



# Heparan sulfate biosynthesis: Interaction partners of the NDST enzymes

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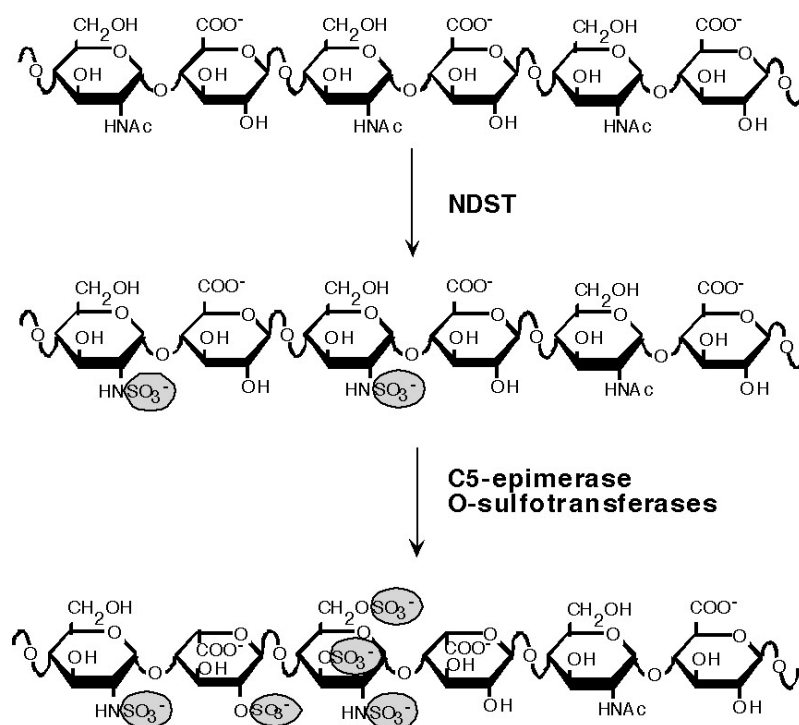
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Lena Kjellén has a PhD from Uppsala University in medical chemistry. Her area of research is heparan sulfate proteoglycans where she has studied both their biosynthesis and more functional aspects. During recent years she has

generated and utilized mice deficient in heparan sulfate biosynthesis enzymes for her studies. From 2001 she is professor in medical glycobiology at the University of Uppsala where she moved from the Swedish University of Agricultural Sciences. She has also spent one and a half year in Birmingham, Alabama, USA.

Fig. 1 Brief outline of the modification reactions in HS biosynthesis



Heparan sulfate (HS) proteoglycans influence embryonic development through interactions with growth factors and morphogens. The interactions depend on HS structure which is largely determined during biosynthesis. Briefly, the biosynthesis occurs in the Golgi compartment and can be divided into chain initiation, polymerization and modification. During chain initiation, linkage tetrasaccharides (glucuronic acid-galactose-galactose-xylose) are assembled on selected serine residues in the core protein. After addition of an *N*-acetylglucosamine residue, polymerization takes place by the alternating addition of glucuronic acid and *N*-acetylglucosamine residues. As the chain grows, modifying enzymes introduce sulfate groups at various positions and some of the glucuronic acid residues are converted into iduronic acid (Fig. 1).

The first modifying enzyme, NDST (glucosaminyl *N*-deacetylase/*N*-sulfotransferase), which replaces the acetyl group in *N*-acetylglucosamine residues with a sulfate group, has a key role in the biosynthesis since all other modifications only occur in the vicinity of *N*-sulfate groups. The final HS biosynthesis product has a molecular design in which clusters of *N*- and *O*-sulfated sugar residues are separated by non-sulfated regions. Four NDST isoforms (transcribed from four different genes) have been identified in vertebrates. They are type II membrane proteins with a short cytoplasmic tail (12 to 18 amino acids), a transmembrane region, and a "stem" region of  $\approx 40$  amino acids followed by the

catalytic domain containing two independent active sites responsible for *N*-deacetylation and *N*-sulfation. It has previously been shown that the HS-polymerases EXT1 and EXT2 form heterodimers, essential for efficient polymerization. Also the 2-*O*-sulfotransferase and the C5-epimerase may interact. We have recently found that NDST1 and EXT2 are coimmunoprecipitated and that overexpression of EXT2 and EXT1, respectively, affects both NDST1 expression and HS sulfation. Our results are compatible with a mechanism where EXT2 acts as a chaperone, carrying both EXT1 and NDST1 to their correct localization in the Golgi compartment. The interacting regions of the interacting proteins have so far not been defined.

In contrast, we have found that the cytoplasmic domain of NDST2 interacts with tubulin. Using a peptide corresponding to the cytoplasmic domain in affinity chromatography of fibroblast lysates, tubulin was the major interacting component. Control experiment with purified tubulin and cytoplasmic peptide established that the binding was due to a direct interaction between tubulin and the peptide, without the requirement for additional bridging proteins. Immunoprecipitation of cell lysates using antibodies against  $\beta$ -tubulin resulted in coprecipitation of NDST2, suggesting that the interaction occurs in the cell. The  $\beta$ -tubulin isoform was identified as mouse  $\beta 5$ . The results are discussed in relation to heparan sulfate biosynthesis and Golgi function.

**Keywords :** Heparan sulfate, *N*-deacetylase/*N*-sulfotransferase, EXT1, EXT2, tubulin