with mRNA from the prostate gland. A cDNA library was constructed from the prostate gland and screened with a probe derived of the putative exon 3 of the LS genomic clone. Two positive hybridizing clones that differ in length and restriction pattern were analyzed. With a PCR method, the 3'RACE (rapid amplification of cDNA ends) protocol, it was demonstrated that β4-GalT related sequences are also present in the albumen gland. Using a primer derived from the putative exon 3 of the genomic LS clone we identified at least four different PCR products of approx. 600 and 700 basepairs.

We conclude that there are several genes present in *Lymnaea stagnalis* that are related to the mammalian β4-GalT. To identify the enzymes they code for the complete cDNAs will be isolated and expressed in COS cells, and the enzymatic properties of the recombinant cells will be studied.

S2.12

Transcriptional Regulation of Murine β1,4-Galactosyltransferase

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The β1,4-galactosyltransferase (β1,4-GT) gene is unusual in that it specifies two mRNAs in somatic cells of 3.9 and 4.1 kilobases (kb). These two transcripts arise as a consequence of initiation at two different sets of start sites that are separated by ~200 base pairs. Translation of each mRNA results in the predicted synthesis of two related protein isoforms that differ only in the length of their NH_2-terminal cytoplasmic domain.

We show that in cells that express low transcript levels, the 4.1 kb transcriptional start site is apparently exclusively used, whereas cells that express high transcript levels preferentially use the 3.9 kb transcriptional start site. To identify the *cis*-acting elements that regulate the use of the two transcriptional start sites, we constructed a series of β1,4-GT/CAT hybrids and carried out transient transfection assays using mouse L cells and Drosophila SL2 cells. These studies have delineated both a distal and proximal regulatory region just upstream of the 4.1 and 3.9 kb transcriptional start sites, respectively. In addition, a negative *cis*-acting regulatory region was identified that represses transcription from the 3.9 kb site. These results suggest a model of transcriptional regulation in which the distal region functions as a housekeeping promoter while the proximal region functions as a mammary cell-specific promoter. Differential initiation from the two promoters is a mechanism for regulation of β1,4-GT enzyme levels. The predictions from this model are consistent with the conclusion that both protein isoforms are functionally equivalent resident *trans*-Golgi membrane-bound enzymes.

S2.13

cDNA Cloning of Three Groups of Sialyltransferase from Chick Embryo

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Sialyltransferases consist of a family of 10-20 enzymes and to date, three groups of sialyltransferase cDNA, Galβ1,4GlcNAc α2,6-sialyltransferase (ST6N)[1], Galβ1,3GalNAc α2,3-sialyltransferase(ST3O)[2], and Galβ1,3(4)GlcNAc α2,3- sialyltransferase(ST3N)[3] have been cloned. Comparison of the amino acid sequences within each sialyltransferase reveal a pattern of homology not observed among other glycosyltransferases. We used this homology to design a cloning strategy to search for additional members of this gene family. Degenerate oligonucleotide corresponding to the highly conserved groups of amino acid among three sialyltransferase genes was constructed and used as PCR primer. DNA sequence of PCR-products obtained from chick embryo cDNA predict an open reading frame with 85-60% homology to the corresponding region of previously cloned sialyltransferase genes. Using these DNA fragments as probe, three groups of chick embryo cDNA(CEA2, CEB1, and CEB3) were cloned. Amino acid sequence of CEA2 showed high homolog to that of rat ST6N. Amino acid sequence of CEB1 and CEB3 showed less homolog to the sequence of previously reported sialyltransferase genes except for the homology to a 55-amino acid residue region. These sequence data suggest that CEB1 and CEB3 encode new groups of sialyltransferase.

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S2.14

Expression of Mouse β-Galactoside α2,6-Sialyltransferase in an Insoluble Form in *Escherichia coli* and Partial Renaturation

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β-galactoside α2,6-sialyltransferase (EC 2.4.99.1) catalyzes the incorporation of sialic acids into terminal positions of glycoconjugate glycans with NeuAc α2,6-Gal linkage. cDNA sequence from rat and human, along with genomic DNA sequence and tissue specific alternative splicing in rat have been described. To gain further insight into the structure and function relationship, we intended to obtain recombinant sialyltransferase synthesized in *E. coli*. An initiation codon and cloning sites were attached by PCR method to mouse brain β-galactoside α2,6-sialyltransferase cDNA which codes polypeptides from 29th a.a. to the c-terminal end of the enzyme. The resulted PCR fragment was incorporated into the expression vector at the down stream of the T7 promoter. The recombinant enzyme, 42k dalton, which lacks cytoslolic and transmembrane domain, was accumulated in the form of insoluble inclusion bodies in *E. coli*. Insoluble fraction of the cell lysate was washed with 1% Triton X-100 and solubilized by 8 M urea. Most of the extracted protein was 42k dalton protein. It was dialyzed to gradually decrease urea concentration in over 48 hours at 40°C. Most of the protein was precipitated in the end. However the supernatant retained