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Review

Specific sides to multifaceted glycosaminoglycans are observed in embryonic development

Kenneth L. Kramer*

Genetics and Developmental Biology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, 10 Center Drive Bldg. 10, Rm 6C103, Bethesda, MD 20892-1583, United States

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ABSTRACT

Ubiquitously found in the extracellular matrix and attached to the surface of most cells, glycosaminoglycans (GAGs) mediate many intercellular interactions. Originally described in 1889 as the primary carbohydrate in cartilage and then in 1916 as a coagulation inhibitor from liver, various GAGs have since been identified as key regulators of normal physiology. GAGs are critical mediators of differentiation, migration, tissue morphogenesis, and organogenesis during embryonic development. While GAGs are simple polysaccharide chains, many GAGs acquire a considerable degree of complexity by extensive modifications involving sulfation and epimerization. Embryos that lack specific GAG modifying enzymes have distinct developmental defects, illuminating the importance of GAG complexity. Revealing how these complex molecules specifically function in the embryo has often required additional approaches, the results of which suggest that GAG modifications might instructively mediate embryonic development.

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1. Introduction

GAGs are long, linear polysaccharides composed of an amino sugar and uronic acid repeating as a disaccharide unit. Variations in disaccharide identity distinguish four classes of GAGs: heparan sulfate/heparin (HS), chondroitin sulfate/dermatan sulfate (CS/DS), keratan sulfate (KS), and hyaluronan (HA, Fig. 1A). Additional differences in disaccharide linkage and modification result in each GAG class assuming a unique macrostructure [1]. While HA is synthesized at the plasma membrane where it is not modified or covalently attached to a core protein, HS, CS/DS, and KS are covalently linked to core proteins and modified by sulfation and epimerization during synthesis in the golgi. Together known as proteoglycans, sulfated GAGs and their attached core proteins are presented on the cell surface, stored in secretory granules, or secreted into the extracellular matrix.

Structurally diverse GAG chains bind and regulate the activity of a wide range of protein ligands. In a well-characterized model, HS

* Tel.: +1 301 451 0257.
E-mail addresses: kramerk2@mail.nih.gov, kramerk2@nhlbi.nih.gov.

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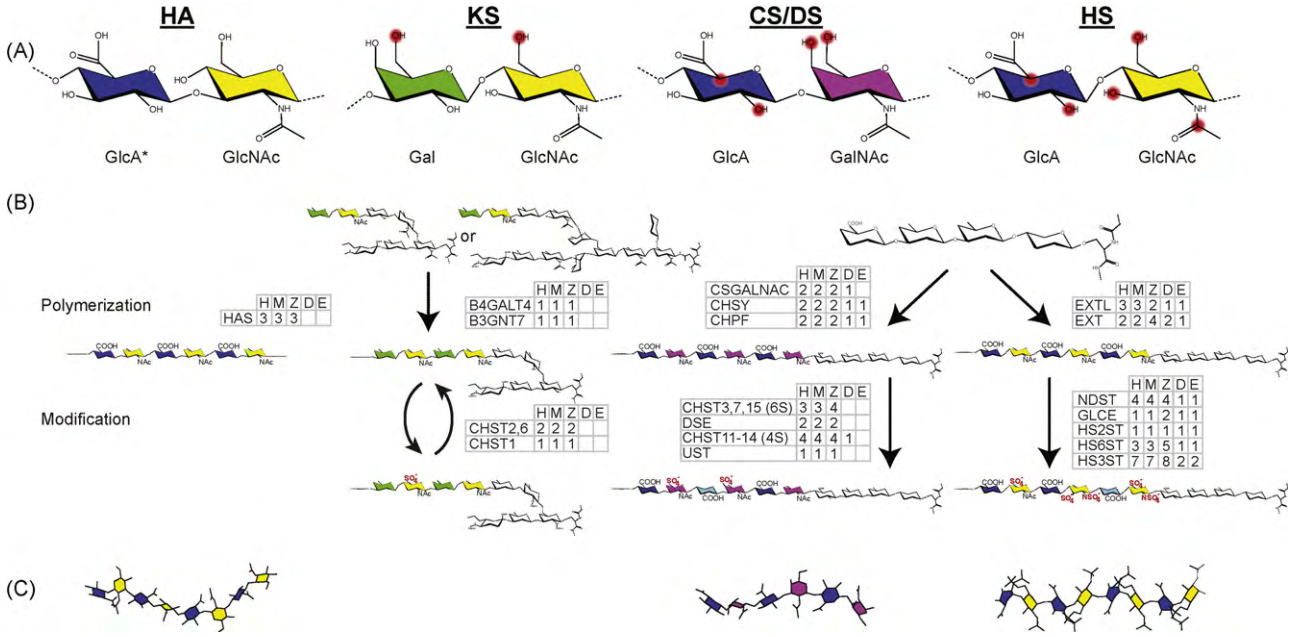

Fig. 1. Interrelated biosynthesis of GAG classes yields similar yet distinct structures. (A) GAGs are classified based on their disaccharide building blocks. Potentially modified positions are highlighted in red. (B) Numerous enzymes catalyze GAG elongation and modification. Families of enzymes involved in each step are listed with the number of homologs from humans-H compared to four models organisms: mouse – M, zebrafish – Z, *Drosophila* – D, and *C. elegans* – E. Saccharides that link KS to core proteins are quite diverse and simplified here for clarity. Descriptions of each gene family along with specific gene IDs are in the Supplemental. (C) GAGs adopt distinct helical conformations. Illustrations were generated using Chem3D Pro with the pdb files 2BVK (HA), 2KQO (CS), and 1HPN (heparin). *Saccharides and modifications are abbreviated using the following nomenclature: GluA, β-D-glucuronic acid; GlcNAc, α/β-D-glucosamine; GalNAc, β-D-galactosamine; Gal, β-D-galactose IdoA, α-L-iduronic acid; NAc, N-acetylation; SO_4^-, sulfate. Specific disaccharides are condensed. For example, the disaccharide containing glucuronic acid linked to a 4-*O* and 6-*O* sulfated galactosamine is represented by GlcA-GalNAc4S,6S (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

binds various cell–cell signaling ligands, regulating both how the cell–cell signaling ligands interact with their receptors and how they form concentration gradients [2]. Recent evidence suggests that CS/DS can employ similar roles as well [3,4]. Furthermore, distinct classes of GAGs can regulate how ligands function in different ways. For instance, all sulfated GAGs interact with collagens and mediate matrix organization: KS increases collagen spacing to aid in light transmission through the cornea [5], CS/DS decreases collagen fibril size to increase the tensile strength of skin [6], and HS remodels collagen matrices to facilitate migration [7]. Modifications on particular GAG chains add an additional level of control as embryos that lack specific GAG modifying enzymes often closely resemble others in which specific developmental signaling pathways have been blocked [8]. While these observations suggest that distinct GAG modifications might provide an extracellular code that helps direct development [8], demonstrating clear structure–function relationships has proven elusive.

A paradigm for GAG-binding specificity is the interaction between antithrombin and a distinct HS pentasaccharide, a target for most heparin-related drugs [9]. Among other modifications, a specific 3-*O* sulfation dramatically increases the affinity of HS/heparin for antithrombin yet can be dispensable for antithrombin activation [10]. The connection between antithrombin–GAG binding and function is important clinically as a number of patient deaths were associated with heparin doses found to be contaminated with chemically oversulfated CS [11], a GAG that can bind antithrombin but cannot stimulate antithrombin-mediated coagulation [12]. Similarly, multiple HS modifications can mediate FGF binding to HS, but specific HS modifications appear to be required to form a functional signaling complex [13]. Thus, GAG modifications that mediate ligand binding can be separated from those that mediate ligand activity. To identify GAG modifications that mediate ligand activity, multiple labs are analyzing the developing embryo, which as Viktor Hamburger suggested, may be "the only teacher who is always right" [14]. This review examines four systems where genetic loss of multiple modifying enzymes results in overlapping phenotypes. A recurring theme emerges: distinct phenotypes are observed when the phenotype can be analyzed as several traits, suggesting that GAG classes and even specific GAG modifications do not appear to be functionally redundant but complement each other.

2. GAG biosynthesis of diverse structures

2.1. Searching for a relationship between GAG fine structures and ligand binding

GAG biosynthesis consists of several polymerization and modification steps (see Supplemental for overview of biosynthesis with enzyme descriptions). The structural variability of sulfated GAG chains is generated by multiple modifying enzymes that expanded in number during early vertebrate evolution (Fig. 1B). Sulfated GAGs are thus endowed with an impressive diversity of possible disaccharides: 4 KS disaccharides, 16 CS/DS, and 48 HS. However, the observed diversity is less than what is theoretically possible as only two-thirds of the HS disaccharides have been identified in nature and homopolymeric repeats tend to predominate, particularly in KS and CS/DS. Synthesis is not template-driven, enzyme reactions do not go to completion, and substrate specificity is generally imprecise, creating heterogeneous chains with unique sulfation patterns that are challenging to characterize. The most common GAG analysis approach is to depolymerize the GAG chain and quantitate the disaccharides, akin to reading a sentence from a list of letters. This technique has revealed that disaccharide content differs between model organisms, between organs, and during different stages of development [15–18]. Curiously, no new disaccharides were observed in vertebrates that might be predicted with the significant increase in the number of modifying enzymes [16]. Since ligand-binding sites typically range from a trisaccharide to a dodecasaccharide [19], disaccharide analysis