EXT1 and EXT2 proteins and heparan sulfate biosynthesis

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Heparan sulfate proteoglycans

- long, unbranched negatively charged sugar chains, glycosaminoglycans, covalently attached to a protein core.
- synthesized by most cells
Heparan sulfate proteoglycans

Sugar chain → Protein

Serglycin

(heparin proteoglycan)

Perlecan

Syndecan

Glypican
Heparan sulfate function: to bind proteins

- storing
- protecting
- co-receptor
- morphogen gradients
Heparan sulfate influences:

- embryogenesis
- angiogenesis
- cell proliferation
- cell differentiation
- lipid metabolism
- tumor metastasis
- inflammatory reactions
- microbial adhesion
- etc
The biosynthesis is catalyzed by transmembrane type II proteins.
Biosynthesis of heparan sulfate

EXT1/EXT2
Polymerization

C5-Epimerization
- IdoA

N-deacetylation
- N-sulfation

O-sulfation

GlcNAc
GlcAGalGal Xyl

HS protein-linkage region
The exostosin (EXT) family

EXT1  EXT1
EXT2  EXTL1
      EXTL2
      EXTL3

EXT1 and EXT2
Associated with hereditary multiple exostoses.
Proposed roles for the EXT family members in heparan sulfate biosynthesis
Aims

To understand the individual roles of EXT1 and EXT2 in heparan sulfate biosynthesis
Effect on HS biosynthesis of mutations in either EXT1 or EXT2
What are the individual roles of EXT1 and EXT2 in HS biosynthesis?

- **In vitro transferase activities**

- **Characterization of HS from embryonic fibroblast with a gene trap mutation in *Ext1***
Transferase activity assays

GlcNAc-T

UDP-[\textsuperscript{14}C]GlcNAc

EXT proteins

GlcA-T

UDP-[\textsuperscript{14}C]GlcA

EXT proteins
Transferase activities of immunopurified soluble EXT1 and EXT2 proteins

$^{14}$C radioactivity (cpm x 10$^{-3}$)

- Black: GlcNAc-T
- Light gray: GlcA-T

EXT1
EXT2
EXT1/EXT2
In vitro polymerase activity assay

GlcA
GlcNAc
$^{3}[$H$_{a}$]Man$_{R}$

UDP-GlcNAc
UDP-GlcA

Affinity purified EXT proteins
In vitro polymerase activity

P10 gel chromatography


• *Ext1* deficient mice generated by gene targeting die around E8.5. Lack heparan sulfate Lin et al. (2000) *Dev. Biol.*
Heparan sulfate structure in Ext1-mutant mice

Metabolically labeled fibroblasts

- Wild-type HS
- Ext1-mutant HS

Yamada et al. (2004) *J.Biol.Chem*
• EXT1 and EXT1/2 can clearly catalyze the *in vitro* polymerization of the HS backbone structure on an oligosaccharide primer, whereas the activity of EXT2 is weaker.

• Reduction of EXT1 transcript result in shorter HS chains.

• The level of EXT2 modifies the catalytic properties of EXT1.
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