Specific sides to multifaceted glycosaminoglycans are observed in embryonic development

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Overview of Glycosaminoglycan Biosynthesis

A short summary of GAG polymerization and modification follows. For more detailed discussion, several excellent reviews should be consulted [1-3].

The longest vertebrate polysaccharide, HA is composed of *N*-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA) repeated to about 10,000 disaccharides (Fig 1). The structure is characterized by a 4-fold helix that is stabilized by weak intramolecular hydrogen bonds, giving HA its well-characterized viscoelastic properties that are critical to its function in eyes and joints [4]. HA synthesis is accomplished by one of three co-polymerases, glycosyltransferases which transfer both monosaccharides to the elongating chain (Supplemental Table). In contrast to the other GAGs, HA is not modified by sulfation, suggesting that it might represent an early evolutionary GAG. However, invertebrates like *Drosophila* or *C. elegans* do not contain homologs of the vertebrate HA co-polymerases.

Galactose (Gal) and GlcNAc are repeated in KS for about 10-50 disaccharide units and are sulfated at the 6-*O* position on both saccharides. KS is not found in invertebrates; a single copy of each polymerizing and modifying enzyme is found in vertebrates. KS can be distinguished from other sulfated GAGs based on its linkages to the core protein, elongation method, and additional modifications. There are two types of KS core protein linkages: KS I (or corneal KS) is found on an *N*-glycan core structure while KS II (or skeletal KS) links via an *O*-glycan core structure, both of which are commonly observed in secreted and membrane-bound glycoproteins. KS polymerization is blocked when one of two closely related sulfotransferases is absent, *Chst2* in brain [5, 6] and *Chst6* in cornea [7], indicating that KS synthesis requires alternating polymerization with 6-*O* sulfation [8]. Finally, the KS chain is not just modified by sulfation, but fucose and sialic acid as well [9].

Invertebrates synthesize CS, but it is not sulfated in *Drosophila* and is undersulfated in *C. elegans* [10]. Addition of *N*-acetylgalactosamine (GalNAc) initiates synthesis of CS on a linker tetrasaccharide that is shared with HS. In vertebrates, CS/DS chain initiation and elongation involves two GalNAc glycosyltransferases and a family of four co-polymerases that each possess GalNAc- and GluA-transferase activity, yet each co-polymerase interacts with another co-polymerase to achieve optimal polymerization [11]. Sulfation at 6-*O* GalNAc occurs in the medial/trans golgi apparatus; epimerizing GlcA to IdoA is dependent on sulfation at the 4-*O*-position and likely takes place later in the golgi [12, 13]. The 2-*O* sulfotransferase prefers 6-*O* and 4-*O* modified CS as substrates, indicating that it is likely the last enzyme in the pathway [14].

HS is the only GAG sulfated in *Drosophila* or *C. elegans* at the same positions as it is in vertebrates [10]. After addition of GlcNAc to the linker tetrasaccharide, GlcA and GlcNAc are polymerized by alternately adding to the nonreducing end by a family of Ext copolymerases. After

elongation or as the chain is elongating, the *Ndst* family of enzymes remove the *N*-acetyl group from selected GlcNAc residues and substitute most of the free amines with sulfates. Subsequent epimerization and sulfation occurs primarily in the *N*-sulfated regions. While *Drosophila* and *C. elegans* only have one gene for each enzymatic step, a similar array of disaccharides is produced [10].

Gene	Description	Human	Mouse	Zebrafish	*Drosophila*	*C Elegans*
HA						
HAS1	Polymerizes the alternate addition of GlcA & GlcNAc	3036	15116	403130		
HAS2		3037	15117	260350		
HAS3		3038	15118	282555		
KS						
B4GALT4	Adds Gal to KS	8702	56375	492760		
B3GNT7	Adds GlcNAc to KS	93010	227327	286748		
CHST6	Sulfates GlcNAc at the nonreducing end at the C6 hydroxy	4166	56773	100170782		
CHST2		9435	54371	561896		
CHST1	Sulfates Gal at the C6 hydroxy	8534	76969	445124		
CS\DS						
Chain initiation and elongation						
CSGALNACT1	Adds GalNAc to linkageoligosaccharide	55790	234356	793671	36079	
CSGALNACT2		55454	78752	560826		
CHSY1	Polymerizes the alternate addition of GlcA & GalNAc	22856	269941	324407	32497	172851
CHSY3		337876	78923	368477		
CHPF	Cooperates with CHSY for chondroitin polymerization	79586	74241	794660	32259	176231
CHPF2		54480	100910	100000654		
Chain modification						
CHST3	Sulfates GalNAc at the C6 hydroxy	9469	53374	556800 559721		
CHST7	Sulfates GalNAc at the C6 hydroxy	56548	60322	563748		
CHST15	Sulfates GalNAc4S at the C6 hydroxy	51363	77590	565523		
DSE	Epimerizes GlcA to IdoA	29940	212898	568713		
DSEL		92126	319901	568099		
CHST14	Sulfates GalNAc in DS at the C4 hydroxy	113189	72136	404732		
CHST11	Sulfates GalNAc at the C4 hydroxy	50515	58250	404232	326156	
CHST12		55501	59031	407076		
CHST13		166012	71797	565671		
UST	Sulfates GlcA at the C2 hydroxy	10090	338362	557218		
HS						
Chain initiation and elongation						
EXTL3	Adds GlcNAc to linkageoligosaccharide	2137	54616	493783	37198	176502
EXTL2		2135	58193	558373		
EXTL1		2134	56219			
EXT1	Polymerizes the alternate addition of GlcA & GlcNAc	2131	14042	497279 497280 497281	36614	178080
EXT2		2132	14043	493780	3772101	
Chain modification						
NDST1	Deacetylates and sulfates GlaNAc	3340	15531	62397* 74936*	38736	177675
NDST2		8509	17423	798226 793524		
NDST3		9348	83398	100317129		
NDST4		64579	64580			
GLCE	Epimerizes GlcA to IdoA	26035	93683	405776 100007670	35569	175562
HS2ST1	Sulfates GlcA and IdoA at the C2 hydroxy	9653	23908	791188	44433	181309
HS6ST1	Sulfates GlcNAc at the C6 hydroxy	9394	50785	553162 791107	42380	189586
HS6ST2		90161	50786	378450		
HS6ST3		266722	50787	569353 792524		
HS3ST1	Sulfates GlcNAc at the C3 hydroxy	9957	15476	557111		
HS3ST2		9956	195646	571246		
HS3ST3		9953 9955	15478 54710	558084 558084	32918	3564879
HS3ST4		9951	628779	563570		
HS3ST5		222537	319415	562344 565201	37161	185573
HS3ST6		64711	328779	100008592		

Supplemental Table: *Diversity of GAG biosynthetic enzymes from 4 model organisms compared to humans.* Gene IDs from NCBI's Entrez Gene database were used to consistently identify glycosyltransferases and sulfotransferases as there are numerous inconsistencies between and within online databases. While the enzymatic activity for many gene products has not been fully characterized,

identification is straightforward as the open reading frame covers few exons and significant homologies exist, particularly in the active sites. The vast majority of genes listed here are specific to GAG synthesis, but there are exceptions. For example, the glycosyltransferase B3GAT1 recognizes a wide substrate specificity that includes branched N- and O-glycans [15] while the sulfotransferase CHST3 has been shown to modify GalNAc in CS along with Gal in KS and in the HS-CS linker tetrasaccharide [16].
* Ensembl gene ID.

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