N-glycans essential for spermatogenesis

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N-glycans of mammalian glycoproteins play numerous roles within the secretory pathway and at the cell surface. To identify roles for N-glycans in spermatogenesis, conditional deletion in spermatogonia was performed using males transgenic for the Stra8-iCre recombinase transgene. Spermatogonia give rise to sperm following differentiation to spermatocytes and spermatids in close association with Sertoli cells. Conditional deletion of the Mgat1 gene, the glycosyltransferase that initiates complex N-glycan synthesis, disrupts spermatogenesis. A block at the post-meiotic, spermatid stage results in male infertility. Interestingly, Mgat1 gene expression is downregulated in spermatocytes, and expression of a physiological inhibitor of the MGAT1 glycosyltransferase, termed GlcNAcT-IIInhibitory Protein (GnT1IP-L), is upregulated. To determine where GnT1IP-L is active in the secretory pathway, a series of chimeric proteins were generated. The stem and luminal domain of GnT1IP-L was attached to the cytoplasmic and transmembrane domain of proteins that are enriched in a specific compartment of the secretory pathway. GnT1IP-L targeted to the endoplasmic reticulum (ER) did not inhibit MGAT1 activity. GnT1IP-L targeted to the trans Golgi or to the Trans Golgi Network, also did not inhibit MGAT1. However, targeting of GnT1IP-L to medial Golgi via the cytoplasmic and transmembrane domain of MGAT1, inhibited MGAT1 activity, and the synthesis of complex N-glycans. This suggested that GnT1IP-L inhibits MGAT1 in the medial Golgi. To investigate the specificity of the interaction between GnT1IP-L and MGAT1, medial Golgi glycosyltransferases MGAT1, MGAT2, MGAT3, MGAT4, MGAT5 and GnT1IP-L, tagged at the C-terminus respectively by Venus or Cerulean GFP, were co-expressed in COS7 cells. GnT1IP-L generated a robust and specific FRET signal solely with MGAT1. The GnT1IP gene is highly expressed in spermatocytes but not spermatids, and is regulated during spermatogenesis. We are testing the hypothesis that high mannose N-glycans expressed on germ cell glycoproteins when the Mgat1 gene is downregulated and the GnT1IP-L gene is expressed, are required for spermatocyte/Sertoli cell interactions; while complex N-glycans, generated when Mgat1 is upregulated and GnT1IP-L is low, are required for spermatids to differentiate into spermatozoa.