EXT1 and EXT2 proteins and heparan sulfate biosynthesis

Marion Kusche-Gullberg

Department of Biomedicine, Division of Physiology, University of Bergen, Norway

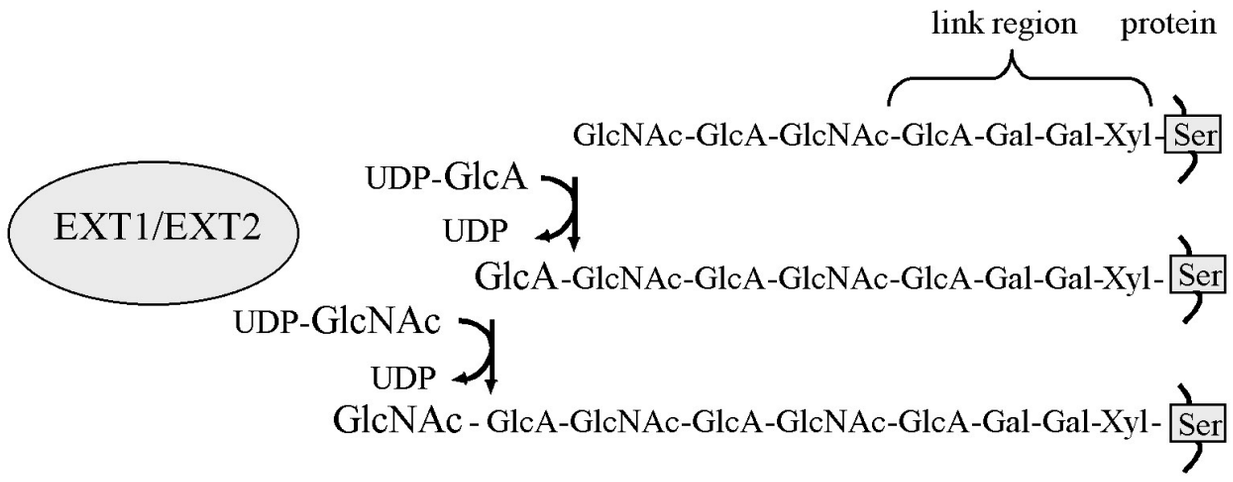
Marion Kusche-Gullberg's research focuses on understanding the molecular mechanisms regulating heparan sulfate biosynthesis. In particular, she is interested in how the activities of the enzymes involved in chain elongation and sulfation reactions affect heparan sulfate structure and function.

Marion Kusche-Gullberg received her PhD in 1990 at the University of Agricultural Sciences, Uppsala, on a thesis dealing with biosynthesis of mast cells proteoglycans. 1990-1992 she did a postdoc with John and Lisa Fessler at the Molecular Biology Institute, UCLA, on a project concerning the molecular biology of basement membrane glycoproteins in the fruitfly *Drosophila melanogaster*. In 1993 she returned to Uppsala and joined the laboratory of Professor Ulf Lindahl, at the Department Medical Biochemistry and Microbiology, Uppsala University, to continue her research in the proteoglycan field. In 2004 she was appointed Professor of Medicine (Physiology) at the Department of Biomedicine, University of Bergen, Norway.

Home page: http://www.uib.no/med/biomed/research/gullberg/

Figure 1. The polymerization process in heparan sulfate biosynthesis

The biosynthesis of HS begins with the formation of the polysaccharide-protein linkage region which consists of the tetrasaccharide: glucuronic acid-galactose-galactose-xylose, where xylose is attached to a serine residue in the core protein After addition of a single N-acetylglucosamine (GlcNAc), chain elongation proceeds by the alternating addition of glucuronic acid (GlcA) and GlcNAc by the EXT1/EXT2 complex.



Heparan sulfate is a complex polysaccharide that plays an important role in several cellular processes, including normal fetal development, wound healing and inflammation. Defects in enzymes involved in heparan sulfate synthesis result in different abnormalities including abnormal skeletal and kidney development. Elongation of heparan sulfate chains occur by the alternat-ing transfer of glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc) units to the nonreducing end of the polysaccharide. Concomitant with elongation, the polymer is modified through a series of reactions that requires the action of several different enzymes. The extent of these reactions varies, giving rise to heparan sulfate chains with different structural properties. The chain elongation reaction has been ascribed to a hetero-oligomeric complex of EXT1 and EXT2 proeins. Mutations in the genes encoding either EXT1 or EXT2 have been linked to the human disorder hereditary multiple exostoses (HME). HME is characterized by the formation of cartilage-capped bony outgrowths, known as osteochondromas or exostoses, at the ends of the long bones. HME is one of the most common skeletal disorders with an estimated frequency of 1/100 000 to 2/100 000. Although EXT1 and EXT2 are expressed ubiquitously, the effects of mutations seem to be limited to the growing bone.

The individual functions of EXT1 and EXT2 in heparan sulfate chain elongation are currently unknown. EXT1 alone has the capacity to elongate heparan sulfate chains *in vitro*. Furthermore, reduced EXT1 expression levels results in the formation of heparan sulfate chains that are shorter than those normally synthesized. The level of EXT2 protein modifies the catalytic properties of EXT1 but the role of EXT2 in heparan sulfate chain elongation is not clear.

To evaluate the effect of EXT2-mutations on heparan sulfate structure, we have generated transgenic mice with a general and constitutive tissue expression of wild-type or mutated EXT2. To understand the individual roles of EXT1 and EXT2, we have overexpressed the proteins or reduced their levels in mammalian cell systems and studied the effects of these manipulations on heparan sulfate structure. Our studies will contribute new information on the mechanism of exostosis formation caused by disturbed HS formation.

Keywords : Heparan sulfate, biosynthesis, chain elongation, EXT1, EXT2