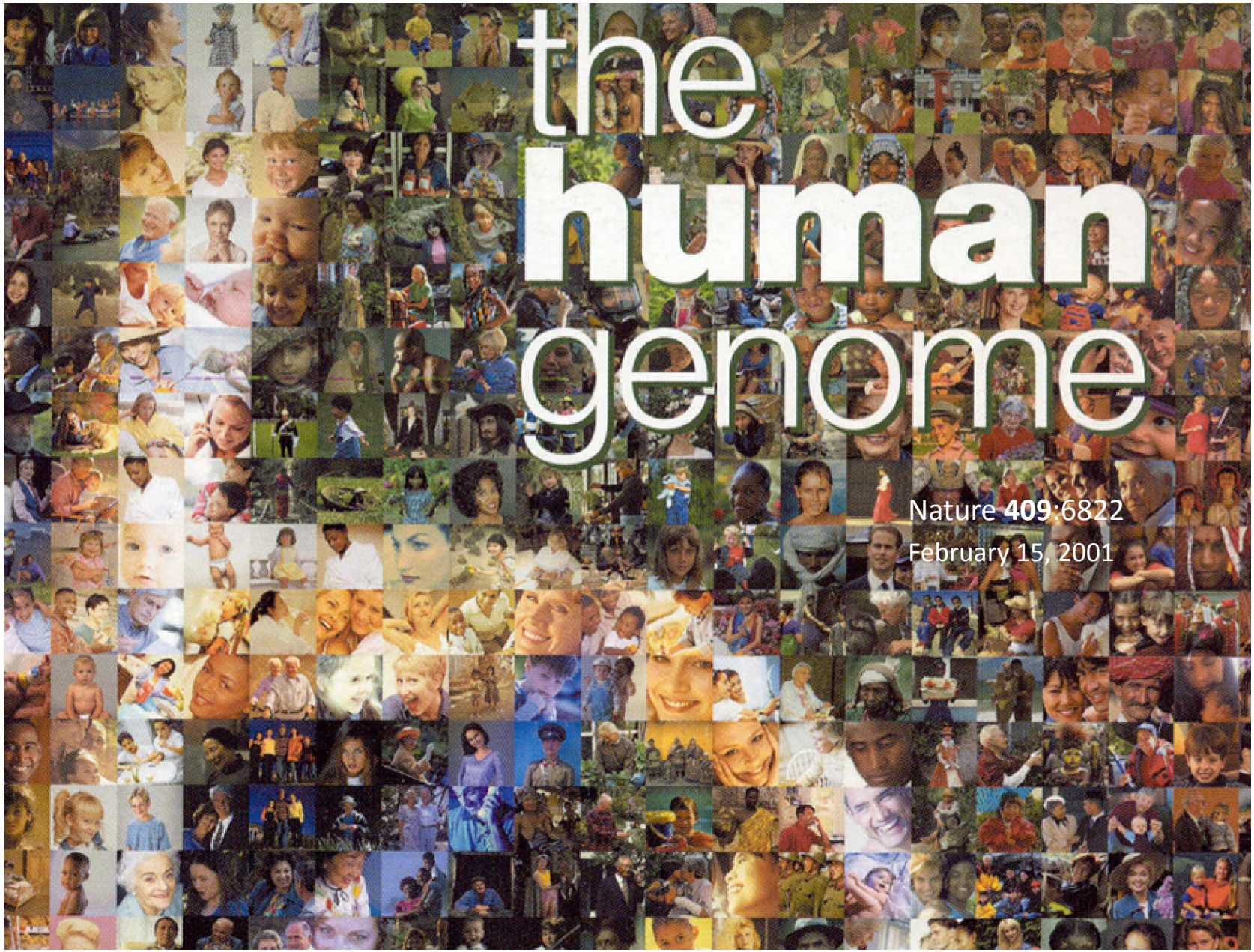
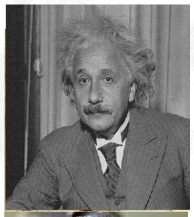
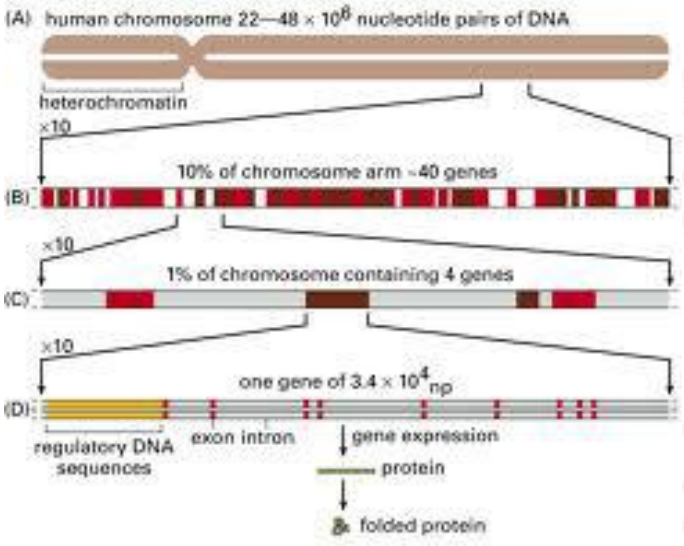

the human genome
Nature 409:6822
February 15, 2001

What do genes offer?

~ number of genes			genetic similarity
30.000	**human**		**99.9%** similarity between humans
30.000	**chimpanzee**		**99%** similarity to humans
30.000	**mouse**		**70%** similarity to humans
13.000	**fly**		**60%** similarity to humans
19.000	**worm**		20% similarity to humans

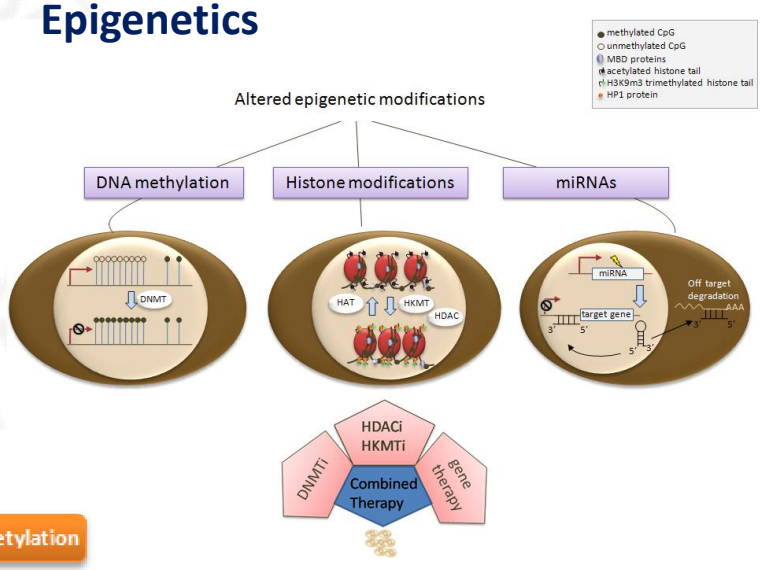
„New biologies”

Non-coding DNA

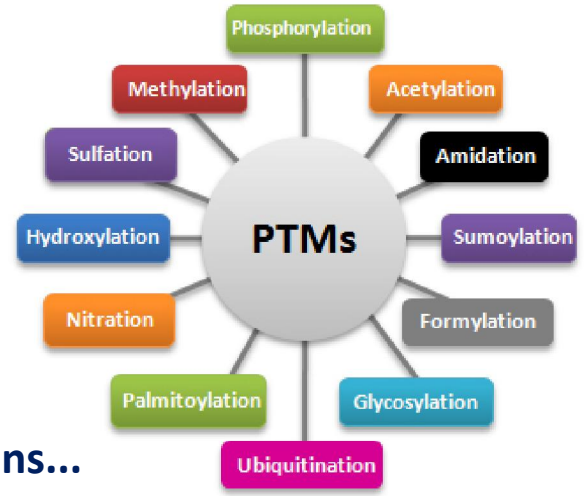


“RNAs”

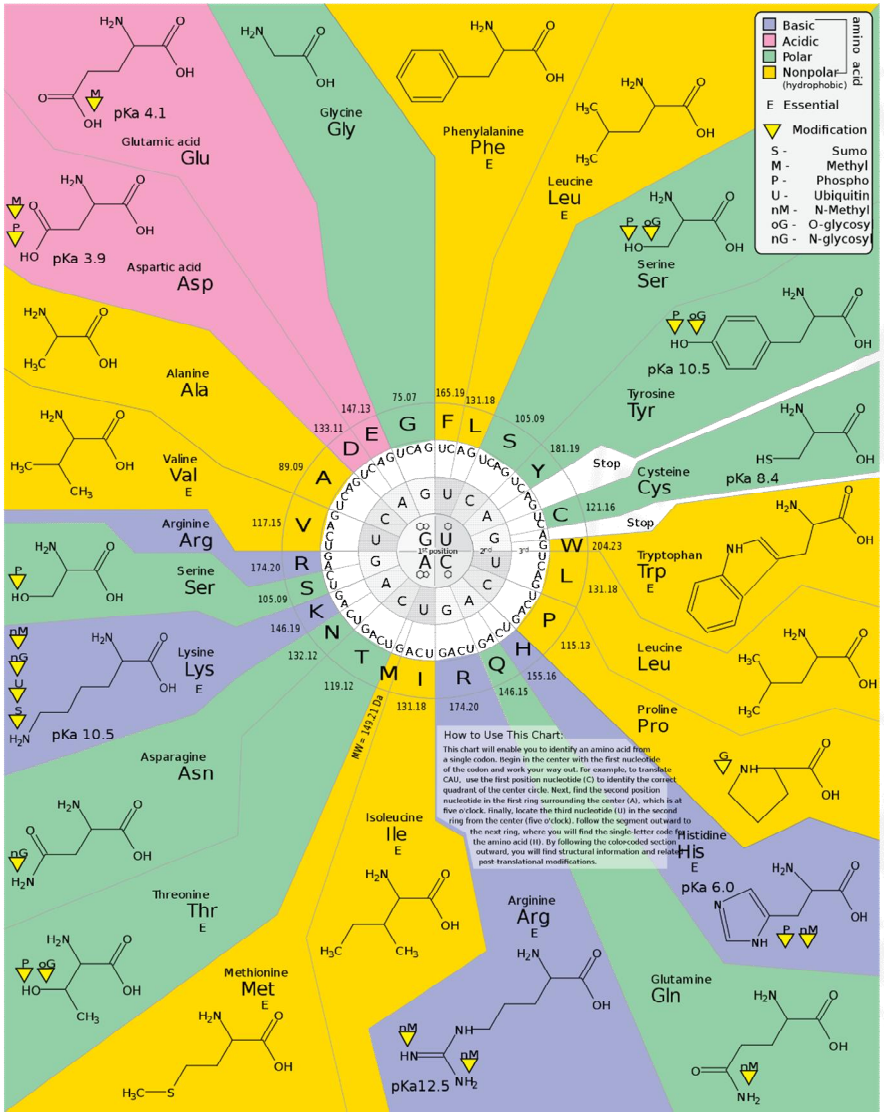
Epigenetics



Posttranslational modifications...



PTMs Greatly Expand Chemical Diversity of the Genetic Code:



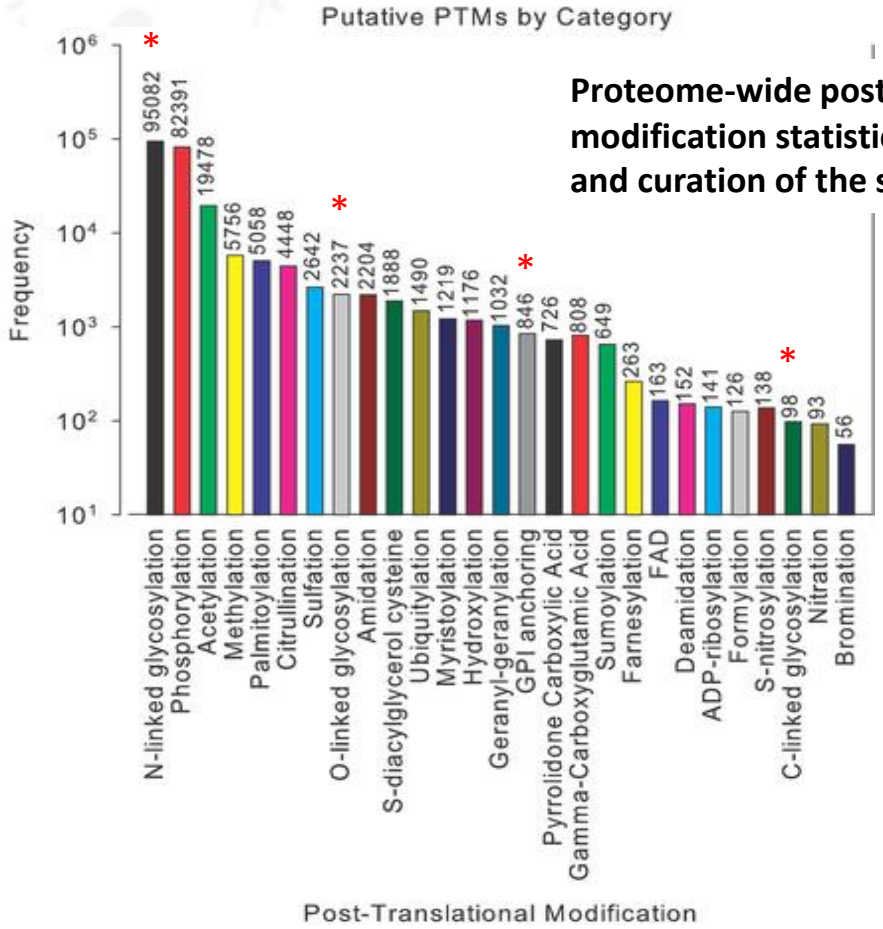
Source: Public Domain: Wikipedia

"~50% of all proteins are glycosylated"

Apweiler et al. Biochim. Biophys. Acta, Gen. Subj. 1473, 4–8 (1999).

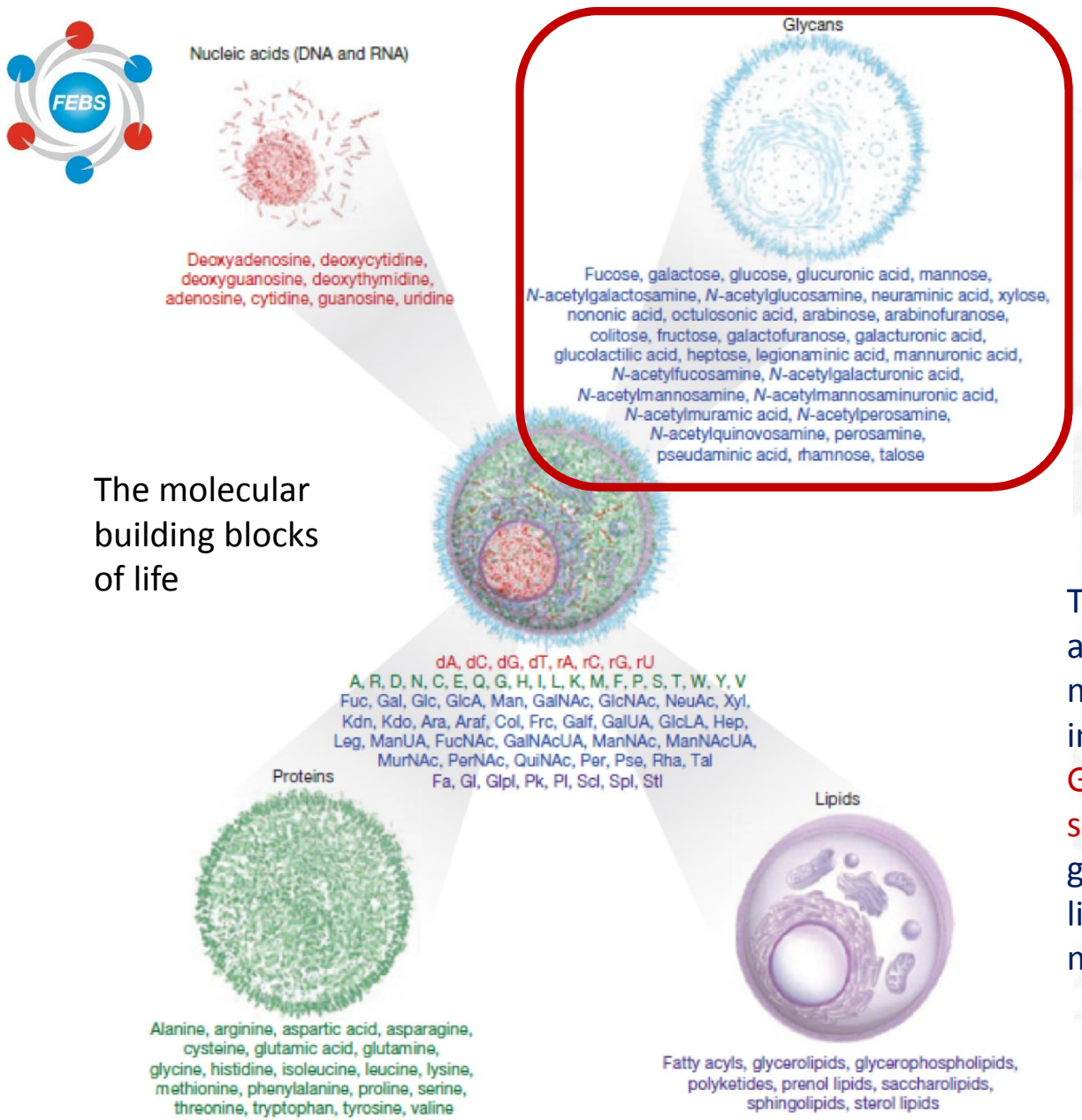
Percentage glycosylated is <u>much</u> higher, if you include O-GlcNAc!

Phosphorylation is <u>not</u> the most common PTM!



Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database

Scientific Reports 1,90
doi:10.1038/srep00090
13 September 2011



The molecular building blocks of life

We still live in a "Protein and Nucleic Acid Centric World"

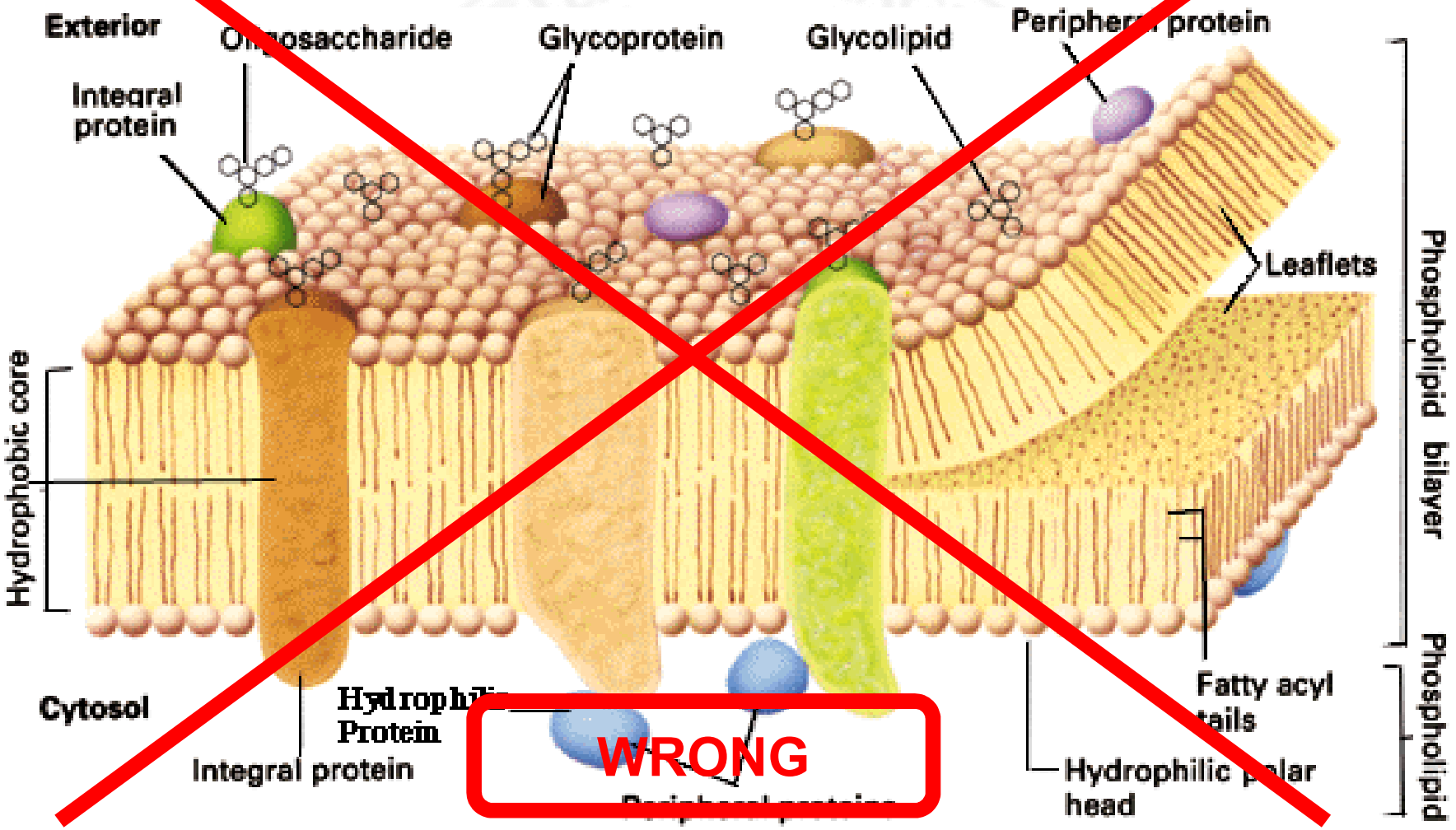
Glycans are one of four principal components of a cell!

There are 68 molecules that contribute to the synthesis and primary structures of the four fundamental macromolecular and structural components of all cells including the - NA, proteins, glycans, and lipids. Glycans derive initially from 34, and possibly more, saccharides used in the enzymatic process of glycosylation and are often attached to proteins and lipids, although some exist as independent macromolecules

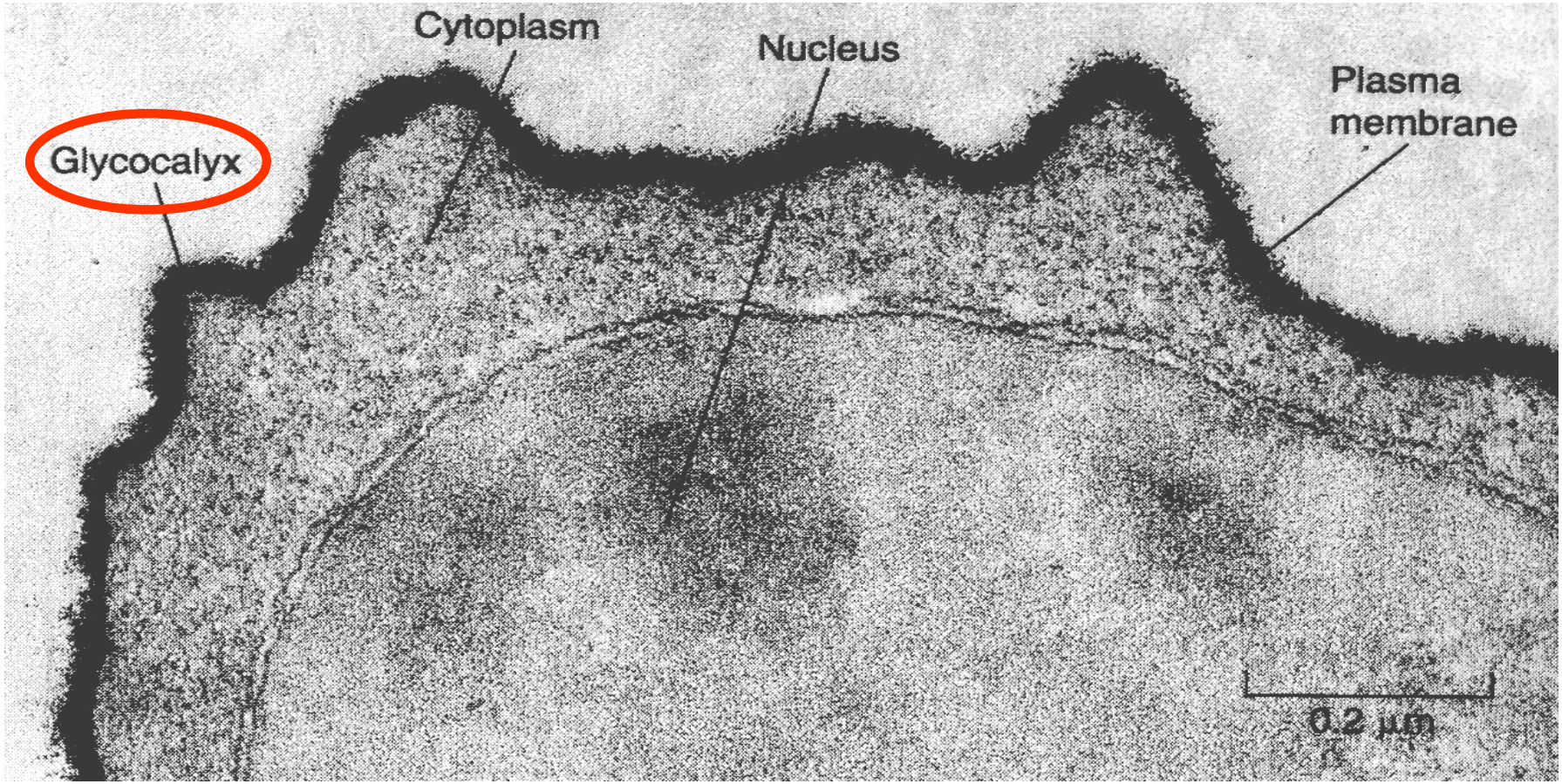
Jamey D. Marth

NATURE CELL BIOLOGY VOLUME 10 | NUMBER 9 | SEPTEMBER 2008

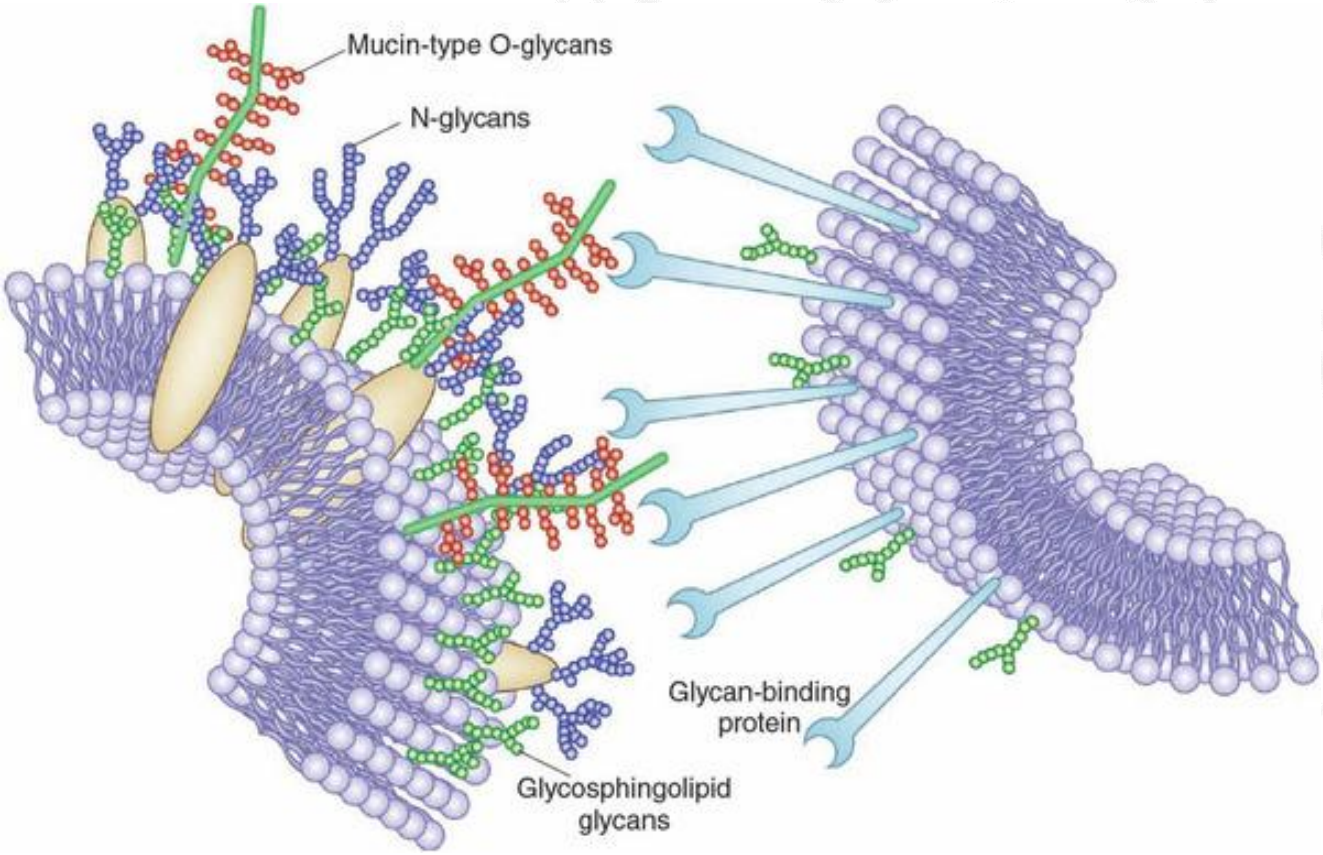
The cell membrane



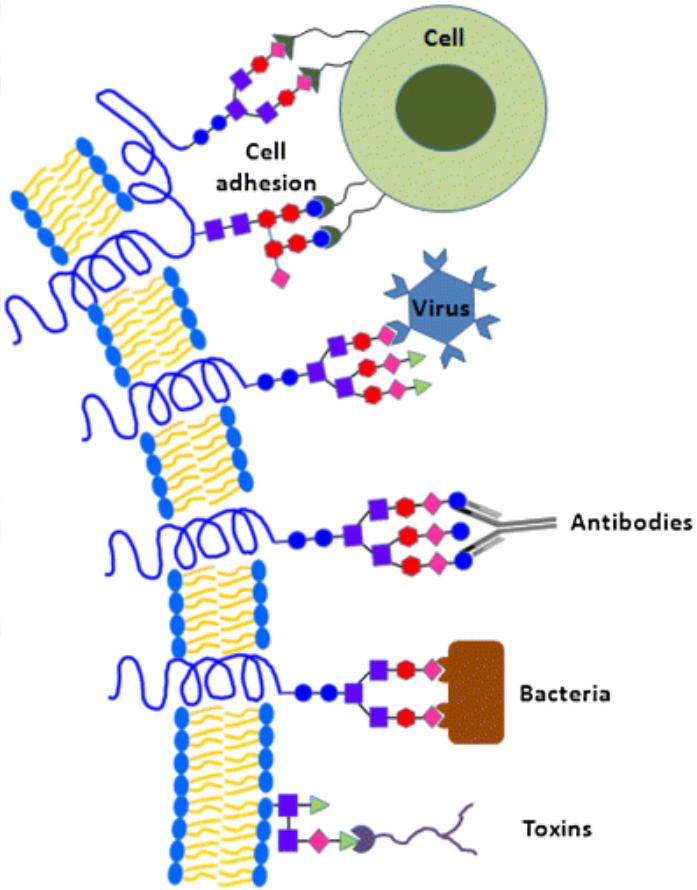
Plasma membranes are covered with glycoconjugates



Cell surface glycans serve as points of attachment for other cells, bacteria, viruses, toxins, hormons, *etc*.

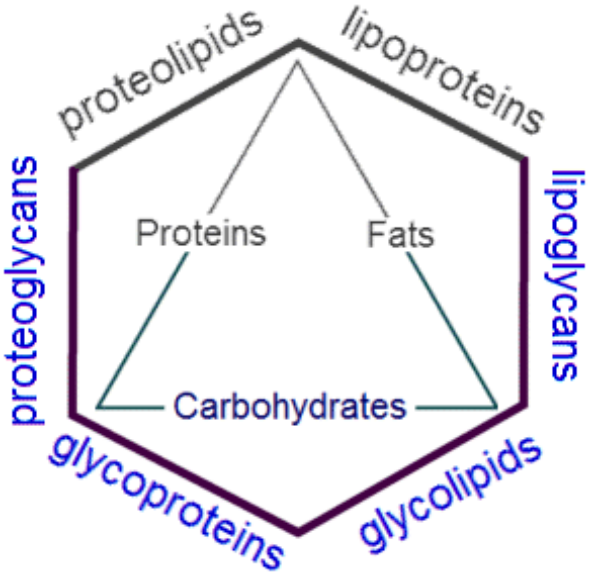


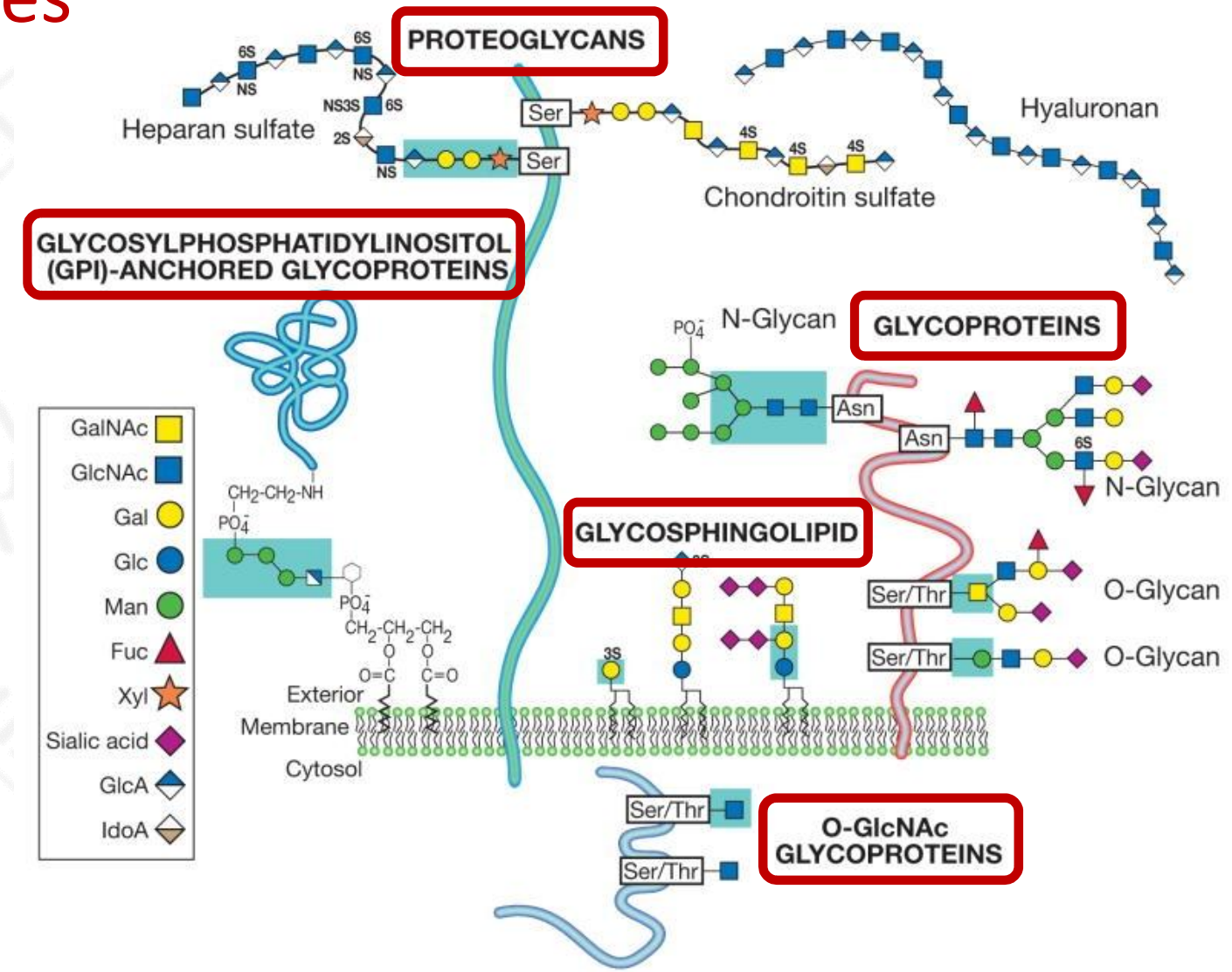
Zaia J, 2011, *Nature Met.*



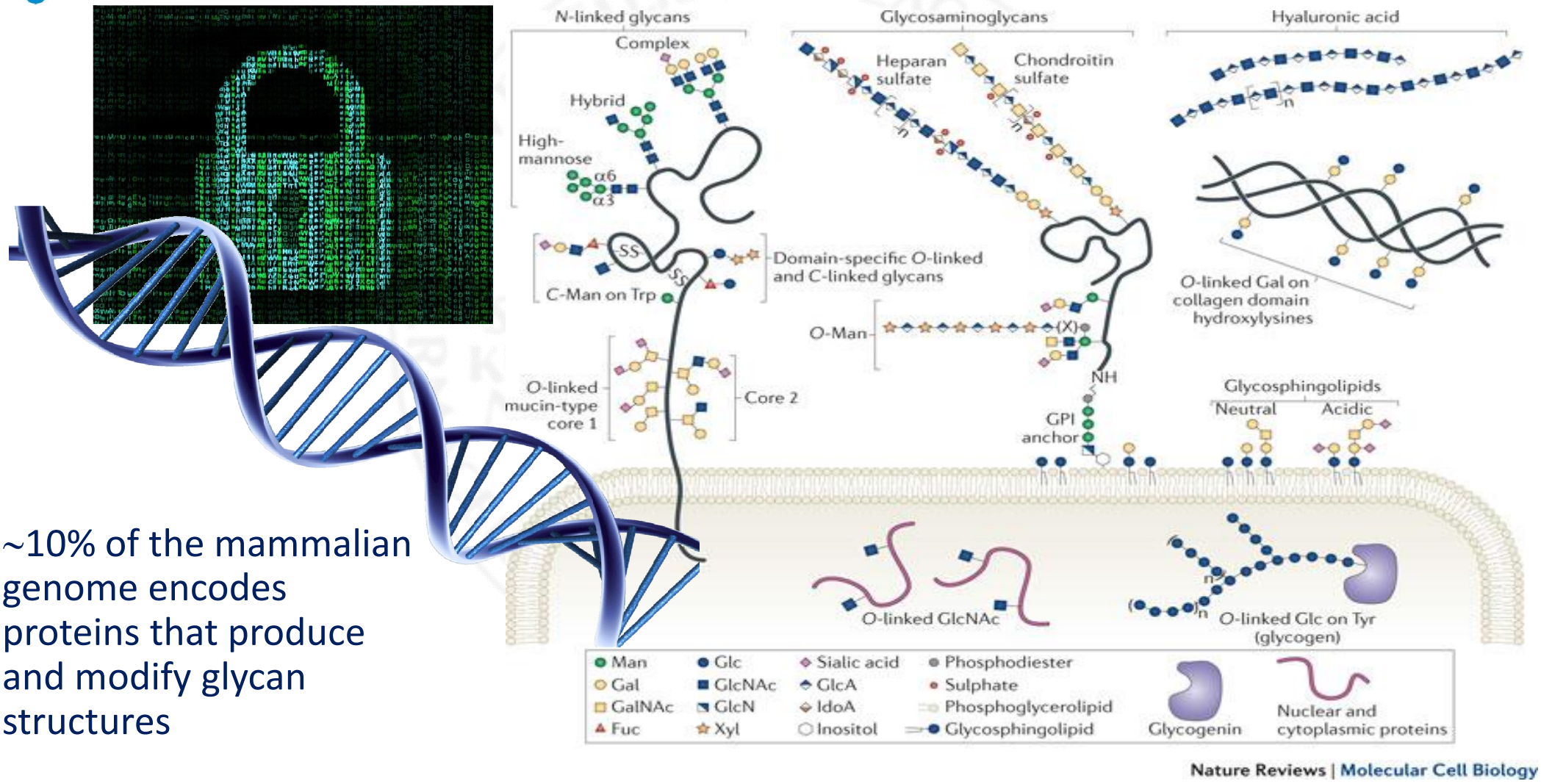
Zeng X. et al., 2012, *Anal. Bioanal. Chem.*

Glycoconjugates



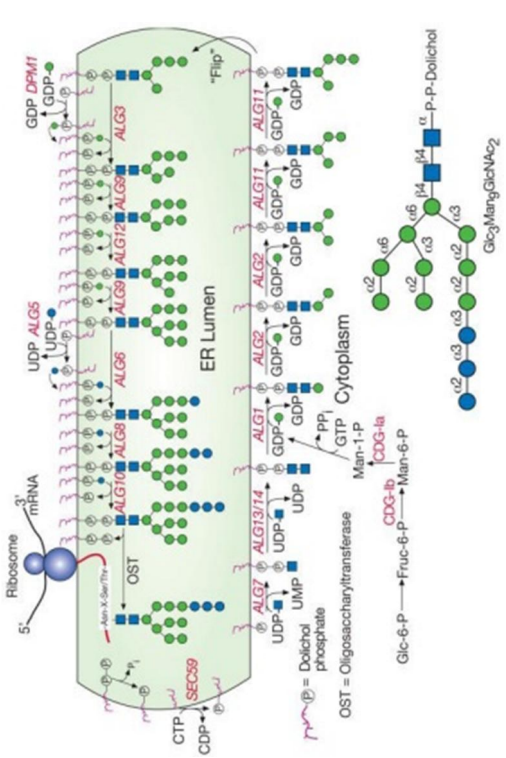


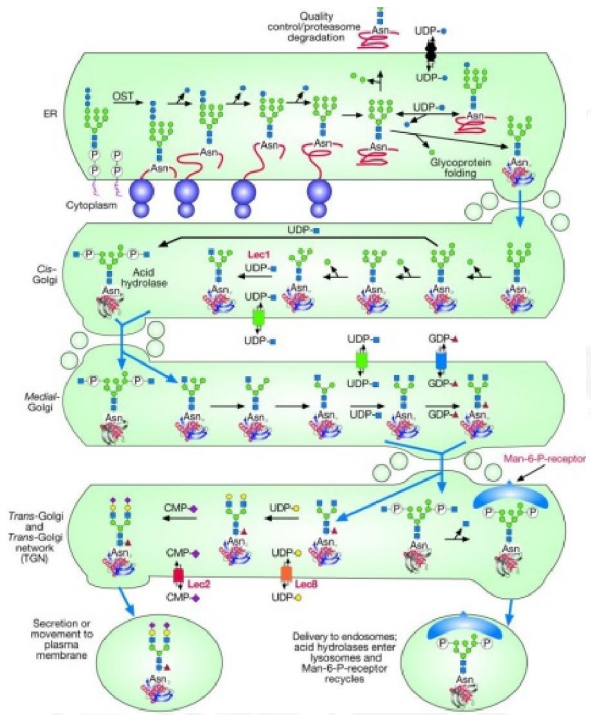
Glycans are encrypted throughout the genome

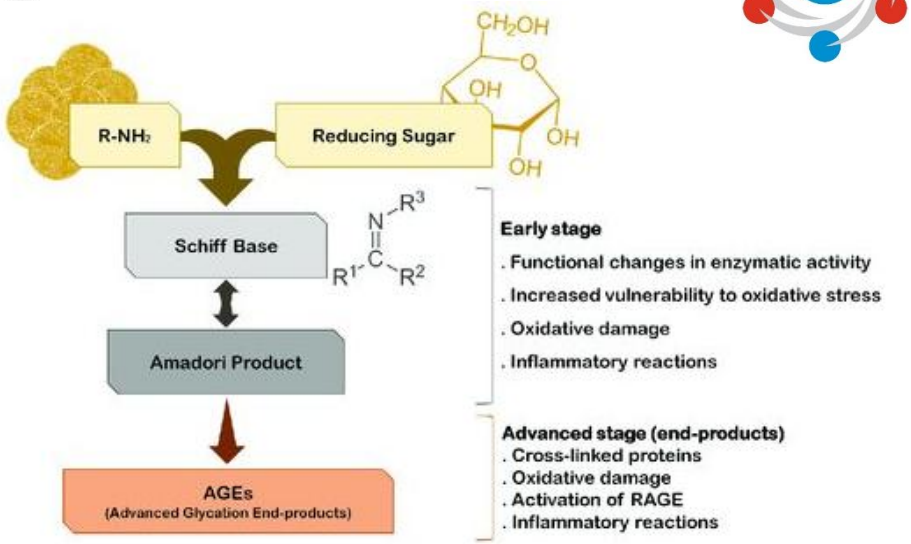


~10% of the mammalian genome encodes proteins that produce and modify glycan structures

Nature Reviews | Molecular Cell Biology







GLYCOSYLATION

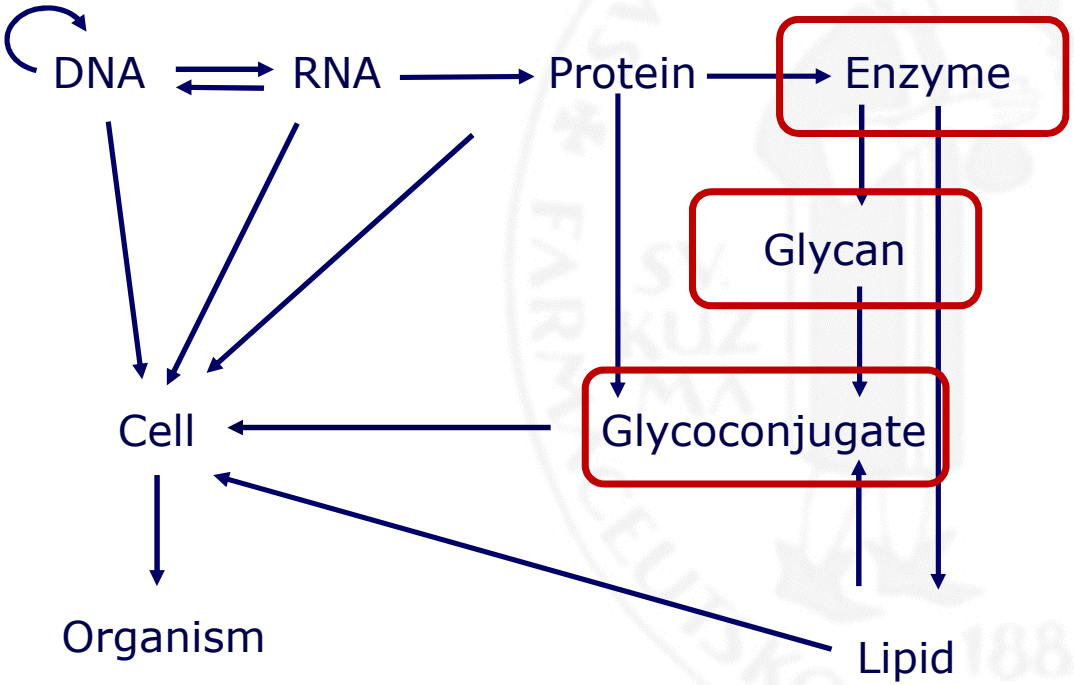
- **complex**, highly **specific** and strictly **regulated** co-translational process
- covalent bonding of **complex sugar structures** on proteins and lipids
- occurs in **ER** and **Golgi** apparatus
- mediated by **enzymes and transporters**
- numerous important **physiological roles**

≠

GLYCATION

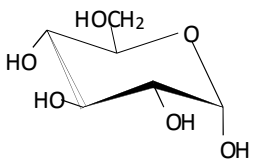
- **random** mechanism
- the reducing ends of **free sugars** (glucose, fructose, galactose) covalently attach to proteins, creating glycated products
- occurs in the **bloodstream**
- **impairs** protein function and stability
- consequence of *diabetes mellitus*

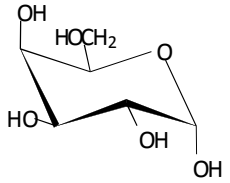
Revised dogma of molecular biology

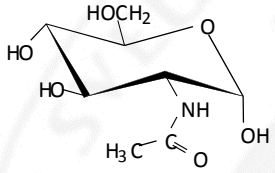


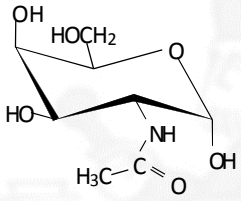
- glycan structures are characteristic for each cell, tissue, organism (GLYCOME)
- there is no template for glycan synthesis
- gycan structure depends on enzyme expression and activity, substrate specificity and availability of the precursors

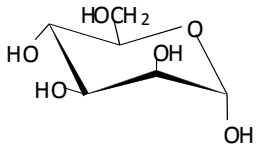
Sources of glycan diversity

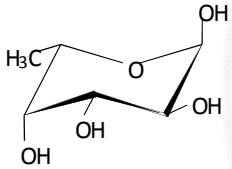

α-D-Glucose (Glc)

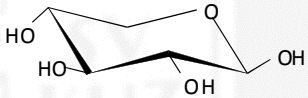

α-D-Galactose (Gal)

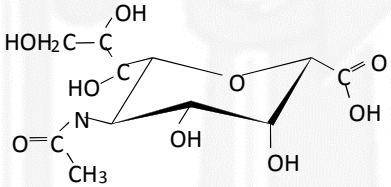

N-Acetyl-α-D-glucosamine (GlcNAc)


N-Acetyl-α-D-galactosamine (GalNAc)


α-D-Mannose (Man)


α-L-Fucose (Fuc)

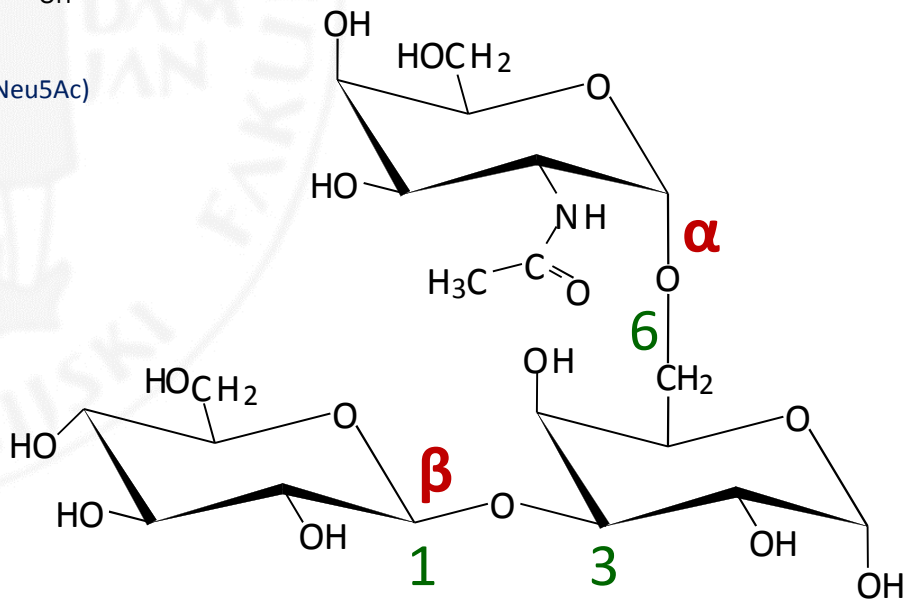

β-D-Xylose (Xyl)


N-Acetylneuraminic acid (Neu5Ac)

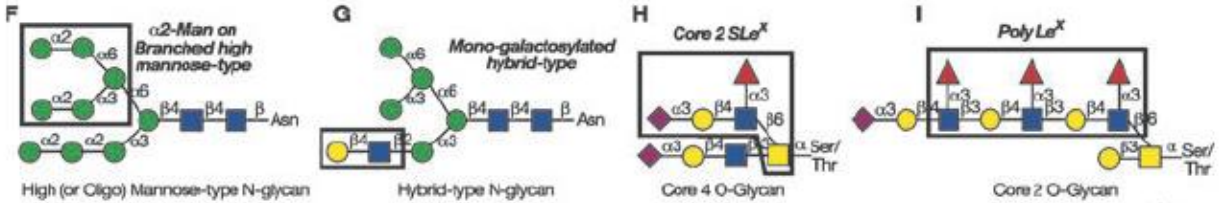
D-Glucuronic acid
L-Iduronic acid
D-Arabinose
D- & L-Rhamnose
D-Galacuronic acid
L-Arabinofuranose
D-Galactofuranose
N-Acetylmannosamine
3-N-Acetyl-D-quinovosamine
Neu5Gc
and a few more including their derivatives

pentamer ABCDE	nuber of isomers
oligosaccharides	**2 144 640**
oligopeptides	**120**

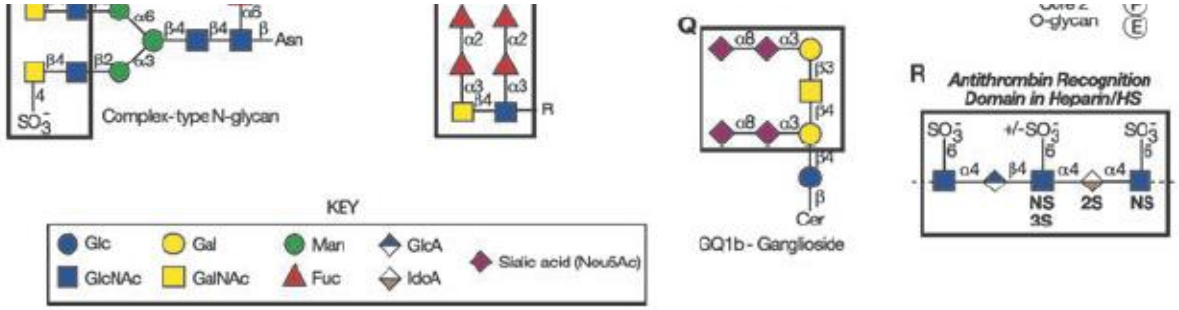
1. Monosaccharide sequence
2. Glycosidic bond position
3. Anomeric configuration (α or β) of glycosidic linkage
4. Number of branching points
5. Position of branching



At least 2000 different glycan determinants are being attached to polypeptide backbones



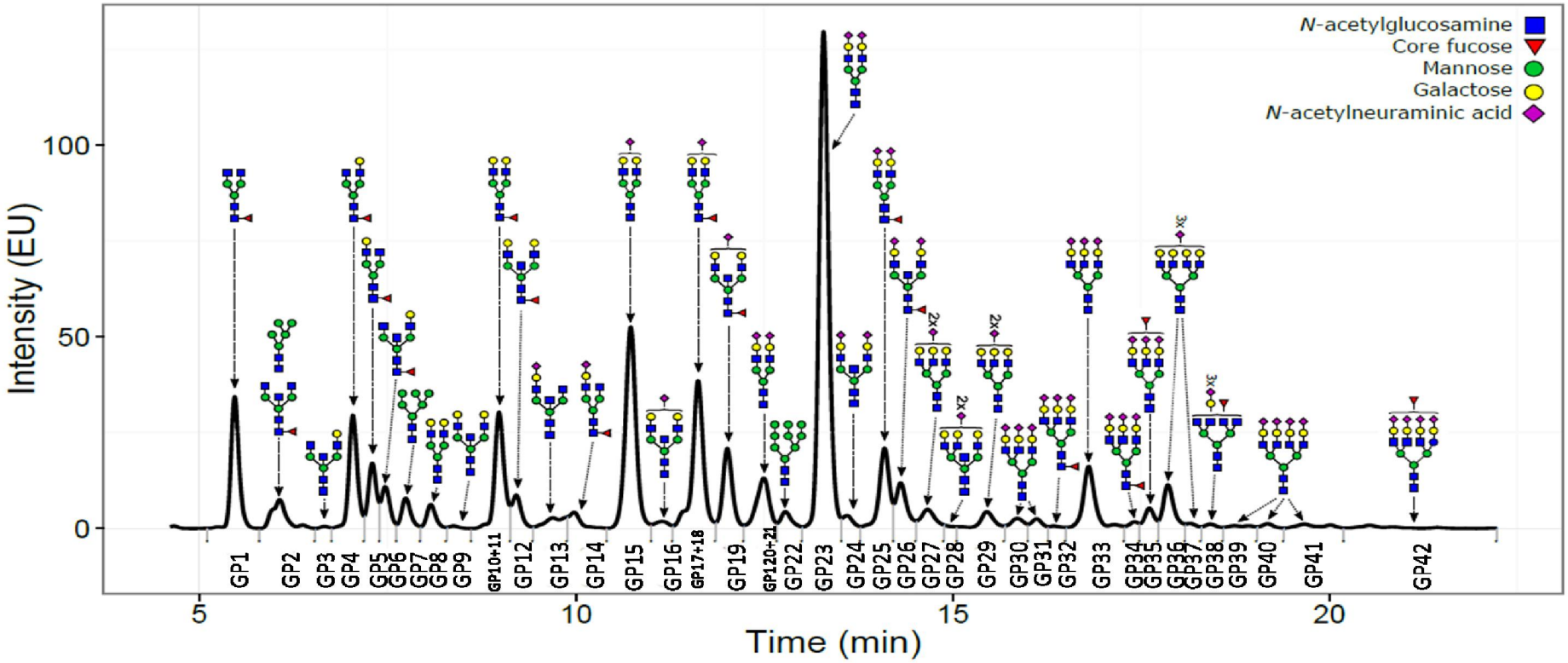
Glycoproteome is several orders of magnitude more complex than the proteome



Problems in glycome solving

- Complex, unlinear structures
- There's no template for their synthesis
- "Assembly-line" system, which includes hundreds of gene products
- Numerous glycosylation sites on which different glycans are attached
- Specific cell, tissue, organ glycosylation patterns, dependent on the state and the activity of the system
- There is no why to synthesize lager amount of glycans *in vitro*
- It is not possible to change specific glycan/glycoconjugate
- Lack of specific, sensitive and user-friendly techniques for glycan analysis

Plasma *N*-glycans separated by HILIC-UPLC

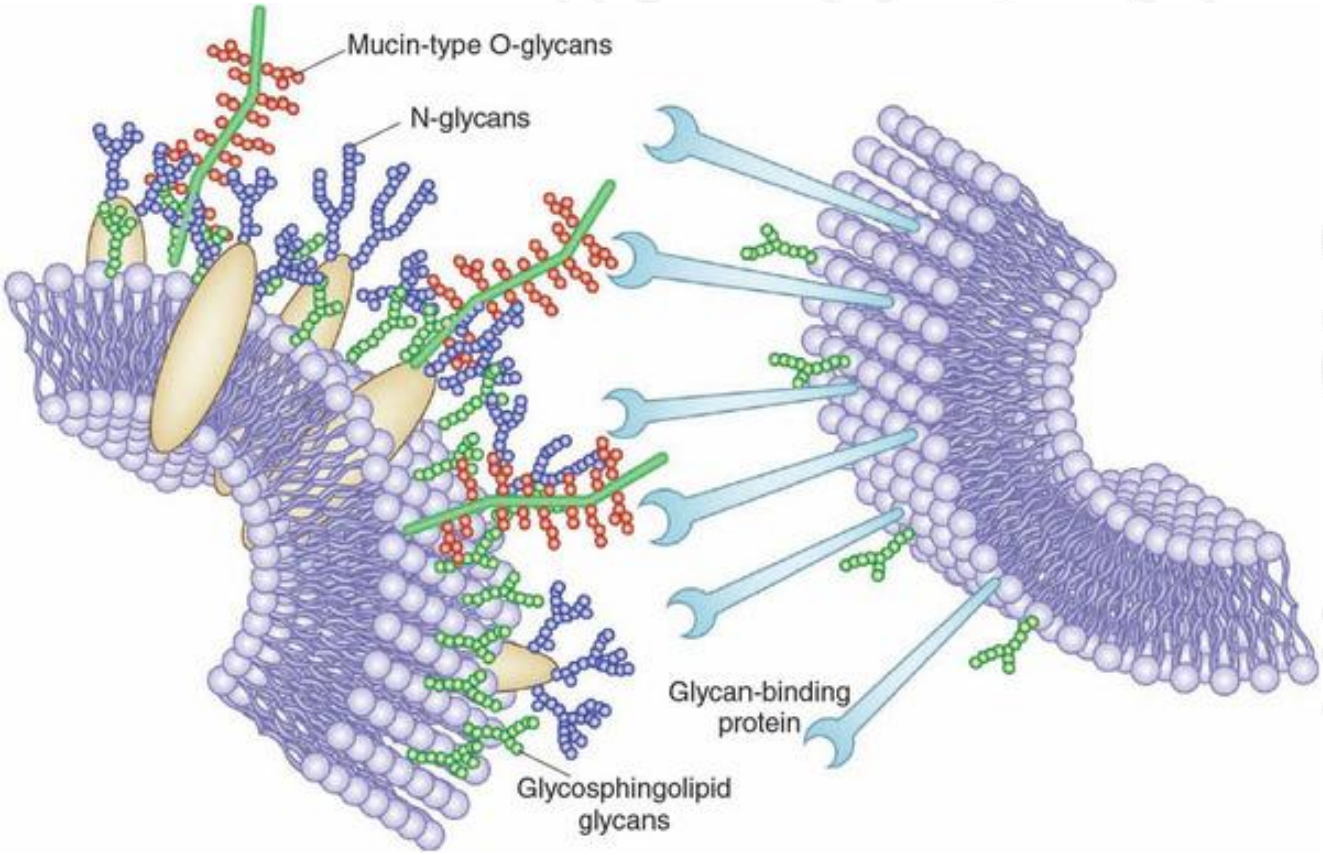


Gordan Lauc's group / University of Zagreb Faculty of Pharmacy and Biochemistry

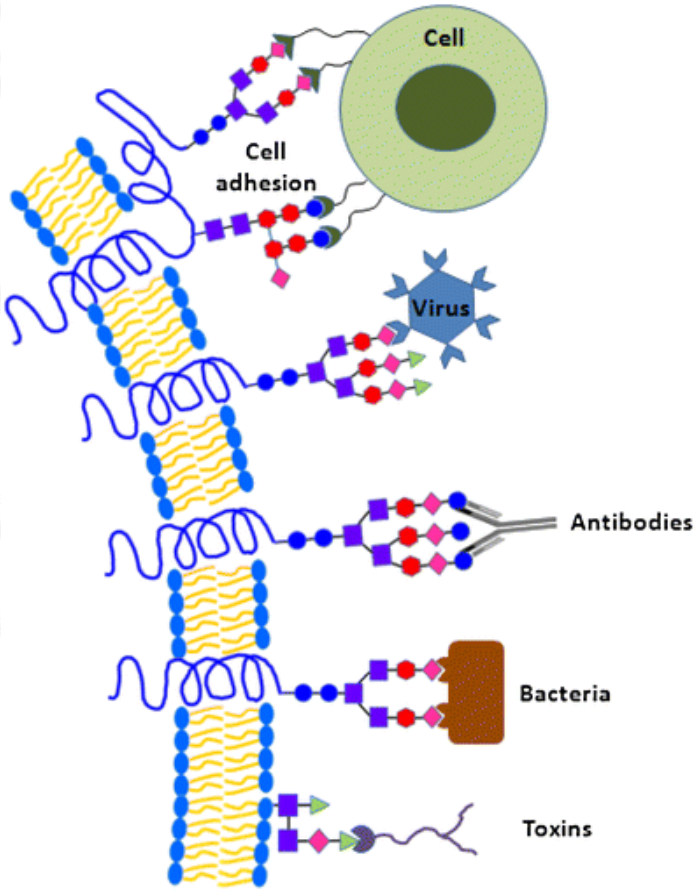
Changes in plasma N-glycom and/or IgG glycosylation

- Aging
- Acute systemic inflammation
- Higher risk of type 2 diabetes
- Features of Inflammatory Bowel Diseases
- higher risk for future diagnosis of rheumatoid arthritis
- Major depressive disorder symptom severity and the antidepressant response
- Colorectal cancer (prognosis)
- HIV persistence during antiretroviral therapy
- Chronic obstructive pulmonary disease (COPD)
- N-glycans discriminate HNF1A-MODY (Maturity onset diabetes of the young) from other subtypes of diabetes
- …

Cell surface glycans serve as points of attachment for other cells, bacteria, viruses, toxins, hormons, *etc*.



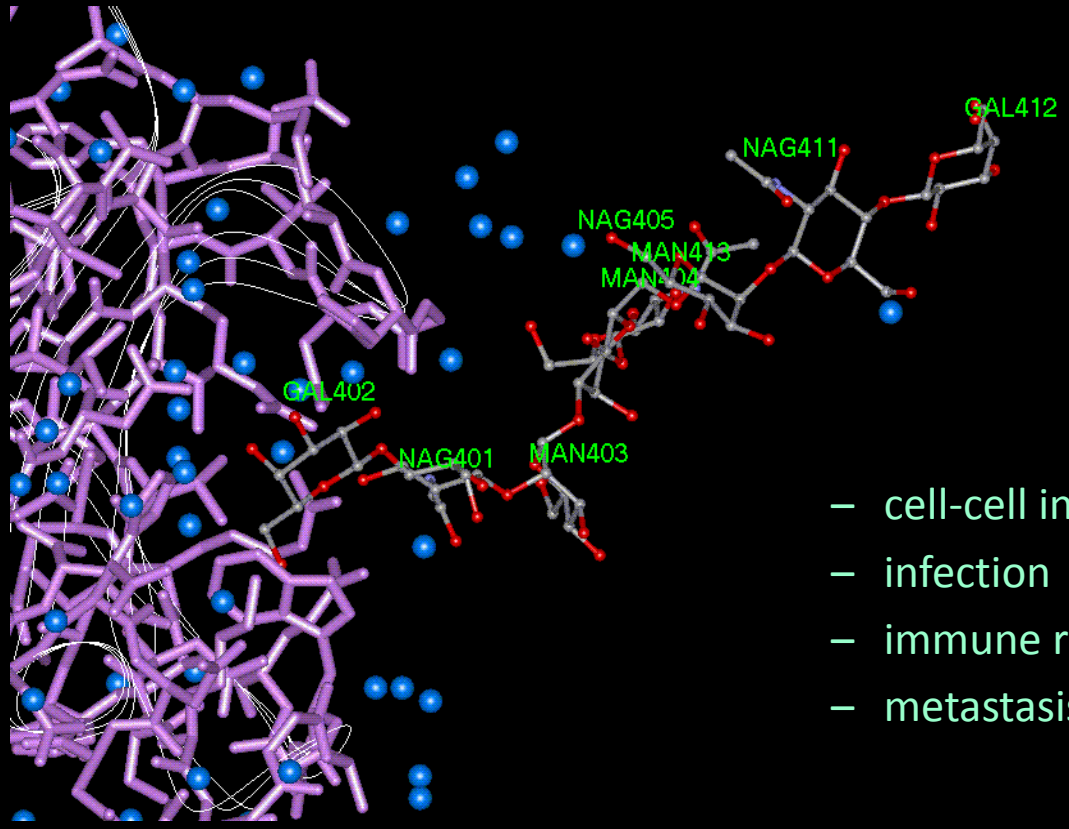
Zaia J, 2011, *Nature Met.*



Zeng X. et al., 2012, *Anal. Bioanal. Chem.*

LECTINS

- “interpreters” of biological informations stored in oligosaccharide structures of glycans

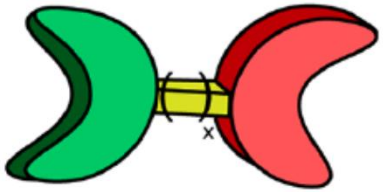


- cell-cell interactions
- infection
- immune reactions
- metastasis formation

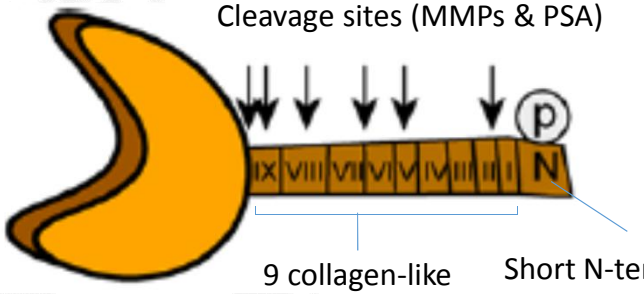
interaction of galectin-3 CRD and βGal14βGlcNAc12αMan13(βGal14βGlcNAc12αMan16)βMan14GlcNAc

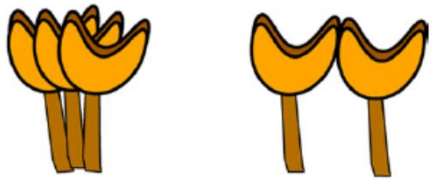
Galectins

proto-type
galectins-1, -2, -5, -7,
-10, -11, -13, -14, - 15



tandem-repeat-type
galectins-4, -6,
-8, -9, -12

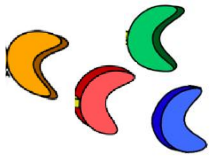




Ability of aggregation
via the N-terminal tail, the CRD, or both

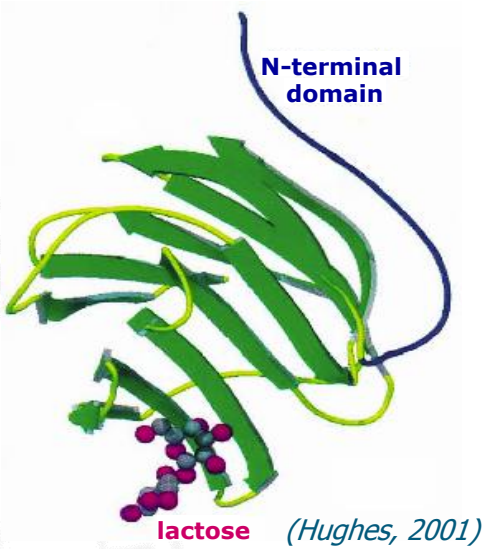
chimera-type
galectin-3

de Jong ZGHM *et al*, 2019, *Cell Mol Life Sci*



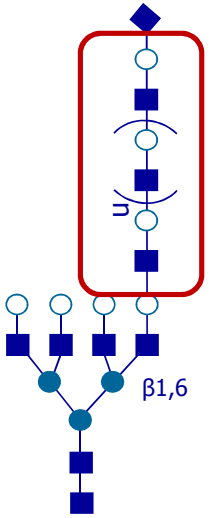
CRD – Carbohydrate Recognition Domain (that specifically recognises β-galactoside structures)

Galectin-3



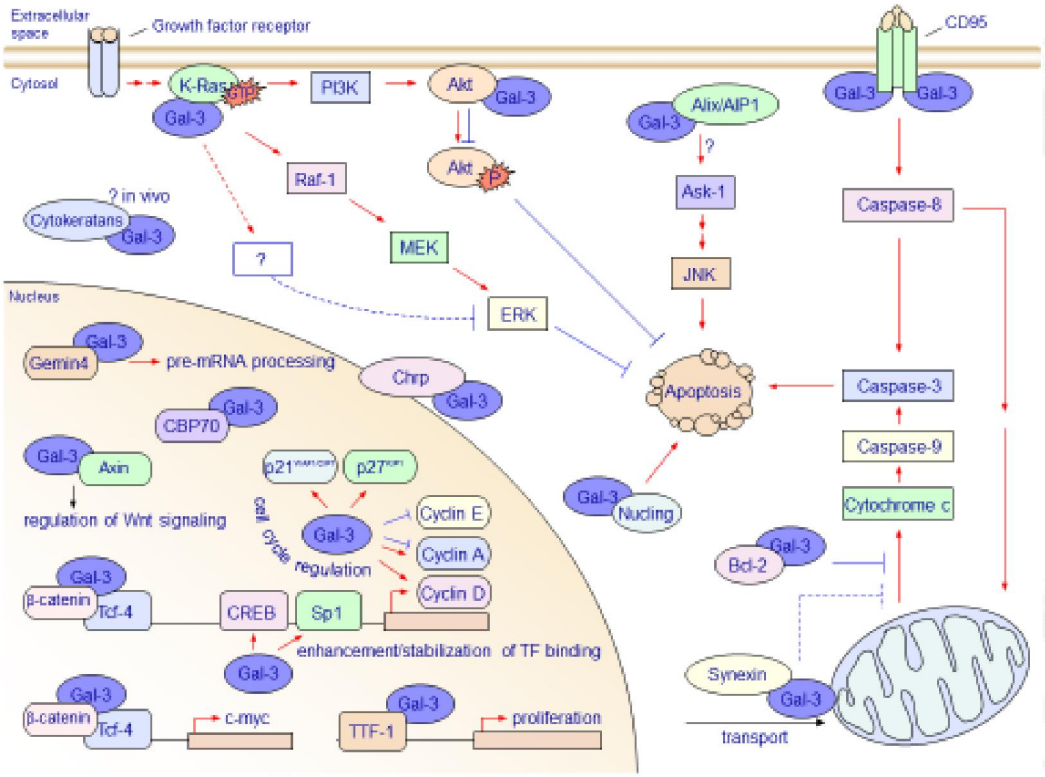
(Hughes, 2001)

- One of 15 galectin family members
- Monomer, Mr 26200-30300, 249 aa
- N-terminal and 9 rich Gly/Pro (collagen-like) domain
- C-terminal carbohydrate binding domain
- Preferentially binds poly-LacNAc chains
- *LGALS3* gene (chromosome 14, locus q21-q22, 17 kb, 6 exons, 5 introns)
- Present in almost all cellular compartments depending on cell type and in extracellular space

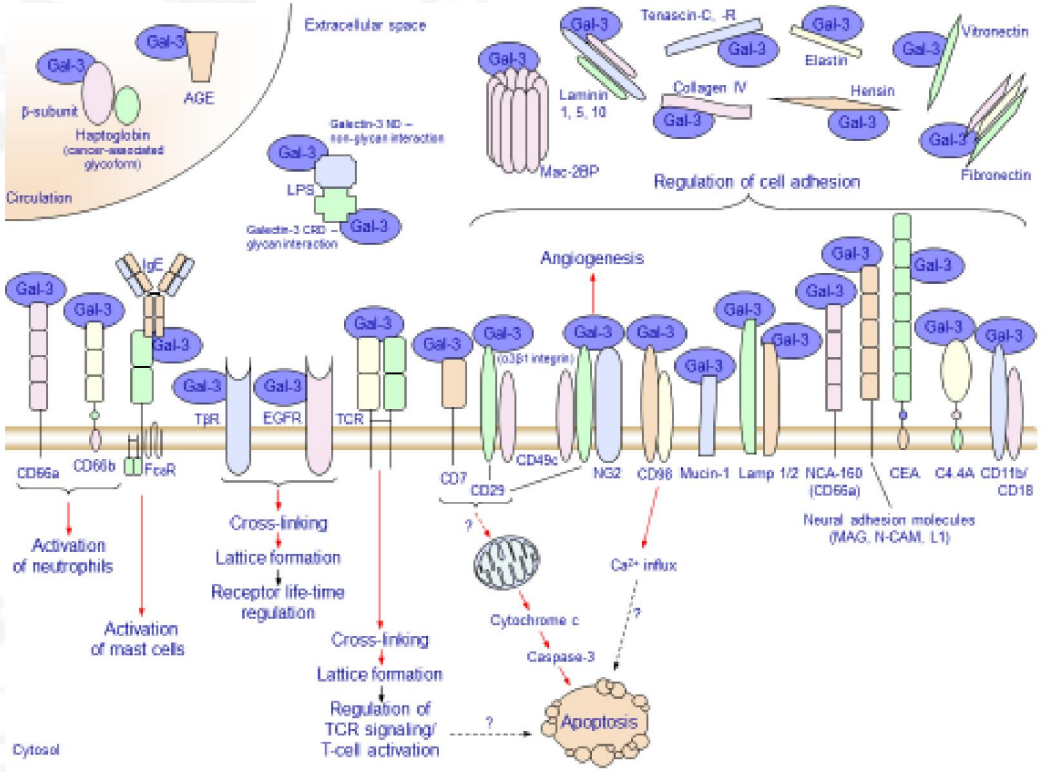


Galectin-3: A protein of 1001 function

Intracellular functions of galectin-3

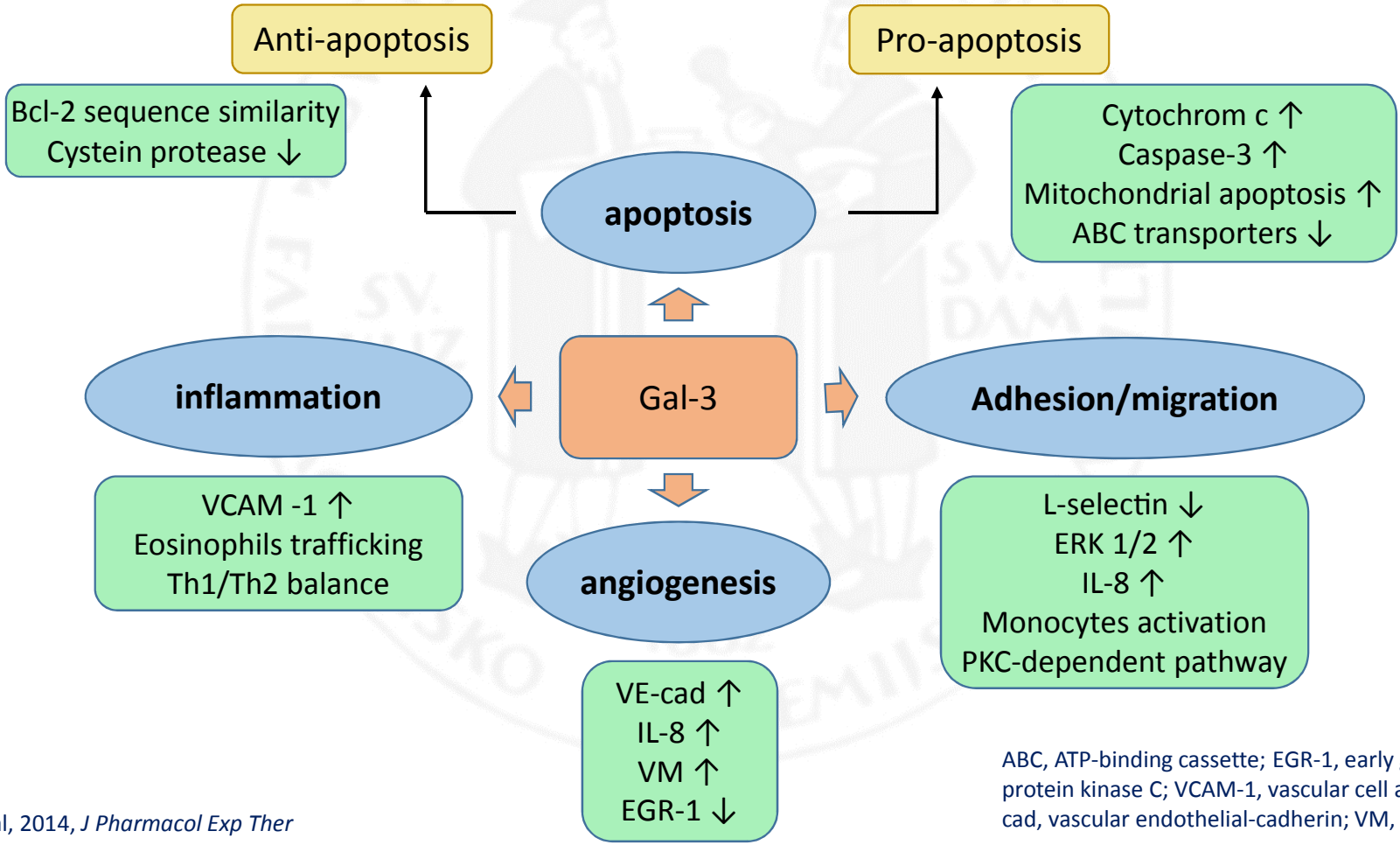


Extracellular functions of galectin-3



Dumić J et al, 2006, *BBA Gen Subj*

The role of Gal-3 in cell apoptosis, adhesion, migration, angiogenesis and inflammation

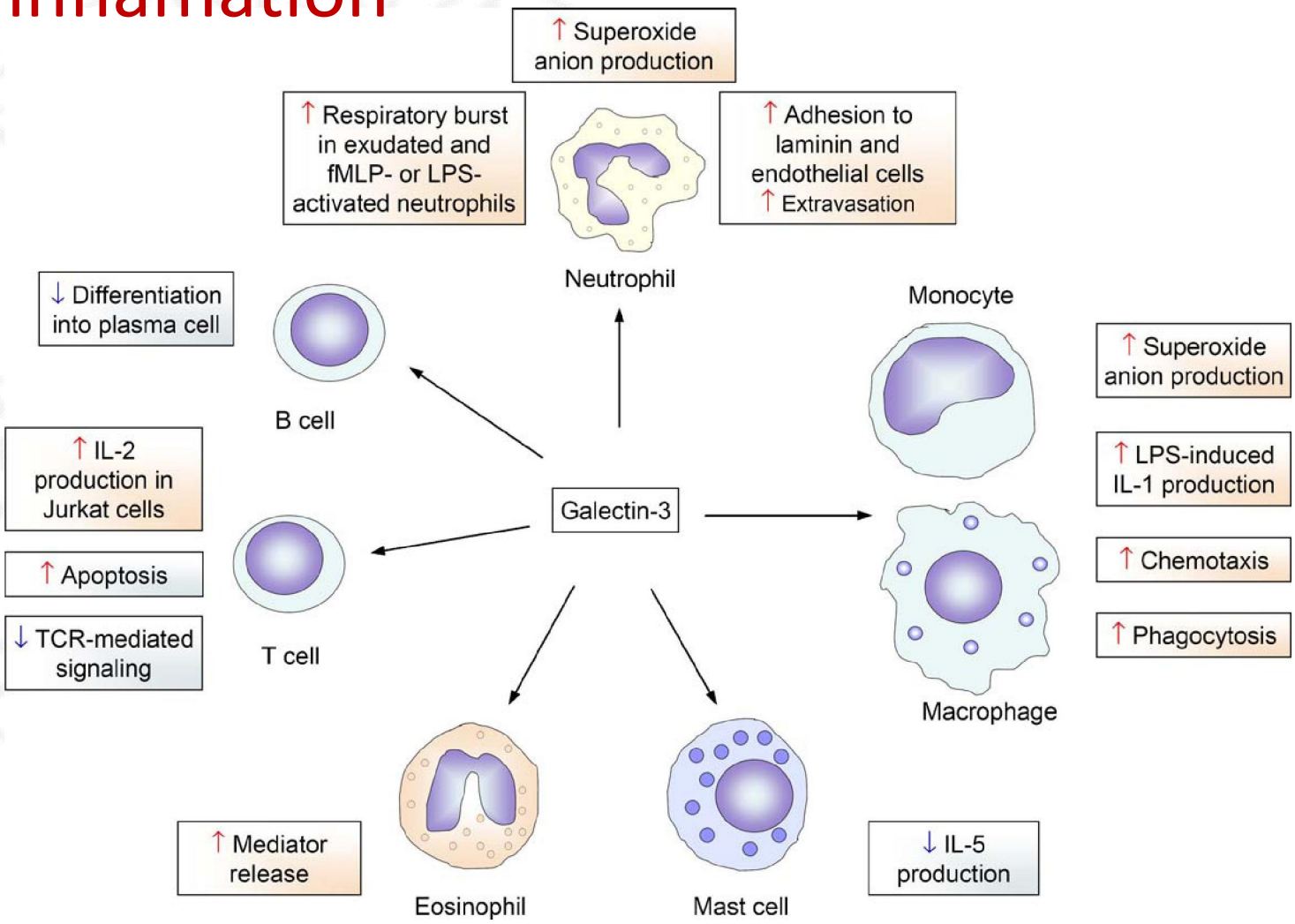


adopted from Li L.-Ch. et al, 2014, *J Pharmacol Exp Ther*

ABC, ATP-binding cassette; EGR-1, early growth response-1; PKC, protein kinase C; VCAM-1, vascular cell adhesion molecule-1; VE-cad, vascular endothelial-cadherin; VM, vasculogenic mimicry

Galectin-3 in inflamation

- Gal-3 - a regulatory molecule acting at various stages along the continuum from acute inflammation to chronic inflammation and tissue fibrogenesis



Dumić J et al. 2006, *BBA Gen Subj*

STRESS
Stress
The International Journal on the Biology of Stress
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Galectin-3 Decreases in Mice Exposed to Immobilization Stress

Jerka Dumić, Karmela Barišić, Mirna Flögel & Gordan Lauc

Acta Clin Croat 2017; 56:673-680 Original Scientific Paper
doi: 10.20471/acc.2017.56.04.14

ASSOCIATION OF PENTRAXIN-3, GALECTIN-3 AND MATRIX METALLOPROTEINASE-9/TIMP-1 WITH CARDIOVASCULAR RISK IN RENAL DISEASE PATIENTS

Milica Miljković[1], Aleksandra Stefanović[1], Nataša Bogavac-Stanojević[1], Sanja Simić-Ogrizović[2], Jerka Dumić[3], Darko Černe[4], Zorana Jelić-Ivanović[1] and Jelena Kotur-Stevuljević[1]

Transfer to *in vitro* Conditions Influences Expression and Intracellular Distribution of Galectin-3 in Murine Peritoneal Macrophages

Jerka Dumić[a], Gordan Lauc[a,*], Mirko Hadžija[b] and Mirna Flögel[a]

Z. Naturforsch. **55c**, 261–266 (2000); received November 25/December 27, 1999

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Biochimica et Biophysica Acta 1760 (2006) 701 – 709
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Galectin-3 in macrophage-like cells exposed to immunomodulatory drugs

Sanja Dabelic *, Sandra Supraha, Jerka Dumic

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ISSN 1330-9862
original scientific paper

Curcumin – A Potent Inhibitor of Galectin-3 Expression

Jerka Dumić, Sanja Dabelić and Mirna Flögel*

CROATICA CHEMICA ACTA
CCACAA 78 (3) 433–440 (2005)
ISSN-0011-1643
CCA-3032
Original Scientific Paper

Effects of Aspirin and Indomethacin on Galectin-3*

Sanja Dabelić,** Mirna Flögel, and Jerka Dumić

Biochimica et Biophysica Acta 1820 (2012) 804–818
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journal homepage: www.elsevier.com/locate/bbagen
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BBA General Subjects

Galectin-3 endocytosis by carbohydrate independent and dependent pathways in different macrophage like cell types

Adriana Lepur [a,*], Michael C. Carlsson [a], Ruđer Novak [b], Jerka Dumić [b], Ulf J. Nilsson [c], Hakon Leffler [a,*]

In Vitro Cellular & Developmental Biology - Animal
September 2012, Volume 48, Issue 8, pp 518–527 | Cite as

Galectin-3 expression in response to LPS, immunomodulatory drugs and exogenously added galectin-3 in monocyte-like THP-1 cells

Authors Authors and affiliations
Sanja Dabelic, Ruder Novak, Sandra Supraha Goreta, Jerka Dumic

Original Paper

Cellular Physiology and Biochemistry

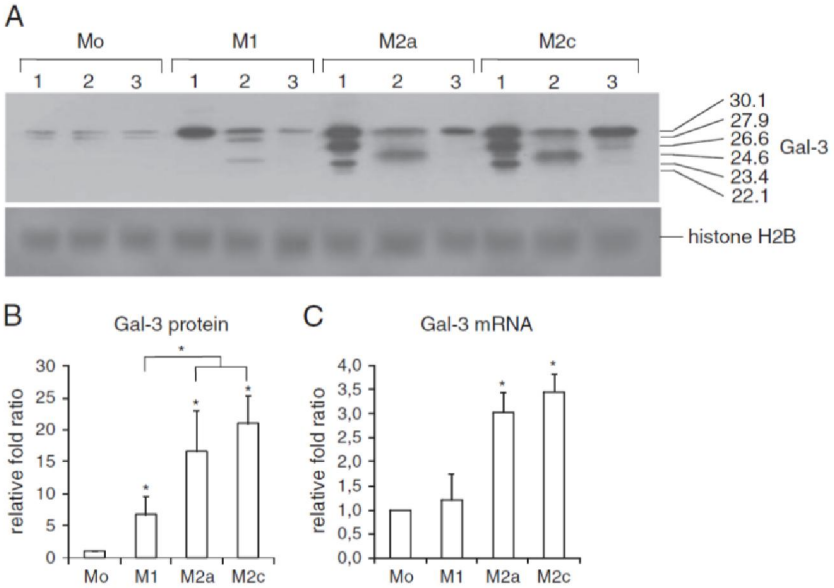
Cell Physiol Biochem 2000;10:149-158 Accepted: May 30, 2000

Expression of galectin-3 in cells exposed to stress - roles of Jun and NF-κB

Jerka Dumic, Gordan Lauc and Mirna Flögel

- EXPRESSION OF GAL-3 IS REGULATED BY TRANSCRIPTION FACTORS NF-κB & AP-1
- *LGALS3* (GAL-3 GENE) IS AN EARLY IMMEDIATE GENE

- GAL-3 IS INVOLVED IN ACTIVATION OF BOTH ALTERNATIVELY (M2) AND CLASICALLY (M1) ACTIVATED Mϕ
- MORE PRONOUNCED EXPRESSION IN M2c THAN IN M2a suggested ITS IMPORTANCE IN TISSUE REMODELING



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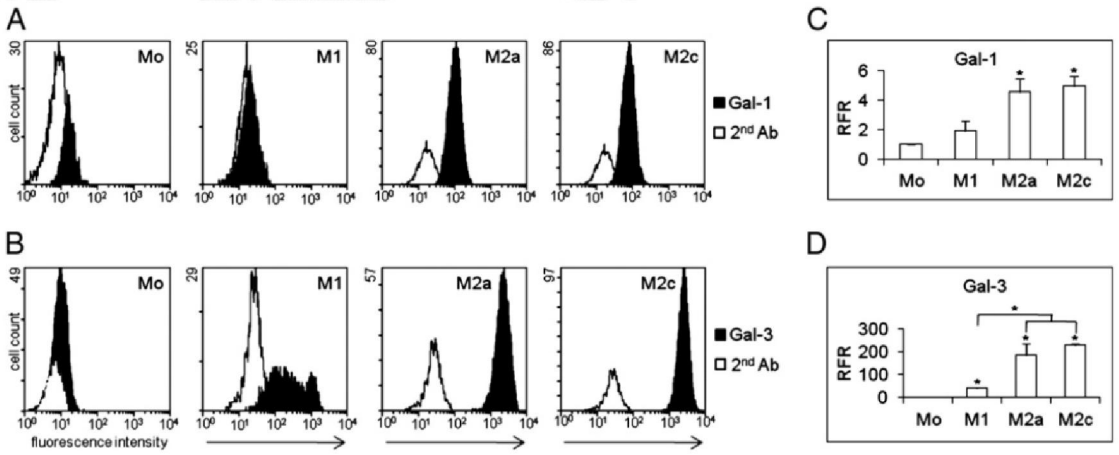
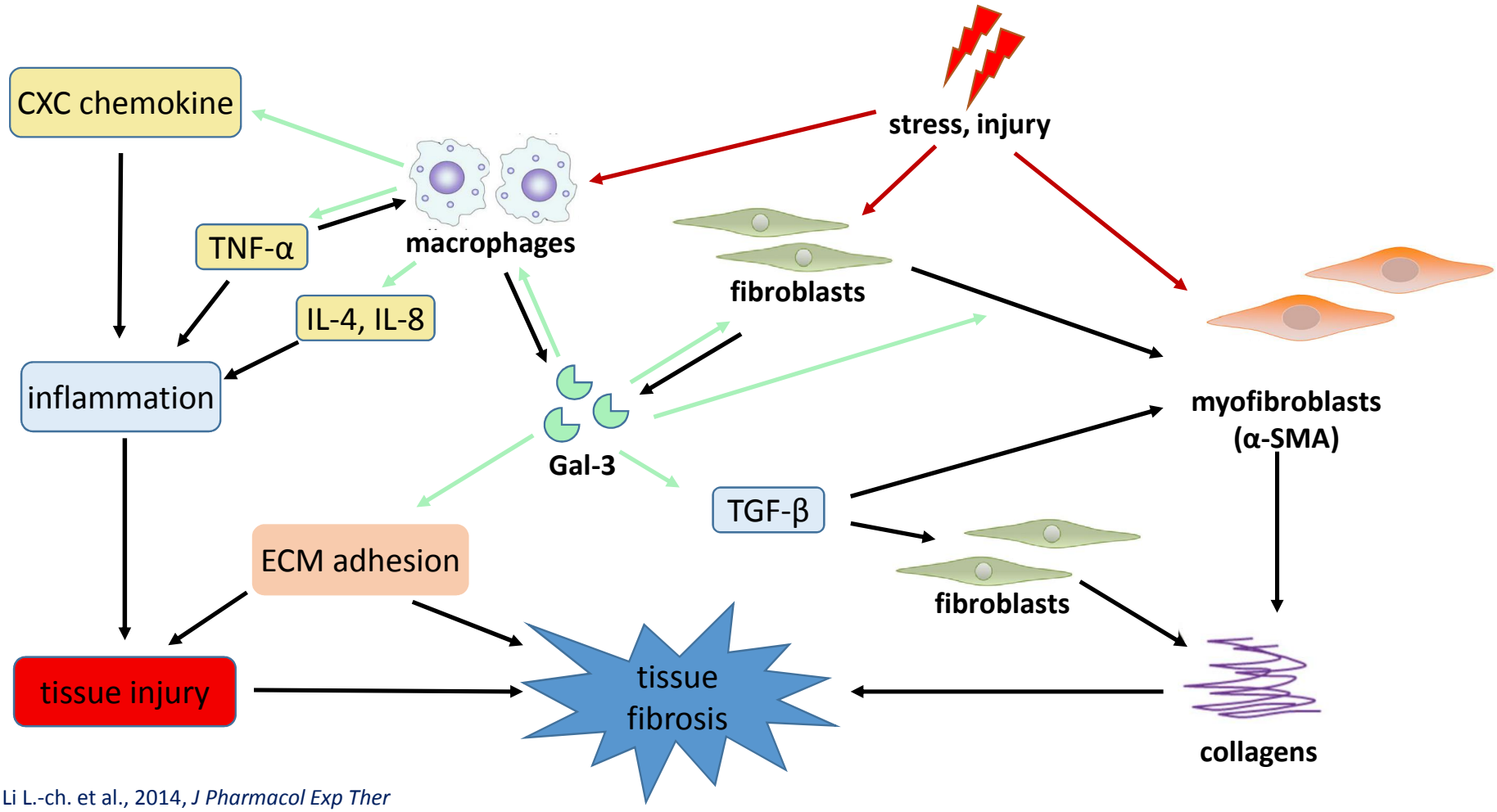
Galectin-1 and galectin-3 expression profiles in classically and alternatively activated human macrophages☆

Ruder Novak, Sanja Dabelic, Jerka Dumic *



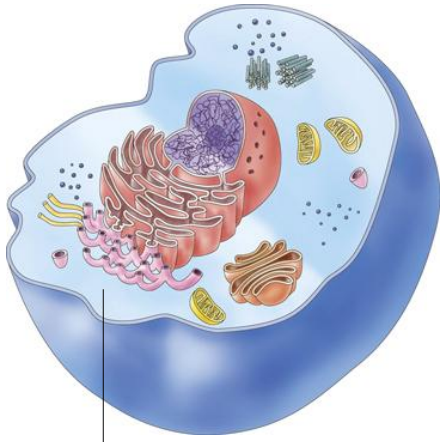
Ruđer Novak

The profibrotic network of Gal-3
secreted by macrophages and fibroblasts in tissue fibrosis



adapted from Li L.-ch. et al., 2014, *J Pharmacol Exp Ther*

Gal-3 is a critical participant in patogenesis of many inflammatory, fibrotic and neoplastic diseases



secreted Gal-3

Secreted in small amounts normally by a number of cells predominantly by macrophages

Bind to cell surface and matrix glycoconjugates

- Modulate cell signalling
- Promote cell-cell interactions
- Promote cell-matrix interactions

Markedly increased in

- Inflammation
- Fibrosis
- Cancer

GALECTIN-3 PROMOTES PATHOLOGY

It is most prominent galectin secreted in disease

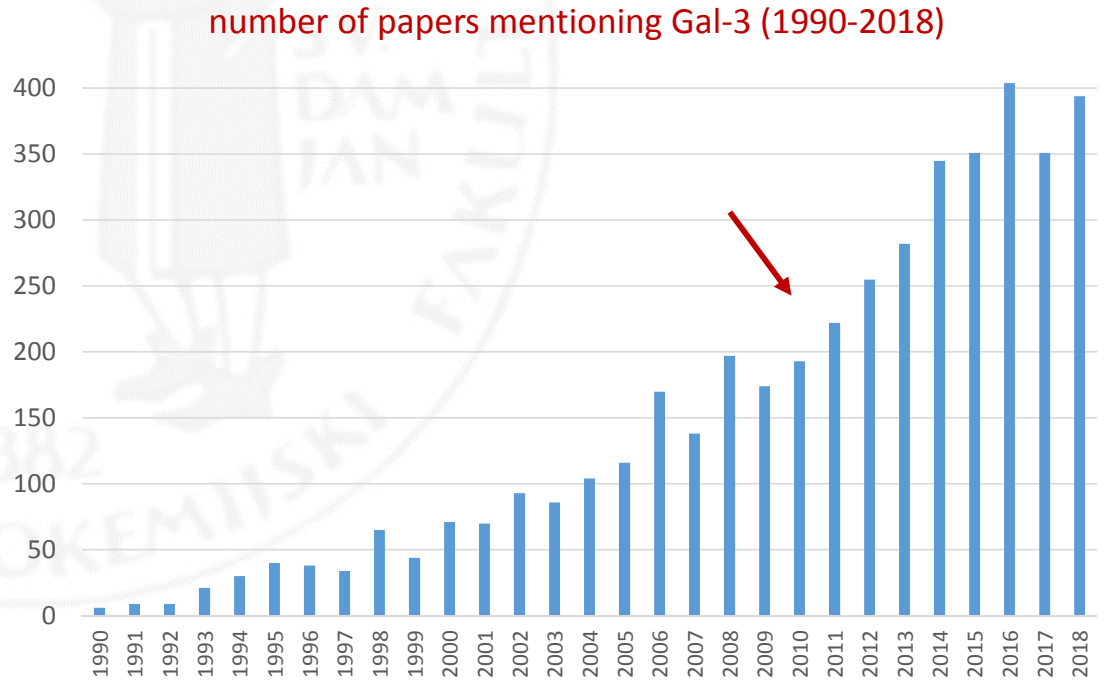
Clinical trials targeting Gal-3

Sponsor	Compound	Proposed target	Origin	Indication	Phase	NCT Number	Status
La Jolla Pharmaceuticals	GCS-100	Galectin-3*	Plant-based	Chronic Kidney Disease	1	NCT01717248	Completed (*n*=29)
				Chronic Kidney Disease	2	NCT00776802	Completed (*n*=120)
				Refractory Solid Tumors	1	—	Completed (*n*=24)
				Chronic Lymphocytic Leukemia	2	NCT00514696	Completed (*n*=24)
				Multiple Myeloma	1	NCT00609817	Terminated
				Diffuse Large B-cell Lymphoma	2	NCT01843790	Withdrawn
Galectin Therapeutics	DAVANAT (GM-CT-01)	Galectins-1&3*	Plant-based	Advanced Solid Tumors	1	NCT00054977	Completed (*n*=40)
				Melanoma	2	NCT01723813	Unknown
				Colorectal Cancer	2	NCT00388700	Withdrawn
				Gallbladder Cancer	2	NCT00386516	Withdrawn
				Colorectal Cancer	2	NCT00110721	Terminated
Galectin Therapeutics	GR-MD-02	Galectin-3*	Plant-based	Metastatic Melanoma	1	NCT02117362	Recruiting (*n*=22)
				NASH Advanced Fibrosis	1	NCT01899859	Completed (*n*=31)
				Melanoma, Lung and Head & Neck Cancer	1	NCT02575404	Recruiting (*n*=22)
				NASH Cirrhosis	2	NCT02462967	Completed (*n*=162)
				NASH Advanced Fibrosis	2	NCT02421094	Completed (*n*=30)
				Psoriasis	2	NCT02407041	Completed (*n*=10)
Massachusetts General Hospital	Modified Citrus Pectin	Galectin-3*	Plant-based	Osteoarthritis	3	NCT02800629	Recruiting (*n*=50)
				High Blood Pressure	3	NCT01960946	Recruiting (*n*=80)
Galecto Bio	TD139	Galectin-3	Synthetic chemistry	Idiopathic Pulmonary Fibrosis	2	NCT02257177	Complete (*n*=60)

Girard A & Magngiani JL, 2018 *Trends Glycosci & Glycotech*

Galectin-3

- First publication on Mac-2 in 1982 (Ho MK, Springer TA. J Immunol. 1982 Mar;128(3):1221-8)
- Until 1994 when the new nomenclature introduced (galectins) <100 scientific papers
- 1994 – 2019 – 3891 papers in PubMed
- 1994 – 2009 – 602 papers in PubMed
- In 2018 – 394 papers in PubMed
- In 2019 – 329 papers in PubMed



Circulation. 2004 Nov 9;110(19):3121-8. Epub 2004 Nov 1.

Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction.

Sharma UC[1], Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, André S, Crijns HJ, Gabius HJ, Maessen J, Pinto YM.

J Am Coll Cardiol. 2006 Sep 19;48(6):1217-24. Epub 2006 Aug 28.

Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure.

van Kimmenade RR[1], Januzzi JL Jr, Ellinor PT, Sharma UC, Bakker JA, Low AF, Martinez A, Crijns HJ, MacRae CA, Menheere PP, Pinto YM.

J Heart Lung Transplant. 2008 Jun;27(6):589-96. doi: 10.1016/j.healun.2008.02.018.

Plasma biomarkers of myocardial fibrosis and remodeling in terminal heart failure patients supported by mechanical circulatory support devices.

Milting H[1], Ellinghaus P, Seewald M, Cakar H, Bohms B, Kassner A, Körfer R, Klein M, Krahn T, Kruska L, El Banayosy A, Kramer F.

Clin Chim Acta. 2009 Nov;409(1-2):96-9. doi: 10.1016/j.cca.2009.09.001. Epub 2009 Sep 10.

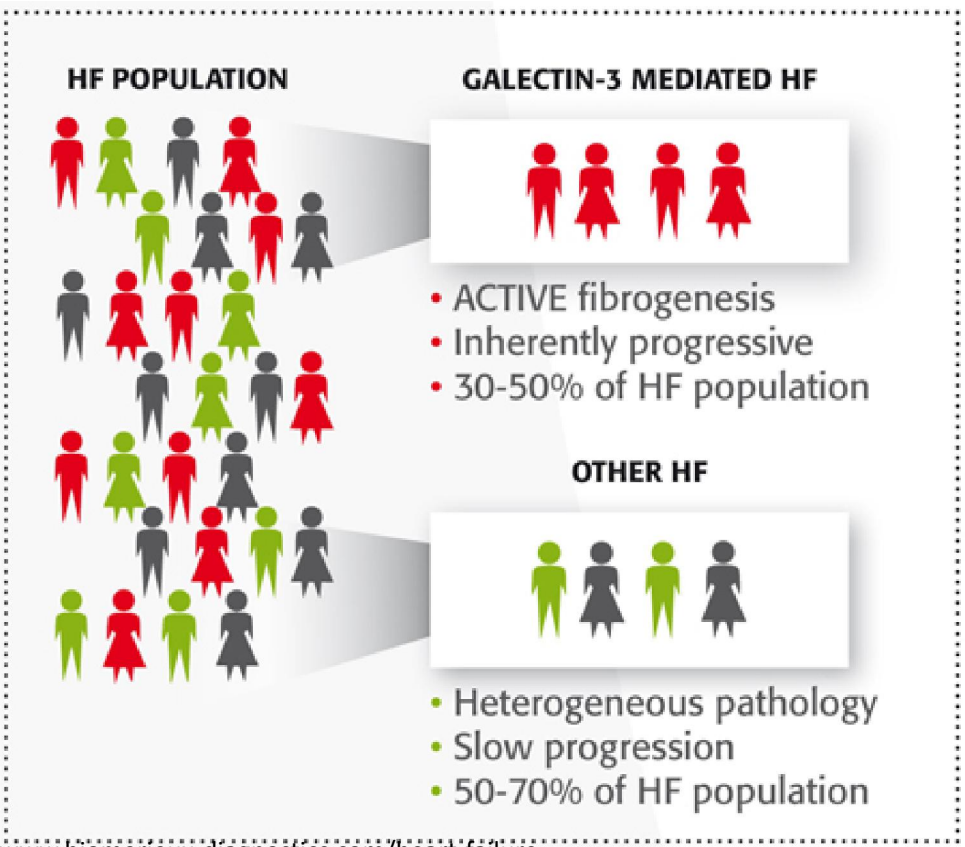
The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients.

Lin YH[1], Lin LY, Wu YW, Chien KL, Lee CM, Hsu RB, Chao CL, Wang SS, Hsein YC, Liao LC, Ho YL, Chen MF.

Galectin-3 - a biomarker of cardiovascular diseases, risk stratification, evaluation of therapy response and predictor of short-term and long-term prognosis

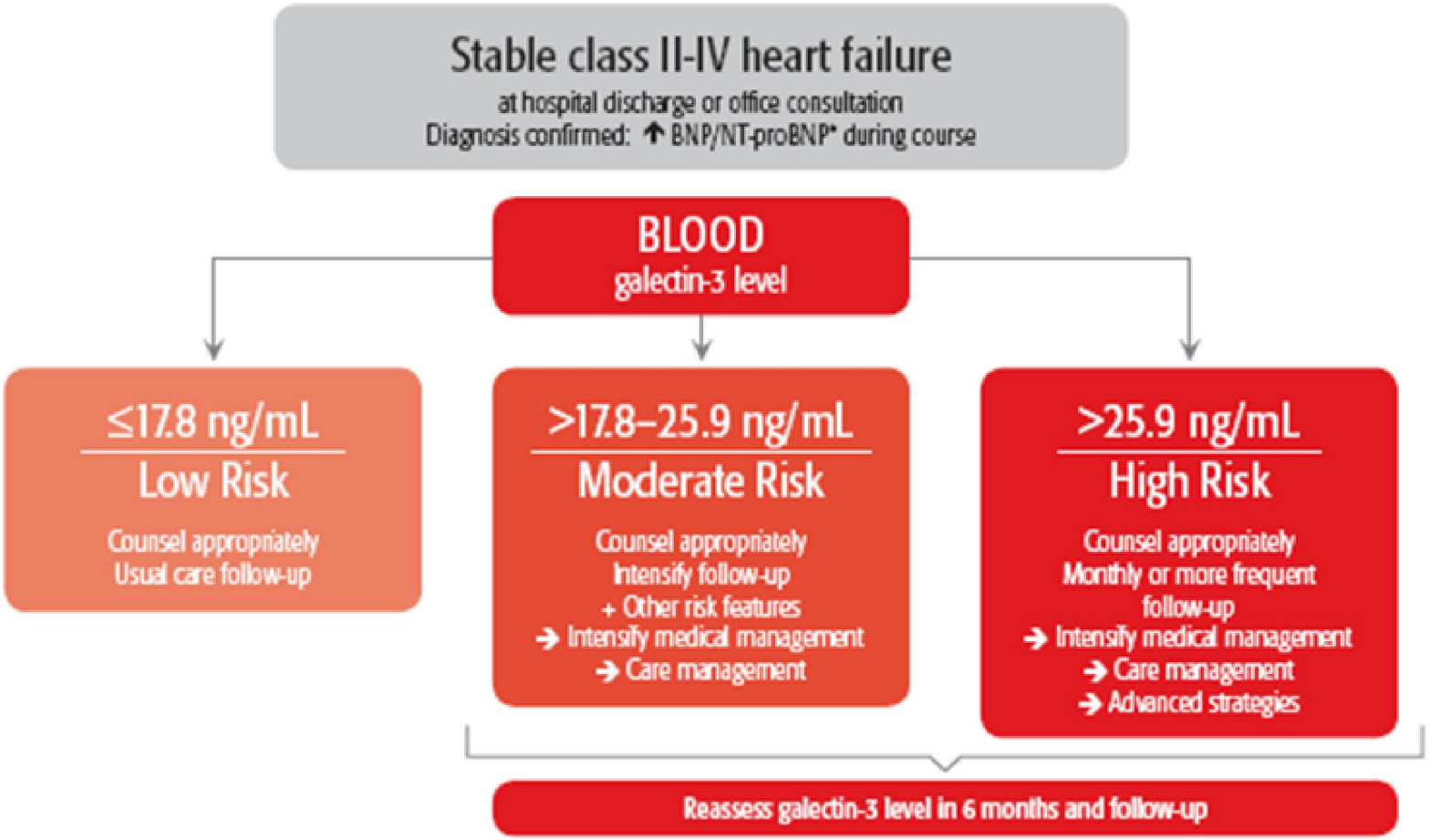
- 30-50% of heart failure patients have an inherently progressive form of the disease mediated by high levels of Gal-3
- measuring Gal-3 can provide information to optimize patient care decisions

- in 2013 the American College of Cardiology Foundation and the American Heart Association Guideline for the Management of Heart Failure recognized the role of Gal-3 testing



http://www.biomerieux-diagnostics.com/heart-failure

Galectin-3 levels & associated risks



McCullough PA *et al*, 2011, *Rev Cardiovasc Med.*

Galectin-3

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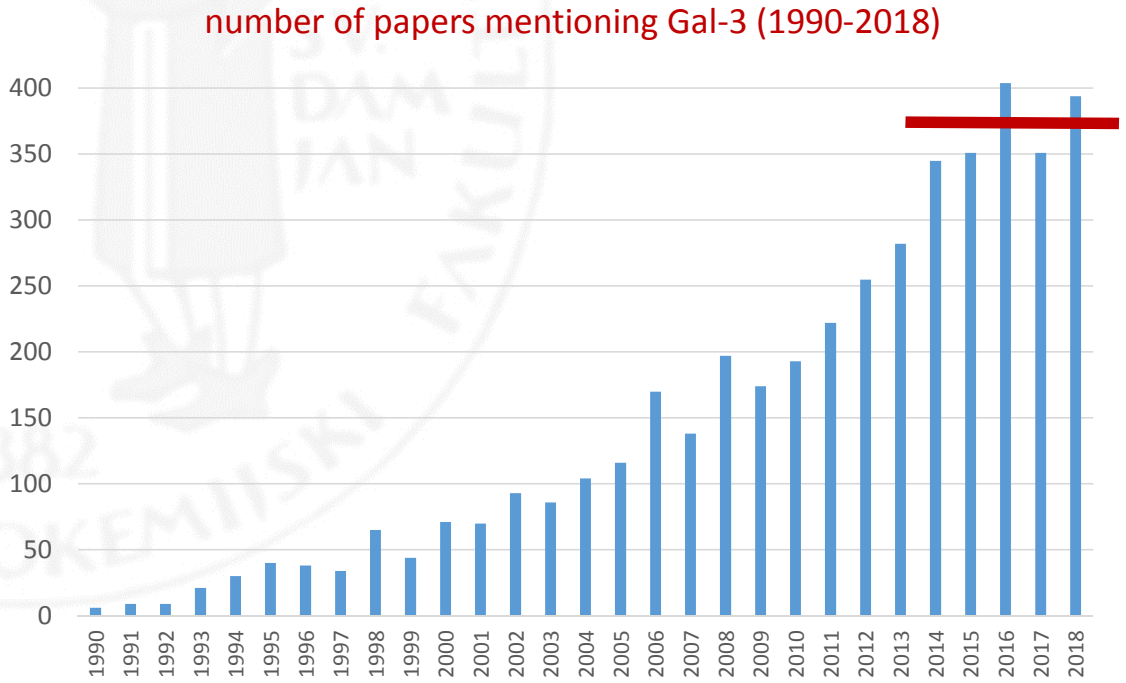
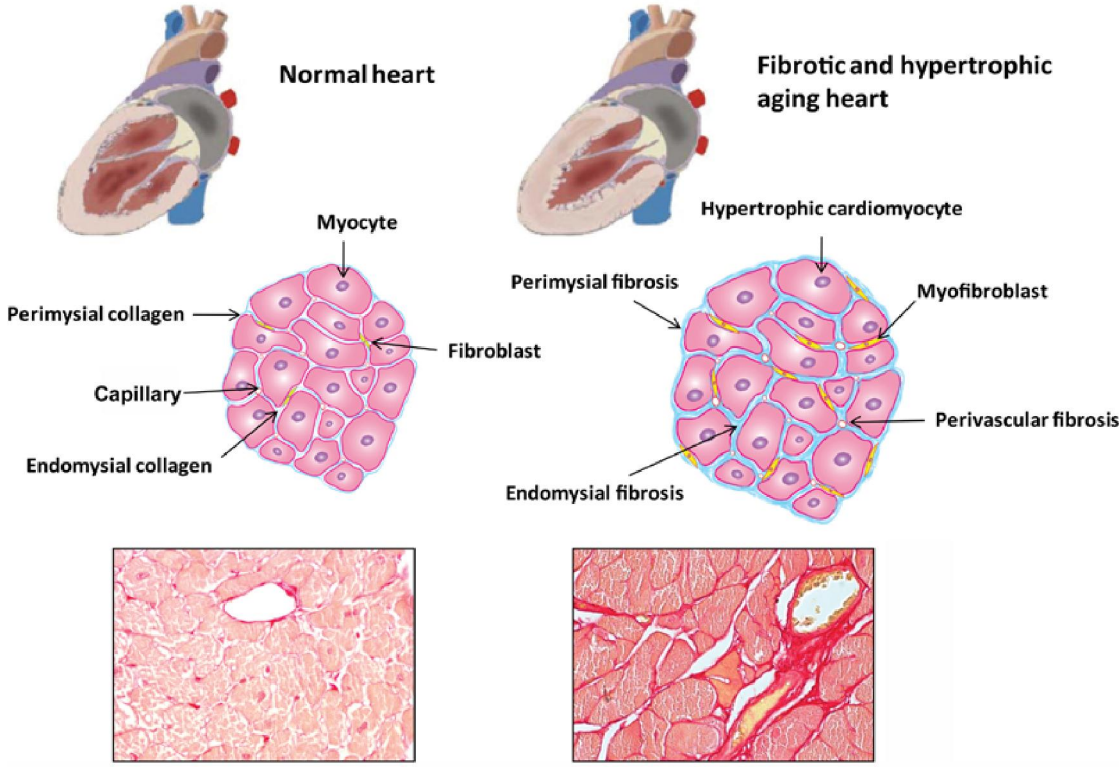
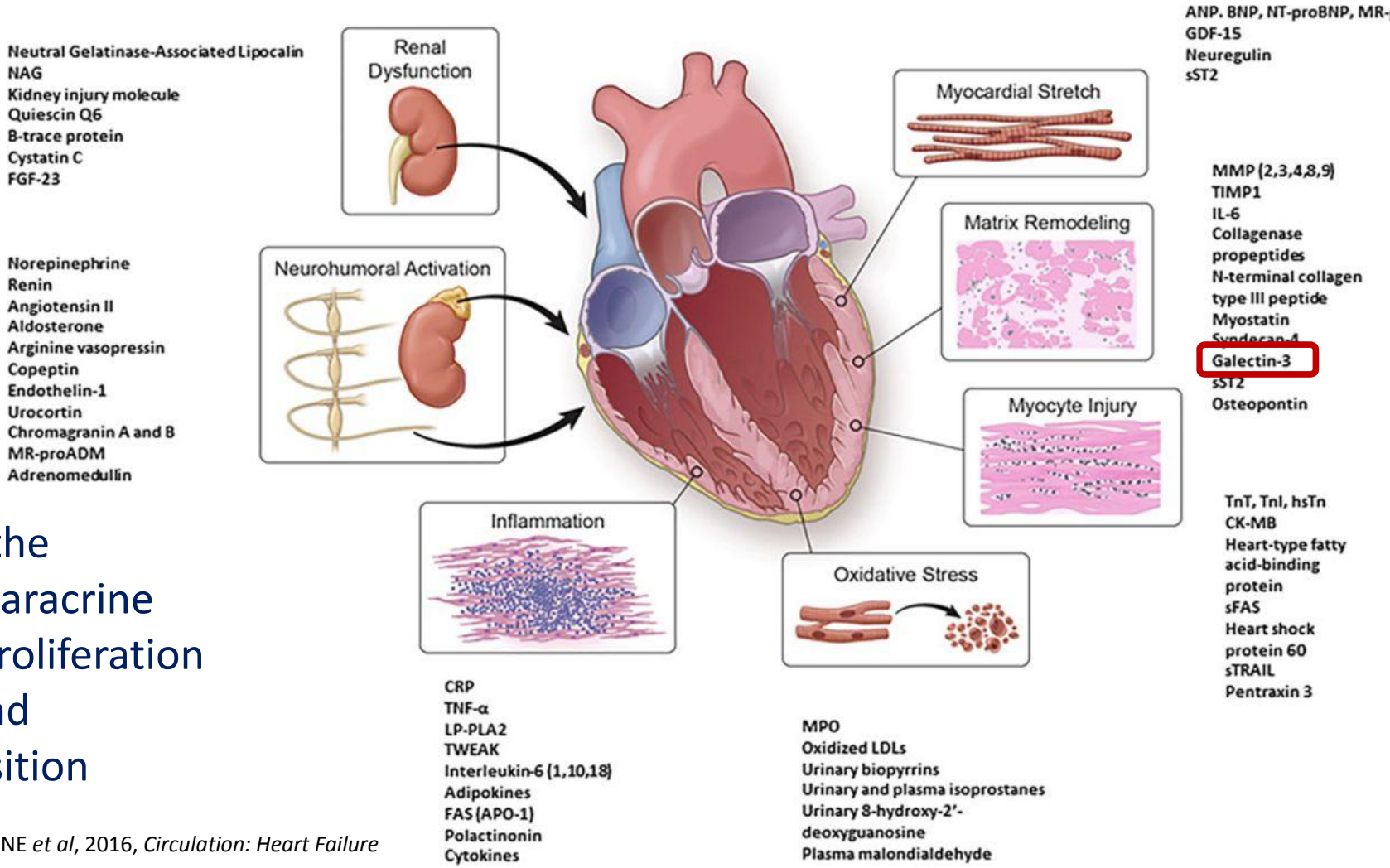


Fibrotic remodelling in the heart

- normal cardiac aging is characterized by morphological and structural changes that increase cardiomyocyte size, increased number of apoptosis with decreased number in myocytes, increased collagen deposition, and functional changes at cellular level → fibrotic remodelling
- cardiac remodelling - one of the main components of heart failure (HF)
- immune cells are recruited to the myocardium after acute or chronic damage or with age



Galectin-3 in heart failure



- Gal-3 is released in the myocardium, *via* a paracrine effect, stimulating proliferation of myofibroblasts and procollagen-1 deposition

Ibrahim NE *et al*, 2016, *Circulation: Heart Failure*

Deep and detailed understanding of molecular processes is prerequisite for revealing biological role(s) of Gal-3 and its (re)evaluation as a biomarker and drug target