

Proteoglycans in stem biology

Heparan Sulphate Proteoglycans

Heparan sulphate (HS), a ubiquitous resident of cell surfaces and the extracellular matrix (Figure 1), has the potential to simultaneously regulate multiple signalling/cell morphogenic events via the binding of ligands to specific saccharide patterns. Chain heterogeneity allows the co-ordinated expression of many specific patterns within one chain thus leading to multifactorial regulation. Alterations in the sulphation patterns within HS chains are known to occur during development and the progression to disease (Figure 2). Previous *in vitro* systems available for the investigation and assay of HS oligosaccharides have limitations since they tend to concentrate on single signalling events (e.g. FGF2) rather than evaluating the more physiologically relevant role of HS in modulating the activity of multiple factors.

We have evaluated the application of embryonic stem (ES) cells to HS research. They provide an *in vitro* system where cells co-ordinate their response to pro- and anti-differentiative influences to select between various outcomes (Figure 3). HS proteoglycans are of critical importance in this process; forming an interactive interface between the cells and their local environment. ES cell differentiation is a well investigated system; markers of pluripotency and specific lineage selection (both at the cell surface and at the transcript level) are therefore established. For many differentiation pathways key inductive factors are already known, and the majority of these are already recognised as being HS dependent or HS binding (FGF4/BMP4/FGF2/HGF). In addition, many changes which occur in early development reflect cellular processes during tumorigenesis, particularly those relating to the initial events in metastasis.

To form a basis for future work, we have detailed the structure of the HS chains synthesised by ES cells. Although ES cell HS shares many characteristics with other HS types found in various tissues, it is of a very low sulphated-type and is organised into unusual multi-component complexes at the cell surface. As ES cells differentiate to form neuroectodermal precursors the structure and localisation of this HS changes. We suggest that this will influence how ES cells and their differentiated progeny interact with signalling factors (Figure 4). We are also investigating the role of specific sulphation patterns within HS by evaluating the altered differentiation potential of ES cell lines from mice mutant for components of the HS biosynthetic/modification pathway.

Figure 1

Heparan Sulphate Proteoglycans

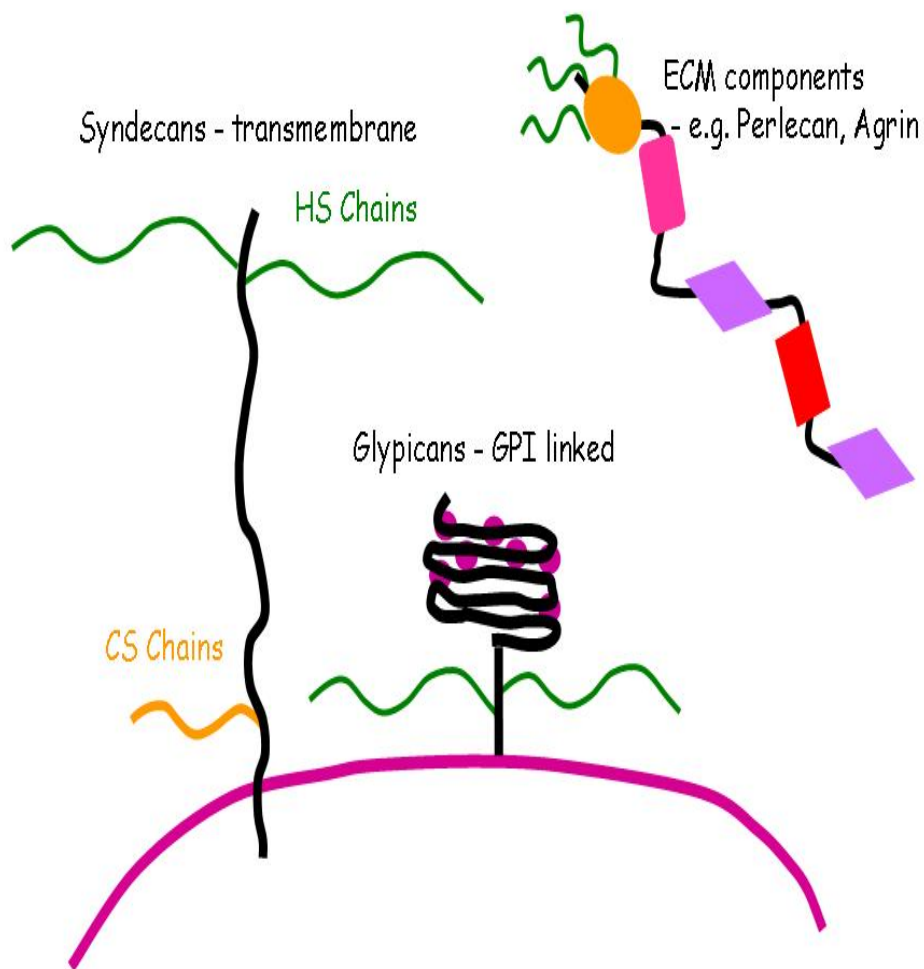


Figure 1

Heparan sulphate proteoglycans (HSPGs) are common constituents of cell surfaces and the extracellular matrix. The HSPG family comprises some of the most abundant proteins on the cell surface, and they are therefore ideally placed to regulate the flow of information between a cell and its environment. The core proteins to which the heparan sulphate (HS) chains are attached dictate their distribution, with both GPI-linked and transmembrane variants present in most cells. The cytoplasmic domains of the transmembrane HSPGs contain recognition domains for intracellular signalling molecules; however the majority of ligand binding events to HSPGs occur via the HS chains themselves. The three major families of HSPGs are the transmembrane syndecans, the GPI-anchored Glypicans and those resident in the extracellular matrix. HSPGs contain 2-3 HS chains which act co-operatively in the binding and activation of growth factors. The core proteins are involved in the organisation of the actin cytoskeleton and in signalling events, acting co-operatively with specific tyrosine kinase membrane receptors which receive growth factors activated by HS.

Figure 2

HS Biosynthesis - An Overview

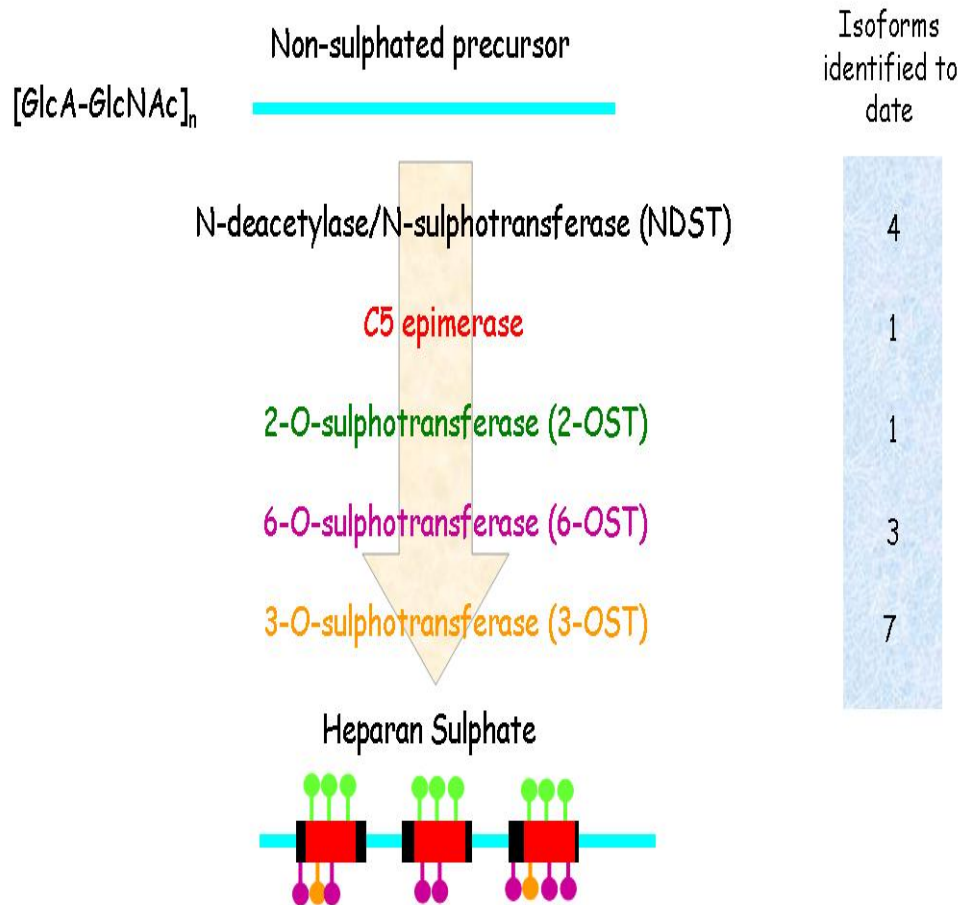
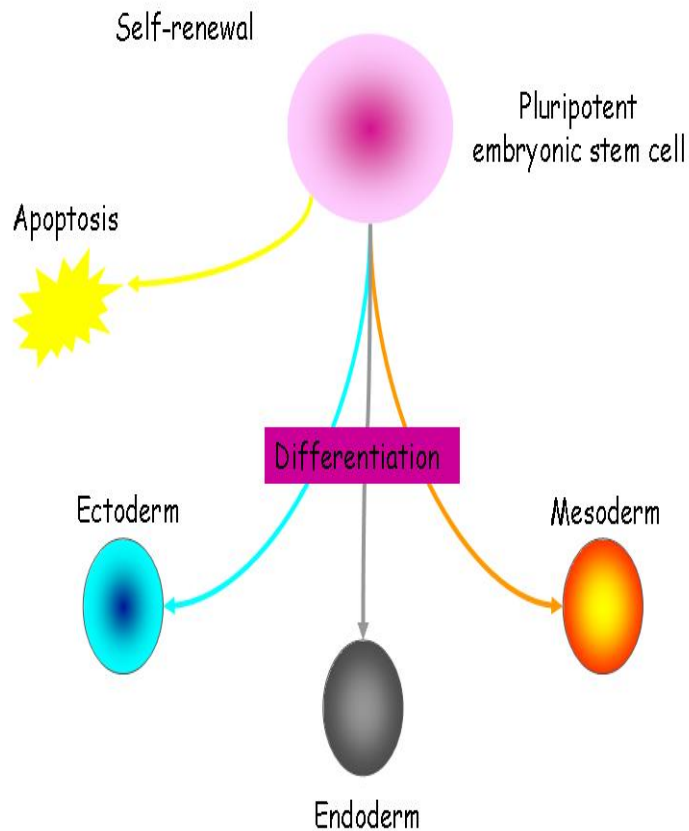


Figure 2

HS biosynthesis is a regulated process by which a uniform oligosaccharide backbone of alternating glucuronic acid (GlcA) and N-acetyl-glucosamine (GlcNAc) residues is modified to a highly heterogeneous mature chain containing regions of high sulphation (sulphated domains) which alternate with extended un-modified sequences. Many of the enzymes which catalyse this process are part of multi-enzyme families which show tight spatio-temporal regulation in their expression.

Figure 3

Embryonic stem (ES) cells



About the group

We work as part of Professor John Gallagher's Cancer Glycobiology Group within the Department of Medical Oncology based at the Paterson Institute.

Department of Medical Oncology
Christie Hospital Research Centre
Wilmslow Road
Manchester
M20 4BX
+44 (0)161 446 3528
Fax: +44 (0)161 446 3269
cmerry@picr.man.ac.uk

Group members

Dr Cath Merry Research Fellow
Mr Graham Rushton Senior Scientific Officer
Ms Claire Johnson PhD student, MRC
Ms Rebecca Baldwin PhD student, Cancer Research UK
Ms Annie Wat PhD student, Dorothy Hodgkin Studentship
Mr Chris Lamanna Visiting PhD student from University of Goettingen

Past group members

Dr Hanae Gouizi
Dr Zoe Scholefield
Dr Stuart Avery
Mr Matt Brown
Ms Nina Remtulla

Key collaborators

Dr Chris Ward	University of Manchester – hES cells and early differentiation
Professor Jeff Esko	UCSD, USA
Professor Toin van Kuppevelt and Dr Gerdy ten Dam	Uni. Nijmegen, The Netherlands
Professor Austin Smith and Dr Valerie Wislon	ISCR, Edinburgh
Drs Valerie Kouskoff and Georges Lacaud	Paterson Institute for Cancer Research
Professor John Gallagher	Professor of Medical Oncology

Publications

Useful reviews

Glycoscience finally comes of age.
Merry, A.H. and Merry, C, L R.
EMBO reports **6**, 10, 900–903 (2005)

Heparan sulfate 2-O-sulfotransferase (Hs2st) and mouse development.
Wilson, V.A., Gallagher, J.T., Merry, C.L.R.
Glycoconj J. 2002 May-Jun;**19**(4-5):347-54.

Heparan sulfate proteoglycans and cancer.
Blackhall, F.H., Merry, C.L.R., Davies, E.J., Jayson, G.C.
Br J Cancer. 2001 Oct 19;**85**(8):1094-8. Review.

Research papers

A new model for the domain structure of heparan sulfate based on the novel specificity of K5 lyase.

Murphy, K.J., Merry, C.L.R., Lyon, M., Thompson, J.E., Roberts, I.S., Gallagher, J.T.
J Biol Chem. 2004 Jun 25;**279**(26):27239-45. Epub 2004 Mar 26.

The molecular phenotype of heparan sulfate in the Hs2st^{-/-} mutant mouse.
Merry CL, Bullock SL, Swan DC, Backen AC, Lyon M, Beddington RS, Wilson VA, Gallagher JT.
J Biol Chem. 2001 Sep 21;**276**(38):35429-34. Epub 2001 Jul 16.

Funding

We are funded by Cancer Research UK via a programme grant to Prof John Gallagher, Dr Malcolm Lyon and Dr Cathy Merry. We receive additional support for PhD students from the MRC, CRUK and the Dorothy Hodgkin Studentship Award (A Wat). Collaborative research visits and conference attendance has been supported by the British Council UK-Netherlands exchange programme, the Royal Society and the Biochemical Society.

Combining the strengths of UMIST and