(aHSPG) are a minor subset of heparan sulfate proteoglycans that endow vascular endothelial cells with anticoagulant activity. These species exhibit a unique sequence of sulfated and non sulfated uronic acid and glucosamine residues, including a 3-*O*-sulfated glucosamine, which are required to complex with the protease inhibitor and induce a conformational change in the protein that accelerate neutralization of blood coagulation enzymes. In the rat ovary, the maturation events leading to the follicule rupture at ovulation are under gonadotrophins control and involve plasminogen activator-mediated proteolytic activities. We have found that the inner follicular granulosa cells synthesize aHSPG in primary cultures using specific ^{125}I-AT binding assays to aHSPG associated to the cell layers or secreted in soluble form into the culture media. Cultured granulosa cells synthesize aHSPG in amounts comparable to microvascular endothelial cells. aHSPG were detected predominantly on granulosa cell layers (associated with the cells membrane and/or extracellular matrix) and in lesser amounts in the culture medium, with a 3:1 ratio between both compartments. Rat granulosa cells can be stimulated *in vitro* by FSH to differentiate and synthesize 17β-estradiol in a dose-dependant manner. We have cultured granulosa cells on protein-coated substrate that allowed them to spread and proliferate without impairing their responsiveness to FSH. Granulosa cells responded to FSH in decreasing by half their cell layer-associated aHSPG while the soluble aHSPG fraction remained unchanged. FSH dose response curves showed that similar concentrations induced both 17β-estradiol synthesis and the decrease of cell-layers associated aHSPG, with a minimal effective FSH dose of 75 pg/ml. The presence of aHSPG in the inner follicle, which remains avascular until ovulation, is surprising and suggests that aHSPG could be involved not only in promoting coagulation inhibition via AT but also in additional physiological functions within the ovary. The hormonal modulation of granulosa cells aHSPG suggests a specific role for these serpin cofactors in the ovulatory process. The decrease of granulosa cells associated aHSPG in response to FSH could facilitate proteolytic attacks of the follicular wall at the time of ovulation.

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Stimulation of Proteoglycan Synthesis by Interleukin-4

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Interleukin-4 (IL-4) is one of the products of T-lymphocytes and mast cells, inflammatory cells which accumulate in connective tissue at early stages of fibrosis. Recently, we have shown that IL-4 stimulated the synthesis of collagen in normal and scleroderma fibroblast cultures. To obtain a further insight on the mode of action of IL-4 on extracellular matrix metabolism we studied the effects of IL-4 on glycosaminoglycan (GAG) and proteoglycan synthesis by fibroblasts in culture.

Cells were incubated with 35-S sulfate and, either 3-H glucosamine or 3-H leucine to study the GAG or the proteoglycan synthesis.

IL-4 induced a dose dependent increase of glucosamine and sulfate incorporation into total GAGs secreted into the culture medium and, to a lesser extent those associated with the cell layer. Maximal incorporation was 30% to 200% above that of control values depending on the fibroblast strain studied and the passage number. Analysis of the GAGs by cellulose acetate electrophoresis indicated a preferential stimulation of culture medium chondroitin/dermatan sulfate synthesis. The proteoglycans secreted to the culture medium were separated on SDS-polyacrylamide gel electrophoresis. IL-4 stimulated the synthesis of the major small chondroitin/dermatan sulfate proteoglycan of Mr 100 k – 120 k (decorin) and slightly modified the molecular mass of the whole molecule.

The stimulation of the synthesis of proteoglycans may be an important factor of IL-4 mediated developement of fibrotic process. IL-4 is the first reported cytokine to increase decorin synthesis.

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S5.17

The Interaction of Heparan Sulphate with Hepatocyte Growth Factor

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Heparan sulphate proteoglycans (HSPGs) interact with many extracellular proteins, including matrix proteins, growth factors and enzymes. These interactions are mediated primarily by the HS chains and it is important to ascertain whether individual interactions require specific sequences within this structurally diverse polysaccharide. Interaction with HS can lead to significant conformational changes within proteins with consequences for their biological activities. This has been clearly demonstrated in the case of basic FGF whose binding to cell surface HS is a prerequisite for subsequent interaction with, and activation of, its signal transducing receptor (Yayon *et al.*, 1991, *Cell* **64**: 841). The structure of a high affinity binding sequence within fibroblast HS has recently been elucidated and corresponds to a specific tetradecasaccharide (Turnbull and Gallagher 1992, *J. Biol. Chem.* **267**: 10337).

We have been investigating the potential interaction between hepatocyte growth factor (HGF) and HS. HGF is unrelated to the FGF family, but also binds to heparin *in vitro* and has been postulated to bind to HSPG *in vivo*. We have demonstrated, using affinity columns substituted with purified cell surface HSPG or recombinant human HGF, that HGF binds to HS chains with an affinity similar to heparin, and requiring 0.8M NaCl for displacement. Digestion of the HS with specific cleavage reagents has indicated some of the structural requirements for interaction, which are distinct from those required for interaction with bFGF. We are at present attempting to elucidate: (a) the sequence of minimal sized HS oligosaccharides which retain high affinity binding to HGF, and (b) whether HS binding modulates the biological activity of HGF.