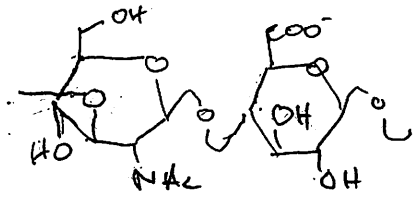
Lecture 10 - Glycosaminoglycans (GAGs)

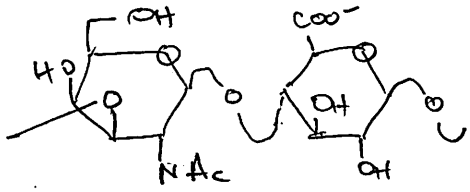
(1) Structure

HA $[GlcNAc\beta4\ GlcA\beta3]_n$



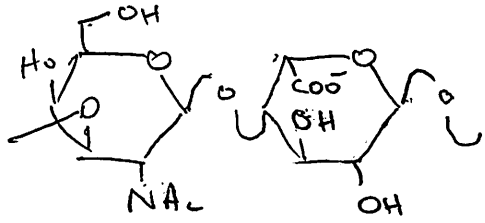
$n \leq 10,000$

CS $[GalNAc\beta4\ GlcA\beta3]_n$ (4S, 6S)



$n \leq 40$

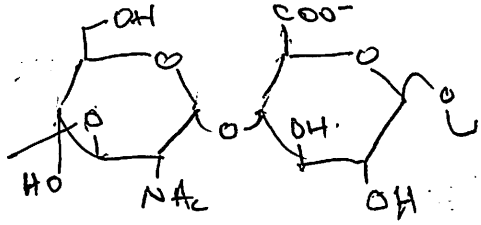
DS $[GalNAc\beta4\ IdoA\alpha3]_n$ (4S, 6S, 2S)



$n \geq 1$

HS $[GlcNAc\alpha4\ GlcA\beta4]_n$

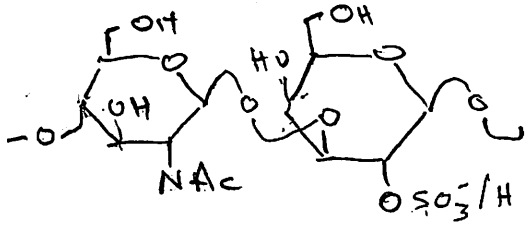
$[GlcNS\ \alpha4\ IdoA\alpha4]_m$ (2S)



$n \leq 150$

$m \leq 20$

Ks $[GlcNAc\beta3\ Gal\beta4]_n$ (6S, 6S)

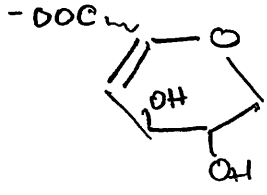


$n \leq 20$

Slide 1 — Explain linear patterns

(2)

- Bacterial lyases - split between Hexosamine and uronic acid
- Generation of 4,5 unsaturated bond



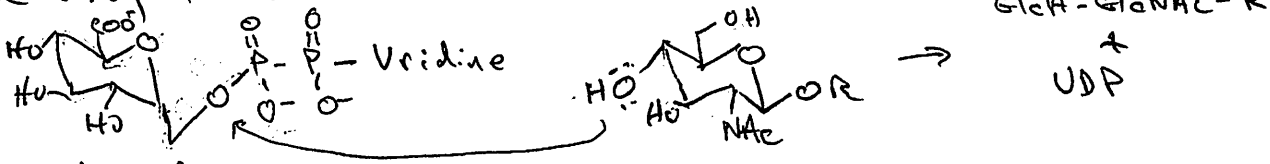
$\Rightarrow$ Disaccharide Composition (SAX or LC/MS after AMACS or aniline tagging)

- Hydrolases
  - Keratinases - endo-β-N-acetylglucosaminidase, endo-β-galactosidase
  - Hyaluronidase - endo-N-acetylhexosaminidase

- Lysosomal
  - Sulfatases
  - exoglycosidases (iduronidase, β-Galactosidase, β-hexosaminidase, etc)
  - Action at NRE

(3) Biosynthesis

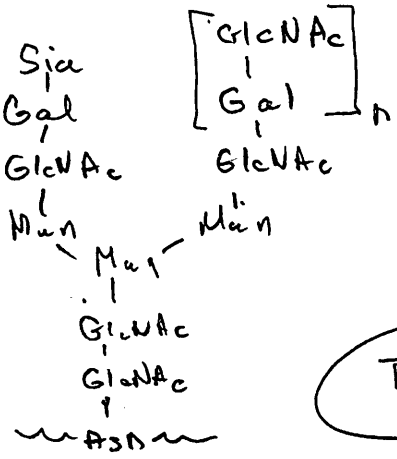
- HA assembly by Hyaluronan Synthase (HAS)
  - Slide 2 HAS embedded in plasma membrane
- Assembly from NRE



- Assembly from RE
  - Slide 3 - Pendulum Model

How would you distinguish these two mechanisms?

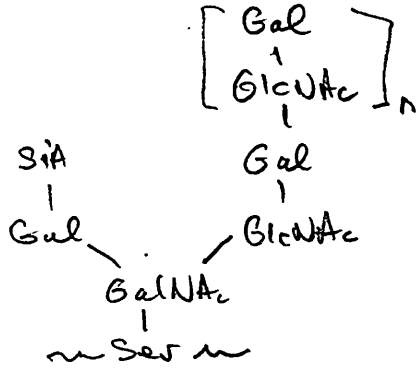
KS



KS I

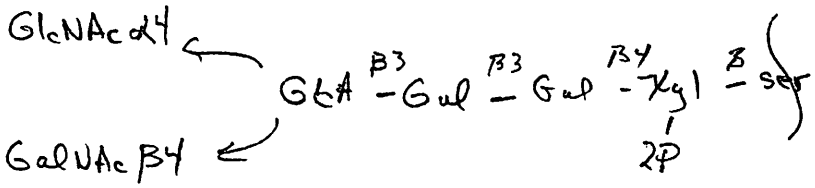
N-linked

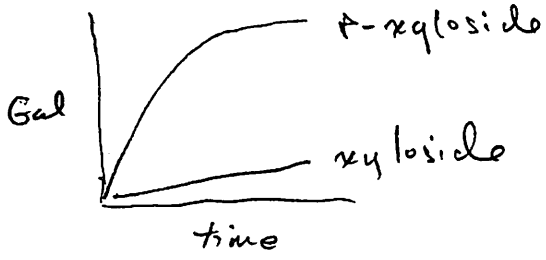
TABLE 1



O-linked

- HS, CS/DS

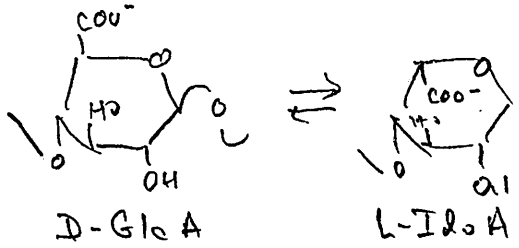




Phosphorylation precedes galactosylation

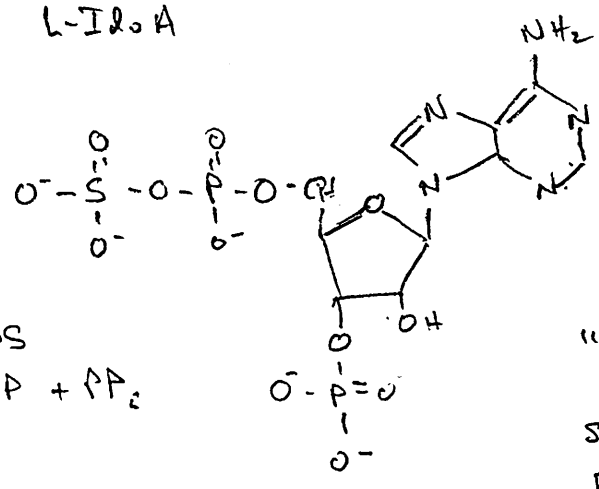
What's the utility of requiring phosphorylation?

✓ Epimerization



What's a reasonable mechanism for performing a polymer level epimerization

✓ Sulfation

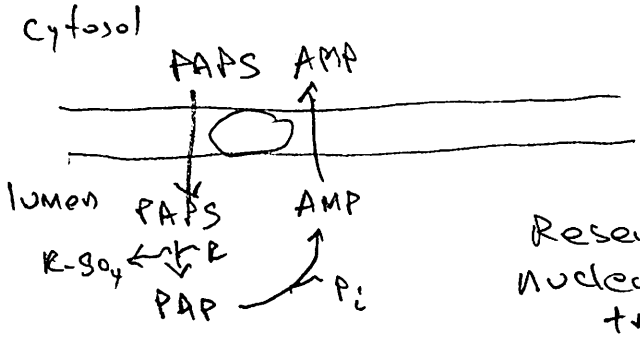


3'-phospho-5' adenyl-phospho-sulfate = PAPS

$2ATP + SO_4^{=} \rightarrow PAPS + ADP + PP_i$

(cytosol)

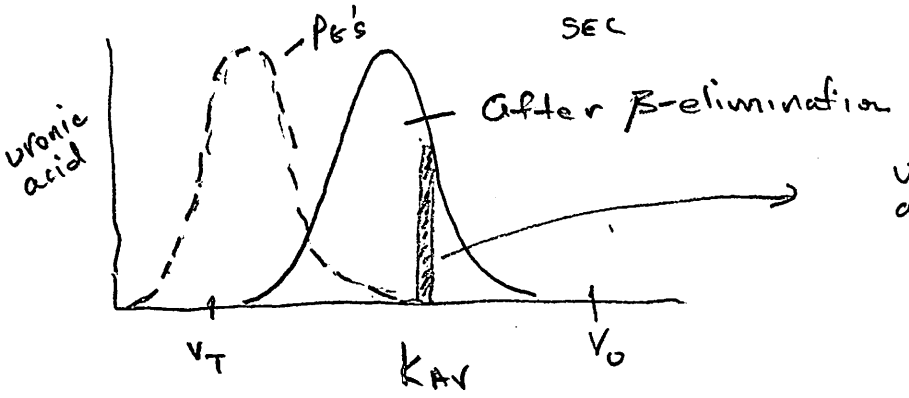
"PAPS synthetase"
sulfurylase
APS kinase

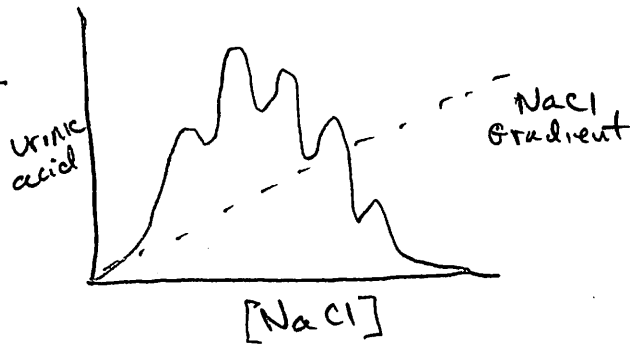


PAPS transporter

Resembles nucleotide sugar transporters

(4) Working with Proteoglycans





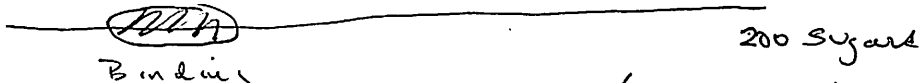
Slide 4 — HS and DS chains

What factors control GAG chain length?

(5) Sequencing GAG chains

How does sequencing a GAG chain differ from sequencing DNA?

- Can't clone a GAG chain → no amplification
- Binding sequence represents only a small portion of chain



~10/200 = ~5%

Discuss affinity chromatography

- More than one sequence might bind, but affinity varies
- Affinity fractionation of partial digests
- Exoenzyme sequencing
- Mass spectrometry (MS^n)

(6) GAG Binding Proteins

HA binding proteins – Hyalectins — Slide 5

HS binding proteins – HSBPs

HA binding proteins considered lectins, but HSBPs are not. Why?

- Binding typically involes electrostatic interactions

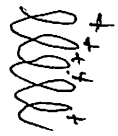
  lysine — sulfate
  arginine — carboxylates
  (histidine)

  hydrogen binding
  hydrophobic interactions

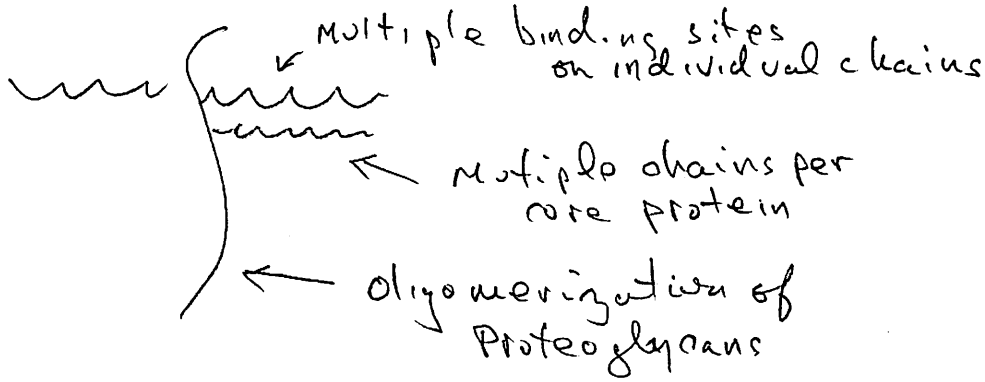
- Binding typically occurs across an electropositive surface of protein or in clefts

Slide 6 HSBPs

amphipathic helies
β-strands
Composite structures



- Affinity - μM to nmolar / Avidity effects



- Rare structures

  3-O-sulfation of HS glucosamine and GlcA in CS
  Contiguous binding sites?
  Free amino groups
  Chain size eg. CD44 responds differently to short HA fragments vs. long chains
  If a particular sequence of sulfated sugars is present in more sulfated region, will it have the same binding properties?