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The enzyme α1,3-galactosyltransferase (α1,3-GT) catalyzes the reaction:

UDP-Gal + Galβ1,4GlcNAc-R→ Galα1,3Galβ1,4GlcNAc-R + UDP,

in which R may be a glycoprotein or a glycolipid (1). cDNAs that encode bovine and murine α1,3-GT have been cloned and characterized (2 – 4), and the organization of the murine gene has been established (4). Bovine α1,3-GT has been expressed as a membrane-bound protein in insect cells (Sf9) after infection with recombinant baculovirus (5).

For the efficient production and purification of large amounts of recombinant α1,3-GT we developed a strategy to express a soluble form of α1,3-GT in insect cells. The DNA sequences that encode the amino terminus and the transmembrane domain of bovine α1,3-GT were deleted from the corresponding cDNA. The DNA fragment thus obtained was ligated in-frame with the cleavable signal peptide of honeybee venom melittin, in the transfer vector pVT-Bac (kindly donated by Dr. T. Vernet, Biotechnology Research Institute, Montreal, Canada). Recombinant baculovirus was prepared by homologous recombination in Sf9 cells, and was used to direct expression of a protein that contained the "stem-region", and the entire catalytic domain (amino acids 28 – 368) of bovine α1,3-GT. Recombinant α1,3-GT accumulated in the growth medium of virus-infected cells to a level of approximately 0.1 U/ml. SDS-PAGE and Western blotting detected a single immunoreactive protein species (≈39 kD), which appeared to be *N*-glycosylated, and could be purified by affinity chromatography on UDP-Sepharose columns.

The strategy as described will be compared with a procedure in which α1,3-GT tagged with a $(His)_6$ sequence is expressed in insect cells, and then purified by metal chelate affinity chromatography. Purification of the recombinant protein from the growth medium should provide sufficient material for protein structural studies.

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S6.4

A Recombinant Approach to Study the Cell Biology and Antigenicity of Human Glycophorin A

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Glycophorin A (GpA) is the most abundant glycoprotein on the surface of human red blood cells (RBC), is highly glycosylated (1 *N*- and 15 *O*-glycans), and encodes several clinically relevant carbohydrate, peptide, and glycopeptide blood group antigens. In particular, the M and N antigens are defined by amino acid polymorphisms at positions one and five:

M: NH2-Ser-Ser-Thr-Thr-Gly-R
N: NH2-Leu-Ser-Thr-Thr-Glu-R

The Ser and Thr residues at positions 2 – 4 are linked to sialylated *O*-glycans and binding of *M*- and *N*-specific antibodies depends on both the peptide sequence and the *O*-glycans. To investigate the cell biology and antigenicity of GpA, we transfected human GpA cDNA (M-allele) into wild-type and glycosylation-defective Chinese hamster ovary (CHO) cells. Stable clones expressing high levels of GpA M antigen were obtained. On immunoblots, monomer (~38 kD) and dimer forms of recombinant GpA co-migrated with human RBC GpA. The recombinant GpA *O*-glycans were primarily NeuAcα2-3Galβ1-3GalNAc, with lower levels of disialylated tetrasaccharide. Using site-directed mutagenesis, we found that the *N*-glycan was not necessary for cell surface expression of GpA; however, studies with IdID CHO cells showed that the *O*-glycans were crucial for this process. By combining site-directed mutagenesis and expression in variant CHO cells, a panel of recombinant GpA blood group antigens was constructed including the M,N,M^c,N^c,T,Tn,Mi.I, and Mi.II antigens. These antigens functioned normally in serological studies with human antisera and were used to investigate the fine specificity of mouse monoclonal antibodies. We also sequenced the V_H and V_L regions of eight mouse monoclonal anti-M and anti-N antibodies in an effort to begin to understand the molecular basis of this antigen-antibody interaction.

S6.5

Heterologous Expression of Membrane-Bound and Soluble Human β1,4 Galactosyltransferase and α2,6 Sialyltransferase in *Saccharomyces Cerevisiae*

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cDNAs coding for the human β1, 4 galactosyltransferase (GT) from HeLa cells and for human α2,6 sialyltransferase (ST) from HepG2 cells were cloned by PCR. The full-length cDNAs coding for the membrane-bound enzymes were inserted into an *E. coli*/yeast shuttle vector between a constitutive yeast PHO5 promoter variant and the PHO5 transcription terminator and expressed in the yeast strain ABYS (deficient in protease A and B and in carboxypeptidase Y and S). Using polyclonal antibodies raised against β-galactosidase-GT and -ST fusion proteins produced in *E. coli*, resp., recombinant enzymes rGT and rST could be immunoprecipitated from extracts of ^{35}S-Met-labelled yeast transformants. Sensitivity to Endo H of both rST and rGT indicated that the rGT and rST entered the secretory pathway in yeast. Both rGT and rST were enzymatically active, affording the conversion of GlcNAc (or Glc in the presence of α-lactalbumin) into LacNAC (or Lac) for GT, and LacNAc into 2,6Sialyl-LacNAc for ST, as shown by H^1-NMR spectroscopy. Soluble forms of both ST and GT were