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Grant Title: **Characterization of the HC2/TSG-6-mediated transfer of HCs between GAGs**

Progress report:

(a) Abstract:

Studies of the interaction between Bikunin proteins, Tumor necrosis factor stimulated gene-6 protein (TSG-6), and glycosaminoglycans have revealed a unique catalytic activity where TSG-6, together with the protein, HC2, transfer proteins between glycosaminoglycan chains [1, 2]. We have recently shown that TSG-6 forms a covalent intermediate complex, mediated by TSG-6's Ser^{28} with the protein to be transferred [3]. However, HC2's role in the transfer reaction is much more unclear. HC2 is a member of a group of HC proteins, but none of the other HCs display the same activity as HC2[2]. These HCs are actually the proteins being transferred to GAGs by TSG-6/HC2. In the present project one of our aims was to elucidate the role of HC2 in the transfer reaction. We have previously shown that the bikunin proteins are structurally unusual proteins, as they are linked by a unique inter-molecular protein-glycosaminoglycan-protein cross-link[4]. This cross-link plays a pivotal role in the protein-transfer reactions mediated by TSG-6/HC2[1], but the structural organization of the bikunin proteins complicates production of recombinant proteins for characterization. In the present project we have successfully established a platform for expressing recombinant forms of bikunin proteins, and we have studied the interaction with TSG-6. We have exploited the protein transfer promoting activity of different variants of HC2 and shown residual chondroitin sulfate mono-saccharides on the C-terminal of HC2 is not required for HC2's role in protein transfer. HC2 contains adjacent cysteine-residues, which is not the case for the other HCs. However, our studies show that these cysteines are also not essential for the transfer activity. After transfer of the HC-proteins to hyaluronic acid (HA) in the extracellular matrix, the HC·HA complexes play a stabilizing role. HC/HC-interactions have been proposed to be involved in this stabilization. We suggest that intra-molecular interactions between HC1 and HC2 in IαI mimic the inter-molecular interactions between HCs in the extracellular matrix. Thus, in the present project we characterized the intra-molecular interactions in IαI using cross-linking technology coupled with LC-MS/MS analyses. Our results show that HCs are interacting and that the chondroitin sulfate is important for intra-molecular interactions in IαI.

1 Sanggaard, K.W., *et al.* (2005) The TSG-6 and I alpha I interaction promotes a transesterification cleaving the protein-glycosaminoglycan-protein (PGP) cross-link. *J Biol Chem* 280, 11936-11942

2 Sanggaard, K.W., *et al.* (2008) The Transfer of Heavy Chains from Bikunin Proteins to Hyaluronan Requires Both TSG-6 and HC2. *J Biol Chem* 283, 18530-18537

3 Sanggaard, K.W., *et al.* (2008) TSG-6 transfers proteins between glycosaminoglycans via a Ser28-mediated covalent catalytic mechanism. *J Biol Chem* 283, 33919-33926

4 Enghild, J.J., *et al.* (1991) Chondroitin 4-sulfate covalently cross-links the chains of the human blood protein pre-alpha-inhibitor. *J Biol Chem* 266, 747-751