Learning Objectives

1. understand the basic structure of GAG's
2. sites and mechanism of synthesis
3. basic functions
4. understand the cause(s) of mucopolysaccharidosis
5. understand the mechanism of GAG's effect on bone regeneration and remodeling

1

Glycosaminoglycans and Proteoglycans

1. long to *very long unbranched polysaccharide* composed of repeating disaccharide units
2. small proportion of a protein component
3. *very highly negatively charged*

2

Glycosaminoglycans Are Anionic Polysaccharide Chains Made of Repeating Disaccharide Units

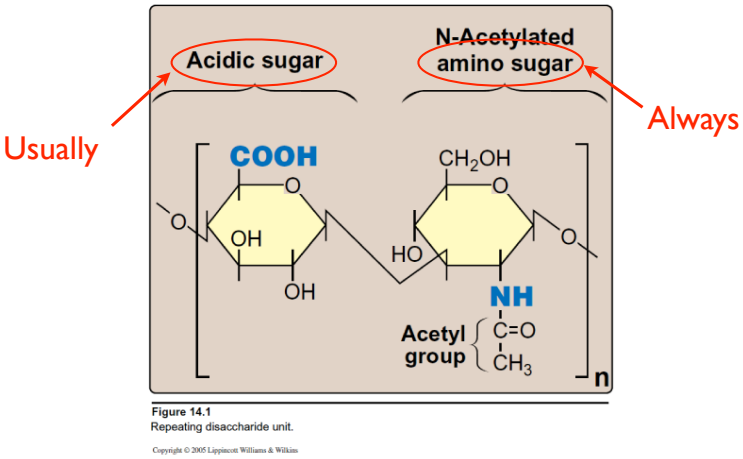
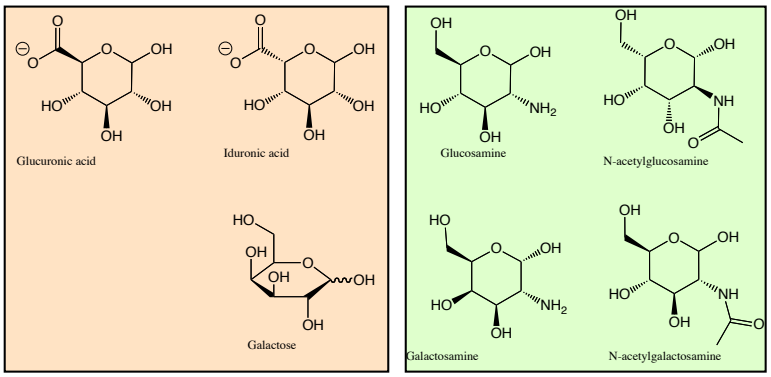


Figure 14.1
Repeating disaccharide unit.

3

Composition of GAG's



4

Glycosaminoglycans are usually attached to proteins to form proteoglycans

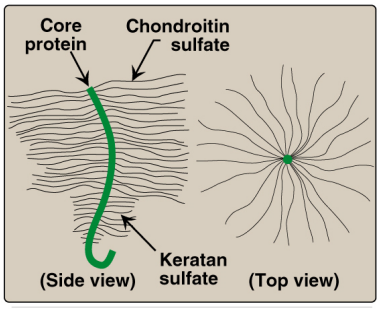


Figure 14.5

"Bottle-brush" model of a cartilage proteoglycan monomer.

1. contain a core protein (as little as 5% by weight)
2. synthesized within ER and Golgi
3. GAG chains are also sulfated in the Golgi (adds additional negative charges)

5

Functions of GAGs and Proteoglycans

1. GAGs form lubricating and/or shock absorbing gels (e.g. Hyaluronan)
2. regulate the activity of signaling molecules (e.g. Perlecan)
   - affect the rates of diffusion
   - affect receptor on/off rates
   - regulate effective concentration
3. function in cell-cell adhesion and are a component of the extracellular matrix

6

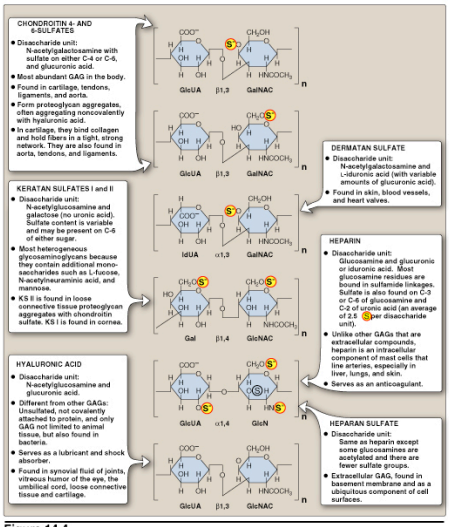


Figure 14.4
Structure and distribution of glycosaminoglycans (GAGs). Sulfate groups (S) are shown in all possible positions. GlcUA = glucuronic acid; IdUA = iduronic acid; GalNAC = N-acetylgalactosamine; GlcNAC = N-acetylglucosamine; GlcN = glucosamine; Gal = galactose.

7

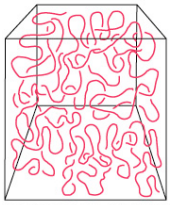
Glycosaminoglycans are usually attached to proteins to form proteoglycans

globular protein (MW 50,000)

glycogen (MW ~ 400,000)

spectrin (MW 460,000)

collagen (MW 290,000)



hyaluronan (MW 8 x 10^6)

300 nm

1. EXCEPT **Hyaluranon**: does **not** contain a core protein
2. is **not** sulfated and includes ~25,000 disaccharide repeats
3. **not** made within golgi or ER: synthesized at the outside surface of the plasma membrane (by transmembrane enzymes)

8

Site of Synthesis of GAGs part 1

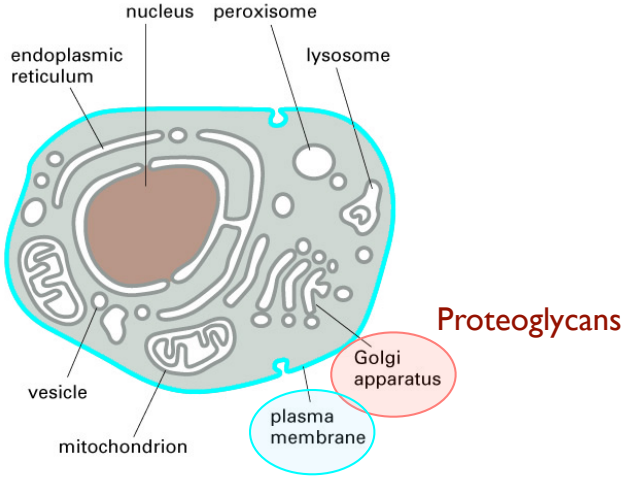


Figure 11-3 Essential Cell Biology, 2/e. (© 2004 Garland Science)

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GAG's are typically synthesized by a single step process

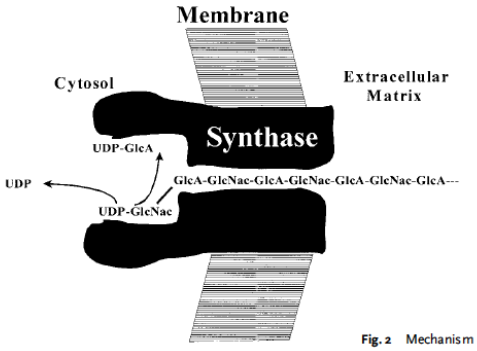
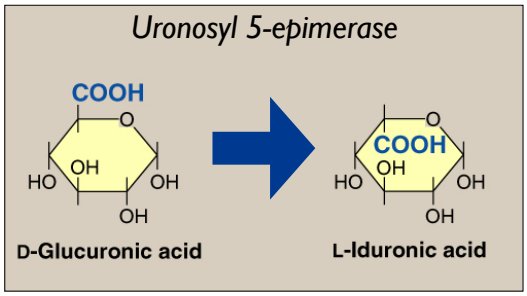


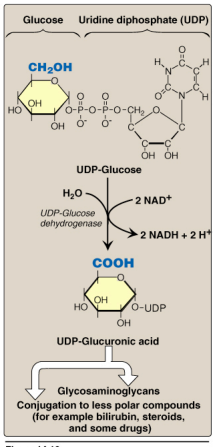
Fig. 2 Mechanism of hyaluranon synthesis.
Biopolymers, A. Steinbuchel, ed. Wiley VCH 2004

e.g. Hyaluronan synthase

10

UDP-glucose-6-dehydrogenase oxidizes UDP-glucose to UDP-glucuronic acid





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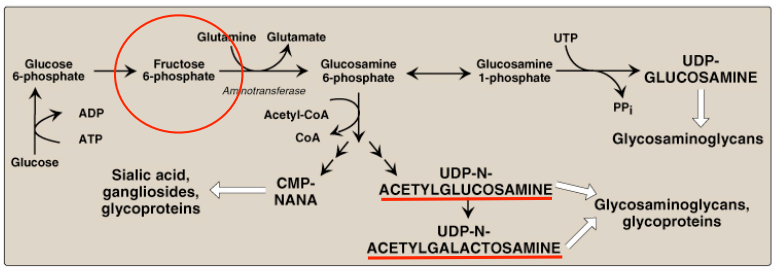

Figure 14.8
Synthesis of the amino sugars.

12

Site of Synthesis of GAGs part 2

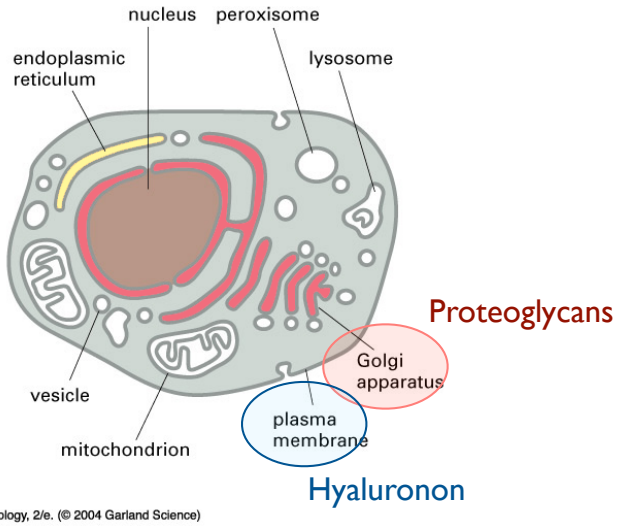

Figure 11-3 Essential Cell Biology, 2/e. (© 2004 Garland Science)

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5 steps in Proteoglycan biosynthesis

1. **translation of the protein into the ER.**
2. **recognition and addition of Xylose to Serine side chain by xylosyltransferase.**
3. **GAG chain elongation by glycosyl-transferases.**
4. **epimerization of glucuronate to iduronate by uronosyl epimerase.**
5. **sulfation of GAG (and protein) by sulfotransferases**

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Step 1

5 steps in Proteoglycan biosynthesis

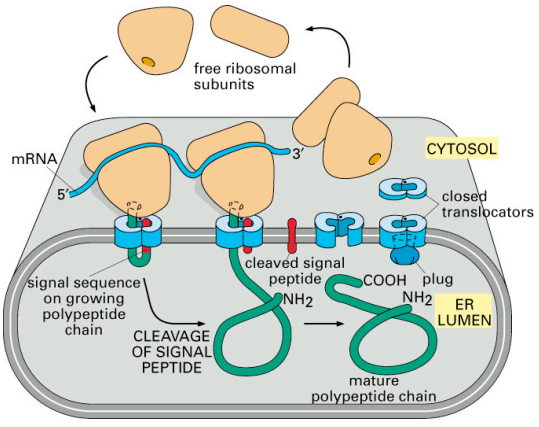

Figure 12–40. Molecular Biology of the Cell, 4th Edition.

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Step 2

Xylosyl transferase adds xylose to core serine sidechain: tetrasaccharide sequence is shared by diverse GAG chains.

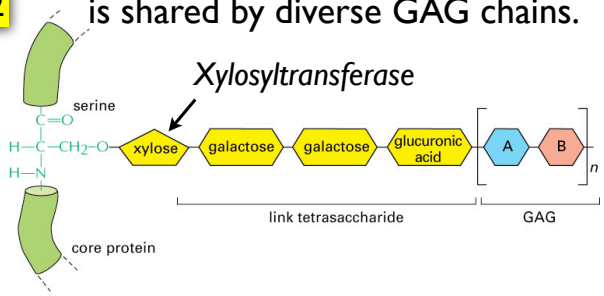

Figure 19–39. Molecular Biology of the Cell, 4th Edition.

specific glycosyl transferases distinguish different link tetrasaccharides by recognition of flanking protein sequence (e.g. Ser-Gly-any-Gly)

16

1: J Biol Chem. 2006 May 19;281(20):14224-31. Epub 2006 Mar 28.

Cloning and recombinant expression of active full-length xylosyltransferase I (XT-I) and characterization of subcellular localization of XT-I and XT-II.

Schon S, Prante C, Bahr C, Kuhn J, Kleesiek K, Gotting C.

Institut fur Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitatsklinik der Ruhr-Universitat Bochum, 32545 Bad Oeynhausen, Germany.

Xylosyltransferase I (XT-I) catalyzes the transfer of xylose from UDP-xylose to serine residues in proteoglycan core proteins. This is the first and apparently rate-limiting step in the biosynthesis of the tetrasaccharide linkage region in glycosaminoglycan-containing proteoglycans. The XYLT-II gene codes for a highly homologous protein, but its physiological function is not yet known. Here we present for the first time the construction of a vector encoding the full-length GFP-tagged human XT-I and the recombinant expression of the active enzyme in mammalian cells. We expressed XT-I-GFP and various GFP-tagged XT-I and XT-II mutants with C-terminal truncations and deletions in HEK-293 and SaOS-2 cells in order to investigate the intracellular localization of XT-I and XT-II. Immunofluorescence analysis showed a distinct perinuclear pattern of XT-I-GFP and XT-II-GFP similar to that of alpha-mannosidase II, which is a known enzyme of the Golgi cisternae. Furthermore, a co-localization of native human XT-I and alpha-mannosidase II could also be demonstrated in untransfected cells. Using brefeldin A, we could also show that both xylosyltransferases are resident in the early cisternae of the Golgi apparatus. For its complete Golgi retention, XT-I requires the N-terminal 214 amino acids. Unlike XT-I, for XT-II, the first 45 amino acids are sufficient to target and retain the GFP reporter in the Golgi compartment. Here we show evidence that the stem regions were indispensable for Golgi localization of XT-I and XT-II.

PMID: 16569644 [PubMed - indexed for MEDLINE]

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Step 3

Glycosyl transferases add each individual sugar moeity to the growing chain

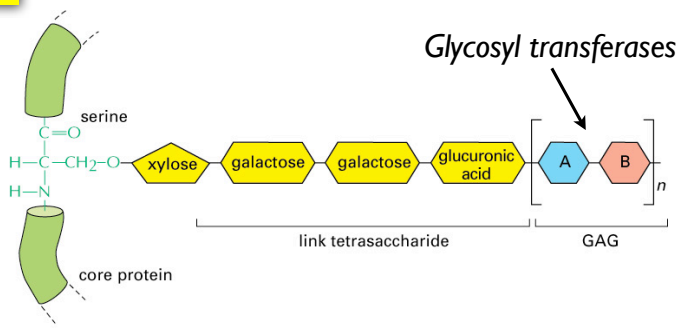

Figure 19–39. Molecular Biology of the Cell, 4th Edition.

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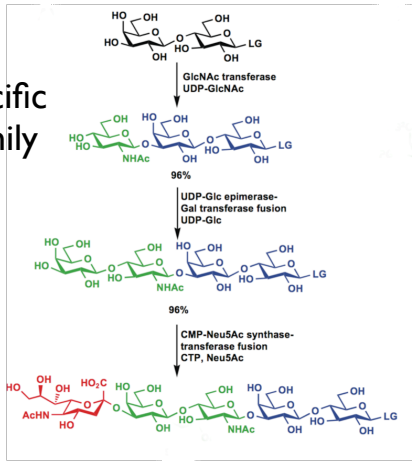
Each sugar is added separately, and by a specific glycosyl-transerfase family member



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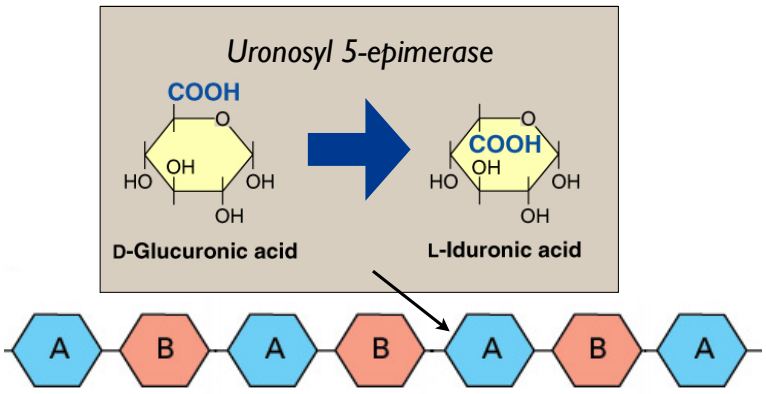
Step 4

Uronosyl-5-epimerase converts glucuronic acid to iduronate after chain formation



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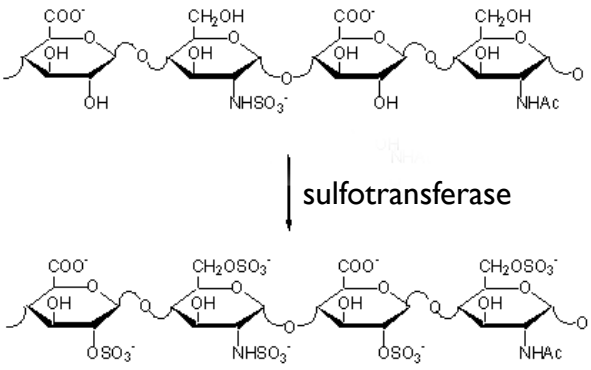
Step 5

The resulting GAG chain can be sulfated by sulfotransferases



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Genetic disorders affecting GAG degradation in lysosomes are common and debilitating

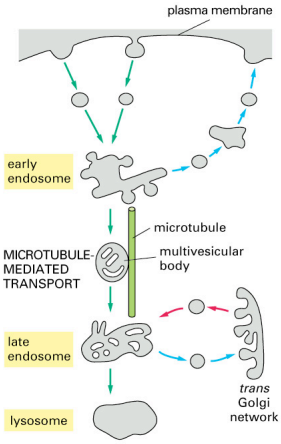

Figure 13–49. Molecular Biology of the Cell, 4th Edition.

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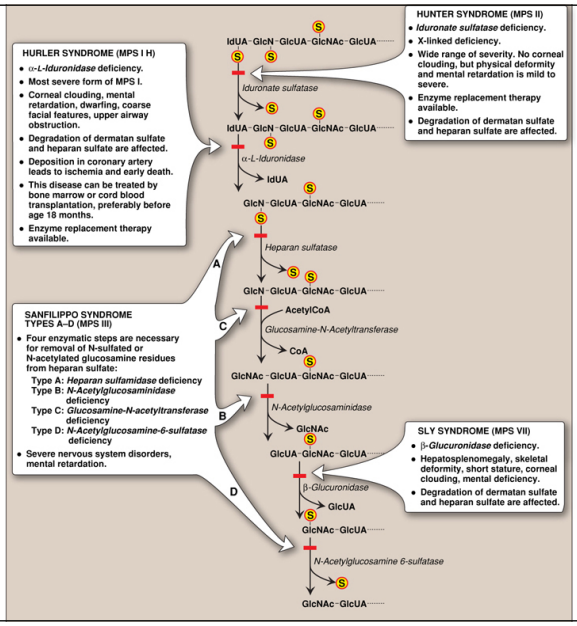

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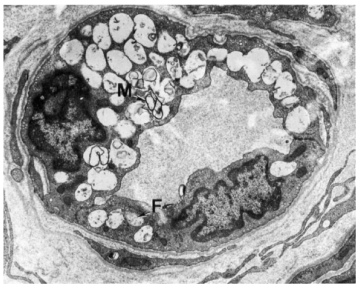
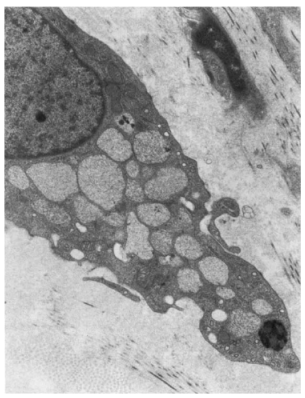
mucopolysaccharidosis





Vogler et al., A. Journal of Med. Gen. Suppl. 3:243-255 (1987)

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National Institute of Neurological Disorders and Stroke

Mucopolysaccharidoses Fact Sheet

Get Web page suited for printing
Email this to a friend or colleague
Request free mailed brochure
Versión en Español

Table of Contents (click to jump to sections)

What are the mucopolysaccharidoses?

The mucopolysaccharidoses are a group of inherited metabolic diseases caused by the absence or malfunctioning of certain enzymes needed to break down molecules called glycosaminoglycans - long chains of sugar carbohydrates in each of our cells that help build bone, cartilage, tendons, corneas, skin, and connective tissue. Glycosaminoglycans (formerly called mucopolysaccharides) are also found in the fluid that lubricates our joints.

People with a mucopolysaccharidosis either do not produce enough of one of the 11 enzymes required to break down these sugar chains into proteins and simpler molecules or they produce enzymes that do not work properly. Over time, these glycosaminoglycans collect in the cells, blood, and connective tissues. The result is permanent, progressive cellular damage that affects the individual's appearance, physical abilities, organ and system functioning, and, in most cases, mental development.

top

Who is at risk?

It is estimated that one in every 25,000 babies born in the United States will have some form of the mucopolysaccharidoses. It is an autosomal recessive disorder, meaning that only individuals inheriting the defective gene from both parents are affected. (The exception is MPS II, or Hunter syndrome, in which the

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ClinicalTrials.gov

A service of the U.S. National Institutes of Health

Search results for *Mucopolysaccharidoses [ALL-FIELDS]* are shown below.

Include trials that are no longer recruiting patients. Search-Within-Results | Query Details | Map of locations

10 studies were found.

1. Recruiting — Intrathecal Enzyme Replacement Therapy for Spinal Cord Compression in Mucopolysaccharidosis (MPS) I
   Conditions: Mucopolysaccharidosis I; Lysosomal Storage Diseases; Spinal Cord Compression
2. Recruiting — Phase II Study of Allogeneic Bone Marrow or Umbilical Cord Blood Transplantation in Patients With Mucopolysaccharidosis I (Hurler Disease)
   Condition: Mucopolysaccharidosis I
3. Recruiting — A Study of the Effect of Aldurazyme® (Laronidase) Treatment on Lactation in Female Patients With Mucopolysaccharidosis I (MPS I) and Their Breastfed Infants
   Conditions: Mucopolysaccharidosis I; Hurler's Syndrome; Hurler-Schei Syndrome; Scheie Syndrome
4. Recruiting — Mucopolysaccharidosis I (MPS I) Registry
   Condition: Mucopolysaccharidosis I
5. Recruiting — A Phase 4 Study Investigating the Relationship Between the Development of Laronidase Antibody and Urinary GAG Levels in Aldurazyme® (Laronidase) Treated Patients.
   Conditions: Mucopolysaccharidosis I; Hurler's Syndrome; Hurler-Scheie Syndrome; Scheie Syndrome
6. Recruiting — Stem Cell Transplantation for Hurler
   Conditions: Mucopolysaccharidosis I; Mucopolysaccharidosis VI; Mannosidosis; Mucolipidosis Type II (I-Cell Disease)
7. Recruiting — Stem Cell Transplant w/Laronidase for Hurler
   Condition: Mucopolysaccharidosis I
8. Recruiting — A Phase 4 Two Dose Level Study of Naglazyme(TM) (Galsulfase) in Infants With MPS VI
   Conditions: Mucopolysaccharidosis VI; Maroteaux-Lamy Syndrome
9. Recruiting — MPS VI Clinical Surveillance Program (CSP)
   Condition: Mucopolysaccharidosis VI (MPS VI, Maroteaux-Lamy Syndrome)
10. Recruiting — Phase II Study of Allogeneic Bone Marrow or Umbilical Cord Blood Transplantation in Patients With Lysosomal or Peroxisomal Inborn Errors of Metabolism
    Conditions: Graft Versus Host Disease; Lysosomal Storage Diseases; Peroxisomal Disorders

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GAG's / proteoglycans in molecular medicine

RHEUMATOLOGY

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Oxford Journals › Medicine › Rheumatology › Volume 45, Number 2 › Pp. 129-138

Rheumatology Advance Access originally published online on November 8, 2005
Rheumatology 2006 45(2):129-138; doi:10.1093/rheumatology/kei171

REVIEW

Chondroprotective drugs in degenerative joint diseases

G. Verbruggen

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Correspondence to: G. Verbruggen, Polikliniek Reumatologie, 0K12, Universitair Hospitaal, De Pintelaan 185, B-9000 Ghent, Belgium. E-mail: gust.verbruggen@ugent.be

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Hyaluronan

25,000

repeating disaccharide

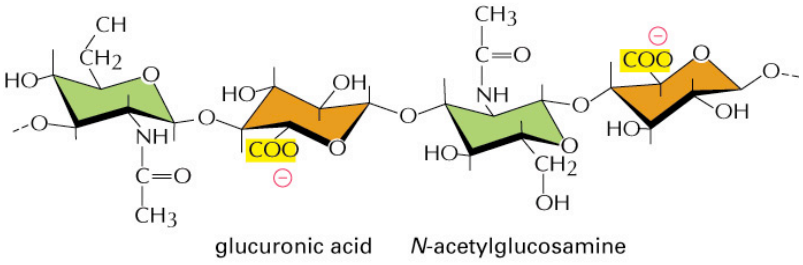

Figure 19–38. Molecular Biology of the Cell, 4th Edition.

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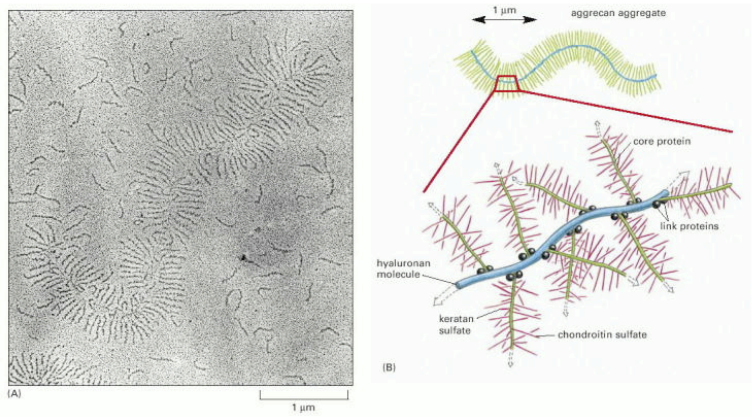

Figure 19-41. An aggrecan aggregate from fetal bovine cartilage. (A) An electron micrograph of an aggrecan aggregate

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Innovations

ORTHOVISC® (High Molecular Weight Hyaluronan)

New Treatment Option is Potential Alternative to OTC Pain Medications For Osteoarthritis of the Knee

Ortho Biotech Products, L.P., recently introduced ORTHOVISC® (High Molecular Weight Hyaluronan), a new treatment option for patients suffering from pain due to osteoarthritis (OA) of the knee. Osteoarthritis is one of the most common causes of physical disability among adults, and the knee is one of the most commonly affected joints. According to data from the First National Health and Nutrition Examination Survey, OA of the knee affects as many as 50 percent of people aged 45 to 74 years in the United States. In severe cases, OA of the knee can lead to disability and may require surgery to replace the knee joint.

ORTHOVISC® is a safe, effective and non-surgical option for knee pain from OA, when over-the-counter pain relievers do not work. Approved by the U.S. Food and Drug Administration in February 2004, ORTHOVISC® is now available to patients through physicians' offices, and is an injectable form of ultra-pure hyaluronic acid that can be given in a series of three weekly injections into the knee joint. The

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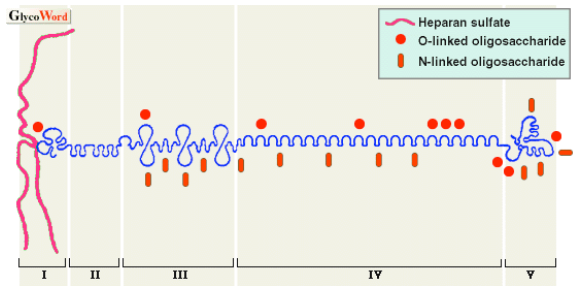
Perlecan proteoglycan regulates tissue development and differentiation



Takashi Saku (Niigata University School of Dentistry)

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CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

The Role of Heparan Sulfate and Perlecan in Bone-regenerative Procedures

A.A. DeCarlo[1]* and J.M. Whitelock[2]

[1]Agenta Biotechnologies, Inc., OADI Technology Center, 2800 Milan Court, Suite 382, Birmingham, AL 35211, USA; and [2]Biomaterials & Tissue Engineering, Graduate School of Biomedical Engineering, University of New South Wales, Australia; *corresponding author, adecarlo@nsu.nova.edu

J Dent Res 85(2):122-132, 2006

ABSTRACT

Tissue engineering, grafting procedures, regeneration, and tissue remodeling are developing therapeutic modalities with great potential medical value, but these regenerative modalities are not as effective or predictable as clinicians

INTRODUCTION

Our improved understanding of the biology of healing and an increasing awareness of the limitations and potential complications of harvesting autogenous bone graft have combined to increase interest in the improvement of bone graft substitutes. Millions of dental and non-dental bone-grafting procedures are performed annually (Bucholz, 2002), but only a small percentage yield the most desirable results. Biological adjuncts to osseous regeneration—such as growth factors, platelet-rich plasma, and enamel-matrix-derived protein—are used today in the clinics. While these offer some improvement in clinical outcome, better control is needed, and a new class of biological adjuncts should be considered. Here, we review the heparan-sulfate-decorated extracellular biomolecule named perlecan, a proteoglycan, and we review the research relating to its potential as an adjunct in bone-regenerative procedures. We begin with an overview of bone-graft

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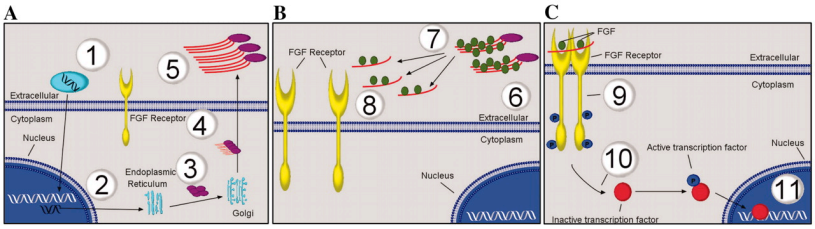
Perlecan can raise the effective local concentration of signalling molecules



DOMAIN I
Cell binding
Inhibits SMC binding
Promotes EC binding
thrombospondin
Angiogenesis
FGF binding & delivery
MMP-binding
Migration +/or Proliferation
Il-8 binding & delivery
Differentiation
FGF binding & delivery
Il-8 binding & delivery

DOMAIN III
Cell binding
Promotes EC binding
Promotes SMC binding
Laminin A chain-like
Beta-1 & -3 integrin binding?

DOMAIN V
Cell binding
Laminin A chain-like
Basement membrane binding
Migration +/or Proliferation
Differentiation
EGF-like repeats

I II III IV V

DOMAIN II
LDL receptor-like
Cysteine-rich

DOMAIN IV
ECM binding

DeCarlo and Whitelock, 2006

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Learning Objectives

1. contrast both structure and function of GAGs and glycoproteins
2. synthesis of N-linked vs O-linked glycoproteins
3. roles for N-linked oligosaccharides
4. roles for O-linked oligosaccharides

Glycoproteins

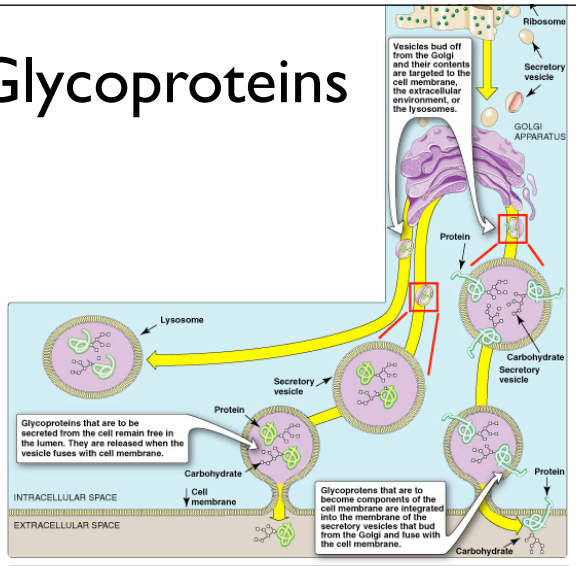

Figure 14.15
Transport of glycoproteins through the Golgi apparatus and their subsequent release or incorporation into a lysosome or the cell membrane.

Proteoglycans vs. Glycoproteins

1. long unbranched chain
2. O-linked glycosidic bonds
3. chains formed of di-saccharide repeats
4. carbohydrates dominate the mass of the average proteoglycan

1. short, often branched
2. O and N-linked glycosidic bonds
3. no disaccharide repeats
4. protein dominates the mass of the average glycoprotein
5. additional functions

Glucose
Fucose
Mannose

Galactose	Galactose
N-acetylglucosamine	N-acetylglucosamine
N-acetylgalactosamine	N-acetylgalactosamine
N-acetylneuraminic acid ⊖	N-acetylneuraminic acid ⊖

Glucosamine
Galactosamine
Glucuronic acid ⊖
Iduronic acid ⊖

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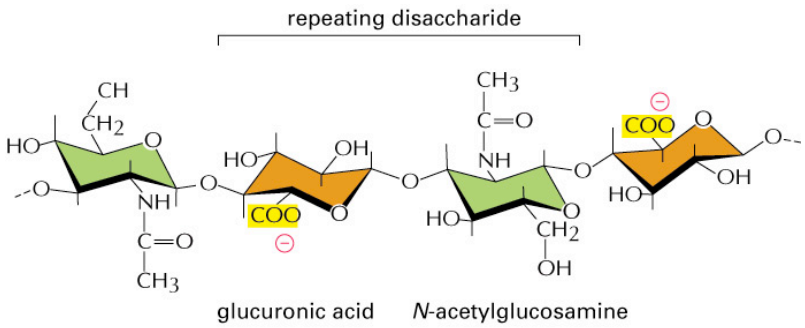

Figure 19–38. Molecular Biology of the Cell, 4th Edition.

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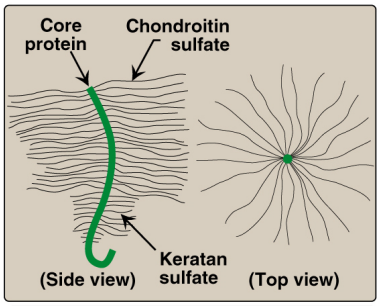
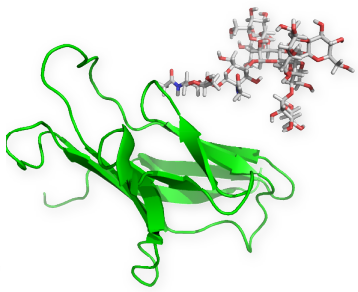


Figure 14.5
"Bottle-brush" model of a cartilage proteoglycan monomer.



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Shared functions with Proteoglycans

1. Effectors and regulators of cell signaling
2. Structural component of Extracellular Matrix
3. Can act as a receptor for a variety of ligands

40

Additional Specialized Functions

1. Regulate solubility of proteins
2. Protection against proteolysis
3. Participate in protein folding and quality control
4. Can function as a 'zip code' for shipping certain proteins to other organelles (e.g. Lysosome)

41

Additional Specialized Functions

1. Regulate solubility of proteins
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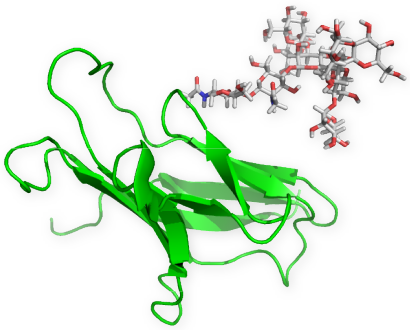
42



43

1. N-linked Glycosylation (Asn)

   (A) prefabricated 'sugar tree' added en bloc

      a. Complex

      b. High Mannose

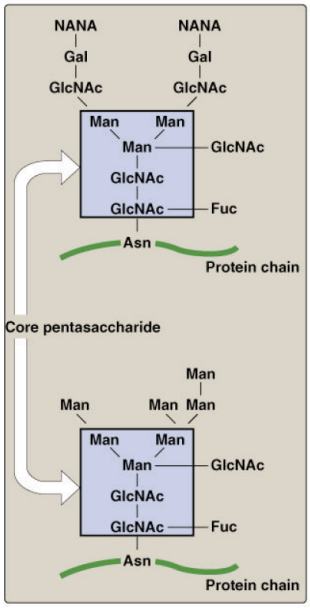
   (B) Added in ER and modified in Golgi

2. O-linked Glycosylation (Ser/Thr)

   (A) Lots of variation, often a single sugar, and no common core

   (B) Occurs in Golgi only **

44



The Golgi contains the enzymes to generate <u>complex oligosaccharides</u> from high-mannose oligosaccharides

High-mannose oligosaccharides are first added to proteins in the endoplasmic reticulum

45

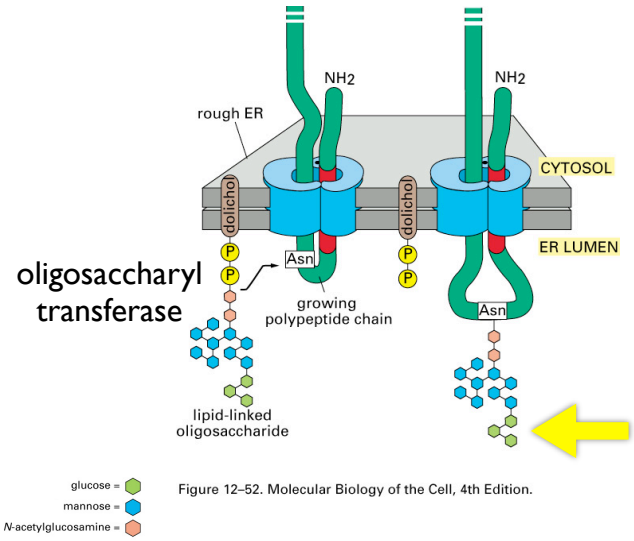
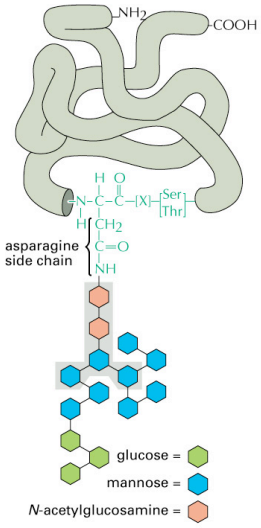


Figure 12–52. Molecular Biology of the Cell, 4th Edition.

glucose is removed from the tree before passage to the Golgi by a **glucosidase**

46



Oligosaccharyl transferase recognizes the sequence {Asn-any residue-Ser/Thre} and attaches the oligosaccharide complex to the terminal amine of Asn side chain

Figure 12–51. Molecular Biology of the Cell, 4th Edition.

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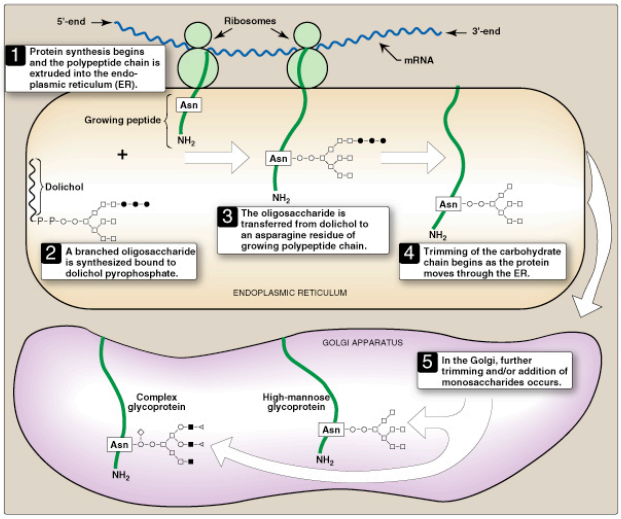

Figure 14.16
Synthesis of N-linked glycoproteins. ○ = N-acetylglucosamine; □ = mannose; ● = glucose; ■ = N-acetylgalactosamine; ◇ or ◁ for example, fucose or N-acetylneuraminic acid.

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Additional Specialized Functions

1. Regulate solubility of proteins
2. Protection against proteolysis
3. Participate in protein folding and quality control
4. Can function as a 'zip code' for shipping certain proteins to other organelles (e.g. Lysosome)

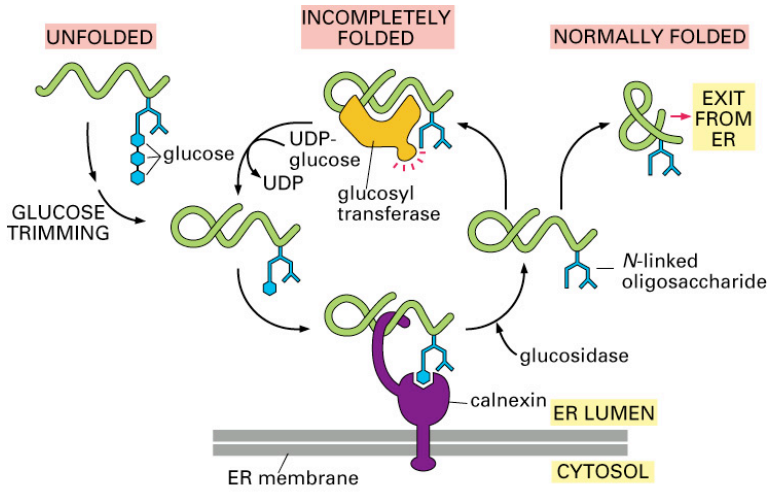
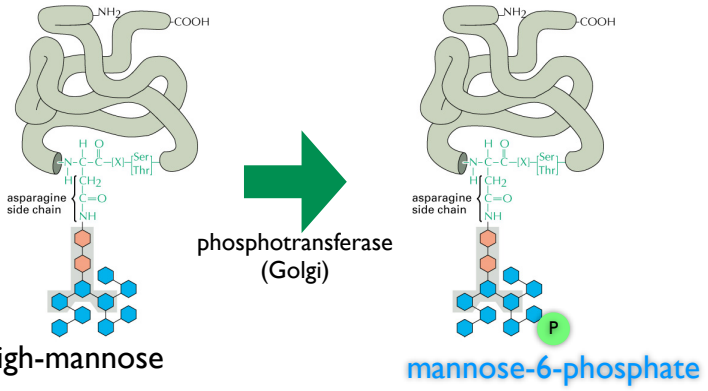


Figure 12–54. Molecular Biology of the Cell, 4th Edition.

Additional Specialized Functions

1. Regulate solubility of proteins
2. Protection against proteolysis
3. Participate in protein folding and quality control
4. Can function as a 'zip code' for shipping certain proteins to other organelles (e.g. Lysosome)



(acid hydrolases)

52

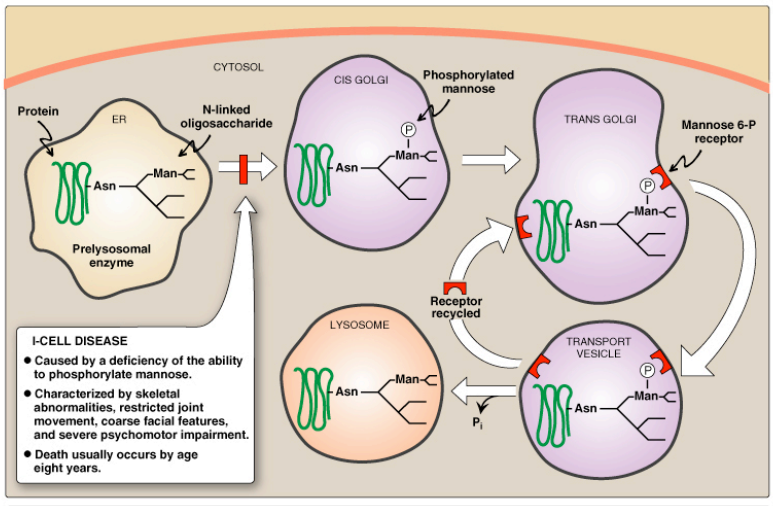


Figure 14.17
Mechanism for transport of N-linked glycoproteins to the lysosomes.

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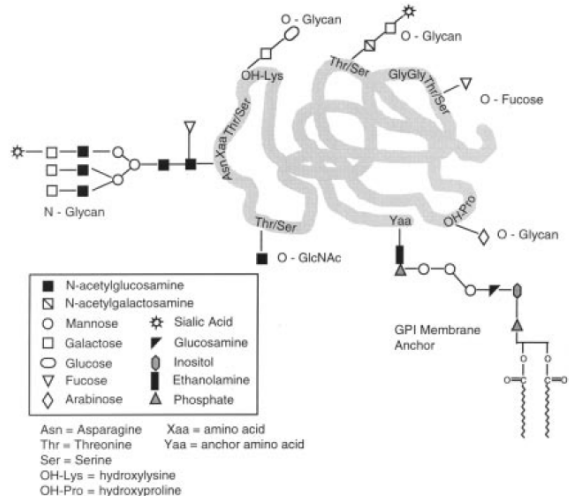

Van Den Steen et al. Critical Reviews in Biochemistry and Molecular Biology, 33(3):151–208 (1998)

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Table 12.1. *Less common types of glycosylation in the Golgi*

Modification	Proteins
O-(α)Fucose	
Fucα-Thr/Ser	urokinase, t-PA, factor XII, factor VII, human notch-1
Sia2–6Gal1–4GlcNAc1–3Fucα1-Thr/Ser	factor IX
O-(β)Glc	
Xylα1–3Xylα1–3Glcβ-Ser/Thr	factors VII, IX, protein 2, human Notch-1
O-(β)Gal	
Glcα-1–2Gal-O-Hyl (hydroxylysine)	collagen, surfactant protein, complement factor Clq, mannan-binding proteins
O-Man	
Man-R-O-Ser/Thr	brain proteoglycan, α-dystroglycan, others in brain and neuronal tissue
O-(α)GlcNAc	
GlcNAcα-Thr	cell adhesion molecule gp80, extracellular matrix protein PST
Phosphoglycosylation	
GlcNAcα-1-P-Ser	*Dictyostelium* lysosomal proteins
Fucβ-1-P-Ser	extracellular matrix proteins, cysteine proteinases
Manα-1-P-Ser	*Leishmania* proteophosphoglycan and filamentous acid phosphatase
Glc-(β)Asn	laminin
C-Mannosylation	RNase 2, interleukin-12

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O-linked glycosylation can regulate Ser/Thr phosphorylation

1: Adv Exp Med Biol. 1995;376:115-23.

O-linked N-acetylglucosamine: the "yin-yang" of Ser/Thr phosphorylation? Nuclear and cytoplasmic glycosylation.

Hart GW, Greis KD, Dong LY, Blomberg MA, Chou TY, Jiang MS, Roquemore EP, Snow DM, Kreppel LK, Cole RN, et al.

Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham 35294-0005, USA. GWHART@BMG.BHS.UAB.EDU

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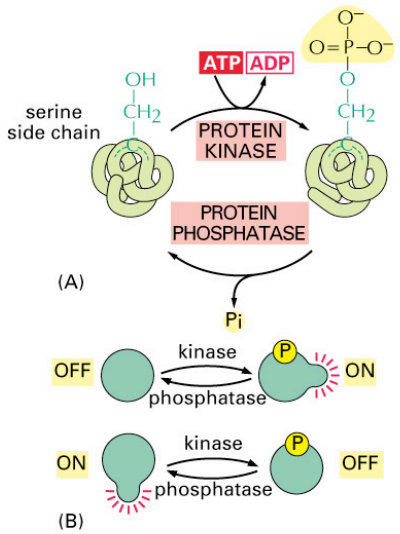

Figure 3–63. Molecular Biology of the Cell, 4th Edition.

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Table 18.1. Defects in glycoprotein degradation

Disorder	Defect	Affects degradation of		Clinical symptoms
		glycoprotein	glycolipid	
α-Mannosidosis types I and II	α-mannosidase	major	none	*type I:* infantile onset, progressive mental retardation, hepatomegaly, death between 3 and 12 years *type II:* juvenile/adult onset, milder, slowly progressive
β-Mannosidosis	β-mannosidase	major	none	severe quadriplegia, death by 15 months in most severe; mild cases have mental retardation, angioker-atoma, facial dysmorphism
Aspartylglucosaminuria	aspartyl-glucosaminidase	major	none	progressive, coarse facies, mental retardation
Sialidosis (mucolipidosis I)	sialidase	major	minor	progressive, severe mucopolysaccharidosis-like features, mental retardation
Schindler types I and II	α-N-acetyl galactosaminidase	yes	?	*type I:* infantile onset, neuroaxonal dystrophy, severe psychomotor and mental retardation, cortical blindness neurodegeneration *type II:* mild intellectual impairment, angiokeratoma corpis diffusum
Galactosialidosis	protective protein/cathepsin A	major	minor	coarse facies, skeletal dysplasia, early death
Fucosidosis	α-fucosidase	major	present	spectrum of severities includes psychomotor retardation, coarse facies, growth retardation
G_{M1} gangliosidosis	β-galactosidase	present	major	progressive neurologic disease and skeletal dysplasia in severe infantile form
G_{M2} gangliosidosis	β-hexosaminidase	present	major	*severe form:* neurodegenerative with death by 4 years

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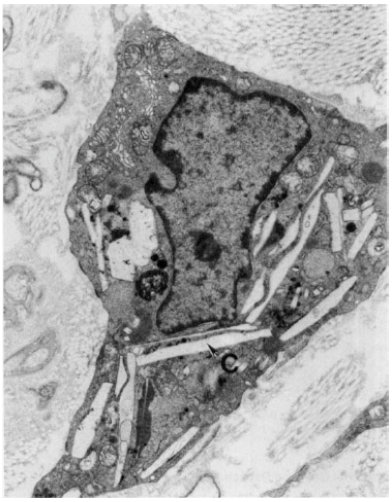

Vogler et al., A. Journal of Med. Gen. Suppl. 3:243-255 (1987)

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