THE SECOND INTERNATIONAL GLYCOSYLTRANSFERASE SYMPOSIUM

GLYCOSYLTRANSFERASES IN THE NEW MILLENNIUM

The Main Auditorium, Hospital for Sick Children, Toronto

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SCIENTIFIC PROGRAMME

Friday, May 12, 2000

S1. Structure-function relationships of enzymes involved in glycan processing.
Chairperson: Jeremy Carver

8:45 - 9:10 a.m. P1.1. Ulug M.Ünligil[1,2], Sihong Zhou[1,2], Sivashankary Yuwaraj[1,2], Mohan Sarkar[3], Harry Schachter[2,3] and James M. Rini[1,2]. Departments of [1]Medical Genetics and Microbiology and [2]Biochemistry, University of Toronto, Toronto, Ontario, M5S 1A8, Canada; [3]Program in Structural Biology and Biochemistry, Hospital for Sick Children, Toronto, Ontario, M5G 1X8, Canada.

X-ray Crystal Structure of Rabbit N-Acetylglucosaminyltransferase I, a Key Enzyme in the Biosynthesis of N-linked Glycans.

9:10 - 9:35 a.m. P1.2. Christophe Bignon and Louis Noel Gastinel. AFMB CNRS UPR 9039 , 31 Chemin Joseph Aiguier 13402 Marseille cedex 20, France.

Crystal structure of the bovine α1,3galactosyltransferase catalytic domain: a glycosyltransferase responsible for the synthesis of the major xenotransplantation antigen and related with ABO blood group glycosyltransferases.

9:35 - 10:00 a.m. P1.3. Simon J. Charnock and Gideon J. Davies. Structural Biology Laboratory, Dept. of Chemistry, Univ. of York, Heslington, York Y010 5DD, United Kingdom.

The structure of the nucleotide-diphospho-sugar transferase, SpsA from *Bacillus subtilis*, in native and nucleotide-complexed forms: implications for other family 2 enzymes

10:20-10:45 a.m. P1.4. Kieron Brown[1], Gerlind Sulzenbacher[1], Caroline Peneff[1], Suzanne Dixon[1], Laurent Gal[1], Frédérique Pompéo[2], Dominique Mengin-Lecreulx[2], Christian Cambillau[1] and Yves Bourne [1]. [1]AFMB, CNRS-UPR 9039, 31 Chemin Joseph Aiguier F13402 Cedex 20 Marseille. [2]CNRS-UMR8619, Université Paris-Sud, Bâtiment 430, F91405 Orsay Cedex.

Enzymes involved in UDP-GlcNAc biosynthesis: the crystal structures reveal a common motif with glycosyltransferases.

10:45 - 11:10 a.m. P1.5. Christelle Breton, Rafael Oriol and Anne Imberty. CERMAV-CNRS, Univ.Joseph Fourier, BP 53, 38041 Grenoble, France.

Structure-function studies of fucosyltransferases

11:10 - 11:35 a.m. P1.6. P.Lynne Howell[1,2], Francois Vallée[1], Patrick Yip[1], Francesco Lipari[3], Barry Sleno[3], Pedro Romero[3], Annette Herscovics[3], Khanita Karaveg[4] and Kelley W.Moremen[4]. [1]Program in Structural Biology and Biochemistry, Hospital for Sick Children, Toronto, Canada; [2]Dept. of Biochemistry, Univ. of Toronto, Toronto, Canada; [3]McGill Cancer Centre, McGill Univ., Montreal, Québec, Canada; [4]CCRC, Univ. of Georgia, Athens GA 30602, USA.

Structure of yeast and human ER Class I α1,2-mannosidases involved in *N*-glycan processing and ER quality control.

11:35 – 11:45 a.m. S1.2. Toshisuke Kawasaki[1], Kazuaki Ohtsubo[1], Uki Sato[1], Takako Nakatani[1], Masamichi Ishiguro[2], Seiichi Imajo[3] and Shogo Oka[1]. [1]Dept.Biol.Chem. and CREST Project, Japan Science and Technology Corp., Graduate School of Pharmaceutical Sciences, Kyoto Univ., Kyoto 606-8501, Japan; Suntory Institutes for [2]Bioorganic Research and [3]Biomedical Research, Shimamoto, Osaka, Japan.

Structure and function of HNK-1 associated glucuronyltransferases (GlcATs).

11:45 - 12 noon. S1.12. Igor Tvaroska, Isabelle André and Jeremy P.Carver. GlycoDesign Inc., 480 University Ave., Suite 900, Toronto Ont. M5G 1V2 Canada.

Catalytic mechanism of inverting *N*-acetylglucosaminyltransferases: *ab initio* calculations of reaction pathways and transition states.

S2. Glycosyltransferase gene families.
Co-chairpersons: Henrik Clausen and Joel Shaper

2:00 - 2:25 p.m. P2.1. Rudi Steffensen[1,2], Karine Carlier[3], Joelle Wiels[3], Steven B.Levery[4], Mark Stroud[5], Bertil Cedergren[6], Birgitta Nilsson Sojka[6], Eric P.Bennett[1], Casper Jersild[2] and Henrik Clausen[1]. [1]School of Dentistry, Copenhagen, Denmark; [2]Regional Center for Blood Transfusion and Clinical Immunology, Aalborg Hosp., Denmark; [3]CNRS UMR 1598 Inst.Gustave Roussy, France; [4]CCRC, Univ. of Georgia, Athens GA, USA; [5]Dept.Cell Surface Biochem., Northwest Hosp., Seattle WA USA; [6]Dept.Transfusion Med., Unea Univ.Hosp., Umea Sweden.

Cloning of the histo-blood group P^k UDP-Gal:Galβ1-4Glcβ1-Cer α1,4-galactosyltransferase: molecular genetic basis of the p phenotype.

2:25 - 2:50 p.m. P2.2. M. Charron, N.L. Shaper, N.W. Lo and J.H. Shaper. The Johns Hopkins University School of Medicine, Baltimore, Maryland 21231, USA, and the Department of Animal Sciences, Tunghai University, Taichung, Taiwan.

A Novel 14-bp Regulatory Element is Essential for *in Vivo* Expression of Murine β4-Galactosyltransferase-I in Late Pachytene Spermatocytes and Round Spermatids.

2:50 - 3:15 p.m. P2.3. Joseph T.Y. Lau, Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263.

Transcriptional control of the murine CMP-Neu5Ac:Galβ1,4GlcNAc-α2,6 sialyltransferase (ST6Gal).

3:15 - 3:40 p.m. P2.4. Theodora de Vries[1], Ten-Yang Yen[2], Rajesh Joshi[2], Janet Storm[1], Dirk van den Eijnden[1], Ronald Knegtel[3], Hans Bunschoten[3], David Joziasse[1] and Bruce Macher[2]. [1]Dept. Medical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands; [2]Dept. Chemistry and Biochemistry, San Francisco State Univ., San Francisco CA; [3]Research and Development Group, NV Organon, Oss, The Netherlands.

Neighbouring cysteine residues in human FucT VII are engaged in disulfide bridges, forming small loop structures. A proposed 3D model based on location of cystines, and threading and homology modeling.

3:40 - 3:50 p.m. S2.15. Bruce A.Macher[1], Ten-Yang Yen[1], Scott Thomas[1], Rajesh Joshi[1], Anton Nguyen[1], Tracy Long[1], Francois Gallet[2], Abderrahman Maftah[2], Raymond Julien[2] and Eric H.Holmes[3]. [1]Dept. Chemistry and Biochemistry, San Francisco State University, San Francisco CA; [2]Faculté des Sciences, Université de Limoges, France; [3]Northwest Hospital, Molecular Medicine, Dept. of Cell Surface Biochemistry, Seattle WA USA.

Human α1,3/4 fucosyltransferases: characterization of highly conserved cysteine residues.

S4. Glycolipids

Chairperson: Subhash Basu

4:10 - 4:35 p.m. P4.1. Subhash Basu, Manju Basu, Sujoy Ghosh, Sara Dastgheib and Shib S. Basu. Dept. of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556.

CARS and HY-CARS Glycosyltransferases in Glycolipid Biosynthesis

4:35 - 5:00 p.m. P4.2. Cliff Lingwood. Hospital for Sick Children, Toronto, Ont. M5G 1X8 Canada.

MDR1 (Multiple Drug Resistance): a role in glycolipid biosynthesis

Five-minute poster talks

Co-chairpersons: Henrik Clausen and Joel Shaper

5:10 - 5:15 p.m. S2.5. Kenji Uchimura[1], Fathy Fasakhany[1], Kenji Kadomatsu[1], Tetsuya Matsukawa[1], Taishi Yamakawa[1,2], Nobuyuki Kurosawa[1] and Takashi Muramatsu[1]. [1]Dept. of Biochem. and [2]the 3rd Dept. of Internal Medicine, Nagoya Univ. School of Medicine, Nagoya 466-8550, Japan.

Identification and molecular characterization of a cDNA encoding a novel N-acetylglucosamine-6-O-sulfotransferase.

5:15 - 5:20 p.m. S2.7. Gustav Fabini[1], Jerka Dumic[1], Dubravko Rendic[1], Jan Mucha[2], Josef Glössl[2], Sabine Müller[2], Marie-Theres Hauser[2] and Iain B.H.Wilson[1]. [1]Institut für Chemie and [2]Zentrum für Angewandte Genetik, Universität für Bodenkultur, Vienna, Austria.

Cloning of glycosyltransferase from genetic model organisms.

5:20 - 5:25 p.m. S2.9. Paul D.Kingsley, Kelly G.Ten Hagen, Kathleen M.Maltby, Jane Zara and Lawrence A.Tabak. Center for Oral Biology, Aab Inst. of Biomedical Sciences, Univ. of Rochester, Rochester NY 14642.

Diverse spatial expression patterns of UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family members during mouse development.

5:25 - 5:30 p.m. S2.12. Shanchun Guo[1,2], Takeshi Sato[1] and Kiyoshi Furukawa[1]. [1]Dept. Biosignal Research, Tokyo Metropolitan Inst.Gerontology, Tokyo 173-0015, Japan; [2]Dept.of Pathology, Beijing Medical Univ., Beijing 100083, P.R.China.
Involvement of β-1,4-galactosyltransferases I-VI in *N*-linked sugar chain biosynthesis.

5:30 - 5:35 p.m. S2.14. Yoshihiro Sakamoto[1], Tomohiko Taguchi[1,2], Koichi Honke[1], Hiroaki Korekane[1], Tomoya Ogawa[2], Ken Kitajima[3], Sadako Inoue[4] , Yasuo Inoue[4] and Naoyuki Taniguchi[1]. [1]Dept.Biochem., Osaka Univ.Medical School, Osaka, Japan; [2]Inst.Phys.Chem.Res. (RIKEN), Wako, Japan; [3]Nagoya Univ., Nagoya , Japan; [4]Academia Sinica, Taiwan.
Purification and cDNA cloning of GnT-VI from hen oviduct.

Saturday, May 13, 2000

S3A. Sialic acids
Chairperson: Ajit Varki

8:30 - 8:55 a.m. P3A.1. Ajit Varki. Glycobiology Research and Training Center, Univ. of California at San Diego, La Jolla CA USA.
Enzymes that Modify Sialic Acids.

8:55 - 9:20 a.m. P3A.2. Rita Gerardy-Schahn[1], Matthias Eckhardt[1], Michaela Windfuhr[1], Arnd Manegold[1], Lihua Wang[1], Martina Mühlenhoff[1], Olena Bukalo[2], Geneviève Chazal[3], Christo Goridis[3], Melitta Schachner[2], Harold Cremer[3], and Alexander Dityatev[2]. [1]Medizinische Mikrobiologie, MHH, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany; [2]Molekulare Neurobiologie, Univ. Hamburg, Martinistrasse 52, 20246 Hamburg, Germany; [3]Laboratoire Génétique et Physiologie du Développement, CNRS, 13288 Marseille France.
Polysialic acid (PSA) synthesis *in vivo*

S3B. Cell biology of glycan processing
Chairperson: Dirk van den Eijnden

9:20- 9:45 a.m. P3B.1. David B.Williams[1], Victoria S.Stronge[1], Yoshito Ihara[2], and Yoshiro Saito[3]. [1]Dept. of Biochemistry, University of Toronto, Toronto, Canada; [2]Dept. of Biochemistry, Nagasaki University School of Medicine, Nagasaki, Japan; [3]Div. of Biochemistry and Immunochemistry, National Institute of Health Sciences, Tokyo, Japan.
Chaperone-assisted folding of glycoproteins within the endoplasmic reticulum.

9:45 - 10:10 a.m. P3B.2. Silvia Schnyder-Candrian[1], Lubor Borsig[1], René Moser[2] and Eric G. Berger[1]. [1]Inst. Physiology, Univ. of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland; [2]IBR Inc., CH-9545 Waengi, Switzerland.
Are Golgi-associated glycosyltransferases always in the Golgi apparatus? The case of fucosyltransferase VI.
Localization of α-1,3-fucosyltransferase VI in *Weibel-Palade* bodies, a storage organelle of endothelial cells.

S5. Congenital diseases with defects in glycoprotein biosynthesis

Chairperson: Eric Berger

10:30 - 10:55 a.m. P5.1. William M. Canfield. Department of Medicine and W.K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Application of basic studies in lysosomal enzyme trafficking to the treatment of lysosomal diseases.

10:55 - 11:20 a.m. P5.2. J Jaeken[1], H Carchon[1], S Grünewald[2] and G Matthijs[2]. [1]Centre for Metabolic Disease and [2]Centre for Human Genetics, University of Leuven, Leuven, Belgium.

Congenital Disorders of Glycosylation: 20 years later.

11:20 - 11:45 a.m. P5.3. Vibeke Westphal[1], Susanne Kjaergaard[2], Joseph A. Davis[1], Sandra Peterson[1], Flemming Skovby[2], and Hudson Freeze[1]. [1]The Burnham Institute, La Jolla, CA; [2]University Hospital Rigshospitalet, Copenhagen, Denmark.

Phosphomannose Isomerase Deficiency: A Few Surprises

11:45 - 12:10 p.m. P5.4. Timo Imbach[1], Barbara Schenk[2], Patricie Burda[2], Stephanie Grünewald[3], Els Schollen[3], Ron Wevers[4], Jaak Jaeken[5], Gert Matthijs[3], Markus Aebi[2], Eric G.Berger[1] and Thierry Hennet[1]. [1]Inst. Physiol., Univ. of Zürich; [2]Inst. Microbiol., Swiss Federal Inst.Technology, Zürich; [3]Center for Human Genetics and [5]Dept.Pediatrics, Univ. Hospital of Leuven; [4]Dept.of Neurology, Univ. Hospital, Nijmegen.

Defective Biosynthesis of lipid-linked oligosaccharides as a cause of congenital disorders of glycosylation (CDG).

Five-minute poster talks

Chairperson: Dirk van den Eijnden

2:20 - 2:25 p.m. S2.17. Tilo Schwientek[1], Steven B.Levery[3], Jiunn-Chern Yeh[2], Birgit Keck[1], Gerard Merkx[4]. Ad Geurts van Kessel[4], Minoru Fukuda[2] and Henrik Clausen[1]. [1]School of Dentistry, Univ. of Copenhagen, Norre Allé 20, 2200 Copenhagen, Denmark; [2]The Glycobiology Program, Cancer Research Center, The Burnham Institute, La Jolla CA 92037; [3]Univ. of Georgia, CCRC, Athens GA 30602; [4]Dept. of Human Genetics, Univ. Hospital Nijmegen, P.O.Box 9101, 6500 HB Nijmegen, The Netherlands.

Molecular cloning and characterization of a novel thymus associated core 2 β1,6-*N*-acetylglucosaminyltransferase.

2:25 - 2:30 p.m. S3A.4. Daisuke Nakata, Takashi Angata, Tsukasa Matsuda and Ken Kitajima. Dept. Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya Univ., Nagoya 464-8601, Japan.

Biosynthesis of deaminoneuraminic acid (KDN) in mammalian cells.

2:30 - 2:35 p.m. S3A.6. Arun K. Datta[1], Chi-Huey Wong[2] and James C. Paulson[1]. [1]Department of Molecular Biology and [2]Department of Chemistry, The Scripps Research Institute, San Diego, California 92037.

N-linked deglycosylation has no significant effect on the enzymatic activity of ST3GalIII.

2:35 - 2:40 p.m. S3A.11. Martina Mühlenhoff, Arnd Manegold, Michaela Windfuhr, Birgit Gotza and Rita Gerardy-Schahn. Medizinische Mikrobiologie, MHH, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany.

Importance of N-glycans for the activity of ST8SiaII and ST8SiaIV.

2:40 - 2:45 p.m. S3B.1. Paul V.Beum, Dhundy Bastola and Pi-Wan Cheng. Dept. of Biochemistry and Molecular Biology, Univ. of Nebraska Medical Center, Omaha NE.

Epidermal growth factor down-regulates mucin core 2 β1,6-*N*-acetylglucosaminyltransferase activity in NCI-H292 airway cells.

2:45 - 2:50 p.m. S3B.2. Eckart Grabenhorst and Harald S. Conradt. Department of Protein Glycosylation, National Research Center for Biotechnology GBF, Mascheroder Weg 1, D-38124 Braunschweig, Germany.

The sequential arrangement and stability of Golgi Glycosyltransferases is mediated by signals contained in their CTS regions

2:50 - 2:55 p.m. S3B.7. C. Zuber[1], M. J. Spiro[2], B. Guhl[1], R. G. Spiro[2] and J. Roth[1]. [1]Div. Cell and Molec. Pathol., University of Zürich, CH-8091 Zürich; [2]Depts. of Biol. Chem. and Med., Harvard Med. Sch. and Joslin Diabetes Ctr, Boston, MA 02215.

Golgi apparatus immunolocalization of endomannosidase suggests post-endoplasmic reticulum glucose trimming implications for quality control.

2:55 - 3:00 p.m. S4.1. Matt S.Anderson[2], Suzanne S.Eveland[2] and Neil P.J.Price[1]. [1]Dept. Chemistry, State Univ. of New York (SUNY-ESF), Syracuse NY 13210; [2]Dept. Enzymology, Merck Research Lab., P.O.Box 2000, Rahway NJ 07065.

Conserved cytoplasmic motifs that distinguish members of the polyprenol-P: *N*-acetylhexosamine-1-P transferase family.

3:00 - 3:05 p.m. S4.3. Joan M.Boggs and Huimin Wang. Program in Structural Biology and Biochemistry, Hospital for Sick Children, Toronto Ont. M5G 1X8 Canada.

Effect of carbohydrate-carbohydrate interactions between glycolipids in apposed membranes of oligodendrocytes and liposomes on the cytoskeleton.

S6. Glycosyltransferases in cancer

Co-chairpersons: Naoyuki Taniguchi and Jim Dennis

3:15 - 3:40 p.m. P6.1. Inka Brockhausen. Depts. of Medicine and Biochemistry, Queen's Univ., Kingston Ont. K7L 3N6 Canada.

Biosynthesis and functions of cell surface O-glycans

3:40 - 4:05 p.m. P6.2.Maria Granovsky, Mike Demetriou, Pam Cheung, Charles Warren and Jim Dennis. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5.

Mgat5 glycans regulate receptor clustering, and affect cancer growth and T cell stimulation.

4:25 - 4:50 p.m. P6.3. Tomoaki Takahashi[1,2], Yoshitaka Ikeda[1], Eiji Miyoshi[1], Yuji Yaginuma[2], Matsuo Ishikawa[2] and Naoyuki Taniguchi[1]. [1]Dept.Biochem., Osaka Univ.Medical School, Osaka, Japan; [2]Dept.Gynecology and Obstetrics, Asahikawa Medical College, Asahikawa, Japan.

Marked and specific expression of α1,6-fucosyltransferase in human ovarian serous adenocarcinomas

4:50 - 5:00 p.m. S6.7. Donald A.Withers, Wendy Smith and Sen-itiroh Hakomori. Pacific Northwest Research Institute and Dept. of Pathobiology, Univ. of Wash., Seattle, WA, USA.

Transcriptional regulatory mechanisms for aberrant glycosylation defining human tumor malignancy.

S7A. O-GlcNAc-transferase
Chairperson: Gerry Hart

5:00 - 5:25 p.m. P7A.1. Gerald W. Hart, Robert N.Cole, Lisa K.Kreppel, C. Shane Arnold, Frank I. Comer, Sai Iyer, Xiaogang Cheng, Jesse Issacs, Glendon J. Parker, Venkat Chinnambally, Yuan Gao, Keith Vosseller, Lance Wells and Natasha Zachara. Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205-2185.

O-GlcNAc Transferase: a ubiquitous enzyme with complex regulation and promiscuous liaisons

Five-minute poster talks
Chairperson: Gerry Hart

5:30 - 5:35 p.m. S4.6. Koichi Furukawa, Tetsuya Okajima, Satoshi Fukumoto, Keiko Furukawa. Department of Biochemistry II, Nagoya University School of Medicine, 65 Tsurumai, Nagoya, Japan 466-0065.

Sialyltransferase genes to synthesize alpha-series gangliosides.

5:35 - 5:40 p.m. S5.1. Vaishali Kothari, AiHua Liu, Thomas F.Scanlin and Mary Catherine Glick. The Cystic Fibrosis Center and Department of Pediatrics, University of Pennsylvania School of Medicine and the Childrens' Hospital of Philadelphia, Philadelphia PA USA.

Fucosyltransferase activity and product localization in Cystic Fibrosis airway cells.

5:40 - 5:45 p.m. S5.2. Deepak Shori[1], Susan Blackett[1], Harsha Kariyawasam[2], Tina Genter[3] and Jasper Hansen[3]. [1]Dept. of Oral Pathology, Rayne Institute, London SE5 9NU, United Kingdom; [2]Imperial College, London, United Kingdom; [3]Rigshospitalet, Copenhagen, Denmark.

Sulphation of salivary mucins is protein specific, is not correlated to sialylation and is unaffected by Cystic Fibrosis.

5:45 - 5:50 p.m. S6.6. Richard A.Steet and Robert D.Kuchta. Dept. Chemistry and Biochemistry, Univ. of Colorado at Boulder, Boulder CO 80309-0215 USA.
Effect of AZT on branching and sialylation of *N*-linked oligosaccharides in cultured cells.

5:50 - 5:55 p.m. S6.8. Alessandro Datti[1], Rob S.Donovan[1], Borzena Korczak[1] and James W.Dennis[2]. [1]GlycoDesign Inc., 480 University Ave., Suite 900, Toronto Ont. M5G 1V2 Canada; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave, Toronto, Ontario M5G 1X5.
A homogeneous cell-based assay to identify *N*-linked carbohydrate processing inhibitors.

5:55 - 6:00 p.m. S7B.12. Barbara Schenk[1], Jeffrey S.Rush[2], Charles J.Waechter[2] and Markus Aebi[1]. [1]Inst. Microbiol., Swiss Federal Inst.Technology, 8092 Zürich, Switzerland; [2]Dept. Biochemistry, Univ. of Kentucky, College Of Medicine, Lexington KY USA.
Dolichols of unusual chain lengths are used for protein glycosylation in yeast.

Sunday, May 14, 2000

S7B. Invertebrate and plant glycosyltransferases
Chairperson: Gerry Hart

8:30 - 8:55 a.m. P7B.1.Sam Turco[1], Anuradha Guha-Niyogi[1], Haiyan Xu[2] and Stephen Beverley[3]. [1]Dept. Biochemistry, Univ.Kentucky Med.Ctr, Lexington KY 40536; [2]Dept. Biol.Chem. and Mol.Pharm., Harvard Med.School, Boston MA 02115; [3]Dept.Mol. Microbiology, Washington Univ. Medical School, St.Louis MO 36110.
The *Leishmania LPG44* gene is essential for mannose-phosphate-transferase activity that controls assembly of phosphoglycan virulence factors.

8:55 - 9:20 a.m. P7B.2. Qinlong Zheng[1], Irma Van Die[2] and Richard D.Cummings[1]. [1]Dept.Biochem.Mol.Biol., Univ.Oklahoma Health Sciences Center, Oklahoma City OK 73104 USA; [2]Dept.Med.Chem., Vrije Universiteit, Amsterdam, The Netherlands.
Molecular cloning and characterization of a novel α1,2-fucosyltransferase (CE2FT-1) from *C.elegans*.

S8. Glycan processing reactions in the endoplasmic reticulum
Chairperson: Andre Verbert

9:20 - 9:45 a.m. P8.1. Fabiana Fernandez, Karin Loeffel, Jonne Helenius, Claude Jakob, Barbara Schenk, Michael Wacker and Markus Aebi. Swiss Federal Inst. of Technology, Inst. of Microbiology. ETH Zentrum, CH-8092 Zürich Switzerland.
From topology to phylogeny: *N*-linked protein glycosylation and the origin of eukaryotes.

9:45 - 10:10 a.m. P8.2. A. Herscovics, McGill Cancer Centre, McGill University, Montréal, Québec, Canada.
Specificity of Class I N-glycan processing α-1,2-mannosidases.

10:30 - 10:55 a.m. P8.3. Andre Verbert[1], Sandrine Duvet[1], Sharon Krag[2], René Cacan[1] and Jean Dubuisson[3]. [1]Laboratoire de Chimie Biologique, UMR no.8576 du CNRS/USTL, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France; [2]School of Hygiene and Public Health, The Johns Hopkins University, Baltimore MD 21205, USA; [3]UMR no.8526 du CNRS, Institut de Biologie de Lille et Institut Pasteur de Lille, Lille, France.

Delayed N-glycosylation of hepatitis C virus E1 glycoprotein.

S9. Glycoimmunology

Co-chairpersons: Minoru Fukuda and John Lowe

10:55 - 11:20 a.m. P9.1. Minoru Fukuda, Jiunn-Chern Yeh and Nobuyoshi Hiraoka. Glycobiology Program, The Burnham Institute, La Jolla CA 92037, USA.

Two distinct core structures that form L-selectin ligands

11:20 - 11:45 a.m. P9.2. Daniel Chui, John Priatel, Lesley Ellies, Kevin Richardson, and Jamey D. Marth. Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, 9500 Gilman Drive - 0625, University of California San Diego, La Jolla, California, 92093.

Sialyltransferases are Specific Mediators of Immune System Function

11:45 - 12:10 p.m. P9.3. JB Lowe[1], JW Homeister[1], B Petryniak[1], AD Thall[1], P Maly[1], C Rogers[1], PL Smith[1], G Smithson[1], G Cheng[2], S Askari[2], UH von Adrian[2] and EP Scheidegger[3]. [1]Howard Hughes Medical Institute, University of Michigan, Ann Arbor MI; [2]Center for Blood Research, Harvard University, Boston MA; [3]Department of Dermatology, University of Zürich, Zürich, Switzerland.

Immune compromise in mice with selectin ligand deficiencies.

Five-minute poster talks

Co-chairpersons: Minoru Fukuda and John Lowe

2:00 - 2:05 p.m. S7B.14. Irina Wenderoth[1,2] and Antje von Schaewen[1]. [1]Universität Osnabrück, Plant Physiology, FB 5, 49069 Osnabrück, Germany; [2]present address: MPB Cologne GmbH, Neurather Ring 1, 51063 Köln, Germany.

Isolation and characterization of different plant *N*-acetylglucosaminyltransferase I (GnT1) cDNA sequences, and generation of potato and tobacco anti-sense plants.

2:05 - 2:10 p.m. S9.3. Sachiko Sato[1,2], Isabelle Pelletier[1,2], Nathalie Ouellette[2], Marie Simard[2], Ann Rancourt[1,2] and Michel G.Bergeron[2]. [1]Glycobiology Laboratory and [2]Research Centre for Infectious Diseases, CRCHUL, Faculty of Medicine, Université Laval, Québec, Canada.

Galectin-3 is one of the adhesion molecules involved in the extravasation of neutrophils in Streptococcal pneumonia.

2:10 - 2:15 p.m. S10.2. Philip E.Pummill[1], E.S.Kempner[2] and Paul L.DeAngelis[1]. [1]Dept. Biochemistry and Molecular Biology, Univ. of Oklahoma HSC, Oklahoma City OK 73190; [2]NIAMS, NIH, Bethesda MD 20892.

Enzymological characterization of a hyaluronan synthase.

2:15 - 2:20 p.m. S11.5. Hisashi Narimatsu, Shoko Nishihara, Takashi Kudo, Hiroko Iwasaki, Fumiaki Nakayama and Mika Keneko. Division of Cell Biology, Instit. Life Science, Soka Univ., 1-236 Tangi-cho, Hachioji, Tokyo 192-8577, Japan.
Analysis of Fut9-knock-out mouse.

2:20 - 2:25 p.m. S12.5. Peng George Wang. Dept. Chemistry, Wayne State Univ., Detroit MI 48202.
α-Gal oligosaccharides: chemistry, biochemistry and biomedical applications.

S10. Proteoglycan biosynthesis
Chairperson: Marion Kusche-Gullberg.

2:30 - 2:55 p.m. P10.1. Claire Senay[1], Thomas Lind[1], Kumi Muguruma[2], Yuko Tone[2], Hiroshi Kitagawa[2], Kazuyuki Sugahara[2], Kerstin Lidholt[1], Ulf Lindahl[1], and Marion Kusche-Gullberg[1]. [1]Department of Medical Biochemistry and Microbiology, University of Uppsala, Uppsala, Sweden; [2]Department of Biochemistry, Kobe Pharmaceutical University, Kobe, Japan.
EXT1 and EXT2 are glycosyltransferases involved in heparan sulfate biosynthesis

2:55 - 3:20 p.m. P10.2. Jeffrey D. Esko and Maria Aparecida S. Pinhal. Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California San Diego, La Jolla, CA
Infrastructure of heparan sulfate assembly.

S11. Glycosyltransferases in development
Chairperson: Pamela Stanley

3:20 - 3:45 p.m. P11.1. Stuart Johnston[1], Jihua Chen[2], Thomas Vogt[1], and Pamela Stanley[2]. [1]Dept. Molecular Biology, Princeton University, Princeton, New Jersey; [2]Dept.Cell Biology, Albert Einstein College Medicine, New York, NY.
Notch signaling is modulated by Fringe in Lec1 but not in Lec13 CHO cells.

3:45 - 3:55 p.m. S11.4. Daniel J.Moloney[1], Vladislav M.Panin[2], Stuart H.Johnston[3], Jihua Chen[4], Li Shao[1], Richa Wilsin[2], Yang Wang[5], Pamela Stanley[4], Kenneth D.Irvine[2], Thomas F.Vogt[3] and Robert S.Haltiwanger[1]. [1]Dept.Biochemistry and Cell Biology, SUNY-Stony Brook, NY; [2]Waksman Instit. and Dept. of Molecular Biology and Biochemistry, Rutgers, The State University; [3]Dept. Molecular Biology, Princeton University, Princeton, New Jersey; [4]Dept.Cell Biology, Albert Einstein College Medicine, New York, NY; [5]Dept. Pharmakokinetics and Metabolism, Genentech Inc.
Fringe is a glycosyltransferase that modifies the *O*-linked carbohydrates on the epidermal growth factor-like repeats of Notch.

4:15 - 4:40 p.m. P11.2. Karen Gentile, Michael Layden, Karie Berbach, Alison Schwartz and Fred Hagen. Center for Oral Biology, Department of Biochemistry and Biophysics, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642 USA.
O-linked glycoproteins and renal tubule development in *C.elegans*.

S12. Chemi-enzymatic oligosaccharide synthesis, bacterial glycosyltransferases and glycotherapeutics

Co-chairpersons: Monica Palcic

4:40 - 5:05 p.m. P12.1. Warren W. Wakarchuk, David C. Watson, Marie-France Karwaski, Anna-Maria Cunningham, and Michel Gilbert, Institute for Biological Sciences, NRCC, 100 Sussex Dr., Ottawa, K1A 0R6.

Bacterial Lipopolysaccharide Sialyltransferases: More than meets the eye.

5:05 - 5:30 p.m. P12.2. Monica M. Palcic[1], Nina O. L. Seto[2] and Suzanne Laferté[3]. [1]Dept. Chemistry, Univ. of Alberta, Edmonton, AB T6G 2G2, Canada; [2]Inst. for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada; [3]Dept. Biochemistry, Univ. of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada.

Blood Group A and B Glycosyltransferases: Synthesis and *in vivo* Inhibition

5:30 - 5:55 p.m. P12.3. Boyan Zhang[1], Monica M. Palcic[1], David C. Schriemer[2], Michael Pierce[3], Gerardo Alvarez-Manilla and Ole Hindsgaul[1]. [1]Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2 Canada, [2]INH Technologies Inc, Calgary, Alberta, Canada, [3]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30605 USA.

Development of Frontal Affinity Chromatography Coupled to Mass Spectrometry (FAC/MS) for the Evaluation of Inhibitor Libraries for GlcNAcT-V

P1.1

X-RAY CRYSTAL STRUCTURE OF RABBIT *N*-ACETYLGLUCOSAMINYLTRANSFERASE I, A KEY ENZYME IN THE BIOSYNTHESIS OF N-LINKED GLYCANS. Ulug M. Ünligil[1,2], Sihong Zhou[1,2], Sivashankary Yuwaraj[1,2], Mohan Sarkar[3], Harry Schachter[2,3], and James M. Rini[1,2]. Departments of [1]Medical Genetics & Microbiology and [2]Biochemistry, University of Toronto, Toronto, Ontario, M5S 1A8, Canada, and [3]Department of Structural Biology and Biochemistry, Hospital for Sick Children, Toronto, Ontario, M5G 1X8, Canada.

The medial-golgi enzyme UDP-N-acetylglucosamine:α-3-D-mannoside β-1,2-N-acetylglucosaminyltransferase I (GnT I, EC 2.4.1.101) is a key enzyme in the asparagine-linked glycosylation pathway; its action serves as the gateway from oligomannose to complex and hybrid glycans. We have solved the x-ray crystal structure of a soluble fragment containing the catalytic domain of rabbit (*Oryctolagus cuniculus*) GnT I in both the presence and absence of UDP-GlcNAc. The structure is formed by two mixed β-sheets: i) an N-terminal 8-stranded β-sheet containing the acceptor and Mn^{2+}/UDP-GlcNAc binding sites, and ii) a C-terminal 4-stranded β-sheet with no obvious function. Both β-sheets are flanked by α-helices. The structure provides insight into the inverting catalytic mechanism, as well as an explanation for the observation that the reaction proceeds in an ordered sequential fashion. Comparison with other glycosyltransferase structures, in conjunction with sequence analysis, suggests a common structural core likely to be found among members of a number of glycosyltransferase families.
Supported by the Medical Research Council of Canada and the Canadian Protein Engineering Network of Centers of Excellence (PENCE).

P1.2

CRYSTAL STRUCTURE OF THE BOVINE α1,3-GALACTOSYLTRANSFERASE CATALYTIC DOMAIN: A GLYCOSYLTRANSFERASE RESPONSIBLE FOR THE SYNTHESIS OF THE MAJOR XENOTRANSPLANTATION ANTIGEN AND RELATED TO THE ABO HISTO-BLOOD GROUP GLYCOSYLTRANSFERASES. Christophe Bignon and Louis Noel Gastinel* AFMB CNRS UPR 9039 , 31 Chemin Joseph Aiguier 13402 Marseille cedex 20, France. *gastinel@afmb.cnrs-mrs.fr.

Xenoantigens that contain the Galα1,3Gal structures (glycotope) are produced by a specific galactosyltransferase present in major mammalian species but not present in Human, Apes and Old World monkeys (1). This enzyme is a type 2 membrane glycoprotein located in the Golgi apparatus named, UDP-Gal:Galβ1,4GlcNAc α1,3-galactosyltransferase (α1,3GalT, EC 2.4.1.87). The enzyme catalyses in the presence of Mn2+ the following reaction: UDP-Gal+Galβ1,4GlcNAc-RGalα1,3Galβ1,4GlcNAc -R+UDP, in which R is a glycoprotein or a glycolipid. In a previous work, we have succeeded to solve the crystallographic structure of the bovine β1,4GalT1 catalytic domain, an enzyme responsible for the synthesis of the lactosamine core (2). We are particularly interested to approach the crystallographic structure of α1,3GalT enzyme because (a) this enzyme as β4GalT1 is a galactosyltransferase which uses UDP-galactose as donor (b) contrary to β4GalT1, α1,3GalT is a retaining enzyme which keeps the anomericity of the transferred sugar (c) the α1,3GalT acceptor substrate is the product of the reaction catalyzed by β4GalT, then the α1,3GalT acceptor site should present some homologies with the β4GalT1 catalytic pocket (d) α1,3GalT is a member of a large family of enzymes which share extensive amino acid sequence homologies with canine Forssman antigen glycosyltransferase and human ABO histo-blood group glycosyltransferases. The catalytic domain of bovine α1,3GalT (E80-V368) was expressed as a fusion protein with a polyHis tag at its N-terminal portion, in *E. coli* by using a pET T7 expression vector as previously described by Wang's group (3). The over-expressed enzyme was purified by using an NiNTA affinity chromatography standard techniques and a specific affinity chromatography step on UDP-hexanolamine-sepharose. The purified protein, eluted from the last chromatography step using UMP or UDP, was demonstrated being as active as the native protein using lactose and lactosamine acceptor substrates. A mass of 36220 daltons was measured using Mass Spectrometer (MALDI), demonstrating the integrity of the purified protein. α1,3GalT crystallizes in space group P4(1)2(1)2, with unit cell dimensions 95x95x117 Å3. Best crystals have dimensions 0.2x0.2x0.3 mm3 and diffract up to 2.8 Å using in house MAR detector. Native data sets were recorded up to 2 Å resolution using the ESRF synchrotron (Grenoble, France). α1,3GalT structure was solved by using seleno-methionine substituted enzyme and MAD diffraction techniques with the ESRF beam line ID14-EH4. The α1,3GalT catalytic domain, residues K82 to T348, has a globular shape. The structure is composed of one N-terminal subdomain with a typical Rossmann fold and a C-terminal domain containing a mixture of five antiparallel β-strands, α-helices and long

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loops. The subdomain from residue Q125 to residue M224, is folded as a typical Rossmann fold composed of a central twisted β-sheet containing four parallel β-strands surrounded by two short α-helices. One face of the protein contains a pocket with an oval shape of dimensions 17 Å x 23 Å, where are concentrated conserved amino acids as well as the D225VD227 motif. Multiple alignements between the amino acid sequences of the α1,3galactosyltransferase family members and the location of UMP bound to the structure, allowed us to locate amino acids important for the sugar transfer catalysis. A model of catalytic mechanism for this retention enzyme and the identification of amino acids probably involved in donor and acceptor substrates will be discussed. Comparison with β4GalT1 structure will be presented. Moreover, a structural basis of the donor and acceptor specificities of the A, B histo-blood group glycosyltransferases (4), and of the Forssman glycosyltransferase (5) will also be presented.

Refs. (1) Joziasse, D.H. et al. (1999) Biochimica et Biophysica Acta, 1455, 403-418. (2) Gastinel , L.N. et al. (1999) The EMBO Journal, 18, 13, 3546-3557. (3) Janczuk, A. et al. (1999) Curr. Med. Chem. 6(2), 155-164. (4) Hakamori, S-I (1999 Biochimica et Biophysica Acta, 1473, 247-266. (5) Haslam, D and Baezinger, J.U (1996) PNAS, 93, 10697-10702.

P1.3

The structure of the nucleotide-diphospho-sugar transferase, SpsA from *Bacillus subtilis,* in native and nucleotide-complexed forms: implications for other family 2 enzymes

Simon J. Charnock and Gideon J. Davies

Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York, Y010 5DD, UK

SpsA is a glycosyltransferase implicated in the synthesis of the spore-coat of *Bacillus subtilis*, whose homologues include cellulose synthase, dolicol-phospho-mannose transferase and many lipopolysaccharide and bacterial *O*-antigen synthases. It is classified into sequence-family GT-2 (Campbell *et al.*, 1997). The 3-dimensional crystal structure of SpsA has been solved by conventional MIR techniques at a resolution of 1.5 Å (Charnock & Davies, 1999). It is a two-domain protein with a nucleotide-binding domain together with an acceptor-binding domain which features a disordered loop spanning the active-site. The structures of SpsA in complex with both Mg-UDP and Mn-UDP and Mg-*d*TDP have also been determined at 1.7 Å resolution. SpsA represents the first structural member for this important family of transferases. Potential roles played by residues invariant in family 2 may be identified, including the determinants of nucleotide specificity (Davies & Charnock, 1999). Implications for some of the mechanisms for polysaccharide synthesis by this family of enzymes will be discussed.

References

Campbell, J. A., Davies, G. J., Bulone, V. & Henrissat, B. (1997). A classification of nucleotide-diphospho-sugar glycosyl transferases based upon amino-acid smilarities. *Biochem J* **326**, 929-942.

Charnock, S. & Davies, G. (1999). Structure of the Nucleotide-Diphospho-Sugar Transferase, SpsA from *Bacillus* subtilis, in Native and Nucleotide-Complexed Forms. *Biochemistry* **38**, 6380-6385.

Davies, G.J & Charnock, S.J. (1999) "Structure of a nucleotide-diphospho-sugar transferase: implications for the synthesis of polysaccharides" in Recent Advances in Carbohydrate Bioengineering (H.J Gilbert, G.J. Davies, B. Henrissat & B. Svensson, eds) Royal Society of Chemistry (Cambridge UK) pp 132-143.

P1.4

ENZYMES INVOLVED IN THE UDP-GlcNAc BIOSYNTHESIS : THE CRYSTAL STRUCTURES REVEALED A COMMON MOTIF WITH GLYCOSYLTRANSFERASES

Kieron Brown[1], Gerlind Sulzenbacher[1], Caroline Peneff[1], Suzanne Dixon[1], Laurent Gal[1], Frédérique Pompéo[2], Dominique Mengin-Lecreulx[2], Christian Cambillau[1] and Yves Bourne[1]
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UDP-GlcNAc, the cytoplasmic nucleotide-activated form of N-acetylglucosamine (GlcNAc), is an essential precursor largely distributed from prokaryotes to eukaryotes. In bacteria, UDP-GlcNAc is essential for both the peptidoglycan and lipopolysaccharide biosynthesis pathways. In eukaryotes, UDP-GlcNAc is a substrate of chitin synthase whose product is essential for fungal cell wall, and the substrate of glycosyltransferase for protein glycosylation and the GPI anchor of cellular proteins. We solved the structures of a truncated and a full-length forms of GlmU, the bacterial bifunctional enzyme responsible for the UDP-GlcNAc biosynthesis, at 2.25 Å and 2.35 Å resolution, respectively. The molecule is composed of two distinct domains connected by a long α-helical arm: (i) an N-terminal domain which resembles the dinucleotide-binding Rossman fold as found in glycosyltransferases; and (ii) a C-terminal domain which adopts a left-handed parallel β-helix structure as found in other bacterial acetyltransferases. We have also investigated the crystal structures of the equivalent enzymes in eukaryotes, for which we have recently obtained crystals. Comparison of these structures will exemplify a three-dimensional template for the development of new antibiotics. These structures will be presented together with the preliminary crystallization results obtained with human GalNAc-T2, a glycosyltransferase involved in the initiation step of O-glycosylation (Collaboration with E. P. Bennett, H. Hassan & H. Clausen, Copenhaghen, Danmark).

P1.5

STRUCTURE-FUNCTION STUDIES OF FUCOSYLTRANSFERASES

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In the light of recent crystal structure determinations and molecular modeling studies, it has been suggested that the catalytic domain of glycosyltransferases (GT) present common features, that include (i) the occurrence of a nucleotide binding domain composed of a α/β/α layer fold, and (ii) the presence of a large pocket on one face, which is lined with residues of conserved motifs identified for each GT family, and that is capable of accomodating both the donor and acceptor substrates. However differences in recognition of nucleotide donor have been reported: In phage T4 βGlcT, direct interactions between phosphate groups of UDP and basic residues (Arg) of the protein are observed whereas in the case of a bacterial GT (SpsA), and presumably in all DxD-containing glycosyltransferases, an aspartate residue of the DxD motif interacts with the phosphate groups through the coordination of a divalent cation.
On the basis of sequence similarities, fucosyltransferases (FucTs) can be classified into four groups: α2-, α3-, mammalian α6- and bacterial α6-FucTs. We previously demonstrated that a highly conserved peptide motif is shared by all the known α2- and α6-FucTs and suggested its involvement in GDP-fucose recognition. The bacterial NodZ protein (α6-FucT) was chosen as a model to investigate the role of the most conserved residues in the catalytic domain of this large enzyme family. Site-directed mutagenesis combined with theoretical approaches (HCA and fold recognition) has shed light on the possible nucleotide donor recognition mode for fucosyltransferases.

P1.6

STRUCTURE OF YEAST AND HUMAN ER CLASS I α1,2-MANNOSIDASES INVOLVED IN N-GLYCAN PROCESSING AND ER QUALITY CONTROL. [1,2]P.Lynne Howell, [1]François Vallée, [1]Patrick Yip, [3]Francesco Lipari, [3]Barry Sleno, [3]Pedro Romero, [3]Annette Herscovics, [4]Khanita Karaveg and [4]Kelley W. Moremen. [1]Program in Structural Biology and Biochemistry, The Hospital for Sick Children, Toronto, Canada. [2]Department of Biochemistry, University of Toronto, Canada. [3]McGill Cancer Centre, McGill University, Montréal, Canada. [4]Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia, 30602, U.S.A.

Trimming of N-glycans in the endoplasmic reticulum (ER) is part of an elaborate quality control system designed to promote the folding and oligomerization of newly formed glycoproteins and to ensure that only properly folded glycoproteins are transported out of the ER to their final destination. Class I α1,2-mannosidases (glycosyl hydrolase family 47) are involved in this quality control and in the complex N-linked oligosaccharide biosynthetic pathway. The ER Class I α1,2-mannosidase catalyzes the removal of a mannose residue from $Man_9GlcNAc_2$ to form $Man_8GlcNAc_2$ isomer B. Structures of the catalytic domain of the ER α1,2-mannosidases from *S. cerevisiae* and *H. sapiens* have been determined at 1.54 and 1.75 Å resolution, respectively. The proteins have a novel $(\alpha\alpha)_7$ barrel fold. In the *S. cerevisiae* structure, an N-glycan from one molecule extends into the barrel of the adjacent molecule interacting with the essential acidic residues and calcium ion (EMBO J., 2000, 19:581-588). The observed protein-carbohydrate interactions provide the first insight into the catalytic mechanism and partially reveals the amino acids responsible for enzyme specificity. The subsequent structure determination of the human enzyme complexed with the potent inhibitor kifunensine further reveals the location and possible mode of binding of the mannose cleaved by the enzyme during the reaction and the role of the essential calcium ion in substrate binding and stabilization. The overall structure of the protein and the proposed catalytic mechanism will be presented.

Research support by NSERC (AH, PLH) and NIH grants GM31265 (AH, PLH) & GM47533 (KM, PLH).

P2.1

CLONING OF THE HISTO-BLOOD GROUP P^k UDP-GAL: GALβ1-4GLCβ1-CER α1,4-GALACTOSYLTRANSFERASE: MOLECULAR GENETIC BASIS OF THE p PHENOTYPE.

Rudi Steffensen[1,2], Karine Carlier[3], Joelle Wiels[3], Steven B. Levery[4], Mark Stroud[5], Bertil Cedergren[6], Birgitta Nilsson Sojka[6], Eric P. Bennett[1], Casper Jersild[2], and Henrik Clausen[1]

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The molecular genetic basis of the P histo-blood group system has eluded characterization despite extensive studies of the biosynthesis of the P_1, P, and P^k glycolipids. The main controversy has been whether a single or two distinct UDP-Gal:Galβ1-R 4-α-galactosyltransferases catalyze the syntheses of the structurally related P_1 and P^k antigens. The P_1 polymorphism is linked to 22q11.3-ter. Database searches with the coding region of an α4GlcNAc-transferase identified a novel homologous gene at 22q13.2 designated α4Gal-T1. Expression of full coding constructs of α4Gal-T1 in insect cells revealed it encoded P^k but not P_1 synthase activity. Northern analysis showed expression of the transcript correlating with P^k synthase activity and antigen expression in human B cell lines. Transfection of P^k negative Namalwa cells with α4Gal-T1 resulted in strong P^k expression. A single homozygous missense mutation, M183K, was found in six Swedish individuals of the rare p phenotype, confirming that α4Gal-T1 represented the P^k gene. Sequence analysis of the coding region of α4Gal-T1 in P_1+/- individuals did not reveal polymorphisms correlating with P_1P_2 typing.

P2.2

A Novel 14-bp Regulatory Element is Essential for *in Vivo* Expression of Murine β4-Galactosyltransferase-I in Late Pachytene Spermatocytes and Round Spermatids.
M. Charron, N.L. Shaper, N.W. Lo and J.H. Shaper. The Johns Hopkins University School of Medicine, Baltimore, Maryland 21231, USA and the Department of Animal Sciences, Tunghai University, Taichung, Taiwan.
The organization of the murine β4-galactosyltransferase-I (β4GalT-I) gene is unusual in that three transcriptional start sites are contained within an ~725 bp contiguous piece of genomic DNA. From the distal end of the β4GalT-I gene, these start sites are termed respectively male germ cell specific, 4.1kb and 3.9kb, where the numbers indicate the size of the transcribed mRNA. The 4.1kb start site, which is used predominantly in somatic tissues, is also used early in murine spermatogenesis in spermatogonia. As spermatogonia develop into early pachytene spermatocytes, transcription of the β4GalT-I gene is essentially turned off. Continued differentiation to late pachytene spermatocytes and round spermatids is coincident with renewed expression, with steady state β4GalT-I mRNA levels comparable to that observed in spermatogonia. However, the β4GalT-I gene is now transcribed exclusively from the distal male germ cell-specific start site. We had shown previously that a 796-bp genomic fragment that flanks the germ cell start site and contains two putative CRE-like (cAMP-responsive element) motifs, directs correct male germ cell expression of the β-galactosidase reporter gene in late pachytene spermatocytes and round spermatids of transgenic mice. From a detailed analysis of this genomic fragment, we show that *in vivo* expression of β4GalT-I in developing male germ cells is not regulated by a CRE-dependent mechanism, but instead requires an essential 14-bp regulatory element (5'-GCCGGTGGAATAGA-3'), which is located 16-bp upstream of the germ cell-specific start site. This cis-element binds a male germ cell protein, that we have termed TASS-1 (Transcriptional Activator in late pachytene Spermatocytes and round Spermatids-1). The presence of the Ets signature binding motif 5'-GGAA-3' on the bottom strand of the TASS-1 sequence (underlined sequence) suggests that TASS-1 regulatory element may be a novel member of the Ets-family of transcription factors. Additional transgenic analyses established that an amazingly short 87-bp genomic fragment containing the TASS-1 regulatory element was sufficient for correct germ cell-specific expression of the reporter gene, β-galactosidase. Futhermore when the TASS-1 motif was mutated by transversion, within the context of the original 796-bp fragment, transgene expression was reduced 12- to 35-fold *in vivo*. [Charron, M., Shaper, N.L., Rajput, B., and Shaper, J.H., Mol. Cell. Biol. 5811-5822 (1999). Shaper, N.L., Harduin-Lepers, A., and Shaper, J.H., J. Biol. Chem. 25165-2517 (1994)].

P2.3

TRANSCRIPTIONAL CONTROL OF THE CMP-Neu5Ac:Galβ1,4GlcNAc-α2,6 SIALYLTRANSFERASE (ST6Gal).
Joseph T.Y. Lau, Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263.
ST6Gal mediates the biosynthetic addition of sialic acid, via an α2,6 linkage, to the non-reducing end of terminal lactosamine structures. Transcription of the murine ST6Gal gene, *Siat1*, is regulated by the selective use of multiple promoters in a tissue- and developmental- specific manner. At least 6 distinct promoters contribute to *Siat1* transcription, generating mature transcripts that are identical to each other except for their untranslated leaders at the 5' termini. These unique segments are encoded by distinct exons dispersed in 70 Kb of genomic sequence. Constitutive *Siat1* expression in most tissues and cells is mediated by the 5'-most distal promoter, P3, resulting in mature transcripts containing unique 5'-UTR sequences from Exons Q and O. Up-modulation of *Siat1* expression in specific tissues and cells is mediated by recruitment of additional promoters. Hepatic expression of ST6Gal is mediated by promoter P1 under the control of glucocorticoids and IL-6. Mice harboring a defect in the P1 region are unable to elevate liver and serum ST6Gal levels in response to inflammatory signals. The degree of sialylation of plasma proteins remains curiously unaffected. In the mammary glands, lactation is accompanied by elevated ST6Gal activity and a 10-fold elevation in ST6Gal mRNA level, the bulk of which is the result of de novo utilization of a unique promoter, P4. P4-mediated transcription generates an mRNA form containing Exon L in the 5'UTR. P4 utilization is apparently restricted to mammary glands during lactation, since Exon L-containing transcripts are not observed in virgin mammary glands or in other mouse tissues examined. Elsewhere, ST6Gal expression in B cells is also exquisitely regulated. B

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lymphocyte development, differentiation, and activation, is also accompanied by programmed recruitment of *Siat1* promoters. While P3-mediated transcription predominates in pre- and immature cells, a number of additional and unique promoters, P2a, P2b, and P2c, are recruited during activation and terminal differentiation of B cells. P2a-, P2b-, and P2c- transcribed mRNA are distinguishable by inclusion of 5'UTR sequences from Exons X_1, X_{2a}-X_{2b}, and X_3, respectively. The implications of this intricate network of transcriptional controls to ST6Gal function is discussed.

P2.4

NEIGHBORING CYSTEINE RESIDUES IN HUMAN FUCT VII ARE ENGAGED IN DISULFIDE BRIDGES, FORMING SMALL LOOP STRUCTURES. A PROPOSED 3D MODEL BASED ON LOCATION OF CYSTINES, AND THREADING AND HOMOLOGY MODELING*

Theodora de Vries, Ten-Yang Yen#, Rajesh Joshi#, Janet Storm, Dirk van den Eijnden, Ronald Knegtel*, Hans Bunschoten*, David Joziasse, and Bruce Macher#
*Depts of Medical Chemistry, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands, #Chemistry and Biochemistry, San Francisco State University, San Francisco, CA, and *Research and Development Group, NV Organon, Oss, the Netherlands*

Among FucTs from most species four cysteine residues appear to be highly conserved. Two of these cysteines are located at the N-terminus and two at the C-terminus of the catalytic domain. FucT VII possesses two additional cysteines in close proximity of each other located in the middle of the catalytic domain. We identified the disulfide bridges in a recombinant, soluble, form of human FucT VII. Our analyses, using mass spectrometry, demonstrated that all six Cys residues of FucT VII are involved in disulfide bonds. The pairing of the cysteines was determined by proteolytic cleavage of non-reduced protein and subsequent analysis by MS of the resulting peptides. The results demonstrated that Cys^{68}-Cys^{76}, Cys^{211}-Cys^{214}, and Cys^{318}-Cys^{321} are disulfide linked. We have used this information, together with a method of fold recognition and homology modeling, using the $(\alpha/\beta)_8$-barrel fold of *E. coli* dihydrodipicolinate synthase as a template, to propose a 3D model for FucT VII.

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P3A.1

ENZYMES THAT MODIFY SIALIC ACIDS. Ajit Varki, Glycobiology Research and Training Center, University of California at San Diego, La Jolla, CA. USA.

The sialic acids are a family of nine-carbon 2-keto acidic monosaccharides typically found at the outer end of glycoconjugates on the cell surfaces of animals of the deuterostome lineage. They are remarkable for their degree of intrinsic structural diversity, which is generated by various subsituents at the 4,5,7,8 and 9 carbon positions. This diversity is amplified by a variety of different glycosidic linkages from the 2-position to the underlying oligosaccharide chain and can determine or modulate specific recognition of glycans by endogenous and exogenous lectins. This talk will provide an update on the current status of attempts to understand the enzymes that modify sialic acids. Particular emphasis will be on the conversion of the 5-N-acetyl group into an N-glycolyl group and on the O-acetylation of the exocyclic side chain. In the former case, attention will be given to the genetic mutation of CMP-sialic acid hydroxylase in humans, and the unexpected finding that trace amounts of the N-glycolyl-sialic acid may still be found in normal humans. With regard to side chain O-acetylation an update will be provided regarding evidence for multiple enzymatic activities that seem to recognize the underlying sialyl linkage, in a cell type and molecule-specific manner. We will also summarize attempts to purify or clone the genes involved in determining O-acetylation. The current status of other enzymes that modify sialic acids will also be briefly mentioned.

P3A.2

PSA-SYNTHESIS *IN VIVO*

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Polysialic acid is an important regulator of NCAM binding activities. Two polysialyltransferases, ST8SiaII and IV, that are differentially expressed during ontogenesis, are responsible for PSA-synthesis. Their individual contribution and catalytic mechanisms are, however, obscure. Genetic approaches were used to address these questions. Mice negative for ST8SiaIV were generated by gene-targeting. Homozygous negative animals develop normal gross anatomical features and are fertile. Contrary to NCAM knockout mice, histology of the hippocampal CA3 region and migration of neural precursor cells in the rostral migratory stream are unchanged. However, adult animals show a dramatic decrease of PSA in most brain areas and an age dependent loss of synaptic plasticity in the Schaffer collateral-CA1 synapses. In a second study the molecular defects that inactivate ST8SiaIV in cells of the genetic complementation group 2A10 were defined. In seven individual clones, the PSA-negative phenotype is caused by point mutations in the catalytic domain. Mutations concern highly conserved amino acid positions in the α2,8-sialyltransferase family and respective changes introduced in ST8SiaII inactivated also this gene. Two mutations were found to singularly interfere with the auto-catalytic self modification of the polysialyltransferases. Our results provide strong evidence that auto-polysialylation is an essential step in the reaction cascade leading to NCAM-polysialylation.

P3B.1

CHAPERONE-ASSISTED FOLDING OF GLYCOPROTEINS WITHIN THE ENDOPLASMIC RETICULUM.

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Calnexin (CNX) and calreticulin (CRT) are homologous membrane-bound and soluble proteins, respectively, of the endoplasmic reticulum (ER). They bind preferentially to newly synthesized glycoproteins by virtue of a lectin site that recognizes the N-linked oligosaccharide processing intermediate, $Glc_1Man_9GlcNAc_2$. Recently, we showed that CRT and the ER luminal domain of CNX (S-CNX) also have the ability to bind to non-glycosylated proteins in vitro and can discriminate between unfolded and folded protein conformers [Saito et al., (1999) *EMBO J.* 18, 6718; Ihara et al., (1999) *Mol. Cell* 4, 331]. This property enables CRT and S-CNX to function as bona fide molecular chaperones in vitro, preventing the thermally-induced aggregation of non-glycosylated proteins and protecting them against thermal denaturation. To assess whether the lectin site of CRT and S-CNX enhances their chaperone functions with glycoprotein substrates, we compared S-CNX's functions to those of the non-lectin ER chaperone, BiP. We found that for non-glycoprotein substrates, S-CNX and BiP were equally effective in suppressing thermally-induced aggregation. In contrast, S-CNX was much more potent than BiP in suppressing the thermal aggregation of a glycoprotein possessing the $Glc_1Man_9GlcNAc_2$ oligosaccharide. This difference in potency was lost upon removal of the oligosaccharide, suggesting that S-CNX's lectin site confers a substantial advantage relative to BiP when interacting with glycoprotein substrates. We also attempted to refold thermally denatured glycoproteins and non-glycoproteins using purified components in vitro. If denaturation was carried out in the presence of S-CNX, subsequent refolding could be accomplished with BiP, ATP, and the J domain of Sec63p (a co-chaperone that stimulates BiP's ATPase activity). No refolding occurred in the absence of S-CNX and S-CNX alone was incapable of promoting refolding. These findings reveal a cooperation between the CNX/CRT and BiP chaperone systems that may be relevant to the folding of proteins and glycoproteins within the ER.

P3B.2

LOCALIZATION OF α-1,3-FUCOSYLTRANSFERASE VI IN WEIBEL-PALADE BODIES, A STORAGE ORGANELLE OF ENDOTHELIAL CELLS

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Leukocytes adhere to endothelia by selectins via recognition of sialylated and fucosylated tetrasaccharide epitopes termed sialyl-Lewis^x. Fucosylation is catalyzed by a family of α-1,3-fucosyltransferases (FT). Type VI (FTVI) has been implicated to play a role in endothelial cells. To study the subcellular localization of FTVI, we generated a monospecific polyclonal antiserum (PEP6B) raised against a 17 amino acid peptide of the variable region of FTVI. This antiserum recognized recombinant FTVI in stably transfected CHO cells in the ER and the Golgi apparatus. Surprisingly, in macrovascular endothelial cells of human umbilical vein, aorta, saphenous vein and mammary artery, staining was restricted to *Weibel-Palade* (WP) bodies as shown by co-localization of the PEB6B antigen with *von Willebrand* factor or P-Selectin, respectively. Electrophoresis and amino acid sequencing confirmed the identity of the antigen isolated by PEP6B antibodies from the endothelial cells as FTVI. Activity measurements in whole cell lysates support the presence of FTVI in the endothelial cells while enriched WP bodies displayed little activity, suggesting the presence of an inactive enzyme form of FTVI. Storage of a FT in WP bodies suggests a need for its rapid recruitment and a stoichiometric rather than a catalytic role.
Supported by the Swiss National Science Foundation grant 3100-46836.96 to EGB and the Hartmann-Müller Foundation.

P4.1

CARS AND HY-CARS GLYCOSYLTRANSFERASES IN GLYCOLIPID BIOSYNTHESIS. Subhash Basu, Manju Basu, Sujoy Ghosh, Sara Dastgheib, and Shib S. Basu. Dept. of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556.

Nearly 18 different glycolipid-glycosyltransferases (GSL-GLTs) have been characterized from the eukaryotic tissues (normal and developing) and from cultured animal cells (normal and tumorigenic) (1,2). All these different GSL-GLTs are classified according to the sugars they transfer (e.g. GalTs, GlcT, FucTs, GalNAcTs, GlcNAcTs, GlcATs, and SATs) Each GLT is specific for its donor sugar nucleotide substrate and catalyzes the transfer of a specific monosaccharide residue from the donor to the nonreducing terminus of the growing chain of an acceptor glycolipid molecule establishing a specific anomeric linkage. The specificity of an acceptor molecule may reside on the sugar chain or on both the ceramide and the sugar moieties. From our continued studies with several GSL-GLTs (purified and recombinant) in the pathway of ganglio-, globo-, and lacto-families it appears most of these glycosyltransferases could be classified in two categories: 1) CARS: the GLTs containing primarily a carbohydrate recognition site; 2) HY-CARS: the GLTs containing recognition sites for hydrophobic moiety (e.g. ceramide) and a carbohydrate recognition site. Substrate specificity studies with a truncated recombinant GalT-4 (UDP-Gal:LcOse3Cer β1-4galactosyltransferase in the lacto-pathway) from ECB suggests (3) it to be a CAR enzyme. On the other hand, using a highly purified GalT-3 (UDP-Gal:GM2 β1-3galatosyltransferase in the ganglio-pathway) and the modified acceptors (GM2-containing C2 and C18 fatty acids) suggest (4) that perhaps this enzyme belongs to the HY-CARS category. Recently GalT-3 genes from rat (5,6) and from embryonic chicken brains (3,7) have been cloned and a similar study is in progress. Further studies with purified GSL-GLTs in both globo-family (GalNAcT-1; UDP-GalNAc:GM3 β1-4GalNAc-transferase and GalNAcT-2; UDP-GalNAc:GbOse3Cer β1-3GalNActransfewrase) and lacto-family (SAT-3; CMP-NeuAc; α2-3sialyltransferase (8,9)) have been completed with their substrate specificity studies. The GSL-GLTs involved in the pathways of all three families of glycolipids (ganglio-, globo-, and lacto-) will be compared and discussed. (Supported by NS-18005 from NIH and a grant-in-aid from Bayer's Pharmaceutical to SB). Refs. (1) Basu, S. and Basu, M. (in press) in Oligosaccharides in Chemistry and Biology-A Comprehensive Handbook (ed.B.Ernst, P. Sinay, and G. Hart), Wiley-VCH Verlag GmbH, Germany. (2) Basu, S. et al. (1999) in "Comprehensive Natural Products Chemistry: (eds.D. Barton, K. Nakanishi, and O. Meth-Cohen) Vol.3, Carbohydrate. Pp 107-128 Pergamon Press, NY. (3) Basu, S. S. et al. (1998) Acta Biochimica Pol.45,451-467. (4) Ghosh, S. et al. (1995) Glycocojugate J., 12, 838-847. (5) Miyazaki, N. et al. (1997) J. Biol. Chem. 272,24794-24799. (6) Amado, M. et al. (1998) J. Biol. Chem. 273, 12770-12778. (7) Dastgheib, S. et al (1997) Glycoconjugate J, 5, page S48. (8) Basu, S. S. et al. (1996) Biochemistry, 35, 5166-5174. (9) Basu, S. S. et al. (1997) Indian J. Biochem. and Biophys. 34, 97-104.

P4.2

MDR1 (Multiple Drug Resistance): role in glycolipid biosynthesis.
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We have previously found that drug resistant variants of Gb_3 positive tumours show increased susceptibility to verotoxin *in vitro*. This increased susceptibility correlated with an increased proportion of globotriaosyl ceramide (Gb_3-verotoxin receptor) containing short chain fatty acids (C:16 and C:18), as opposed to the parental drug sensitive cells which have higher levels of longer chain fatty acid Gb_3 isoforms (C:22 and C:24). Similarly, in samples from primary multi-drug resistant tumours, we have found that the Gb_3 isoforms preferentially expressed are those which contain shorter chain fatty acids. In order to determine whether MDR1, which provides the major mechanism for the generation of multiple drug resistance, plays any direct role in VT sensitivity or glycolipid biosynthesis we have analyzed VT sensitivity and Gb_3 expression in MDCK cells transfected with the MDR1 gene. The parental cells were found to contain little or no Gb_3 whereas the MDR1 transfected cells showed higher levels of Gb_3 and its precursors, lactosylceramide and glucosylceramide. These upregulated glycolipids were preferentially of the short fatty acid isoforms. Sensitivity to VT was increased by approximately one million-fold, as compared to the parental cell type. Cell sensitivity and Gb_3 synthesis was inhibited when the cells were cultured with cyclosporin, a selective inhibitor of the Pgp pump. Similarly, inhibition of glycolipid biosynthesis in the presence of PPMP, resulted in the loss of sensitivity to verotoxin. In the MDR1 transfected cells the Pgp glycoprotein was for the most part expressed in the Golgi and we therefore proposed that the Golgi, located MDR1 is involved in the translocation of the Gb_3 precursor, glucosylceramide, which is synthesized on the cytosolic surface of the Golgi into the lumen for subsequent elongation by a lactosyl ceramide synthetase and subsequently Gb_3 synthase. Thus MDR1 plays a direct role in the biosynthesis of Gb_3 and its subsequent cell surface expression results in the increased sensitivity of multiple drug resistant cells to verotoxin. This provides a molecular explanation for the increased sensitivity of drug resistant cells to verotoxin and also indicates an additional potential of verotoxin as a clinical antineoplastic.

P5.1

APPLICATION OF BASIC STUDIES IN LYSOSOMAL ENZYME TRAFFICKING TO THE TREATMENT OF LYSOSOMAL STORAGE DISEASES
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Lysosomal enzymes are targeted to the lysosome by a mannose 6-phosphate (M6P) dependent lysosomal targeting pathway. In this pathway, lysosomal enzymes are specifically and uniquely modified to contain a M6P modification of high mannose oligosaccharides. The M6P modification is synthesized in two steps, the first step being the transfer of GlcNAc-phosphate from UDP-GlcNAc catalyzed by the enzyme UDP-*N*-Acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (abbreviated GlcNAc-phosphotransferase). The second step is catalyzed by the enzyme *N*-Acetylglucosamine phosphodiester α-GlcNAcase (abbreviated phosphodiester α-GlcNAcase), which removes the covering GlcNAc exposing a terminal M6P group. Once the M6P targeting determinant is synthesized, the lysosomal enzymes bind to M6P receptors in the *trans*-Golgi network and are targeted to the endosome and ultimately the lysosome. Once in the lysosome, lysosomal phosphatases remove the phosphate, rendering the targeting irreversible.
In the last several years our group has first purified and subsequently cloned the cDNAs and genes of GlcNAc-phosphotransferase and phosphodiester α-GlcNAcase. GlcNAc-phosphotransferase is encoded by two genes, the first gene which encodes the α- and β-subunits is found on chromosome 12q while the second gene which encodes the γ-subunit is on chromosome 16p. The location of the phosphodiester α-GlcNAcase gene is as yet unknown. To generate a highly phosphorylated preparation of recombinant human acid α–glucosidase (rh-GAA) for therapy of Pompe disease, an over-expressing CHO cell line was generated using gene amplification. Culture of this cell line in media buffered to pH 7.2, containing 5 mM deoxymannojirimycin resulted in the secretion of rh-

(continued)

GAA containing predominantly high-mannose type oligosaccharides. Treatment *in vitro* with purified bovine GlcNAc-phosphotransferase and rh- phosphodiester α-GlcNAcase produced a highly phosphorylated preparation of rh-GAA. Cell uptake studies using cultured Pompe disease fibroblasts demonstrated this preparation is internalized about 3-fold more efficiently than bovine testis GAA and ~1200-fold more efficiently than human placental GAA. These studies demonstrate *in vitro* phosphorylation strategies can produce rh-GAA preparations which can be more effectively internalized by cells via the M6P-receptor.

P5.2

Congenital Disorders of Glycosylation: 20 years later.

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The Congenital Disorders of Glycosylation (CDG) are genetic defects in the assembly or processing of the carbohydrate moiety of glycoproteins. They were first described in 1980. In the course of 20 years, in man eight members have been identified in this large family of metabolic diseases. They fall into two groups: CDG-I are defects in the assembly of N-glycans (in cytosol and ER) while CDG-II are defects in the processing of N-glycans and the assembly of O-glycans (in Golgi). CDG with unexplained defect is CDG-x. The basic diagnostic test remains isoelectrofocusing (IEF) of serum transferrin (Tf). CDG-x can have a serum transferrin IEF type 1 (increase of di-(and a)sialo Tf) or type 2 pattern (increase also of three-(and mono)sialoTf. Six disorders have been identified in CDG-I: CDG-Ia (phosphomannomutase 2 deficiency), CDG-Ib (phosphomannose isomerase deficiency), CDG-Ic (dolichyl-P-Glc:Man_9 $GlcNAc_2$ –PP-dolichol glucosyltransferase deficiency), CDG-Id (dolichyl-P-Man:Man_5 $GlcNAc_2$–PP-dolichol mannosyltransferase deficiency), CDG-Ie (dolichol-P-Man synthase-1 deficiency) and leukocyte adhesion deficiency II syndrome. Two disorders are known in CDG-II: CDG-IIa (N-acetylglucosaminyl-transferase II deficiency) and deficient import of GDP-fucose in the Golgi.
The clinical spectrum of all known CDG comprises important neurological features (besides variable dysmorphy and impairment of other organs) except for CDG-Ib that is a hepatic-intestinal disease and the only treatable CDG (mannose).
CDG should be considered in children or adults with an unexplained clinical syndrome.

P5.3

PHOSPHOMANNOSE ISOMERASE DEFICIENCY: A FEW SURPRISES

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Most of the seven known defects that cause Congenital Disorders of Glycosylation (CDG) reduce N-glycosylation by limiting the amount of correctly synthesized lipid-linked oligosaccharide precursor or its efficient transfer to protein. Patients with phosphomannose isomerase deficiency (PMI, CDG-Ib, Fru-6-P↔Man-6-P) develop normally, but have hepatic fibrosis, coagulopathy, and protein-losing enteropathy. Dietary mannose is an effective therapy for these patients because it bypasses the metabolic block. Several young CDG-Ib patients died before diagnosis and therapy became available in 1998. A paper from 1980 described a pair of young sibs with the hallmarks of CDG-Ib. One died at 5 years of age. We located the surviving sib, now 33 years old. She is completely healthy, has a normal diet, and 3 normal children. She had hypoglycosylated transferrin, a facile indicator of CDG, and reduced PMI antigen and activity (10-20% normal). She and her deceased sib have two missense mutations in PMI, confirming the CDG-Ib diagnosis. Three months of mannose therapy normalized transferrin the transferrin isoelectric focusing pattern and antithrombin III level, but they returned to a pre-therapy state when mannose was discontinued. Ultrasound analysis of the liver appeared normal. The absence of any clinical symptoms suggests that the residual PMI activity is sufficient to maintain normal health in an adult, but that transferrin, and perhaps other plasma proteins, may remain abnormally glycosylated. This may mean that PMI-deficient patients might expect normal adult lives, depending on other factors such as environment and genetic background. On the other hand, persistent, long-term hypoglycosylation of multiple serum glycoproteins could lead to hepatopathology in later life. For now, these results provide a positive prognosis for at least some young CDG-Ib patients, who are currently on mannose therapy. (Supported by RO1DK55615)

P5.4

DEFECTIVE BIOSYNTHESIS OF LIPID-LINKED OLIGOSACCHARIDES AS A CAUSE OF CONGENITAL DISORDERS OF GLYCOSYLATION (CDG).

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Prior to the transfer to nascent glycoproteins, the oligomannose core is constructed in the endoplasmic reticulum by subsequent addition of GlcNAc, Man and Glc provided by nucleotide-sugar and dolichol-P-sugar donor molecules. Defects along this biosynthetic pathway have been identified as cause of CDG. The analysis of lipid-linked oligosaccharides in CDG cells, in which CDG-Ia and -Ib have been excluded, unraveled various forms of altered biosynthesis. The majority of those findings were characterized by an accumulation of the precursor structure dolichyl pyrophosphate (Dol*PP*) $GlcNAc_2Man_9$. Genetical investigation revealed that the corresponding CDG patients carried mutant alleles of the *ALG6* α1,3 glucosyltransferase gene. Complementation analysis in *Δalg6* yeasts confirmed the deleterious effect of the mutations onto the *ALG6* protein function, thereby establishing a novel type of CDG referred to as CDG-Ic. Another form of CDG, designated CDG-Ie, was characterized by an accumulation of Dol*PP*-$GlcNAc_2Man_5$ and a low dolichol-P-Man synthase (DPM) activity. Mutations were identified in the *DPM1* gene encoding the catalytic subunit of DPM. Additional abnormal profiles of lipid-linked oligosaccharides were also identified, such as a concomitant accumulation of Dol*PP*-$GlcNAc_2Man_9$ and Dol*PP*-$GlcNAc_2Man_9Glc_1$. Further investigations are presently aimed at understanding the molecular defects underlying these novel types of CDG.

P6.1

BIOSYNTHESIS AND FUNCTIONS OF CELL SURFACE O-GLYCANS

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Cell surface glycoproteins are responsible for the expression of many carbohydrate and peptide epitopes and the functions of cell surfaces. The structures of mucin type O-glycans of cell surface glycoproteins are altered in many cancer cells, and change during differentiation and activation of cells. These structural alterations have been shown to be associated with increased aggressivenss and metastatic behaviour of cancer cells, as well as with apoptosis and altered cell adhesion. In a number of different cell models the biosynthetic pathways of O-glycans and the mechanisms of structural changes could be elucidated. Although each cancer model showed unique mechanisms for structural alterations, a number of enzymatic changes were frequently observed. Several possible mechanisms can lead to similar cell surface expressions of cancer-associated antigens. A number of enzymes were shown to be responsible for the overall structures of cell surface glycans. For example, α3-sialyltransferase and core 2 β6-GlcNAc-transferase activities, both acting on Galβ1-3GalNAc- (core 1) substrates, were altered in most cancer models, and this could explain the structural and antigenic alterations observed. These two enzymes were found to effectively compete for the core 1 substrate, resulting in sialylated core 1 or in complex branched core 2 structures, respectively. α3-Sialyltransferase and core 2 β6-GlcNAc-transferase activities are also altered in endothelial cells undergoing apoptosis. The role of these glycosyltransferases therefore appears to be the control of cell surface O-glycan functions.

This research was supported by the Heart and Stroke Foundation and the Canadian Cystic Fibrosis Foundation of Canada.

P6.2

MGAT5 *N*-GLYCANS REGULATE INTEGRIN AND T CELL RECEPTOR FUNCTIONS AFFECTING CANCER DEVELOPMENT AND IMMUNE RESPONSES *IN VIVO*.

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Golgi β1-6N-acetylglucosaminyltransferase V (Mgat5) produces β1-6GlcNAc-branched complex N-linked N-glycans, which are commonly increased in cancers and in T cell following activation. To examine the role of GlcNAc-TV in development and cancer, mice deficient in Mgat5 were generated by targeted mutation of the *Mgat5* locus. *Mgat5*$^{-/-}$ mice lacked Mgat5 glycan products and appeared normal, but differed in their responses to certain extrinsic conditions. We show that mammary tumor growth and metastases induced by the polyomavirus middle T oncogene was markedly reduced in *Mgat5*$^{-/-}$ mice compared to transgenic littermates expressing Mgat5. Furthermore, we demonstrate that Mgat5 glycan products stimulate membrane ruffling and PI3 kinase-PKB/Akt activation, fueling a positive feedback loop that amplifies oncogene signaling and tumor growth *in vivo*.

We also observed that T cell of *Mgat5*$^{-/-}$ mice are hypersensitive to stimulation via the T cell receptor, and this is associated with lymphocyte infiltrates in liver and kidney, and enhanced delayed-type hypersensitivity skin reactions. The Mgat5 glycans on leukocytes enhance cell migration into sites of inflammation while on T cell they decrease response to antigens. Our finding suggest that Mgat5 glycans impede agonist induced clustering of T cell receptor and of fibronectin receptors, thereby dampening T cell activation and enhancing cell migration, respectively. Mammalian and *C. elegans* Mgat5 (ie *gly-2*) share catalytic specificity, as well as a predominantly neuronal expression pattern. The molecular functionality of Mgat5 glycans in cell-cell communications may be conserved.

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P6.3

MARKED AND SPECIFIC EXPRESSION OF α1,6FUCOSYLTRANSFERASE IN HUMAN OVARIAN SEROUS ADENOCARCINOMAS

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An elevated level of α1,6fucosylation in *N*-glycans is one of the cancer-related alterations of oligosaccharides, as found in patients with malignant diseases such as hepatomas, and this aberrant glycosylation involves α1,6fucosyltransferase (α1,6FucT). We have cloned cDNAs for pig and human α1,6FucTs, and revealed by the transfection with the gene that an increase of α1,6fucose is associated with the metastatic potential of hepatoma cells. Although we have suggested biological significance of α1,6fucosylated glycoproteins in hepatomas as well as its clinical application as a tumor marker, the expression of α1,6FucT and the resulting alteration of oligosaccharides have not been intensively explored in other malignant tumors. We examined the expression of α1,6FucT in various types of epithelial ovarian carcinoma tissues, as well as normal ovary, benign and borderline ovarian tumors. The activity assay showed that α1,6FucT is highly and specifically elevated in serous adenocarcinomas but not in normal and other ovarian tumor tissues. This elevation was found to be due to an enhancement in the mRNA expression, as evidenced by Northern blot analysis. Furthermore, it was shown by an immunohistochemical study that the distribution of α1,6FucT expression is predominantly localized in cancer cells. Lectin blot analysis using LCA, which preferentially recognizes α1,6fucose, suggests that several glycoproteins were likely glycosylation targets modified by α1,6fucosylation in serous adenocarcinoma tissues. These findings suggest that the elevated expression of α1,6FucT and the resulting modification of *N*-glycans are distinctive features of this type of ovarian cancer, and are directly related to the progression of this malignancy.

P7A.1

O-GlcNAc-TRANSFERASE: A UBIQUITOUS ENZYME WITH COMPLEX REGULATION AND PROMISCUOUS LIAISONS

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We are continuing to elucidate the functions of the dynamic glycosylation of nuclear and cytoskeletal proteins by O-linked N-acetylglucosamine (O-GlcNAc). O-GlcNAc is a dynamic protein modification that is as abundant as protein phosphorylation in virtually all higher eukaryotes. Some recent highlights: 1) The O-GlcNAc Transferase (OGT) is encoded by a centromeric X-linked gene localized to Xq13 of the human, and Marth et al. have shown that at least one functional allele is essential for viability of embryonic stem cells. 2) O-GlcNAc Transferase is regulated by multimerization, and by its variable affinities for UDP-GlcNAc; 3) The tetratricopeptide repeat domains of OGT regulate its dimerization; 4) Capping of O-GlcNAc to prevent its rapid cycling by transfection of a protein that binds the saccharide is lethal to CHO cells; 5) A CE-laser Fluorescence assay capable of detecting a few thousand molecules of saccharide modified peptides has been developed; 6) Facile mass spectrometric methods for mapping sites of O-GlcNAcylation/ phosphorylation have been developed and applied to synaptic vesicle regulatory proteins; 7) TATA-binding protein has been demonstrated to bear O-GlcNAc; 8) Insulin promoter factor –1 (IPF-1), the glucose-sensitive transcription factor regulating insulin synthesis, is O-GlcNAcylated; 9) The tau microtubule-associated protein associates with OGT, perhaps bridging it to tubulin; 10) OGT has been found to associate with phosphatases (including MAP kinase phosphatase II), consistent with its proposed 'yin-yang relationship with phosphorylation; 12) SR RNA-splicing proteins are extensively O-GlcNAcylated and/or phosphorylated, with differential localization in the cell; 13) Yeast two-hybrid analyses have identified several proteins which appear to specifically interact with OGT. Some of these OGT-interacting proteins appear to play a role in RNA processing. We are taking an eclectic approach in several systems to elucidate O-GlcNAc's functions. Supported by NIH Grants HD13563, CA42486, a grant from the Juvenile Diabetes Fdn, and a grant from American Health Assistance Fdn. GWH is a consultant for Monsanto and serves on the scientific advisory board of Oxford Glycosciences.

P7B.1

THE *LEISHMANIA LPG4A* GENE IS ESSENTIAL FOR MANNOSE-PO_4-TRANSFERASE ACTIVITY THAT CONTROLS ASSEMBLY OF PHOSPHOGLYCAN VIRULENCE FACTORS.

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Leishmania parasites have evolved a unique family of glycoconjugates, the phosphoglycans that enable them to survive and proliferate in harsh, typically microbiocidal venues. The minimal structure of these phosphoglycans is a Gal(β1,4)Man(α1)-PO_4 repeat unit. The phosphoglycans can either be attached to a GPI anchor and be expressed on the surface of the parasites or appear as carbohydrate chains of secreted proteins. The number of repeat units (avg. n=15 in procyclics, n=30 in metacyclics) doubles during metacyclogenesis, a process of differentiation that is crucial in interactions of the parasite with the mammalian host and the sand fly vector. Thus, regulation of the number of repeat units is a critical part of the parasite's infectious cycle. An "elongation" specific mannosylphosphoryltransferase (MPT) is believed to be intimately involved in controlling the number of repeat units. We have identified the *LPG4A* gene by functional complementation of an *lpg-* mutant of *L. donovani*, which synthesizes only a single repeat unit on glycoconjugates and cannot elongate the glycan. Transfection of the mutant with *LPG4A* restores repeat unit assembly as well as MPT activity, indicating that *LPG4A* encodes either the MPT or a factor required for its activity. We are attempting to express the gene heterologously for characterization of the protein and to study its regulation during development.

P7B.2

MOLECULAR CLONING AND CHARACTERIZATION OF A NOVEL α1,2-FUCOSYLTRANSFERASE (CE2FT-1) FROM *C. ELEGANS*. Qinlong Zheng[1], Irma Van Die[2] and Richard D. Cummings[1]. [1]Dept. Biochem. Mol. Biol., Univ.Oklahoma Health Sciences Center, Oklahoma City, OK 73104 U.S.A., [2]Dept. Med. Chem., Vrije Universiteit, Amsterdam, The Netherlands.

Fucose is a critical component for several carbohydrate-binding proteins, but the broad biological functions of fucosylated glycoconjugates are unclear. To further examine their biological roles, we have begun studies in *Caenorhabditis elegans*, a free-living nematode whose genome of 10^8 bp has been recently deduced. Searches of the *C. elegan.* genome revealed that dozens of putative fucosyltransferase genes are present and the genome contains at least 18 different putative α1,2-fucosyltransferases (α1,2FucTs) with some homology to mammalian α1,2FucTs. To define the activity and function of these putative α1,2FucTs we cloned the cDNAs for some of these enzymes, analyzed the activity of recombinant proteins, and examined the expression of the enzymes in *C. elegans*. One putative α1,2FucT, designated CE2FT-1, contains an open reading frame encoding a predicted protein of 355 amino acids with the type 2 topology and domain structure typical of other glycosyltransferases. The recombinant CE2FT-1 transiently expressed in human 293T cells shows high α1,2FucT activity toward the Galβ-O-phenyl acceptor with GDP-Fuc as the donor. A comprehensive analysis of the acceptor specificity of CE2FT-1 was performed, indicating that this enzyme is completely different from known mammalian α1,2FucTs in its acceptor specificity. For example, the recombinant CE2FT-1 prefers the acceptor Galβ1-4Xylβ-O-benzyl, yet is inactive with the acceptor Galβ1-4Glcβ-O-*p*-nitrophenyl. Promoter analysis of the CE2FT-1 gene using GFP reporter constructs demonstrate that the CE2FT-1 gene is expressed in early stage embryos and only in gut cells of L1-4 and adult worms. Preliminary studies on other members of the putative α1,2-FucT family in *C. elegans* suggest they differ from CE2FT-1 in acceptor specificity. These unexpected findings suggest that that each of the fucosyltransferases in *C. elegans* may have a unique enzyme activity and role in development.

P8.1

FROM TOPOLOGY TO PHYLOGENY: N-LINKED PROTEIN GLYCOSYLATION AND THE ORIGIN OF EUKARYOTES.

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Several components of the eukaryotic N-glycosylation pathway are conserved in archaea. In particular, proteins with a high similarity to the oligosaccharyltransferase component STT3 are found in the archaeal kingdom, where N-linked protein glycosylation can be required for the assembly of the cell wall. The structure of the oligosaccharide transferred to protein seems to be specific for individual archaeal species. Based on the analysis of different archaeal genomes, the N-glycosylation process in archaea and the structure of the lipid-linked oligosaccharide in primitive eukaryotes, it is proposed that N-linked protein glycosylation originates from the archaeal ancestor of eukaryotic cells. This ancestor transferred the oligosaccharide $Man_5GlcNAc_2$, now the intermediate in oligosaccharide biosynthesis that is flipped form the cytoplasm to the lumen of the ER, from the lipid dolichylpyrophosphate to asparagine residues of specific S-layer polypeptides. Within the framework of the hypothesis, this lipid-linked $Man_5GlcNAc_2$ oligosaccharide structure represents a unique feature common to the archaeal ancestor and all eukaryotic cells. In eukaryotes, extension of the oligosaccharide structure to $Glc_3Man_9GlcNAc_2$ as well as glycosylation of many different secretory proteins occurred in the course of evolution. At the same time, the oligosaccharyltransferase changed from a single polypeptide enzyme (STT3) in archaea to the multisubunit complex in higher eukaryotes, a complex that is now able to accommodate the big diversity of the polypeptide substrates in the ER.

P8.2

SPECIFICITY OF CLASS 1 N-GLYCAN PROCESSING α1,2-MANNOSIDASES

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Class I α1,2-mannosidases are type II membrane proteins of the ER and Golgi that are important in the biosynthesis of glycoproteins containing N-glycans (reviewed in 1, 2). This enzyme family has been conserved through eukaryotic evolution. They are calcium-dependent inverting glycosidases inhibited by 1-deoxymannojirimycin and kifunensine, but not by swainsonine. The yeast and human ER α1,2-mannosidases, that remove a single mannose from Man9GlcNAc2 to form Man8GlcNAc2 isomer B, are implicated in ER quality control. Other α1,2-mannosidases that convert Man9GlcNAc2 to Man5GlcNAc2 are essential for complex/hybrid N-glycan synthesis in mammalian cells. The different enzymes that form Man5GlcNAc2 exhibit a distinct order of mannose removal from Man9GlcNAc2, and produce different high mannose oligosaccharide isomers (3-5). Studies on the yeast ER α1,2-mannosidase have served as a model of this enzyme family. It is the first family member whose structure has been determined by X-ray crystallography (6). The structure represents an enzyme/product complex in which the N-glycan of one enzyme molecule extends into the barrel of the adjacent symmetry related molecule. Mutation of a single amino acid interacting with the oligosaccharide alters the specificity of the yeast enzyme. The resulting yeast α1,2-mannosidase mutant can trim Man9GlcNAc to Man5GlcNAc (7). These studies provide insight into the specificity determinants of Class I α1,2-mannosidases, as well as a model explaining site-specific processing of N-glycans.

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1. Herscovics, A. (1999) *Biochim Biophys Acta* **1426**, 275-285
2. Herscovics, A. (1999) *Biochim Biophys Acta* **1473**, 96-107
3. Lal *et al.* (1998) *Glycobiology* **8**, 981-995
4. Kawar *et al.* (2000) *Glycobiology, in press*
5. Tremblay, L.O. and Herscovics, A., *in preparation*
6. Vallée *et al.* (2000) *EMBO J.* **19**, 581-588; Howell *et al.*, this meeting
7. Romero *et al.* (2000) *J. Biol. Chem., in press*

P8.3

DELAYED N-GLYCOSYLATION OF HEPATITIS C VIRUS E1 GLYCOPROTEIN.

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N-glycosylation is usually described as a co-translational event. In a few examples, namely in yeast, it has been described that the protein backbone can be synthesized in the cytoplasm and further translocated to ER lumen to be glycosylated. Since the hepatitis C virus E1 envelope glycoprotein has been shown to be strictly retained in the rough ER, we used this model to examine whether post-translational N-glycoslation could occur in mammalian cells. By pulse chase experiments, we have followed the progression of the glycosylation of the four N-glycosylation sites of E1 glycoprotein expressed in CHO cells. We observed that the fourth glycan was added once the protein synthesis was completed. This post-translational N-glycosylation is more pronounced by using Man-P-Dol deficient CHO cells (B3F7) since only one or two glycans are added co-translationally and the addition of the other glycans requires more than one hour after completion of the protein synthesis. The relationship between glycosylation, folding and ER retention of newly synthesized glycoproteins will be discussed.

P9.1

TWO DISTINCT CORE STRUCTURES THAT FORM L-SELECTIN LIGANDS.

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Lymphocyte homing is important for the surveillance of foreign pathogens. Extravasation of lymphocytes in peripheral lymph nodes is mediated through L-selectin binding to L-selectin ligands, sulfated sialyl Lewis^x present on high endothelial venules (HEV). To determine the roles of sulfation in L-selectin ligand formation in HEV, we and others have recently cloned L-selectin ligand sulfotransferases (LSST or HEC-GlcNAc6ST) that form core2-based selectin ligand functional in rolling assays (ref. 1, 2). The expression of LSST is highly restricted to HEV while another sulfotransferase, GlcNAc6ST, is more widely present and less specific in acceptor substrate requirement. Analysis of core2GnT-leukocyte knockout mice indicates that lymphocyte homing and expression of MECA-79 antigen persist even after the gene for core2GnT-leukocyte has been inactivated. Structural analysis of L-selectin ligands in HEV of the knockout mice demonstrated that the major oligosaccharides remaining are based on extended core1 structure such as NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα→Ser/Thr. The expression of the oligosaccharides on CHO cells by a novel core1 extension enzyme and human LSST resulted in L-selectin dependent rolling and MECA-79 reactivity. (Supported by P0l CA 71932, R01 CA48737 and R37 CA33000)

1. Hiraoka, N., Petryniak, B., Nakayama, J., Tsuboi, S., Suzuki, M., Yeh, J. C., Izawa, D., Tanaka, T., Miyasaka, M., Lowe, J. B. and Fukuda, M. (1999) *Immunity* **11,** 79-89
2. Bistrup, A., Bhakta, S., Lee, J. K., Belov, Y. Y., Gunn, M. D., Zuo, F. R., Huang, C. C., Kannagi, R., Rosen, S. D. and Hemmerich, S. (1999) *J. Cell. Biol.* **145,** 899-910

P9.2

SIALYLTRANSFERASES ARE SPECIFIC MEDIATORS OF IMMUNE SYSTEM FUNCTION. Daniel Chui, John Priatel, Lesley Ellies, Kevin Richardson, and Jamey D. Marth.
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The vertebrate sialyltransferase family has expanded recently to over sixteen members encoded by distinct genes. By using in vivo mutagenesis strategies involving both somatic or germ cells of the intact mouse, we continue to explore the functions of these enzymes. All sialyltransferase mutants produced and studied thus far function in controlling processes crucial to the hematopoietic and immune systems. ST6Gal and ST3Gal-I sialyltransferases have been found to provide unique immunologic functions in specific lymphocyte cell types. ST6Gal generates the trisaccharide structure (Siaα2-6Galβ1-4GlcNAc; 'Sia6LacNAc'), which is the ligand for the CD22 lectin found on B lymphocytes. Absence of ST6Gal results in an immunodeficiency due to attenuated B lymphocyte function. ST6Gal deficient B cells fail to generate high titers of antibody and exhibit reduced phosphotyrosine accumulation on key cellular glycophosphoprotein signal transducers. We continue to study the mechanism involved and have also identified aberrant intracellular kinase activation profiles. Our current data suggest a role for ST6Gal in modulating antigen receptor complex assembly at the cell surface. This phenotype is in contrast to that obtained in studies of ST3Gal-I deficient mice. ST3Gal-I generates α2-3 sialic acid linkages on the galactose of the Galβ1-3GalNAc disaccharide, an event highly conserved in vertebrate T cell ontogeny. Absence of ST3Gal-I results in a deficiency of cytotoxic $CD8^+$ T lymphocytes by apoptosis. T cell-specific mutagenesis of ST3Gal-I using Cre-loxP recombination, recapitulates the phenotype, indicating that the deficit of cytotoxic $CD8^+$ T cells is due to loss of ST3Gal-I function in T cells. Naive $CD8^+$ T cells are especially susceptible to the loss of ST3Gal-I and those few $CD8^+$ T cells remaining in ST3Gal-I deficient mice are primarily of the memory phenotype. While activation responses of ST3Gal-I deficient $CD8^+$ T cells are normal, they are susceptible to CD43-induced apoptosis by a caspase 3-independent mechanism. Additionally, loss of ST3Gal-I induces core 2 O-glycan biosynthesis without a change in core 2 GlcNAcT specific activity, revealing the unexpected presence of a competition in vivo between core 2 GlcNAcT and ST3Gal-I for the same glycoprotein substrate(s). Our findings indicate that the ST3Gal-I sialyltransferase regulates $CD8^+$ T cell homeostasis by modulating O-glycan biosynthesis. These studies reveal distinct roles for sialyltransferase genes and their glycoprotein substrates in immune system regulation.

P10.1

EXT1 AND EXT2 ARE GLYCOSYLTRANSFERASES INVOLVED IN HEPARAN SULFATE BIOSYNTHESIS.

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Multiple exosotoses is a dominantly inherited bone disorder caused by defects in either one of the two genes EXT1 and EXT2. Recent studies have shown that EXT1 and EXT2 relate to the glycosyltransferase reactions involved in heparan sulfate chain elongation [Lind et al. (1998) *J. Biol. Chem.* 26265-8]. Heparan sulfate polymerization occurs by the alternating addition of glucuronic acid and N-acetylglucosamine units to the nonreducing end of the polysaccharide, a process catalyzed by a single protein with dual activities, a glucuronyl/N-acetylglucosaminyltransferase [Lind et al. (1993) *J. Biol. Chem.* 20705-8]. To determine if, indeed, both EXT1 and EXT2 harbour both transferase activities, we overexpressed EXT1 and EXT2 in COS-1 cells and in the yeast strain *Pichia pastoris*. Yeast does not synthesize HS and thus is suitable for determination of the individual functions of EXT1 and EXT2. The data obtained clearly showed that each of the two EXT proteins possesses both glucuronyl- and N-acetylglucosaminyltransferase activities. Remarkably, coexpression of EXT1 and EXT2, in yeast as well as in mammalian cells, lead to substantially higher glycosyltransferase activity than expression of EXT1 or EXT2 alone. Coexpression in yeast also lead to increased N-linked glycosylation of EXT1 and EXT2. These observations suggest that the biological functional HS polymerization unit is a complex of EXT1 and EXT2 and that complex formation induces a relocalization from the endoplasmic reticulum to the Golgi compartment. Furthermore, the potentiation of the two catalytic activities does not depend on the membrane-bound state of the EXT1 and EXT2 proteins, as a similar increase in glycosyltransferase activties was observed after coexpression of soluble EXT1 and EXT2.

P10.2

INFRASTRUCTURE OF HEPARAN SULFATE BIOSYNTHESIS

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Many of the enzymes involved in heparan sulfate biosynthesis are type II membrane proteins thought to reside in the Golgi. However, the precise distribution of these enzymes within the Golgi or other subcellular compartments are unknown. We focused our studies on the location and physical interaction of iduronosyl-2-O-sulfotransferase (2OST), glucuronosyl C5 epimerase, glucuronosyl transferase I (GlcAT-I), and galactosyltransferase I (GalT-I). Chimeric forms of the enzymes were prepared by fusing them at their C-termini with green fluorescent protein (GFP) or c-myc tags. After transfection into Chinese hamster ovary cells (CHO), the chimeras exhibited a typical juxtanuclear Golgi localization. To test if these enzymes physically interact, we took advantage of a retargeting method originally described by Nilsson et al. (EMBO J 13:562-574, 1994). These investigators showed that a type II ER membrane protein, p33 invariant chain, contains an ER retention signal on its N-terminus. Fusion of this segment to Golgi GlcNAc transferase I resulted in its redistribution to the ER, causing relocation of α-mannosidase II from Golgi to ER, suggesting that the two proteins physically interact (kin recognition). With this idea in mind, we constructed fluorescent or myc-tagged chimeric enzymes that contain the ER retention sequence and we observed that the ER form of epimerase can relocate the 2OST. We also observed colocalization between p33-2OST-myc and 2OST-GFP, suggesting oligomerization. However, no colocalization was observed between epimerase or 2OST with GlcAT-I and GalT-I. Assays of each chimeric enzyme *in vitro* showed that both Golgi and ER forms of GlcAT-I and GalT-I have enzymatic activity. On the other hand, p33-2OST-GFP has no enzymatic activity *in vitro*. When we analyzed the chimeric 2OST containing an ER retrieval sequence (KDEL), the enzyme was active, suggesting that 2OST must pass through the Golgi compartment to become active. Finally, we followed the synthesis of heparan sulfate using mutant CHO cells transfected with different chimeric enzymes. All chimeric enzymes containing GFP at the C-terminus were able to correct the respective mutant CHO cell deficiency, while the ER retained forms of 2OST and GlcAT-I were not able to restore heparan sulfate synthesis in the respective mutants, pgsF-17 and pgsG. However, the ER form of galactosyltransferase I corrected the deficient mutant, pgsB, suggesting that the first galactose residue that forms the linkage region of heparan sulfate may be added in the ER compartment.

P11.1

NOTCH SIGNALING IS MODULATED BY FRINGE IN Lec1 BUT NOT Lec13 CHO CELLS.

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Notch glycoproteins are cell membrane receptors that transduce cellular differentiation signals when stimulated by ligand on adjacent cells. Notch receptors function in metazoans to effect cell fate and cell growth control. Upon ligand binding, a fragment of the Notch intracellular domain is released by proteolysis and transported to the nucleus where it may activate the transcriptional regulator CBF1. Notch signaling can therefore be assayed in co-cultures in which one cell expresses Notch and a transiently transfected reporter gene downstream of CBF1 DNA binding sequences, and the other cell expresses a Notch ligand. In this type of assay, when CHO cells containing endogenous Notch 1 and transiently expressing a CBF1-luciferase reporter construct were cultured with L cells stably expressing the Notch ligand Jagged1 (J1 cells) or with L cells, significantly higher luciferase activity was obtained in cultures with J1 cells. Fringe proteins can positively and negatively modulate the ability of Notch ligands to activate the Notch receptor. When CHO cells were stably expressing either Manic or Lunatic fringe proteins (Mfng or Lfng), the J1 induced Notch signal was suppressed. The same level of Notch signaling by J1 was obtained with Lec1 CHO cells and was also reduced in Lec1 cells stably expressing Mfng. Since complex and hybrid N-glycans are not synthesized by Lec1 cells, the results show that these N-glycans are not required in this assay, either for Notch signaling or for modulation of Notch signaling by Mfng. By contrast, Lec13 CHO cells transiently expressing the CBF1-luciferase reporter construct, responded poorly to Jagged 1 stimulation and this low level response was not suppressed in Lec13 cells stably expressing Mfng. Lec13 CHO cells are severely deficient in GDP-fucose due to a lack of GDP-Man-4,6-dehydratase activity. Partial rescue of Notch signaling and of Mfng modulation of Notch signaling was obtained in Lec13 cells by inclusion of 1 mM fucose in the co-culture medium, a condition that increases GDP-fucose levels by a salvage pathway. Thus, optimal signaling and fringe modulation of Jagged 1 induced Notch signaling in CHO cells do not require complex or hybrid N-glycans but do require fucose on a distinct glycan structure. This glycan structure is shown in the abstract of Moloney et al to be O-linked fucose on the EGF-repeats of Notch to which N-acetylglucosamine is added by the novel N-acetylglucosaminyltransferase activity of fringe.

P11.2

O-LINKED GLYCOPROTEINS AND RENAL TUBULE DEVELOPMENT IN *C. ELEGANS*. Karen Gentile, Michael Layden, Karie Berbach, Alison Schwartz, and Fred Hagen. Center for Oral Biology, Department of Biochemistry and Biophysics, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642 USA. fred_hagen@urmc.rochester.edu.

The *C. elegans* renal excretory/secretory cell is a large mono-nucleated cell, which undergoes drastic alterations in cell shape and growth cone migration during development to produce renal tubules or canals that run the length of the body. Mutations in the *let-653* mucin glycoprotein gene result in a cystic defect in the canals, which resembles the cytology observed for human polycystic kidney disease. To examine the role of O-glycosylation in renal canal development, transgenic animals were designed to express inhibitory antisense RNAs against either the let-753 glycoprotein or the glycosyltransferases that initiate mucin-type O-glycosylation. The *C. elegans* genome encodes for a family of nine polypeptide GalNAc transferase (ppGaNTase) sequence homologs, which exhibit high sequence similarity to mammalian ppGaNTases. Antisense inhibition of all nine ppGaNTases was targeted to specific cells of the nematode renal system using cell-specific promoter-driven expression constructs. *Let-653* antisense RNA expression pheno-copied the let-653 mutant defect, resulting in embryonic lethality and huge renal cysts in the canal cell. Transgenic expression of all nine ppGaNTase antisense RNAs simultaneously, driven by the *let-653* promoter, resulted in a more severe phenotype, including embryonic lethality and larval arrest. Arrested larva were significantly shorter in size. Viable larva and adults displayed variable canal cysts and epithelial cell defects, resulting in animals with improper tissue organization in the head, tail and body wall. The defects involved cells expressing a co-injected let-653 promoter-GFP transgene marker. Current efforts are directed at modulating the severity of O-glycosylation defects by regulating the levels of antisense RNA through inducible promoters that express antisense RNA in defined cells at defined times in development. This work was supported in part by NIH grant R03 DE12827 (FKH).

P12.1

BACTERIAL LIPOPOLYSACCHARIDE SIALYLTRANSFERASES: MORE THAN MEETS THE EYE

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We have been investigating the sialyltransferases produced in various human bacterial pathogens that express sialylated lipopolysaccharides. These organisms use the LPS sialyltransferases to produce mimics of mammalian glycolipids. We have identified and characterized enzymes from *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Campylobacter jejuni* and recently in *Haemophilus influenzae*. During the course of this work it became apparent that these enzymes have some interesting properties very distinct from those sialyltransferases described in mammals. First, the enzyme Lst from *N. meningitidis* is involved in making an α-2,3-sialyl-Lacto-*N*-neotetraose as well as α-2,6-sialyl-globotriose structure. Second, the enzyme Cst-II from *C. jejuni* is involved in making both the α-2,3 and α-2,8 linkages in ganglioside mimics. We have looked at naturally occurring variants of these enzymes and noticed sequence heterogeneity which we used to guide our dissection of the bi-functional nature of these enzymes. Using site-directed mutagenesis we have shown that a single amino acid change in both Lst from *N. meningitidis* and Cst-II from *C. jejuni* is responsible for the bi-functional nature of these enzymes.

P12.2

BLOOD GROUP A AND B GLYCOSYLTRANSFERASES: SYNTHESIS AND *IN VIVO* INHIBITION

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The development of glycotherapeutics is a three-stage process. In the first stage a biological target for inhibition is identified, e.g., selectin, glycosidase, glycosyltransferase. The design and discovery of potent inhibitors for the target follows. The last stage is large-scale production of the inhibitor. Glycosyltransferases are of particular interest in glycotherapeutics since they are both targets for inhibition and they can also be used for the large-scale synthesis of inhibitory glycoconjugates. The human blood group A and B glycosyltransferases that biosynthesize the ABO antigens will be used as examples of these dual roles in therapeutic development.

P12.3

DEVELOPMENT OF FRONTAL AFFINITY CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY (FAC/MS) FOR THE EVALUATION OF INHIBITOR LIBRARIES FOR GlcNAcT-V

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FAC/MS has previously been used to estimate the binding constants of individual compounds present in a mixture (Angew. Chem. 37, 3384, 1998). In the present study, recombinant human GlcNAcT-V was biotinylated and adsorbed on immobilized streptavidin. The enzyme was shown to be catalytically active, converting βGlcNAc(1,2)αMan(1,6)βGlc-OR to the expected product βGlcNAc(1,2)[βGlcNAc(1,6)]αMan(1,6)βGlc-OR. The kinetic parameters K_m and V_{max} could be determined for the immobilized enzyme and were comparable to those measured in solution. Analysis of a mixture of 8 potential trisaccharide-based inhibitors in a single run yielded K_d values for the individual members ranging from 0.3 -36 μM. These K_d values were 2-10 times lower than the K_i values measured in solution using a radioactive assay. By including a known specific inhibitor of GlcNAcT-V in the analysis, it could be established that the mode of inhibition was competitive.

S1.1

CHANGING THE DONOR COFACTOR OF BOVINE ALPHA1,3-GALACTOSYLTRANSFERASE BY FUSION WITH UDP-GALACTOSE 4-EPIMERASE: MORE EFFICIENT BIOCATALYSIS FOR ALPHA-GAL SYNTHESIS

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Two fusion enzymes consisting of uridine diphosphogalactose 4-epimerase (UDP-galactose 4-epimerase, GalE, EC 5.1.3.2) and α1,3-galactosyltransferase (α3GT, EC 2.4.1.151) with an *N*-terminal hexo-histidine tag and an intervening three-glycine-linker were constructed by in-frame fusion of *E. coli galE* gene either to the 3'-terminus (f1) or to the 5'-terminus (f2) of a truncated bovine α1,3-galactosyltransferase gene respectively. Both fusion proteins were expressed in cell lysate as active, soluble forms as well as in inclusion bodies as improperly folded proteins. Both f1 and f2 were determined to exist as a homodimer based on a single band observed at about 67 kDa in sodium dodecyl sulfate polyacrylamide gel electrophoresis and on a single peak with a molecular weight around 140 kDa determined by gel filtration chromatography. Without altering the acceptor specificity of the transferase, the fusion with the epimerase changed the donor requirement of α1,3-galactosyltransferase from UDP-galactose to UDP-glucose and decreased the cost for the syntheses of biomedically important Galα1,3Gal terminated oligosaccharides (α-Gal epitopes) by more than 40-folds. For enzymatic synthesis of Galα1,3Galβ1,4Glc from UDP-glucose and lactose, the fusion enzymes f1 and f2 showed kinetic advantages with reaction rates about 300% and 50% faster respectively than that for a equal amount of GalE and α3GT mixture, indicating that the active sites of the epimerase and the transferase were in proximity in fusion enzymes. The kinetic parameters suggested a random mechanism of the substrate binding for the α1,3-galactosyltransferase. This work demonstrates a general approach that fusion of a glycosyltransferase with an epimerase can change the required, but expensive sugar nucleotide to a cheaper one.

S1.2

STRUCTURE AND FUNCTION OF HNK-1 ASSOCIATED GLUCURONYLTRANSFERASES (GlcATs)

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GlcAT is a key enzyme in the biosynthesis of the HNK-1 epitope (HSO_3-3GlcAβ1-3Galβ1-4GlcNAcβ1-R). The epitope is specifically expressed on neural cell adhesion molecules such as NCAM and P0 and on some glycolipids (SGGL) and is suggested to be involved in cell to cell adhesion and cell migration during development of the neural system. Recently, two GlcATs, GlcAT-P and GlcAT-S, were cloned. These GlcATs contain four conserved regions (modules I-IV) in the catalytic domain. The functional significance of these modules was studied by combination of site-directed mutagenesis and computer aided modeling of GlcAT-P. Mutation studies suggested that all 4 modules are associated with the formation of the active site structure. This is consistent with the computer aided three-dimensional (3-D) model of the enzyme, which indicates a Rossmann fold-type topology consisting of several α-helix and β-sheet structures. GlcAT-P has a high specificity to N-acetyllactosamine residues on bi-, tri and tetra antennary N-linked sugar chains. The activity of GlcAT-S was much lower than that of GlcAT-P and preferred tri antennary sugar chains. Sphingomyelin enhanced markedly the activity of the recombinant soluble form of GlcAT-P to glycoprotein substrates. In addition, GlcAT-P exhibited a significant activity to glycolipid substrates when phosphatidylinositol is added to the incubation mixture. In contrast, these membrane phospholipids did not show any significant effects on GlcAT-S.

S1.3

MOLECULAR COMPLEX OF *Thermus caldophilus* GLUCOSYLTRANSFERASE WITH ADP-GLUCOSE PYROPHOSPHORYLASE ENHANCES α-GLUCAN SYNTHESIS

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Glucosyltransferase gene (glgA) and glycogen biosynthesis-related genes were identified from the genome analysis of *Thermus caldophilus* GK24. The glucosyltransferase gene was cloned and expressed in *E. coli* (MV1184), which appeared to be sticky each other, forming rough cell surface. α-Glucan synthesis was attempted by the recombinant glucosyltransferase in the presence of ADP-glucose and maltooligosaccharides as acceptor. It turned out to be that glucosyltransferase itself has critical limitation for high degree of glucose polymerization, which might be caused by feedback inhibition of ADP and PPi. However, two enzyme reaction having glucosyltransferase and ADP-glucose pyrophosphorylase gave higher degree of glucose polymerization. It indicates that the molecular complex is formed, suitable to two sequential reactions for α-glucan synthesis without having feedback inhibition.

S1.4

A COMBINED EXPERIMENTAL AND THEORETICAL STUDY OF THE SOLUTION CONFORMATION OF UDP-GLUCOSE IN THE PRESENCE OF CATIONS

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Glycosyl esters of nucleoside di or mono-phosphates, generally referred to as "sugar nucleotides", serve as a sugar donors during the biosynthesis of oligo- and polysaccharides; they are therefore of a primary importance in carbohydrate metabolism in the living world. Conformational behavior of a nucleotide sugar, UDP-glucose (UDP-Glc) in solution has been studied using combined NMR experiemnts and molecular dynamics simulation. Homonuclear and heteronuclear coupling constants, together with NOE data have been measured. Molecular dynamics calculations were performed using the AMBER program package together with energy parameters especially developed for nucleotide sugars [1]. Experimental and theoretical studies in aqueous solution, were carried out for increasing concentrations of monovalent K^+ and divalent Mg^{2+} ions. For the monovalent ion, it is revealed that its presence and concentration is crucial for the conformational behavior, resulting in the stabilization of the extended conformation. The preferred location of K^+ is in close proximity to the negatively charged phosphate oxygens, but the ion moves freely and can occupy other sites. The analyses of the trajectories provide new insight on the role of the metal ion in the catalytic mechanism of glycosyltransferases.

1. P. Petrova, J. Koca, and A. Imberty (1999) *J. Am. Chem. Soc.*, 121, 5535-5547.

S1.5

INITIAL STEPS TOWARDS SOLVING THE STRUCTURE OF BOVINE α-1,3-GALACTOSYLTRANSFERASE

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The minimal catalytic domain of bovine α-galactosyltransferase (80-368aa) was amplified by PCR and inserted into NdeI/BamHI site of pET15b expression vector, which provides the protein with an N-terminal His_6-tag for purification as well as a thrombin cleavage site for subsequent removal of the His_6-tag. The resulted plasmid pET15b-αGalT was transformed into BL21(DE3) and the recombinant strain was evolved to grow and overexpress α-galactosyltransferase in modified M9 medium containing ^{15}N-labeled ammonium sulfate. The α-galactosyltransferase was purified by Nickel-column to about 90% homogeneity and the active fraction was subjected to 80% ammonium sulfate precipitation. The precipitate was dissolved in 20mM Tris-HCl (pH8.5) containing 150 mM NaCl and dialyzed against the same buffer, followed by thrombin cleavage for 12 hrs at room temperature. The cleaved product was applied to Superdex200 FPLC gel filtration, which resulted in a homogenous protein as judged by SDS-PAGE. About 1mg/L pure enzyme was achieved with a specific activity of 0.35 unit/mg. Mass spectrometry revealed a molecular weight of 34872.21, quite similar to the theoretical value of 34863.50. N-terminal sequencing showed an N-terminus of GSHMESKLKL, indicating that the digestion of thrombin is complete and produces homogenous N-terminus. The NMR study of ^{15}N-labeled α-galactosyltransferase is under going. ^{13}C-labeled enzyme will also be used for structural determination in solution by NMR. In addition, work is in progress to produce single crystals from the purified enzyme.

S1.6

DISULFIDE BOND LOCATIONS IN THE GOLGI ENZYME GANGLIOSIDE GM2 SYNTHASE. Jianghong Li[1], M. Laura Allende[1], Ten-Yang Yen[2], Rajesh K. Joshi[2], Bruce A. Macher[2], & William W. Young, Jr.[1]. Univ. of Louisville, Louisville, KY[1] and San Francisco State Univ., San Francisco, CA[2].

GM2 synthase is a disulfide-bonded homodimer which produces gangliosides that are functionally important to nervous system maintenance. The goal of this study is to determine the role of specific cysteine residues in the dimerization and activity of this enzyme by defining the locations of the disulfide bonds. The locations of free and disulfide bonded cysteine residues were determined by tryptic digestion and analysis of the peptides by mass spectrometry. All Cys residues were found to be disulfide bonded. LC/MS of a tryptic digest of unreduced GM2 synthase revealed a triply charged ion at m/z 1373.5 (monoisotopic $(M+H)^+$ ion at 4117.3) which is the result of a disulfide bonded tryptic dipeptide containing the amino acids 418-442 and 473-486. MS/MS analysis of this species provided a complete verification of the proposed disulfide bonded tryptic peptide pair; thus, Cys 429 and 476 are disulfide bonded. Evidence for a second disulfide bonded set of tryptic peptides came from the presence of ions with 3 & 4 positive charges at m/z 1950.2 & 1462.9 (average monoisotopic $(M+H)^+$ ion at 5848.6). These ions are consistent with the presence of a tripeptide consisting of the amino acid sequences 69-95, 398-414, and 527 to the C terminus. MS/MS analysis of the ion with 4 positive charges was consistent with the proposed disulfide-bonded tripeptide, thus suggesting that Cys 80 and 82 are disulfide bonded in some combination to Cys 412 and 529. Current studies are aimed at determining which disulfides form the intersubunit linkages necessary to produce the homodimer. Supported by GM42698 and the Mizutani Foundation for Glycoscience.

S1.7

APPLICATION OF 3D-QSAR IN THE DEVELOPMENT OF CPI DRUGS: MANNOSIDASE II INHIBITORS

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The design, development and commercialization of a new drug is a tedious, time-consuming and expensive process. In the last decade, the use of computer-aided drug design has appeared to be an attractive alternative to accelerate the design of potent and specific drugs. This methodology implies the use of an explicit or implicit knowledge of the three-dimensional arrangement of the participating molecules, the target receptor and the ligand. 3D-QSAR is one of the new methods which have emerged from the efforts to improve the lead optimization process, that is, the conversion of lead compounds into drug candidates with improved potency and specificity. This approach, based on the correlation of the measured inhibitory power against an enzyme with the computed molecular and structural properties, might allow the prediction of the biological activity of an analogue well before envisaging its synthesis and hence weeding out poor candidates early in the drug development cycle. We report here an application of the 3D-QSAR method, currently used at GlycoDesign Inc, for the design of a 'second generation' of Carbohydrate Processing Inhibitors (CPIs) for Mannosidases. Analogues of swainsonine, a naturally occurring inhibitor of Mannosidase II, were synthesized and the broad range of inhibitory activities found in these analogues enabled us to establish 3D-QSAR models, which have proved to be useful in our lead optimization strategy.

S1.8

STRUCTURAL STUDIES ON GnT V: HOMOLOGY PROTEIN MODEL AND BINDING MODES OF THE UDP AND UDP-GlcNAc

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N-acetylglucosaminyltransferase-V (GnT V, E.C. 2.4.1.155) transfers GlcNAc from the UDP-GlcNAc donor to the O6 hydroxyl group of the α-(1-6) linked mannose of the core oligosaccharide. GnT V is an enzyme of particular interest because β-(1-6) branched oligosaccharide structures are involved in many physiological and pathological processes. Although the amino acid sequence of GnT V has been known for several years and the minimal catalytic domain has also been recently determined the 3D structure of this enzyme has not been solved yet. Knowledge of the 3D structure of the active site of GnT V and a description of the interaction profile between GnT V and its natural substrates are prerequisites for the rational design of inhibitors. State of the art homology modeling can help us to get insight on the topology of the active site of enzymes lacking X-ray structural data. The 3D structure of GnT V, based on the X-ray structure of GnT I and results of such modeling are presented in this study. Molecular docking was performed to understand the driving forces of protein-ligand interactions between GnT V and its natural substrates. Our intent was to describe and present the most important intermolecular interactions responsible for recognition and stabilization of the natural substrate UDP-GlcNAc and of its competitive inhibitor UDP at the active site of GnTV.

S1.9

STRUCTURE OF THE BOVINE α-1,3-GALACTOSYLTRANSFERASE AND ITS COMPLEX WITH UDP INFERRED FROM MOLECULAR MODELING

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Oligosaccharide structures with a terminal Galα1-3Galβ sequence (α-galactosyl epitopes) are xenoactive antigens and are considered to be the major cause for hyperacute rejections in xenotransplantation. The unique enzyme responsible for the formation of α-galactosyl epitopes is α-1,3-galactosyltransferase (α-1,3-GalT), which catalyzes the transfer of galactose from UDP-Gal to a specific hydroxyl of the acceptor sugar with a retention of the anomeric configuration. This enzyme is absent in humans and, conversely, large quantities of anti-Gal antibodies exist in the human body which react with the α-Gal epitope and thus providing a barrier to xenotransplant. An understanding of the reaction mechanism and the knowledge of a three-dimensional structure of α–1,3-GalT are, therefore, of great interest. Recently, the structure and fold of the pig α-1,3-GalT has been predicted by means of knowledge-based molecular modeling. In this paper we describe our efforts in producing a homology model for α-1,3-GalT based on the published SpsA glycosyltransferase structure. Structural features of the α-1,3-GalT model will be compared with available structural data on this class of enzymes and protein-UDP complexes were studied using a docking approach.

S1.10

MODELING OF GLYCOSYLTRANSFERASES AND THEIR COMPLEXES.

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Glycosyltransferases comprise a group of enzymes that are involved in the biosynthesis of the complex oligosaccharides. The result of the reaction catalyzed by these enzymes is the formation of a new glycosidic linkage and it appears that there is at least one distinct glycosyltransferase for every type of glycosidic linkage. *N*- and *O*-linked oligosaccharide structures are involved in many cell processes, e.g., cell adhesion and migration, vascular development, angiogenesis and tumor cell metastasis. Glycosyltransferases represent therefore potential targets for drug development. Analysis of the published X-ray structures of GnT I, SpsA, β-1,4GalT, and β-GlcT from bacteriophage T4 suggests a structural similarity at the UDP sugar donor recognition sites. In this work we describe our efforts in modeling Core2L GnT, Core2b/M GnT, Core3 GnT, GnT II, GnT V and α-1,3-GalT using the crystal structures of GnT I and SpsA. In an attempt to elucidate the structural features accounting for the specificity of each of these enzymes, complexes of the glycosyltransferases with their respective nucleotide-sugar donor (UDP-sugar) have been generated using an automated docking approach. Comparison of the active site architecture reveals a structural similarity in all the enzymes studied. Details of the constructed protein models and their interactions with UDP ligands are described.

S1.11

STRUCTURAL MODELING OF Core2L GnT AND ITS COMPLEXES WITH UDP-GlcNAc AND UDP

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Glycosyltrasferase Core2L is involved in the biosynthesis of *O*-linked oligosaccharides. Under physiological conditions Core2L GnT transfers a GlcNAc residue from the donor UDP-GlcNAc to the hydroxyl group of the acceptor. To understand this biologically important process in atomic detail and to selectively inhibit this reaction, it is essential to have the three-dimensional structure of the Core2L GnT and its complexes with UDP-GlcNAc. Unfortunately, no structural information is available yet on this enzyme. Recently the high-resolution crystal structure of GnT I complexed with UDP has been determined. Comparison of this structure with earlier reported GnT structures such as SpsA and β-GlcT from bacteriophage T4 clearly shows that the nucleotide binding domain is highly conserved in terms of amino acid composition, properties and shape. The sequence similarity and structural homology between these enzymes prompted us to use the GnT I structure as a template model to generate a homology model for Core2L GnT. The docking approach was used to model complexes of Core2L GnT with UDP and UDP-GlcNAc ligands and to characterize the predicted active site of Core2L. The well-defined binding sub-site for UDP-GlcNAc has been identified and the relevant interactions with the amino acids in the active site have been determined.

S1.12

CATALYTIC MECHANISM OF INVERTING *N*-ACETYLGLUCOSAMINYLTRANSFERASES: *AB INITIO* CALCULATIONS OF REACTION PATHWAYS AND TRANSITION STATES.

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N-acetylglucosaminyltransferases catalyze the transfer of a GlcNAc residue from a sugar-donor molecule, UDP-GlcNAc, to one of the hydroxyl groups located at a particular position of an acceptor oligosaccharide. High-level *ab initio* calculations were used to gain some insight into the enzymatic reaction catalyzed by these enzymes. The structural model used in this investigation consists of all essential molecules or their fragments assumed to be involved in the mechanism: a complete sugar-donor molecule, UDP-GlcNAc, a hydroxyl group of the oligosaccharide-acceptor modeled by methanol, a divalent metal cofactor represented by Mg^{2+}, as well as the essential parts of the catalytic acid (A) and catalytic base (B) modeled by acetic acid and acetate molecules. Different possible mechanisms of reaction have been followed by means of several two-dimensional potential energy maps calculated as a function of predefined reaction coordinates. A detailed description of the different possible reaction pathways that includes energetic evaluations and the structural modifications occurring along the catalytic process has been performed. The feasibility, consequences, and implications of the different reaction pathways on the catalytic mechanism will be discussed.

S2.1

THE REGULATION OF POLY-N-ACETYLLACTOSAMINE BIOSYNTHESIS IN N-GLYCANS OF HUMAN INTERFERON-γ

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In our previous report, we have determined the antennary structure of N-glycans in human interferon-γ (hIFN-γ) produced by Chinese Hamster Ovary (CHO) cells (HIIF-D from ATCC). HIIF-D cells produced hIFN-γ with biantennary oligosaccharides mainly, which is comparable to that of natural hIFN-γ (1). In this study, we demonstrate the regulation of poly-N-acetyllactosamine ((Galβ1→4GlcNAcβ1→3)n) biosynthesis of N-glycans in hIFN-γ. HIIF-D cells were transfected with N-acetyl-glucosaminyltransferase V (GnT-V) gene and α2,3-sialyltransferase (2,3SiaT) gene or α2,6-sialyltransferase(2,6SiaT) gene. We selected a clone overexpressing 2,3SiaT and a clone overexpressing 2,6SiaT. We found that the terminal structures of N-glycans of hIFN-γ produced by these clones were highly sialylated and that N-glycans terminating with poly-N-acetyllactosamine were reduced compared with a clone only overexpressing GnT-V. These results showed sialyltransferase expression suppressed poly-N-acetyllactosamine biosynthesis. This work was performed as part of the Research and Development Project of the Industrial Science and Technology Frontier Program supported by NEDO.

Reference: 1. Glycobiology, (2000), **10**, (4)

S2.2

HUMAN UDP-GlcNAc:α-3-D-MANNOSIDE β1,2-N-ACETYLGLUCOSAMINYLTRANSFERASE I.2 (GnT I.2), A HOMOLOGUE OF GnT I, IS ENZYMATICALLY ACTIVE. Doron Betel[1,2], Wenli Zhang[1] and Harry Schachter[1,2].
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The human EST database contains a UniGene (Hs.183860) encoding a protein similar (64% over 109 amino acids) to human GnT I (UniGene Hs.151513). This gene (MGAT I.2) maps to chromosome 1 (D1S2843-D1S417) whereas GnT I maps to 5q35. The mouse EST database contains an orthologue (UniGene Mm.2069) with 97% identity to human GnT I.2 over 361 C-terminal residues. GnT I.2, like GnT I, is widely expressed (liver, spleen, adrenal, brain, breast, colon, oesophagus, small intestine, heart, skeletal muscle, kidney, lung, lymph node, ovary, pancreas, pancreatic islet, parathyroid, placenta, prostate, spleen, testis). GnT 1.2 cDNA (2.7kb; unpublished data from our group and NEDO human cDNA sequencing project, Japan, AN AK000284) has a 1980 bp ORF encoding a 660 amino acid protein; human GnT I has 445 amino acids. GnT I.2 has the type 2 domain structure typical of glycosyltransferases, i.e., a 37-residue N-terminal cytoplasmic domain, a 24-residue non-cleavable hydrophobic signal-anchor domain, a relatively long stem region (~240 aa) and a catalytic domain (~360 aa). Several regions of GnT I (containing residues identified as substrate contact regions in the X-ray crystal structure of rabbit GnT I; UM.Ünligil, S Zhou, S Yuwaraj, M Sarkar, H Schachter, JM Rini, in preparation) are highly homologous to GnT I.2, e.g., the GnT I EDDL sequence involved in binding UDP-GlcNAc-Mn^{2+} (conserved in all GnT I sequences cloned to date) corresponds to EEDL (residues 393-396) in both human and mouse GnT I.2. The C-terminal region of human GnT I.2 (amino acid residues 227-660) was expressed in the baculovirus/Sf9 system as a secreted protein containing a $(His)_6$ tag. Protein purified by adsorbtion to nickel-NTA beads converted Manα1-6(Manα1-3)Manβ-octyl (M_3-octyl) to Manα1-6(GlcNAcβ1-2Manα1-3)Manβ-octyl with K_m values of 0.23 and 0.25 mM for UDP-GlcNAc and M_3-octyl respectively. Assays for GnT II to VIII were negative. Northern analysis of GnT I.2 showed a 3.3 kb message with a tissue distribution similar to GnT I. We thank Dr.Henrik Clausen for his help during the early parts of this investigation, Dr.Erika Staudacher for enzyme substrates and the Medical Research Council of Canada for financial support.

S2.3

MOLECULAR CLONING AND EXPRESSION OF A MOUSE PROTEIN WITH HOMOLOGY TO *DROSOPHILA* BRAINIAC AND THE MAMMALIAN BETA-1,3-GALACTOSYLTRANSFERASE FAMILY.

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BLAST searches of the mouse DNA databases with the *Drosophila* Brainiac protein sequence allowed us to clone a novel murine cDNA with high homology to both *Drosophila* Brainiac and the β1,3-galactosyltransferase family (β3GalT). The predicted protein encoded by this cDNA has 397 amino acids and contains 7 cysteine residues that are all conserved in a human orthologous cDNA (AK002009.1) and 5 of which are conserved in the β3GalT family. The protein showed a type II membrane protein domain structure, typical of the Golgi glycosyltransferases. Northern blot analysis revealed the presence of a single transcript in all adult mouse organs tested, with highest levels expressed in the liver and kidney. Preliminary gene mapping located the human orthologue of this gene on chromosome 2p15. We have expressed a truncated form of the mouse protein (residues 83-397, lacking the N-terminal cytoplasmic and transmembrane domains) in the baculovirus/Sf9 system. This protein contains an N-terminal leader sequence, which resulted in secretion into the Sf9 cell media, and a $(His)_6$ epitope tag downstream of the leader sequence cleavage site. Recombinant protein was detected with antibody to the epitope tag. Preliminary enzyme assays with affinity-purified protein using low molecular weight glycosides as acceptor substrates showed galactosyltransferase activity indicating that the protein belongs to one of the GalT families, presumably the β3GalT family. We thank the Medical Research Council of Canada for support.

S2.4

A NOVEL VARIANT FORM OF MURINE β-1,6-*N*-ACETYLGLUCOSAMINYLTRANSFERASE FORMING BRANCHES IN POLY-*N*-ACETYLLACTOSAMIN.

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A novel form of murine β-1,6-*N*-acetylglucosaminyltransferase that form branches in poly-*N*-acetyllactosamines (IGnT B) was cloned based on sequenc homology to the known IGnT (IGnT A). The C-terminal 1/4 of IGnT B was identical to that of IGnT A, while the rest of the predicted sequences showe 63 % identity. Genomic analysis indicated that IGnT A and IGnT B were derive by alternative splicing; the unique portion was encoded by exon 1, and th common portion was encoded by exons 2 and 3. The substrate specificity o IGnT B was indistinguishable from that of IGnT A. However, IGnT B showe higher enzymatic activity than IGnT A.

S2.5

Identification and molecular characterization of a cDNA encoding a novel N-acetylglucosamine-6-O-sulfotransferase.

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N-acetylglucosamine-6-O-sulfotransferase (GlcNAc6ST) catalyzes the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate to C-6 position of the non-reducing N-acetylglucosamine (GlcNAc) residue. Three genes encoding GlcNAc6ST isozymes has been isolated so far, namely GlcNAc6ST/CHST2 (GlcNAc6ST-1), HEC-GlcNAc6ST/LSST (GlcNAc6ST-2), and I-GlcNAc6ST (GlcNAc6ST-3). Based on the sequence homology to the previously cloned mouse GlcNAc6ST-1, we cloned and identified human and mouse cDNA encoding a novel GlcNAc6ST, designated GlcNAc6ST-4. Both human and mouse GlcNAc6ST-4 were predicted to be a type II transmembrane protein composed of 486 and 484 amino acid residues, respectively. Among the human organs examined, expression of the mRNA was detected in the heart, pancreas, spleen, ovary and peripheral blood leukocytes, while mouse GlcNAc6ST-4 mRNA was strongly expressed in the kidney. Transfection and expression of the GlcNAc6ST-4 cDNA in CHO cells yielded GlcNAc6ST activity. Core 2 oligosaccharide, mannosyl linked GlcNAc and N-acetyllactosamine oligomer in which GlcNAc residue was exposed at non-reducing end served as substrates for the expressed enzyme, suggesting that GlcNAc6ST-4 may play roles in the biosynthesis of various glycoconjugates harboring N-linked and/or O-liked oligosaccharides.

S2.6

THE C-TERMINAL N-GLYCOSYLATION SITES OF THE HUMAN α1,3/4-FUCOSYLTRANSFERASE III, -V and –VI ARE NECESSARY FOR THE EXPRESSION OF FULL ENZYME ACTIVITY

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The α1,3/4-fucosyltransferases are involved in the synthesis of fucosylated cell surface glycoconjugates. Human α1,3/4-fucosyltransferase III, -V and –VI (hFucTIII, -V and –VI) contain two conserved C-terminal N-glycosylation sites. We have analyzed the functional role of these potential N-glycosylation sites, laying the main emphasis on the sites in hFucTIII. Tunicamycin treatment completely abolished hFucTIII enzyme activity while castanospermine treatment diminished hFucTIII enzyme activity to approximately 40 % of the activity of the native enzyme. To further analyze the role of the conserved N-glycosylation sites in hFucTIII, -V and –VI, we made a series of mutant genomic DNAs in which the asparagine residues in the potential C-terminal N-glycosylation sites were replaced by glutamine. Subsequently the hFucTIII, -V and-VI wild type and the mutants were expressed in COS-7 cells. All the mutants exhibited lower enzyme activity than the wild type and elimination of individual sites had different effects on the activity. The mutations did not affect the protein level of the mutants in the cells, but reduced the molecular mass as predicted. Kinetic analysis of hFucTIII revealed that lack of glycosylation at Asn185 did not change the K_m values for the oligosaccharide acceptor and the nucleotide sugar. The present study demonstrates that hFucTIII, -V and -VI requires N-glycosylation at the two conserved C-terminal N-glycosylation sites for expression of full enzyme activity.

S2.7

CLONING OF GLYCOSYLTRANSFERASES FROM GENETIC MODEL ORGANISMS

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The completion of the *Drosophila* genome project and the impending completion of the *Arabidopsis* genome project offers a new opportunity to extend our knowledge of glycosyltransferase families and of the glycobiology of insects and plants. We have recently identified a large number of glycosyltransferase homologues in these organisms. We then predicted reading frames (partly in combination with examination of identical or similar expressed sequence tags) encoding these homologues and have successfully used RT-PCR or λ-library screening to isolate cDNAs for a number of glycosyltransferases. We will present preliminary data on the cloning and expression of cDNAs encoding β1,4-galactosyltransferase homologues from *Drosophila* and α1,3-fucosyltransferase homologues from *Arabidopsis*.

S2.8

CHARACTERIZATION OF MURINE UDP-GALNAC: POLYPEPTIDE N-ACETYLGALACTOSAMINYLTRANSFERASE-T2,

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UDP-GalNac: polypeptide N-acetylgalactosaminyltransferases (ppGaNTases) are a family of enzymes which initiate mucin-type O-glycosylation, through the transfer of a GalNac to serine or threonine. In the present study we have cloned, expressed and characterized murine ppGaNTase-T2. Murine ppGaNTase-T2 displays 58 to 62% aa similarity to the conserved region of the previously identified mouse isoforms, ppGaNTases-T1, -T3 and –T4. Northern blot analysis reveals that the ppGaNTase-T2 transcript is widely expressed in adult tissues and organs. While ppGaNTase-T1 is also expressed broadly, the transcripts do not overlap completely. Analysis of late stage embryos by in situ hybridization reveals that ppGaNTase-T2 and ppGaNTase-T1 display "complementary" expression in several organ systems. For example, ppGaNTase-T1 is expressed in the retina, whereas ppGaNTase-T2 is localized to the eye lens. ppGaNTase-T2 transfers GalNAc, in vitro, to a panel of nine peptide substrates in a manner which is distinct from murine ppGaNTases-T1, -T3 and –T4. Previous reports demonstrated that human ppGaNTase-T2, but not human ppGaNTases-T1 or –T3, could utilize UDP-Gal as a sugar donor. In contrast, we find that all three mouse isoforms are promiscuous in that each are capable of utilizing UDP-Gal as a sugar donor. Supported, in part, by USPHS grant DE08108. M.E.F. was supported by USPHS T32 DE07202.

S2.9

DIVERSE SPATIAL EXPRESSION PATTERNS OF UDP-GALNAC:POLYPEPTIDE N-ACETYLGALACTOSAMINYLTRANSFERASE FAMILY MEMBERS DURING MOUSE DEVELOPMENT, Paul D. Kingsley, Kelly G. Ten Hagen, Kathleen M. Maltby, Jane Zara and Lawrence A. Tabak, Center for Oral Biology, Aab Institute of Biomedical Sciences, University of Rochester 601 Elmwood Ave., Rochester, NY 14642

Cell migration and adhesion during embryonic development are complex processes which likely involve interactions among cell-surface carbohydrates. The initial step of mammalian mucin-type O-glycosylation is catalyzed by a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (ppGaNTases). The spatial expression patterns of the messenger RNAs of seven ppGaNTase family members were investigated from gastrulation through organogenesis stages of mouse development. All seven glycosyltransferases were expressed in unique patterns during embryogenesis. ppGaNTase-T1, -T2, -T4 and –T9 were expressed more ubiquitously than ppGaNTase-T3, -T5 and -T7. Organ systems with discrete accumulation patterns of ppGaNTase family members include the gastrointestinal tract (intestine, liver, stomach, submandibular gland), nervous system (brain, eye), lung, bone, yolk sac and developing craniofacial region. The pattern in the craniofacial region included differential expression by family members in developing mandible, teeth, tongue and discrete regions of the brain including the pons and migratory, differentiating neurons. The unique spatio-temporal expression of the different ppGaNTase family members during development suggests unique roles for each of these gene products. Supported, in part, by USPHS DE08108.

S2.10

EXPRESSION OF MURINE UDP-GALNAC: POLYPEPTIDE N-ACETYLGALACTOSAMINYLTRANSFERASE-T1, T2, T3 IN *PICHIA PASTORIS* Hua Mao, Michele E. Forsythe, Gurrinder S. Bedi, Brian C. Van Wuyckhuyse, and Lawrence A. Tabak, Center for Oral Biology, Aab Institute of Biomedical Sciences, University of Rochester, 601 Elmwood Avenue, Box 611, Rochester, NY 14642.

Mucin-type *O*-glycosylation is initiated by a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (ppGaNTases). Biochemical analysis of these glycosyltransferases has been hampered by the limited quantities of enzymes that can be prepared. Murine ppGaNTase -T1, -T2 and -T3 cDNAs were cloned into expression vector pPIC9 and expressed in methylotrophic *Pichia pastoris* strains GS115 and SMD1168. Expression of recombinant enzyme was induced using methanol as the sole carbon source in the yeast growth media; the transferases were engineered to be expressed as secreted proteins. Each transferase was purified to electrophoretic homogeneity and was characterized by western-blotting using isoform-specific antisera. We found no significant difference in kinetic parameters obtained for the recombinant enzymes produced in yeast compared to those expressed from COS7 cells, using the peptide EA2 (PTTDSTTPAPTTK) and the sugar donor UDP-GalNAc. However, the *Pichia pastoris* system produces 10- to 100-fold higher levels of enzyme, thus making it possible to obtain mg quantities of purified ppGaNTases. This work was supported, in part, by USPHS Grant DE08108. M.E.F was supported by USPHS DE07202.

S2.11

CLONING AND CHARACTERIZATION OF A NINTH MEMBER OF THE UDP-GALNAC:POLYPEPTIDE N-ACETYLGALACTOSAMINYLTRANSFERASE FAMILY, PPGANTASE-T9*, Kelly G. Ten Hagen[1], Gurrinder S. Bedi[1], Daniel Tetaert[2], Paul Kingsley[1], Fred K. Hagen[1], Marlene M. Balys[1], Thomas M. Beres[1], Pierre Degand[2] and Lawrence A. Tabak[1], [1]Center for Oral Biology, Aab Institute for Biomedical Sciences, University of Rochester, Rochester, New York 14642[2], UniteINSERM377, Biologie et Physiopathologie de Cellules Mucipares, Place de Verdun, 59045 Lille Cedex, France.

We have cloned, expressed and characterized the gene encoding a ninth member of the mammalian UDP-GalNAc:polypeptide N-acetylgalactosaminyl-transferase (ppGaNTase) family, termed ppGaNTase-T9. Northern blot analysis revealed significant levels of the 5 kb and 4.2 kb transcripts in rat sublingual gland, testis, small intestine, colon and ovary, with lesser amounts in heart, brain, spleen, lung, stomach, cervix and uterus. In situ hybridization to mouse embryos (E14.5) revealed significant hybridization in the developing mandible, maxilla, intestine and mesencephalic ventricle. Constructs expressing this gene transiently in COS7 cells resulted in no detectable transferase activity in vitro against a panel of unmodified peptides. However, when incubated with MUC5AC tri-glycopeptide (obtained by the prior action of ppGaNTase-T1 on MUC5AC), additional incorporation of GalNAc was achieved, resulting in a new hydroxyamino acid modification. This isoform thus represents the second example of a *glyco*peptide transferase and is distinct from the previously identified form in expression in both embryonic and adult tissues. This work was supported in part by USPHS grant DE08108.

S2.12

Involvement of β-1,4-galactosyltransferases I-VI in N-linked sugar chain biosynthesis

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To date seven members were found in a β-1,4-galactosyltransferase (β-1,4-GalT) family and named β-1,4-GalTs II, III, IV, V, VI and VII according to the homology distances closer to previously known β-1,4-GalT as β-1,4-GalT I. Preliminary studies showed that β-1,4-GalTs I, II, IV and V are involved in the biosynthesis of glycoproteins while β-1,4-GalTs III, IV, V and VI in the biosynthesis of glycolipids and β-1,4-GalT VII in the biosynthesis of glycosaminoglycans. Since membrane glycoproteins from Sf-9 cells contain hybrid-type sugar chains mainly terminated with *N*-acetylglucosamine, the cells could be used for analysis of β-1,4-GalT function *in vivo*. Human β-1,4-GalT I, II, III, IV, V or VI cDNA with the full coding sequence was transfected into Sf-9 cells, and then the galactosylation of membrane glycoproteins was examined by lectin blot analysis using RCA-I which interacts with sugar chains terminated with β-1,4-linked galactose residues. RCA-I-positive bands were found after transfection of each β-1,4-GalT cDNA into the cells although the number and intensity of lectin-reactive bands differed among the samples. Since all lectin-positive bands disappeared upon pretreatment of blots with diplococcal β-galactosidase or N-glycanase, β-1,4-GalTs I-VI can galactosylate N-linked sugar chains *in vivo*. Differences between *in vitro* and *in vivo* acceptor specificities of them remain to be elucidated. Differences were also found in their acceptor specificities toward branched N-linked sugar chains.

S2.13

MOLECULAR CLONING AND CHARACTERIZATION OF A NOVEL β-GALACTOSIDE SULFOTRANSFERASE ACTING ON GLYCOPROTEINS

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We previously cloned GalCer 3'-sulfotransferase (cerebroside sulfotransferase, CST). In this study, we identified a novel functional sulfotransferase (termed GP3ST) gene acting on glycoproteins. We have probed the GenBank™ DNA data base with the open reading frame (ORF) of CST gene to reveal a candidate gene in a human minisatellite, CEB1 cosmid, located on the human chromosome 2q37.3. A full-length cDNA was isolated from human colon by reverse transcription-PCR and 5'- and 3'-RACE based on the candidate gene's sequence. The isolated cDNA clone contained an ORF that predicts a type II transmembrane protein composed of 398 amino acid residues. Amino acid sequence exhibits 33% identity to that of the human CST. A recombinant protein expressed in COS-1 cells showed no CST activity but sulfotransferase activity toward oligosaccharides containing nonreducing β-galactoside such as N-acetyllactosamine, lactose, lacto-N-tetraose (Lc4), lacto-N-neotetraose (nLc4), Galβ1-3GalNAcα-Bzl (*O*-glycan core 1 oligosaccharide). In order to characterize the cloned sulfotransferase, we developed a sulfotransferase assay method using pyridylaminated Lc4 and nLc4 (PA-Lc4 and PA-nLc4) as an enzyme substrate. The optimal pH was found to be between 6.0 and 6.5. The cloned sulfotransferase essentially does not require divalent cations. Kinetics studies suggested that GP3ST is able to act on both type 1 chain and type 2 chain with a similar efficiency. GP3ST was found to be ubiquitously transcribed.

S2.14

PURIFICATION AND cDNA CLONING OF GnT-VI FROM HEN OVIDUCT

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Six different N-acetylglucosaminyltransferases (GnT-I ~ VI) are supposed to be involved in the branch formation on the core structure of N-linked complex-type sugar chain. So far, GnT-I through V have been purified and their corresponding genes have been cloned. Only GnT-VI has not been purified and its gene structure has been unknown. GnT-VI catalyzes the transfer of GlcNAc to position 4 of the Manα1-6 arm of the core structure of N-glycan, forming the most highly branched N-linked complex-type glycan, pentaantennary glycan with a bisecting GlcNAc. GnT-VI has been purified 64,000-fold in 16% yield from a homogenate of hen oviduct by successive column chromatographies involving Q-Sepharose FF, Ni^{2+}-chelating Sepharose FF, and UDP-hexanolamine-agarose. This enzyme required divalent cations such as Mn^{2+}. The apparent molecular weight of the purified enzyme was 72,000 under nonreducing conditions. The purified GnT-VI could not act on biantennary oligosaccharide but on β1-6N-acetylglucosaminylated Manα1-6 arm (GnT-V product). Based on the partial amino acid sequence of the purified enzyme, we have isolated a cDNA clone of GnT-VI from a hen oviduct cDNA library. A recombinant protein of the cloned cDNA showed GnT-VI activity. The deduced protein is a type II transmembrane protein composed of 464 amino acid residues and has two potential N-glycosylation sites.

S2.15

Human α1,3/4 Fucosyltransferases: Characterization of highly conserved cysteine residues.

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Human α1,3 fucosyltransferases (FucTs) contain four highly conserved cysteine (Cys) residues. Their functional significance and participation in disulfide bonds were characterized in the present study by site directed mutagenesis and mass spectrometry analyses, respectively. Mutagenesis of each Cys residue to Ser resulted in proteins lacking enzymatic activity. Three of the four mutants proteins have molecular weights similar to wild type enzyme and maintained an ability to bind GDP, whereas the other mutant protein produced a series of lower molecular weight bands when characterized by Western blot analysis, and did not bind GDP. The four Cys residues were found to be involved in disulfide bonds and occur between Cys^{81} to Cys^{338} and Cys^{91} to Cys^{341} of FucT III. Thus, amino acids located at the N- and C-termini, are brought close together in space. These include amino acids at both ends of the catalytic domain that we have shown to be involved in acceptor substrate specificity. These results will be discussed with respect to the disulfide bond pattern of human FucT VII.

S2.16

A GDP-FUCOSE PROTECTED, PYRIDOXAL-5'-PHOSPHATE/$NaBH_4$ SENSITIVE LYS RESIDUE COMMON TO HUMAN α1,3/4FUCOSYLTRANSFERASES CORRESPONDS TO LYS^{300} IN FUCT-IV

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Human α1-3/4fucosyltransferases (FucT's) contain a common, essential pyridoxal-5'-phosphate (PLP)/$NaBH_4$ reactive, GDP-fucose protectable Lys. For identification, site directed mutants at lysines of FucT-IV and -VII were prepared and tested. Non-conserved lysine mutants, K119Y and K394Q, were similar to wild type FucT-IV. However, mutants of conserved lysines K228R and K300R were distinct. The specific activity of K228R was 2- to 3-fold lower but retained K_m values for both substrates as wild type FucT-IV. The specific activity of K300R was reduced over 400-fold with an apparent K_m for GDP-fucose over 200 μM. FucT-VII mutants K169R and K240R (equivalent to K228R and K300R for FucT-IV, respectively) were inactive. No change in PLP/$NaBH_4$ sensitivity occurred with K119Y, K228R, and K394Q compared to wild type FucT-IV. These and previous results (Sherwood, et al. (1998) J. Biol. Chem., 273, 25256-25260), demonstrate that of three conserved lysines in FucT-IV, two (Lys^{228} and Lys^{283}) are not involved in substrate binding but perhaps in catalysis. The third site, Lys^{300}, is involved in GDP-fucose binding and PLP/$NaBH_4$ inactivation.

S2.17

MOLECULAR CLONING AND CHARACTERIZATION OF A NOVEL THYMUS ASSOCIATED CORE 2 β1,6-*N*-ACETYLGLUCOSAMINYLTRANSFERASE

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Core 2 branching catalyzed by UDP-*N*-acetyl-α-D-glucosamine: acceptor β1-6-*N*-acetylglucosaminyltransferases (β6GlcNAc-Ts) is an important step in *O*-glycan biosynthesis. Core 2 complex-type *O*-glycans are involved in selectin mediated adhesion events, and *O*-glycan branching appears to be highly regulated. Two homologous β6GlcNAc-Ts functioning in *O*-glycan branching have previously been characterized, and here we report a third homologous β6GlcNAc-T designated C2GnT3. C2GnT3 was identified by BLAST analysis of human genome survey sequences. The catalytic activity of C2GnT3 was evaluated by *in vitro* analysis of a secreted form of the protein expressed in insect cells. The results revealed exclusive core 2 β6GlcNAc-T activity. The product formed with core 1-*para*-nitrophenyl was confirmed by ^{1}H-NMR to be core 2-*para*-nitrophenyl. *In vivo* analysis of the function of C2GnT3 by coexpression of leukosialin (CD43) and a full coding construct of C2GnT3 in CHO cells confirmed the core 2 activity, and failed to reveal I activity. The C2GnT3 gene was located to 5q12, and the coding region was contained in a single exon. Northern analysis revealed selectively high levels of a 5.5 kilobase C2GnT3 transcript in thymus with only low levels in other organs. The unique expression pattern of C2GnT3 suggests that this enzyme serves a function different from other members of the β6GlcNAc-T gene family.

S2.18(L1)

Fuc-TIX AND THE DISTINCT FUCOSYLATION PHENOTYPES OF LEC12 AND LEC29 CHO GLYCOSYLATION MUTANTS.

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Gain-of-function glycosylation mutants provide access to glycosylation pathways, glycosylation genes, and mechanisms that regulate expression of a glycotype. Previous studies have shown that the gain-of-function Chinese hamster ovary (CHO) mutants LEC12 and LEC29 express an N-ethylmaleimide-resistant α(1,3)fucosyltransferase (α(1,3)Fuc-T) activity that is not detected in CHO cells and that generates the Lewis(X) but not the sialyl-Lewis(X) determinant. The two mutants differ, however, in lectin resistance properties, expression of fucosylated antigens, and *in vitro* α(1,3)Fuc-T activities. We have shown that the only known α(1,3)Fuc-T expressed by each mutant is Fuc-TIX. LEC12 cells have an *in vitro* α(1,3)Fuc-T activity of ~100 nml/hr/mg protein and bind the αSSEA-1, VIM-2 and SH-1 monoclonal antibodies, whereas LEC29 cells bind only the αSSEA-1 antibody and have an ~ 40-fold lower α(1,3)Fuc-T activity. Mixing experiments do not detect Fuc-TIX inhibitory activity in LEC29 cell extracts, and CHO cells expressing a transfected Fut9 cDNA behave like LEC12 cells. LEC29 cells transfected with a *Fut9* cDNA express the VIM-2 epitope, suggesting that they have the appropriate acceptors for internal α(1,3) fucosylation. Mouse Fuc-TIX has been shown to preferentially fucosylate terminal N-acetylglucosamine residues of tri-lactosamine acceptors *in vitro* and it is possible that LEC29 cells lack internally fucosylated glycans on the cell surface because of their low Fuc-TIX activity. Interestingly, the *in vitro* α(1,3)fucosylation activities of LEC12 and LEC29 cells do not correlate with the relative abundance of their *Fut9* gene transcripts (LEC29 >> LEC12). Genomic Southerns do not show any *Fut9* gene rearrangement and *Fut9* cDNAs from the two mutants are identical in coding region sequence. It is proposed that a difference in the untranslated region (UTR) of the *Fut9* gene is responsible for the low Fuc-TIX activity in LEC29 CHO cells.

S3A.1

CATALYTIC ACTIVITY FOR POLYSIALYLATION IS LOCATED IN CARBOXYL TERMINAL REGION OF POLYSIALYLTRANSFERASES. Kiyohiko Angata and Minoru Fukuda, Glycobiology Program, The Burnham Institute, La Jolla, California, USA

Polysialic acid is a homopolymer of α-2,8-linked sialic acid and play an important role in neural development. Two polysialyltransferases, PST (ST8Sia IV) and STX (ST8Sia II), have been shown in differential and cooperative manners to synthesize polysialic acid of neural cell adhesion molecule (NCAM). However, how polysialyltransferases form polysialic acids has been unknown. In order to analyze which part(s) of enzyme is involved in elongation of α-2,8-linked sialic acid, we made various chimeric enzymes between PST and ST8Sia III, which is another α-2,8-sialyltransferase that forms oligosialic acid. First, PST entirely lost its activity by replacing its portion with ST8Sia III. Second, ST8Sia III lost its activity when its amino terminal region of catalytic domain including sialylmotif L was replaced with corresponding region of PST. In contrast, chimeric ST8Sia III enzymes, in which a carboxyl terminal quarter was replaced with that of PST, exhibited polysialic acid synthesis on those chimeric enzymes themselves (autopolysialylation) but not on NCAM. The results suggest that NCAM recognition and catalytic activity could be separated in PST. We also found substitutions of Cys to Ala in the sialylmotifs and at 4th amino acid from carboxyl terminal of PST abolished polysialyltransferase activity, indicating these Cys residues are important for enzymatic activity. These results, taken together, suggest that the amino terminal and carboxyl terminal regions of PST confers its NCAM recognition and elongating activity, respectively. (Supported by CA33895)

S3A.2

Cloning and characterization of *N*-acetylgalactosaminide α2,6-sialyltransferase (ST6GalNAc II) responsible for sialylation of rainbow trout egg polysialoglycoprotein

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The occurrence of polysialic acid (poly Sia) structure in vertebrates was first found in rainbow trout egg polysialoglycoprotein (PSGP). Rainbow trout egg PSGP is composed of tandem repeats of a glycotridecapeptide, to which three *O*-linked glycan chains with an α2,8-linked oligo/polyNeu5Gc group are attached. Three sialyltransferase activities were shown to be responsible for the biosynthesis of the oligo/polySia chain of PSGP: (i) *N*-acetylgalactosaminide α2,6-sialyltransferase, which catalyzed transfer of α2,6-linked Sia residue to the proximal GalNAc residue in asialo-PSGP; (ii) α2,6-sialoside α2,8-sialyltransferase, which catalyzed transfer of α2,8-linked Sia residue to the α2,6-linked Sia residue; and (iii) α2,8-polysialyltransferase, responsible for the synthesis of the α2,8-linked oligo/polySia chain in PSGP. Our objective is to reveal the biosynthetic mechanism of the oligo/polySia chain on *O*-glycans. In this study, a cDNA for ST6GalNAc II homologue has been cloned from rainbow trout ovary during oogenesis by PCR-based strategy. Based on the developmental expression of the mRNA, it is suggested that this ST6GalNAc II homologue is responsible enzyme for catalyzing the reaction (i).

S3A.3

Molecular cloning and expression of a novel CMP-sialic acid synthetase which can activate both deaminoneuranimic acid (KDN) and N-acetylneuranimic acid (Neu5Ac) to the CMP-sialic acid

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2-Keto-3-deoxy-D-*glycero*-D-*galacto*-nononic acid (KDN) is a new member of sialic acids that is expressed in vertebrate cells and tissues during normal development and tumorigenesis. Differential recognition of KDN and Neu5Ac by various enzymes utilizing sialic acids may be critical for biological functions that these sialic acid residues are implicated in. It has been shown that mammalian CMP-*N*-acetylneuraminic acid (Neu5Ac) synthetases have very low activity to synthesize CMP-KDN from KDN and CTP, while the corresponding enzyme from rainbow trout testis was shown to have high activity to synthesize both CMP-KDN and CMP-Neu5Ac from KDN and Neu5Ac, respectively, using a partially purified enzyme preparation. In order to elucidate structural requirements of this class of enzyme for recognition of KDN and Neu5Ac, cDNA cloning of the trout CMP-Sia synthetase was carried out by PCR-based strategy. The cloned cDNA encoded 432 amino acid residues with two functional nuclear localization signals. The amino acid sequence displayed 54% identity to that of the murine enzyme. Based on the k_{cat}/K_m values, the recombinant trout enzyme had high activity toward both KDN and Neu5Ac (1.5×10^5 versus 0.89×10^5 $min^{-1}M^{-1}$). In contrast, the recombinant murine enzyme had 15 times lower activity towards KDN than Neu5Ac (0.027×10^5 versus 0.40×10^5 $min^{-1}M^{-1}$), indicating that the murine enzyme can produce CMP-KDN with low efficiency if KDN is provided. This enzyme is the first KDN-recognizing protein that has ever been cloned.

S3A.4

Biosynthesis of deaminoneuraminic acid (KDN) in mammalian cells

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KDN is now acknowledged to be a ubiquitous component of vertebrate glycoconjugates. In mammalian cells, the expression of KDN is very low, about a hundred times lower compared with that of Neu5Ac, although it is developmentally regulated, tumor-associated and age-dependent. Recently we have shown that Man plays an important role as a precursor in the synthesis of KDN (Angata *et al.* (1999) *J. Biol. Chem.* 274, 22949; *Biochem. Biophys. Res. Commun.* 261, 3261). It is proposed that the biosynthetic pathway for KDN residues consists of the following sequential reactions: (i) Man + ATP → Man-6-P + ADP; (ii) Man-6-P + phosphoenol pyruvate → KDN-9-P + Pi; (iii) KDN-9-P → KDN + Pi; (iv) KDN + CTP → CMP-KDN + PPi; (v) CMP-KDN + HO-R → KDN-R + CMP. Our objective is to elucidate biosynthetic mechanism for differential expression of KDN and Neu5Ac in mammalian cells. Here we present the recent results suggesting that KDN-specific enzyme(s) or protein(s), such as CMP-KDN transporter and KDN-transferase, are necessary for the efficient expression of KDN residues on the surface of mammalian cells. (1) In mouse melanoma B16 cells, the level of intracellular free KDN increased from 1.2 μM up to about 79 μM, when the cells were transferred from basal medium into Man-rich medium containing 20 mM Man. (2) The increase of intracellular free KDN did not lead to a concomitant increase of bound forms of KDN. (3) B16 cells were transfected with cDNA for trout testis CMP-Sia synthetase that catalyzes the reaction (iv) and cultured in the Man-rich medium. The expression level of bound forms of KDN did not change in cells before and after the transfection.

S3A.5

Identification and biosynthesis of a new oligoSia-containing glycoprotein (adipoQ) in bovine and murine serum

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Recently, α2→8-linked oligoSia residues with 2-4 Sia residues on various glycoproteins in mammalian adult and embryonic tissues have been demonstrated using newly developed sensitive chemical and immunochemical methods: fluorometric C_7/C_9 analysis (1); mild-acid hydrolysis-fluorometric HPLC analysis (2); and a series of anti-oligoSia antibodies in combination with exo- and endo-sialidases (3). We now hypothesize that these oligoSia moieties on glycoproteins may have similar important functions in common with those proposed for oligoSia-containing gangliosides. To gain an insight into the biological roles of these oligoSia residues on glycoproteins, it is important to know the occurrence and regulatory mechanism for the expression of oligoSia residues on glycoproteins. Previously, we showed that α_2-macroglobulin and fetuin from fetal bovine serum contained di- and oligoSia structures (4). In this study, we identified an adipocyte-specific protein (adipoQ) as a new member of oligoSia-glycoprotein in fetal bovine serum using the chemical and immunochemical analyses described above. We also showed that adipoQ isolated from adult murine serum contained oligoSia structure. Furthermore adipoQ secreted from mouse 3T3-L1 cells which had been differentiated into adipocytes by treatment with differentiation-inducing reagents was shown to be oligosialylated. Thus, the expression of oligoSia residues on adipoQ appeared to be expressed in a differentiation stage-dependent manner. In order to identify enzymes responsible for the synthesis of the oligoSia structure on adipoQ, the gene expression of five α2,8-sialyltransferases (ST8Sia I-V) and adipoQ in 3T3-L1 cells during differentiation was also examined.

(1)C. Sato *et al.* (1998) *Anal. Biochem.* 261, 191-197; (2)C. Sato *et al.* (1999) *Anal. Biochem.* 266, 102-109; (3) C. Sato *et al.* (2000) *J. Biol. Chem. in press*; (4)K.Kitajima et al. (1999) in the *Sialobiology and Other Novel Forms of Glycosylation*, pp69-76

S3A.6

N-LINKED DEGLYCOSYLATION HAS NO SIGNIFICANT EFFECT ON THE ENZYMATIC ACTIVITY OF ST3Gal III.

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The mammalian ST3Gal III (for nomenclature, see Tsuji, S., Datta, A.K., and Paulson, J.C. *Glycobiology* **6**: v – vii, 1996) transfers sialic acid from CMP-NeuAc to form NeuAcα2,3Galβ1,3/4GlcNAc oligosaccharide sequence found in the terminal carbohydrate groups of glycoproteins and glycolipids. This enzyme was cloned from rat liver (Wen, D.X., Livingston, B.D., Medzihradszky, K.F., Kelm, S., Burlingame, A.L., and Paulson, J.C., *J. Biol. Chem.* **267**: 21011 – 21019, 1992) and the analysis of the 374-amino acid sequence deduced from its cDNA indicated the presence of two potential N-linked glycosylation sites at N79 and N170. To investigate their functional role, the *N*-terminal truncated ST3Gal III gene was fused in frame with a glucoamylase sequence with a kex2 site (LysArg) and has been expressed as the secreted form in *Aspergillus niger*. The protein collected from the culture medium of the growth was purified using ammonium sulfate precipitation followed by SP-Sepharose column chromatography. SDS-polyacrylamide gel electrophoresis showed it as heterogeneous due to high mannosylation. These high mannose *N*-linked carbohydrates were removed by Endo H treatment under a condition to retain the native conformation. The deglycosylated protein was purified by a combination of Con A-agarose, Mono Q-Sepharose, SP-Sepharose and Superdex-75 column chromatography. SDS-polyacrylamide gel electrophoresis showed the presence of a single band of approximately 32 kD. Enzymatic assay indicated that the deglycosylation did not affect the ST3Gal III activity. Additionally, comparative kinetic studies of the native secreted form and the deglycosylated form showed no significant difference in Km values for either the donor CMP-NeuAc or the acceptor LNT.

S3A.7

CLONING, EXPRESSION AND CHARACTERIZATION OF HUMAN ST6Gal (EC 2.4.99.1)

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Sialyltransferases are recognised as an important class of glycosyltransferses however detailed studies c their catalytic mechanism, regulation and tertiary structure have been hampered by their relatively lo abundance in tissues. Despite several attempts, production of these enzymes in *E.coli* has bee unsuccessful. Here we report the cloning of ST6Gal from human liver cDNA using specific PCR prime and subsequent expression in insect cells. PCR primers were designed to amplify a truncated form (ST6Gal (amino acids 46-406) which was cloned into the vector pMelBacB (Invitrogen). The resulta plasmid was used to make recombinant virus by homologous recombination which was then used to infe Sf9 insect cells. Preliminary experiments have shown that the yield of truncated human ST6Gal approximately 12U L^{-1} of culture media. We monitored the expression and activity of ST6Gal using a rap semi-automated method for detecting the product of ST6Gal activity. The NeuAcα2-6Gal-R specific lect *Sambucus nigra* agglutinin (SNA), immobilised to the carboxymethyl dextran surface of a BIAcore™ sensor chip, is used to detect the formation of the NeuAcα2-6Gal-R epitope on the acceptor substrat Generally we use the glycoprotein acceptor asialofetuin and we can monitor rection progress curves ar enzyme kinetics using this method. Analysis of the sialylated glycoproteins produced by recombina hST6Gal indicated that the correct anomeric linkage is being formed by the enzyme.

S3A.8

THE SIALYLTRANSFERASE ST8SIA IV GOVERNS NCAM POLYSIALYLATION AND IS SPECIFICALLY UP-REGULATED IN RETINOIC ACID-INDUCED DIFFERENTIATION

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The sialyltransferases ST8SiaII (STX) and ST8SiaIV (PST) are independently capable to induce polysialic acid (PSA) on the neural cell adhesion molecule NCAM. Despite a distinct regulation during rat brain development, many PSA-positive neurons appear to express mRNAs of both enzymes (Hildebrandt H. et al. 1998, J. Neurochem. 71:2339). Coexpression of ST8SiaII and ST8SiaIV was also detected in a human neuroblastoma tumor (Hildebrandt H. et al. 1998, Cancer Res. 58:779). Asking for their differential contribution to polysialylation of the same substrate, PSA-positive human tumor cell lines were screened for expression of ST8SiaII and ST8SiaIV at different mRNA levels. The divergent patterns observed in some cell lines suggest that the polysialyltransferases are independently regulated at the transcriptional level. Moreover, ST8Sia IV is specifically up-regulated during neuronal differentiation of SH-SY5Y neuroblastoma cells induced by retinoic acid. In subsequent analyses the different mRNA levels of ST8SiaII and ST8SiaIV in these tumor cells were correlated with the degree of PSA expression and the cellular capacity to synthesize PSA. Our data indicate that ST8SiaIV is the major regulator of NCAM polysialylation in the cooperative action of both enzymes *in vivo*.

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S3A.9

ST6Gal I CLEAVAGE-SECRETION DIFFER IN DIFFERENT CELL TYPES

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ST6Gal I that adds Sia residue to the N-linked glycan chains of glycoproteins is localized in the trans Golgi and trans Golgi network in rat liver. After localizing to the Golgi, part of ST6Gal I is cleaved and secreted. Previously we constructed the mutant ST6Gal I in which the whole stem plus a small portion of active domain were deleted. The mutant protein showed significantly higher cell surface expression and was neither cleaved nor secreted. These results suggested that the secretase that acts on ST6Gal I could control how much ST6Gal I gets to the surface and to the extracellular fluid as a soluble form. Here we studied the cleavage and secretion of ST6Gal I in various cell types, and found that the proteloytic cleavage site differs from cell to cell, suggesting that cell type specific secretases act on ST6Gal I cleavage-secretion. Using different cell types, we also studied the relationship between ST6Gal I Golgi retention and its cleavage-secretion.

S3A.10

STRESS EXCERTS TISSUE - SPECIFIC EFFECTS ON SIALYLTRANSFERASE ACTIVITY

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Metabolic response to psychological stress is a very complex and demanding physiological process that involves numerous organs and organ systems. Though it is a highly important for survival in ever changing environment, its excessive activation is associated with various detrimental effects. A number of epidemiological and experimental studies clearly demonstrated a link between stress and the development and course of many diseases from simple virus infections and gastric ulcers to cardiovascular diseases and cancer. Previously we have shown specific changes in protein glycosylation and expression of lectins that occur during stress response. Here we report that stress also affects activity of sialyltransferases (STs). Using asialofetuin as an acceptor and ^{14}C-CMP-NeuAc as a donor of sialic acid we have measured total ST activity in rat liver, kidney, heart, skeletal muscle, adrenal gland, and medulla spinalis. Rats were exposed to immobilization stress, either acutely (one 2 h episode), or chronically (multiple immobilization, swim, and footshock stress for eight days), and the activity of STs in their tissues was compared to ST activity in tissues of control rats. Significant effects of stress were found in livers and spleens, while in other analyzed tissues there were no significant changes. In both liver and spleen acute stress caused approximately 40% increase in ST activity. However, chronic stress was found to have opposite effects in liver in spleen. While ST activity in spleen nearly doubled in rats exposed to chronic stress (193% of control), in liver it decreased to 82% of the activity in control rats. Interestingly, despite changes in ST activity in both liver and spleen, we observed no changes in glycoprotein profiles.

S3A.11

IMPORTANCE OF N-GLYCANS FOR THE ACTIVITY OF ST8SIAII AND ST8SIAIV

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Poly-α2,8-N-acetyl neuraminic acid (polysialic acid, PSA) is a specific and highly regulated posttranslational modification of the neural cell adhesion molecule (NCAM). PSA has been implicated in the plasticity of cell-cell interactions and plays a crucial role in neural development, neural regeneration and plastic processes in the vertebrate brain [1]. Two different polysialyltransferases are able to catalyze NCAM-polysialylation: ST8SiaII and ST8SiaIV. Recently, we identified a second enzymatic activity of ST8SiaIV: the ability to catalyze autopolysialylation [2]. This unusual self-modification, which could also be detected for ST8SiaII, seems to be a prerequisite for NCAM-polysialylation. Autocatalytically synthesized PSA is linked to the enzyme by N-glycan chains. Using limited PNGase F digests, we could demonstrate for both enzymes that all of the potential N-glycosylation sites (five in ST8SiaIV and six in ST8SiaII) are glycosylated *in vivo*. To investigate the role of individual N-glycans in the process of autopolysialylation and NCAM-polysialylation the N-glycosylation sites were systematically eliminated in various combinations using site directed mutagenesis, to form a panel of mutants in which the role of each carbohydrate chain could be assessed. Using transfection studies and *in vitro* assays, we analysed these mutants for their ability to catalyze autopolysialylation and NCAM-polysialylation. In ST8SiaIV a single N-glycosylation site could be identified, which is sufficient for enzymatic activity, while for ST8SiaII two N-glycans are important for an active enzyme. Loss of autopolysialylation activity due to the elemination of N-glycosylation sites did always lead to a complete loss of enzymatic activity, including NCAM-polysialylation.

[1] Mühlenhoff *et al.* (1998) *Curr. Opin. Struct. Biol.* **8**:558. [2] Mühlenhoff *et al.* (1996) *EMBO J.* **15**:6943

S3A.12

MUCIN-TYPE SIALYLATION OF A GLYCOPEPTIDE

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O-Glycosylation is an abundant post-translational modification of proteins, but analogous structures in neoglycoconjugates are still not easily achieved either chemically, enzymatically or biotechnologically. We have focussed on the synthesis of short-chain mucin-type derivatized glycopeptides. As several of the shorter glycosequences are of special medical interest. And the length of the oligosaccharide seems to influence only marginally effects on a peptide backbone, such as peptidase protection, break-up of folding structures and exposition to a hydrophilic outer sphere. Sialylation of glycoconjugates were sometimes described as a possible method to suppress fast clearance from the blood stream. Mucin-type sialylated sequences such as the siaTn (Siaα2,6GalNAcαSer/Thr), the siaT (Siaα2,3Galβ1,3GalNAcαSer/Thr) and the DSGGn (Disialo-Gal-GalNAc) structure were designated as target structures. We were able to synthesize GalNAc-α-Ser, GalNAc-α-Thr and mucin-type core 1 (Galβ1,3GalNAcαSer/Thr) in a gram-scale using α-*N*-acetyl-galactosaminidase from *A. niger* and recombinant β-galactosidase III from *B. circulans*. However, further elongation by sialidase assisted methods suffered by low yields, which limited their use to readily available acceptors. Therefore, it was necessary to have a look also on sialyltransferases. Sialylation toward SiaTn and SiaT structures was tried on the level of glycopeptide building blocks as well as glycosylated derivatives of a bioactive LSQVHR peptide fragment.

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S3A.13

TRYPANOSOMAL TRANSSIALIDASE; A MULTI-TALENTED "GLYCOSYLTRANSFERASE"

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Sialidases hydrolyse glycosidically linked sialic acid, in which a water molecule is the acceptor. Sialyltransferases catalyse the transfer of sialic acid from its activated CMP-glycoside to a carbohydrate acceptor. In contrast, trans-sialidases (TS) catalyse the transfer of, preferably, an α2,3-carbohydrate-linked sialic acid to another carbohydrate forming a new α2,3-glycosidic linkage to galactose or *N*-acetylgalactosamine [1]. TS has been found, so far, only in trypanosomes, such as *Trypanosoma brucei brucei* and *Trypanosoma congolense*, which are the agents of Nagana disease, as well as *Trypanosoma cruzi*, the pathogen of Chagas disease [1,2]. Trypanosomes do not synthesise sialic acid, but utilise TS to acquire it from the mammalian host [3]. Sialic acid transferred, *via* this reaction, to surface glycoproteins may be required for the survival and pathogeniety of the parasites inside the vector and host [4]. Currently, there are no inhibitors against TS or potent vaccines against trypanosomes available. Taking this into consideration, as well as the biotechnological importance of the enzyme, investigating trans-sialidase is of major scientific significance. Here we report on the cultivation of *Trypanosoma congolense* and present purification and characterisation experiments of the TS from culture medium. In addition we outline a molecular biological approach which is hoped will lead to the expression of recombinant trans-sialidase from African trypanosomes.

[1] Schenkmann, S., Jiang, M.S., Hart, G.W., Nussenzweig, V., (1991) *Cell*, **65**, 1117-1125
[2] Engstler, M., Reuter, G., Schauer, R., (1992) *Hoppe Seyler's Z. Physiol. Chem.*, **373**, 843
[3] Schauer, R., Reuter, G., Mühlpfordt, H., Andrade, A.F., Pereira, M.E.A., (1983) *Hoppe Seyler's Z. Physiol. Chem.*, **364**, 1053-1057
[4] Pereira-Chioccola, V.L. and Schenkmann, S., (1999) *Biochem. Soc. Trans.*, **27**, part 4

S3A.14

GENOMIC STRUCTURES AND EXPRESSION CHARACTERISTICS OF GANGLIOSIDE GM3 SYNTHETIC SIALYLTRANSFERASE IN HUMANS AND MICE.

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The transfer of sialic acid from the donor substrate CMP-NeuAc to the terminal galactose residue of lactosylceramide is mediated by GM3 synthetic sialyltransferase (SAT-I/ST3 SiaV). SAT-I is one of the key enzymes catalyzing the biosynthesis of complex glycosphingolipids since it acts at the branch point in the metablic pathway. Recently, we cloned human, mouse, rat and monkey cDNAs encoding GM3 synthase, and demonstrated that SAT-I is a novel sialyltransferase showing several features common in the other sialyltransferases, although an invariant aspartic acid in the sialylmotif L's of all other sialyltransferases is replaced by histidine. The expression profile of the genes displayed a tissue- and species-specific manner, indicating that the transcription of these genes might be differentially regulated in a variety of animals. We analyzed a BAC clone containing the entire human GM3 synthase gene and six independent clones harboring mouse SAT-I gene. Human GM3 synthase gene spanned over 50 kb of genomic DNA, and consisted of seven exons ranging from 112 to 1242 bp, with intron sizes of about 1.3-28 kbp. The first exon coded 5'-noncoding region, and the following 6 exons did code the sequence of the enzyme. 5'-RACE analyses using human brain and skeletal muscle poly(A)-RNA supported a single 5' most exon in human SAT-I gene. On the other hand, 5'-RACE analyses with poly(A)-and total RNAs from mouse brain and liver indicated the existence of at least 3 variants of transcripts with different 5'-noncoding region, and the three transcriptional products of mouse SAT- I gene were expressed at different levels in several tissues.The characterization of mouse SAT-I genomic clones suggested that GM3 synthase gene consists of 9 exons and the intron positions whithin the coding region were identical to the human GM3 synthase gene. The structural difference in the noncoding exon of GM3 synthase gene between humans and mice could explain, in part, the tissue- and species-specific expression of SAT-I gene. FISH analyses revealed that the human GM3 synthase gene is located near the centromere of the short arm on chromosome 2, an area which corresponds to band 2p11.2, whereas the mouse SAT-I gene was mapped to chromosome 6, region C, that exhibited the conserved synteny with human chromosomal localization.

S3A.15

KIDNEY TRANSPLANT PATIENTS WITH DELAYED GRAFT FUNCTION HAVE INCREASED SERUM LEVELS OF GALβ1-4GLCNACα 2-6SIALYLTRANSFERASE PRE-TRANSPLANT. Gro Thorne-Tjomsland[1], T. Hosfield[1], J.C. Jamieson[1], B. Liu[2], P. Nickerson[2,3], J.C. Gough[4], D.N. Rush[2], J.R. Jeffery[2] and R.M. McKenna[4]. Departments of Chemistry[1], Medicine[2] and Immunology[3], University of Manitoba, Winnipeg, MB R3T 2N2; Department of Pathology and Laboratory Medicine[4], University of Calgary, Calgary, AB T2N 4N1.

Cytokines cause release of Galβ1-4GlcNAcα2-6sialyltransferase (ST6Gal I) from hepatocytes and endothelial cells. Patients with chronic renal failure have high serum cytokines. We thus investigated whether patients on a renal transplant waiting list have increased serum ST6Gal I levels which predict post-transplant outcome. Serum ST6Gal I was measured immediately pre-renal transplant in 70 patients (49 male, 64 first transplant, 47 cadaver donors). Mean serum ST6Gal I was significantly higher in the patients (3162 +/- 97) than in 19 age and sex matched controls (2569 +/- 125, $p< 0.003$). There were no correlations between pre-transplant ST6Gal I levels and post-transplant serum creatinine levels or number of rejection episodes. But pre-transplant ST6Gal I levels were significantly higher in patients who required dialysis for treatment of delayed graft function (n=20; 3735 +/- 228) than in those who did not (2933 +/- 83, $p< 0.0001$). ST6Gal I overlapped in patients with or without DGF, but all patients (n=5) with ST6Gal I levels ≥ 4700 had DGF. Regression analysis showed that pre-transplant ST6Gal I and cold ischemic time were independent risk factors for DGF and in a model that also included donor age accounted for 30 % of the DGF predicting variables. We are currently tracking longitudinal post-transplant levels of ST6Gal I in 10 patients with and 10 patients without DGF.

S3A.16

CONFORMATIONAL INVESTIGATION OF CYTIDINE 5'-MONOPHOSPHATE *N*-ACETYLNEURAMINIC ACID USING NMR SPECTROSCOPY AND MOLECULAR MODELLING; Fengyang Yan, Jean-Robert Brisson, James C. Richards, and Dennis M. Whitfield*; *Institute for Biological Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, ON K1A 0R6, Canada*

Sialyltransferases have been shown to be valuable for the synthesis of sialylated oligosaccharides. Such sugars serve important biological roles as receptors for lectins involved in cell adhesion as well as in toxin, viral, and bacterial binding. As part of our efforts to better understand the molecular basis of substrate recognition and the mode of action of sialyltransferase, we have initiated studies of the enzymes from *Nessieria meningitidis* responsible for the activation and attachment of sialic acid to the lacto-*N*-neotetraose analogue. In this presention, we report results of a conformational investigation of cytidine 5'-monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) and CMP-Neu5Ac-Mg^{2+} complex bound to the synthetase by NMR spectroscopy and by molecular modelling. ^{1}H, ^{13}C, and ^{31}P NMR spectra of CMP-Neu5Ac in absence and presence of Mg^{2+} ions show that no major conformational changes occur. ^{31}P NMR titration data suggest that Mg^{2+} binds predominantly to the phosphate group. A set of NOE build-up curves was obtained from one-dimensional transient NOE experiments in absence and presence of synthetase. The very small Rib H-1 to Cyt H-6 NOE indicates that the Rib-Cyt conformation is *anti* in both cases. New NOEs in the presence of synthetase were observed most notably a NOE between Rib H-4 and Neu5Ac H-6. The molecular modelling was performed using *Hyperchem* and the AM1 semi-empirical method, and a low-energy conformer which has Rib H-4 near Neu5Ac H-6 was found.

S3B.1

EPIDERMAL GROWTH FACTOR DOWNREGULATES MUCIN CORE 2 β1,6-N-ACETYLGLUCOSAMINYLTRANSFERASE ACTIVITY IN NCI-H292 AIRWAY CELLS Paul V. Beum, Dhundy Bastola and Pi-Wan Cheng Dept. of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE

The carbohydrate portion of mucin plays a critical role in the structure and function of mucin, and thus it is important to understand how mucin carbohydrate structure is regulated in the cell. Recently, epidermal growth factor (EGF) was shown to upregulate MUC5AC in H292 airway cells (Takeyama et al. (1999) Proc. Natl. Acad. Sci. USA 96, 3081). This led us to examine the effects of EGF on mucin glycosyltransferases in these cells. EGF suppressed the activity of the branching enzyme core 2 β 1,6 N-acetylglucosaminyltransferase (C2GnT) in a time-dependent and dose-dependent fashion, but had no effect on other enzymes assayed, including core 1 β 1,3 galactosyltransferase and polypeptide N-acetylgalactosaminyltransferase. TGFα mimicked the effects of EGF on C2GnT activity, implicating the EGF receptor (EGFR) in C2GnT suppression. The EGFR tyrosine kinase inhibitor AG1478 prevented C2GnT suppression by EGF, confirming the role of EGFR. PD98059, a specific inhibitor of MEK1/2 in the Ras-MAPK pathway, completely blocked the EGF suppressive effect, while inhibitors of Src kinase and p38 MAP kinase were without effect. This indicates that the suppression of C2GnT occurs predominantly via the Ras pathway. Furthermore, northern blotting analysis showed that EGF preferentially inhibits the M isoform of C2GnT, which forms core 2, core 4 and I β1,6 branched carbohydrate structures, rather than the L isoform, which forms only the core 2 structure. In conclusion, EGF stimulation of H292 cells triggers an intracellular signal via the Ras pathway, leading to inhibition of C2GnT activity. This is the first report to describe the details of an intracellular signalling pathway involved in regulation of C2GnT by growth factors.

S3B.2

THE SEQUENTIAL ARRANGEMENT AND STABILITY OF GOLGI GLYCOSYLTRANSFERASES IS MEDIATED BY SIGNALS CONTAINED IN THEIR CTS REGIONS

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We show that the CTS (cytoplasmic, transmembrane and stem) region of type II Golgi membrane proteins contains signals mediating their proteolytic turnover, their retention and subcompartmental localization (*E.Grabenhorst and H.S.Conradt, 1999, J. Biol. Chem. 274, 36107-16)* . Thus, the CTS region is essential for maintainance of a sequentially organized glycosylation machinery of cells and the distribution of the enzymes within the early, medial or the late Golgi subcompartments. The *in vivo* activity of chimeric constructs of human FT6 containing the CTS regions of different donor glycosyltransferases toward a coexpressed reporter glycoprotein enabled us to map their *in vivo* functional localization from medial Golgi to the trans Golgi network as follows: GnT-I < (ST6Gal I, ST3Gal III) < GnT-III < ST8Sia IV < GalT-I < (FT3, FT6) < ST3Gal IV < FT7. The CTS regions of GnT-III and FT7 were found to be resistant towards intracellular proteolysis, and our results obtained with all chimeric transferases showed that the proteolytic sensitivity or resistance is transferred to these enzyme forms together with the CTS regions. The catalytic domain of FT6 was also fused to the type I transmembrane anchor and flanking (CTS) region of human TGN46 (N-terminus oriented towards the Golgi lumen) resulting in intracellular active enzyme forms of FT6 with a high *in vivo* fucosylation efficiency towards coexpressed ß - trace protein similar to wild-type (type II transmembrane-anchored) FT6. This further corroborates the significance of the transmembrane / anchoring plus flanking regions for a proper *in vivo* functional activity of the transferases, since soluble forms of glycosyltransferases lacking the CTS region do not show significant *in vivo* activity. From the sialyl-Lewis^x:Lewis^x ratio measured in the reporter glycoprotein, we hypothesize that TGN46 localizes in an earlier subcompartment than wt-FT6, but later than ST6Gal I or ST3Gal III.

S3B.3

QUALITY CONTROL OF GLYCOPROTEIN FOLDING IN THE ENDOPLASMIC RETICULUM (ER): A PROPOSED MODEL

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Intervention in the ER quality control glycosylation mechanism has been demonstrated *in vitro* to affect the infectivity of several viral diseases, including HIV and Hepatitis B. In the ER, the glycan Glc3Man9Gn2 is added to the nascent polypeptide chain and the three glucoses (Glc) are then trimmed. The Man9Gn2 of an incompletely folded glycoprotein can then be selective reglucosylated by UDP-glucose:glycoprotein glucosyltransferase (GT) to form GlcMan9Gn2. The GlcMan9Gn2 is bound by the chaperone calnexin and retained in the ER during folding through deglucosylation by glucosidase II and reglucosylation by GT. We have modeled GlcMan9Gn2 and Man9Gn2 using Genetic Algorithm and molecular dynamics simulations. For Man9Gn2, an anomalous (i.e. not the most favorable for a disaccharide model) torsion angle was observed. A hydrogen bond formed linking OH6 on α1,2-Man of the α1,6-α1,6 arm to the carboxylic oxygen of the *core* N-acetyl glucosamine (Gn). We are hypothesizing that hydrogen bonding is stronger when the glycoprotein is in an unfolded state and weaker in a folded state.

S3B.4

ROLE OF THE HNK-1 CARBOHYDRATE EPITOPE IN CELL-CELL ADHESIVE INTERACTIONS

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The HNK-1 carbohydrate epitope, which is recognized by the monoclonal antibody HNK-1, is characteristically expressed on a series of cell adhesion molecules and on some glycolipids in the nervous system. To investigate the function of the HNK-1 carbohydrate epitope on cell-cell interactions, we established stable transformant cell lines (C6/GlcAT-P and COS-1/GlcAT-P cells) of C6 glioma and COS-1 cells with GlcAT-P cDNA, which is a key enzyme of the HNK-1 carbohydrate biosynthesis. These cell lines turned to be expressed the HNK-1 carbohydrate epitope on their cell surface glycoproteins. Cell aggregation analysis revealed that the parental C6 and COS-1 cells showed a formation of cell aggregates in time-dependent manner, whereas C6/GlcAT-P and COS-1/GlcAT-P cells markedly reduced the formation of the cell aggregates. Using antibodies specific for NCAM and L1, we demonstrated that these cell adhesion molecules are mainly involved in aggregates formation of the C6 glioma cells and the HNK-1 carbohydrate epitope was preferentially expressed on these cell adhesion molecules in the C6/GlcAT-P cells. We also provided the evidence that NCAM homophilic binding is negatively regulated by the presence of HNK-1 carbohydrate epitope. These lines of evidence suggested that the expression of the HNK-1 epitope expressed on cell adhesion molecules such as NCAM and L1 negatively regulates their homophilic interactions and resulted in reducing the cell-cell adhesion.

S3B.5

Spontaneous galactosylation of agalactoglycoproteins in colostrum. A possible role of milk β1,4-galactosyltransferase.

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We have found that spontaneous galactosylation of GlcNAc residues occurs in bovine colostrum, but not in dialyzed colostrum, without adding UDP-Gal as a donor substrate. UDP-Gal was shown to be present in bovine colostrum at a level ranging from 200 to 600 μM, which is much higher than the K_m for GalT. When UDP-[^{14}C]Gal was added to the colostrum as a tracer, and incubated for 2h, no radioactivity was incorporated into any milk protein as determined by SDS-PAGE/autoradiography. In contrast, when a Gal β1,4-specific bacterial β-galactosidase was added to the colostrum, a marked incorporation of radioactivity into 18-25 kDa and 33 kDa RCA1-positive glycoproteins took place in colostrum. Such spontaneous galactosylation potential of colostrum to glycoprotein acceptors was also confirmed by galactosylation of 100-140 kDa agalactoglycoproteins from a CHO mutant cell line, Lec8, blotted on PVDF membrane during the incubation with the colostrum supplemented with UDP-[^{14}C]Gal. These results suggest a possible role of colostrum GalT in continuing the galactosylation of milk glycoproteins in the lumen of the mammary gland at late gestation and early lactation stages.

S3B.6

TOWARDS UNDERSTANDING THE PHOSPHORYLATION OF β1,4-GALACTOSYLTRANSFERASE

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Past evidence indicates that β1,4-galactosyltransferase (GalT) can be phosphorylated and that there is potentially a 58 kDa cdc2-like kinase that co-purifies with GalT from milk. Perplexingly, this cdc2-like kinase was later found to be a part of the PITSLRE kinase family and appears to have functions in the cell cycle. In recent years, however, the association with β1,4-galactosyltransferase appears to have been neglected. We have now begun to approach this problem and have assayed protein kinases in bovine milk whey using MALDI-TOF-MS. Of the four peptides tested, the two which are substrates for protein kinase C were phosphorylated by whey. Experiments with two other peptides, substrates for Golgi casein kinase and $p34^{cdc2}$ kinase respectively, were negative. This result is interesting since GalT has three potential sites for protein kinase C. In addition we have tried to investigate further the 58 kDa protein present in a commercial preparation of bovine milk GalT. Comparison of its MALDI peptide map against the databases (including translated bovine ESTs) has not yet proven that the 58 kDa protein is really a cdc2-like kinase. However, further experiments to clarify this problem are currently being performed.

S3B.7

GOLGI APPARATUS IMMUNOLOCALIZATION OF ENDOMANNOSIDASE SUGGESTS POST-ENDOPLASMIC RETICULUM GLUCOSE TRIMMING: IMPLICATIONS FOR QUALITY CONTROL. C. Zuber, M. J. Spiro,† B. Guhl, R. G. Spiro, † and J. Roth
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Trimming of N-linked oligosaccharides by ER glucosidase II is implicated in protein folding quality control. An alternate glucosidase II-independent deglucosylation pathway exists in which endo-α-mannosidase cleaves internally the glucose-substituted mannose residue of oligosaccharides. By immunogold labeling, we detected most endomannosidase in *cis*/medial Golgi cisternae (83.8% of immunogold labeling) and few in the intermediate compartment (15.1%), but none in *trans* Golgi apparatus and ER including its transitional elements. Double immunogold labeling established a mutually exclusive distribution with glucosidase II and presence of calreticulin in endomannosidase-reactive sites in addition to the ER. Endomannosidase, compared to Golgi mannosidase II and the p58 intermediate compartment marker, exhibited a behavior typical for Golgi residential proteins when exposed to 15oC, or to 20oC in the presence of caffeine following brefeldin A treatment and 15oC shift. Inhibition of glucosidase I and II activity by deoxynojirimycin did not affect endomannosidase and Golgi mannosidase II distribution. Our results demonstrate that glucose trimming of N-linked oligosaccharides is not limited to the ER and that protein deglucosylation by endomannosidase in the Golgi apparatus additionally ensures that processing to complex-type oligosaccharides can proceed. We therefore propose that endomannosidase functions in quality control of N-glycosylation and that this represents a mechanism in addition to those for control of DNA replication translation and protein folding to guarantee the fidelity of synthetic processes and the biological function of their products.

S4.1

CONSERVED CYTOPLASMIC MOTIFS THAT DISTINGUISH MEMBERS OF THE POLYPRENOL-P: N-ACETYLHEXOSAMINE-1-P TRANSFERASE FAMILY.
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WecA/GPT, MraY and WbcO are conserved members of the polyprenol phosphate:*N*-acetylhexosamine-1-phosphate transferase family that catalyze reactions between a polyprenol phosphate acceptor and a cytoplasmically-located UDP-D-aminosugar donor substrate, UDP-GlcNAc, UDP-MurNAc-pentapeptide or UDP-FucNAc, respectively. How these enzymes discriminate between sugar-nucleotide substrates is unknown, but domain differences between MraY, WecA/GPT, and WbcO that are potentially involved in this discrimination are likely to occur on the cytoplasmic side of the membrane, adjacent to conserved domains involved in catalysis and with access to cytosolic aminosugar nucleotides. Conserved C-terminal domains have been identified that satisfy these criteria and uniquely characterize members of the transferase family. Topological analysis of these domains indicates that they form the highly basic (theoretical pI > 10.0) fifth cytoplasmic loop between TM regions IX and X. In order to determine whether these sequences were determinants of substrate specificity, we cloned *E. coli* WecA and MraY, and *Y. enterocolitica* WbcO into the T7-directed vector pET11. These clones were induced in a WecA-null background and membrane extracts were assayed for both MurNAc-pentapeptide-1-P and GlcNAc-1-P transferase activities. Whereas only MraY allows transfer of MurNAc-pentapeptide 1-P, the data demonstrate that the WecA and WbcO Cytoloop 5 domains may bear overlapping specificity.

S4.2

TUNICAMYCIN BIOSYNTHESIS IN RESISTANT AND NON-RESISTANT STREPTOMYCES: EIGHT NEW TUNICAMYCINS.

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Several *Streptomyces* and *Clavibacter* species produce tunicamycin-like antibiotics (tunicamycin, streptoviridins, corynetoxins, etc) that are inhibitors of the polyprenol phosphate:N-acetylhexosamine-1-P transferase family. However, the mechanisms of biosynthesis of these toxins and of resistance by the producing bacteria are largely unknown. We have developed electrospray ionization (ESI) and matrix assisted laser desorption (MALDI) mass spectrometric techniques to structurally assign tunicamycin variants in the picomolar range. Application of these techniques coupled to in-line reverse-phase high performance liquid chromatography (HPLC) has resulted in the finding of eight new tunicamycins that differ in the attached N-acyl chain. However, we were unable to confirm the production of corynetoxins in culture by phage-infected *Clavibacter toxicus*. Several strains of tunicamycin-producing *Streptomyces lysosuperificus* were selected with different sensitivities to their toxin using a tunicamycin agar diffusion assay. The application of HPLC-ESI-MS to culture supernatants and cellular extracts of these lines indicate that tunicamycin resistance can be uncoupled from its biosynthesis.

S4.3

EFFECT OF CARBOHYDRATE-CARBOHYDRATE INTERACTIONS BETWEEN GLYCOLIPIDS IN APPOSED MEMBRANES OF OLIGODENDROCYTES AND LIPOSOMES ON THE CYTOSKELETON, Joan M. Boggs and Huimin Wang, Research Institute, Hospital for Sick Children, Toronto, Canada M5G 1X8

Oligodendrocytes (OLs) and the myelin produced by them are enriched in two glycosphingolipids, galactosylceramide (GalC) and its sulfated form, cerebroside sulfate (SGC). A requirement for these glycolipids for myelin function and stability was recently demonstrated in a mutant mouse lacking UDP-galactose:ceramide galactosyltransferase[1]. We have shown that these two glycolipids in apposed liposomal membranes or in methanol solution can adhere to each other via a Ca^{2+}-mediated carbohydrate-carbohydrate interaction[2,3]. Here we have examined the potential effect of an interaction between GalC/SGC in apposed membranes of myelin or OLs by incubating cultured OLs with GalC/SGC-containing liposomes and observing the effect on the OL membrane sheets using confocal microscopy. The GalC/SGC-containing liposomes caused a decrease in the density of anti-GalC and anti-myelin basic protein staining but no apparent change in the area of the membrane sheets. In addition, they caused depolymerization of microtubules and actin filaments. The latter resembles the effects of anti-GalC antibodies on OLs reported by Dyer[4]. Control liposomes lacking GalC/SGC had no effect. The effect of the GalC/SGC-containing liposomes was reversible. The fact that these two glycolipids adhere to each other, plus the fact that anti-GalC antibodies have a similar effect suggests that a glycolipid-glycolipid interaction between apposed liposomal and OL membranes occurs as shown recently by Hakomori[6] for other cells. The fact that this interaction with GalC/SGC on the extracellular surface of the OL has effects on the cytoskeleton indicates that it causes transmission of a signal across the membrane. (Supported by MS Society of Canada).

1. Coetzee et al, Cell 86 (1996) 209; 2. Stewart & Boggs, Biochemistry 31 (1993) 10666; 3. Koshy & Boggs, J Biol Chem 271 (1996) 3496; 4. Dyer, Mol Neurobiol 7 (1993) 1; 5. Hakomori et al, Glycobiology 8 (1998) xi.

S4.4

PRESENCE AND ROLE OF MAMMALIAN SPERM SURFACE ARYLSULFATASE-A (AS-A) IN ZONA PELLUCIDA BINDING. E Carmona[1], W Weerachatyanukul[1], T Soboloff[1], A Fluharty[4], D White[1], L Promdee[1], M Ekker[1,3], M Buhr[5] and N Tanphaichitr[1,2], [1]Loeb Health Res. Inst., [2]Depts. Obst./Gyn., Bioch./Microb./Immu., [3]Med. and Mol. Med., Univ Ottawa, Ont, Canada; [4]Dept Psych./Behav. Sci., Univ California Los Angeles, U.S.A.; and [5]Dept Animal/Poultry Sci., Univ Guelph, Ont., Canada.

P68 is a plasma membrane protein isolated from pig sperm, which is involved in mammalian zona pellucida (ZP) binding (Tanphaichitr et al., *Mol Reprod Dev* 1998, 49:203). In this report, we described P68's identity as AS-A, based on the following results. First, three P68 tryptic peptides have >80% homology to the known sequence of human testis AS-A (Stein et al., *JBC* 1989, 264:1252). Second, the P68 peptide sequences matched perfectly with regions of the translated cDNA sequence of the pig testis AS-A, constructed in our lab. A P68 crude extract was obtained by treating sperm with a sucrose solution containing ATP/EDTA, which only removed peripheral plasma membrane proteins, leaving the inner and outer acrosomal membranes intact, as observed by transmission electron microscopy (TEM). P68, further purified by chromatofocusing followed by Mono-Q column chromatography, exhibited desulfation activity on an AS-A aqueous-soluble artificial substrate (*p*-nitrocatecholsulfate) with a pH optimun of 4.5, as well as on a natural substrate, sulfogalactosylceramide (SGC) in a detergent-containing buffer. Furthermore, immunoblotting using an anti-AS-A antibody (produced against human liver AS-A) revealed cross-reactivity with the P68 band. The presence of AS-A on the sperm head surface was demonstrated conclusively by immuno-gold TEM as well as the detection of AS-A activity of intact sperm. Like P68, biotinylated AS-A bound to the ZP. This binding was reduced by co-incubated sulfated monosacharide (mM range) or polyssacharide (μM range), suggesting that ZP sulfated sugar residues may be AS-A's binding ligands. Our finding adds AS-A to the list of ZP-binding sperm surface enzymes. *Funded by MRC, Canada; the Rockefeller Foundation; and National Sci.Technol.Develop.Agency of Thailand.*

S4.5

PHASE BEHAVIOR OF GLYCOLIPIDS

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Thermotropic Fourier transform infrared spectroscopy (FTIR) was used to study the polymorphic phase behavior of the male germ cell specific sulfogalactosylglycerolipid (SGG) and its structural anolog cerebroside sulfate (SGC). The effect of the α-OH group of galactosylceramide (GC) on the acyl chain melting transition and interfacial hydrogen bonding was also investigated. Heating of SGG and SGC bilayers showed that their lamellar crystalline (L_c)/liquid crystalline (L_α) transition was centered at 46 and 41 °C, respectively. Subsequent cooling revealed that the lamellar gel (L_β)/L_α transition was centered at 45 for SGG and 40 °C for SGC. Non α-OH and α-OH GC showed a similar behavior with L_c/L_α transitions centered at 80 and 71 °C and L_β/L_α transitions at 59 and 65 °C, respectively. The OH group reduced the T_m of GC, destabilizing the hydrogen bonding network and the packing of the acyl chains of the glycolipid. The three lamellar phases (L_β, L_c, L_α) of the glycolipids studied are spectroscopically distinguishable; the patterns of the ester C=O band of SGG and amide I band of SGC, non α-OH and α-OH GC revealed that the L_c phase is crystal-like and poorly hydrated. Reconstituted bilayers of dimyristoylglycerophosphocholine (DMPC) or ram testis phospholipids with SGG were homogeneous, with of the lipid components being molecularly dispersed in the bilayer plance. SGG modified the lipid bilayer's interfacial hydrogen bonding network and increased membrane stabilization. The presence of SGG in the sperm head plasma membrane may provide stablization required for the initial binding of sperm to the egg via carbohydrate-carbohydrate mediated interactions. *(Funded by NSERC and MRC)*

S4.6

SIALYLTRANSFERASE GENES TO SYNTHESIZE ALPHA-SERIES GANGLIOSIDES

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Sialylation of glycoconjugates is critical for their chemical and biological properties. There have been reports of 16 sialyltransferase genes to date. Recently, we have isolated two novel mouse sialyltransferase cDNAs which are responsible for the synthesis of alpha-series gangliosides based on BLAST analysis of expressed sequence tags. One is GD1α synthase (ST6GalNAc V), and the other is GD1α/GT1aα/GQ1bα synthase gene. They showed homology to the previously cloned ST6GalNAc III and IV. ST6GalNAc V gene encoded type II membrane protein. A fusion protein with protein A and extracts from transfectants showed enzyme activity of α2,6-sialyltransferase almost exclusively for GM1b, resulting in the synthesis of GD1α. Interestingly, eleven CAG repeats were found in the stem region. Northern blotting revealed that three transcripts of ST6GalNAc V were expressed specifically in brain. ST6GalNAc VI gene also coded type II membrane protein. This gene product showed enzyme activity of α2,6-sialyltransferase for GM1b, GT1b, and GD1a, forming GD1α, GQ1bα and GT1aα, respectively. In contrast to ST6GalNAc V, ST6GalNAc VI was expressed in a wide range of mouse tissue including the brain. This gene was strongly expressed in cerebellum, suggesting its role in GD1α expression in Purkinje cells. Alpha-series gangliosides have been attractive, since they were shown to play important roles in cancer metastasis and in the development of nervous system. It seems of particular interest to investigate the expression of these two genes in human tissues.

S4.7

MOLECULAR CLONING AND EXPRESSION OF CD77/GB3 SYNTHASE GENE THAT INITIATES THE SYNTHESIS OF GLOBO-SERIES GLYCOSPHINGOLIPIDS

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CD77/Gb3 is globotriaosylceramide expressed in a subgroup of Burkitt lymphoma cells and in immature B cells at the germinal center of tonsil. It is also known as a receptor of E. coli O157 verotoxin (Shiga-like toxin) to mediate apoptosis. To further analyze the biological function of CD77/Gb3, we have isolated CD77/Gb3 synthase (alpha1,4-galactosyl-transferase) by expression cloning. The cDNA library in pCDM8 vector was prepared from a human melanoma line SK-MEL-37, and a mouse fibroblast line L cell was used as a recipient cell of transfection. A cDNA clone predicted a type II membrane protein with 353 amino acids. Homology search revealed no significantly homologous cDNAs, indicating that this alpha1,4-galactosyltransferase gene is of different entity from known beta1,4- or beta1,3-galactosyltransferase genes.Substrate specificity analysis revealed that this enzyme could synthesize Gb3 and Gal-Gal-ceramide from lactosylceramide and galactosylceramide, respectively. This gene was strongly expressed in human heart, kidney, placenta and spleen etc. Introduction of this cDNA into L cells resulted in the induction of high-sensitivity to cytotoxicity of verotoxin, confirming that CD77/Gb3 itself is the verotoxin receptor. Analysis on the gene expression in a wide variety of human hematopoietic cell lines have been performed, and very restricted expression pattern has been elucidated. Further analysis on the meaning of the gene expression in some restricted cell lines is now on-going.

S5.1

FUCOSYLTRANSFERASE ACTIVITY AND PRODUCT LOCALIZATION IN CYSTIC FIBROSIS AIRWAY CELLS. Vaishali Kothari, AiHua Liu, Thomas F. Scanlin, and Mary Catherine Glick. The Cystic Fibrosis Center and Department of Pediatrics, University of Pennsylvania School of Medicine, and The Children's Hospital of Philadelphia, Philadelphia, PA. USA.

The airways are the site of the most lethal pathology in cystic fibrosis (CF). Glycoconjugates of CF material have a glycosylation phenotype of increased fucosylation and/or decreased sialylation when compared with non-CF (1). A major increase was observed in fucosyl residues linked α1,3 to antennary GlcNAc when CF airway epithelial cells were compared to non-CF cells (2). Importantly, this increase was modulated with the expression of wild type CFTRcDNA under conditions which brought about a functional correction of the Cl^- channel defect in CF cells. In contrast, examination of fucosyl residues in α1,2 linkage by a specific α1,2 fucosidase showed that the non-CF cells had a higher percentage of fucose in α1,2 linkage than the CF cells (15% vs. 2%, respectively). Airway epithelial cells in primary culture had a similar reciprocal relationship of α1,2- and α1,3-fucosylation when CF and non-CF surface membrane glycoconjugates were compared. In striking contrast, the enzyme activity and mRNA of α1,2 fucosyltransferase did not reflect the difference in glycoconjugates observed between the CF and non-CF cells. We conclude that mutated CFTR may cause faulty compartmentalization in the Golgi so that the processing glycotypes are retained in the compartment of α1,3FucT, thus permitting excess α1,3 fucosylation in CF. In subsequent compartments, no or little terminal glycosylation can take place since the sialyl- or α1,2 fucosyl-transferases may not utilize a substrate, which is fucosylated in α1,3 position to antennary GlcNAc. (*Supported by CF Foundation*)

1. Scanlin, T.F. and Glick, M.C. (1999) *Biochim. Biophys. Acta.* 1455, 241-253.
2. Rhim, A., Kothari, V., Park, P., Mulberg, A., Glick, M.C., and Scanlin, T.F. (2000) Submitted.

S5.2

SULPHATION OF SALIVARY MUCINS IS PROTEIN SPECIFIC, IS NOT CORRELATED TO SIALYLATION, AND IS UNAFFECTED BY CYSTIC FIBROSIS. Deepak Shori[1], Harsha Kariyawasam[2], Tina Genter[3] and Jesper Hansen[3].[1]Dept of Oral Pathology, Rayne Institute, London SE5 9NU, UK, [2]Imperial College, London, UK and [3]Rigshospitalet, Copenhagen, Denmark.

Defective acidification of intracellular organelles, particularly the trans-Golgi network, was proposed by Barash et. al. (Nature 252:70-3 (1991)) to explain findings of decreased sialylation and increased sulphation on secreted proteins in cystic fibrosis (CF). To test this hypothesis we have compared expression of sulphate and sialic acid on three salivary 'mucins' namely MG1, MG2 and Gl. Proteins in whole mouth saliva (WMS) from 4 individuals were separated by FPLC on a Superdex 200 column and the purified 'mucins' were slot-blotted and assayed for sulphate content using the dye alcian blue. Sulphation varied with individual and with mucin: MG1 was found to be the most sulphated, it contributing to almost the entire sulphate content of WMS; light sulphation was seen on MG2 or Gl but only in some individuals. These results allowed us to test small volumes of WMS from twenty CF patients and age- and sex-matched controls for reliable estimates of sulphate on MG1. Wherever possible sulphate on MG1 was also visualised by staining washed SDS-PAGE gels with alcian blue at pH 1. For sialic acid content of salivary mucins, electroblots of SDS-PAGE gels were probed with labelled *Triticum vulgaris* agglutinin. In summary, our results show that MG1 is the main sulphated protein in whole mouth saliva and that there are large differences in the expression of sulphate and of sialic acid on MG1 in both the control and the CF groups. Cystic fibrosis leads to neither a decrease in sialylation nor an increase in sulphation and direct comparisons of sialic acid content with sulphate on MG1 failed to reveal any obvious link between the two in health and in disease. Overall our data do not support the defective acidification hypothesis but point instead to inter-individual differences in expression of terminal glycosyltransferases for the observations. We thank the European Union Biomed Programme for support.

S6.1

EXPRESSION OF THE HEPATIC TRANSCRIPT OF β-GALACTOSIDE α2,6 SIALYLTRANSFERASE (ST6Gal.I) IN HUMAN COLON CANCER

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Previous work from our and other groups have demonstrated an increased expression of β-galactoside α2,6 sialyltransferase (ST6Gal.I) and an increased reactivity with the α2,6-linked sialic acid-specific lectin from *Sambucus nigra* (SNA) in human colon cancer. Several lines of evidence suggest that these modifications are associated with increased malignancy of cancer cells. In this work we have investigated the molecular bases of these phenomena. Transcription of ST6Gal.I gene generates three major transcripts, differing in the 5' untranslated (5'-UT) regions. A first type containing the 5'-UT exons Y and Z (Y+Z form), is thought to represent the housekeeping expression; a second form, lacking exons Y+Z but containing the 5'-UT exon X, is specific for mature B-lymphocytes and a third form, lacking exons Y, Z and X, but containing a short, specific 5'-UT sequence is expressed by liver cells. RT-PCR analysis of a panel of colon cancer cell lines revealed that the Y+Z form is expressed by all the cell lines but that some lines express, in addition, also the hepatic transcript. Surprisingly, the same analysis performed on surgical samples indicated that the hepatic transcript is generally expressed by both cancer and normal tissues, although at an apparently lower level in the latter. SNA-western blot analysis of tissue homogenates revealed an increased extent of α2,6-sialylation of glycoproteins in cancer but the size of α2,6-sialylated glycoproteins appears to be different among specimens. Together, our results suggest that colon carcinogenesis is associated with an altered transcriptional regulation of the ST6Gal.I gene, which is at the basis of the enhanced α2,6-sialylation of membrane glycoproteins.

S6.2

ALTERATIONS IN METASTASIS-RELATED PHENOTYPE AND SUSCEPTIBILITY TO APOPTOSIS OF HUMAN HEPATOCARCINOMA CELLS TRANSFECTED WITH ANTISENCE c-DNA OF N-ACETYLGLUCOSAMINYLTRANSFERASE V

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ABSTRACT(S5): The antisense cDNA of N-acetylglucosaminyltransferase V (GnT-V) was constructed and transfected into human hepatocarcinoma 7721 cell line. The transfection was confirmed with Northern blot. It was found that the cells transfected with antisense GnT-V (GnT-V-AS/7721) expressed less GnT-V activity and β-1,6 GlcNAc branching in the cell glycoproteins as compared with the cells mock-transfected with the vector (pcDNA3/7721). The growth rate of GnT-V-AS/7721 was decreased in serum-containing medium, while the cell death was accelerated in serum-free medium. The GnT-V-AS/7721 showed an increase of cell adhesion to Fn or Ln, and decrease of cell migration and matrigel-penetrating invasion. The GnT-V-AS/7721 cells were more susceptible to the apoptosis induced by all-trans retinoic acid than the mock-transfected cells. This was evidenced by the obvious appearance of a hypoploid sub-G_1 fraction in the DNA histogram using FCM analysis, the more condensed new moon type nuclei under morphological observation, and the more intensive TUNEL reaction for assaying the fragmented DNA. It was concluded that GnT-V contributed to the alterations in metastasis-related phenotypes and the susceptibility to apoptosis.

Key words: N-acetylglucosaminyltransferase V; antisense cDNA; human hepatocarcinoma cell; metastasis-related phenotypes; apoptosis

S6.3

CHANGES IN THE EXPRESSION OF LEWIS ANTIGENS, α1,3 FUC-T AND BIOLOGICAL BEHAVIORS OF 7721 CELL AFTER TRANSFECTION OF CANCER PROMOTING GENE C-*ERB*B-2/*NEU*

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ABSTRACT(S5): The pCMV4 plasmid containing cancer promoting gene, *c-erb*B-2/*neu* was cotransfected with pcDNA3 vector to 7721 human hepatocarcinoma cells, and several clones with stable expression of *c-erb*B-2/*neu* mRNA and protein p185 were established. The results showed that in the mock-transfecteded cells, the expression of SLe^x was high, but $SDLe^x$, Le^x and SLe^a were low, which were in consistant with the high expression of α1,3 FucT-VII and low expression of α1,3 FucT-III/VI . After transfection of *c-erb*B-2/*neu*, the expression of SLe^x and α1,3 FucT were elevated. The expressions of SLe^x and α1,3 FucT in different clones were compatible with the expression of c-erbB-2/neu expression. In addition, the adhesion of 7721 cells to human umbilical vein endothelial cells (HUVEC) and P-selectinas as well as the motility and invasive ability of the 7721 cells were also increased after *c-erb*B-2/*neu* transfection. The increase of above metastasis-related behaviors were positively proportional to the expression intensity of c-erbB-2/neu, SLe^x and α1,3 FucT-VII. The adhesion of 7721 cells to HUVEC was significantly abolished by the specific monoclonal antibody of SLe^x, KM93, but not by the other monoclonal antibodies of Le^x, $SDLe^x$ and SLe^a. The above results indicated that there were closed relationships between the expression of α1,3 FucT, its specific product SLe^x and the ability of cell adhesion, motility and invasion. Moreover, the metastasis promoting effect of *c-erb*B-2/*neu* is partially mediated by the increased expression of SLe^x. Key words: Cancer promoting gene; 7721 human hepatocarcinoma cells; Lewis antigens; α1,3 fucosyltransferase (α1,3 FucT), Metastasis-related behaviors

S6.4

INTRACELLULAR INHIBITION OF BLOOD GROUP A GLYCOSYLTRANSFERASE.

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We report the intracellular inhibition of blood group A N-acetylgalactosaminyltransferase in the human colorectal carcinoma cell line HT29 by 3-amino-3-deoxy-[Fucα(1-2)]Galβ-$O(CH_2)_7CH_3$. Inhibition was demonstrated with a novel capillary electrophoresis assay that monitored decreased intracellular conversion of fluorescently labeled Fucα(1-2)Gal-R acceptor to the corresponding A epitope, GalNAcα(1-3)[Fucα(1-2]Galβ-R. Growth of HT29 cells with either the amino-inhibitor or a competitive substrate, Fucα(1-2)Galβ-$O(CH_2)_7CH_3$, also resulted in decreased expression of blood group A determinants on cell-associated glycoproteins, as detected by immunoprecipitation analysis using A-specific monoclonal antibodies. Furthermore, exposure of these cells to the amino-inhibitor or competitive substrate resulted in significant reduction of cell-surface expression of blood group A determinants. Since integrin α3β1, a cell-surface receptor mediating cell-cell and cell-extracellular matrix interactions, was shown previously to be a major carrier of blood group A determinants on HT29 cells, the studies described herein highlight the potential usefulness of these compounds for elucidating the role of blood group A determinants in biological phenomena.

S6.5

EXPRESSION OF *N*-ACETYLGLUCOSAMINYLTRANSFERASE V IN COLORECTAL CANCER CORRELATES WITH METASTASIS AND POOR PROGNOSIS

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N-Acetylglucosaminyltranferase V (GnT-V) is an enzyme that catalyses β1-6 branching on asparagine-linked oligosaccharides of cell proteins. Malignant transformation of various cells has been shown to correlate with the increase of GnT-V activity and concomitant β1-6 branching. However, the protein expression and localization of GnT-V in human cancer tissue has not yet been demonstrated. We established a monoclonal antibody against GnT-V and performed immunohistochemical study of GnT-V in 103 human colon cancer tissues to clarify the possible relationship between malignant potential and GnT-V expression. Out of 103 cases, 26 cases (25.2%) showed specific expression of GnT-V in cancer tissues. The expression of GnT-V was strongly correlated with distant metastasis ($p<0.05$, Chi-square test). Overall five-year survival rate was 52.8% for GnT-V positive patients and 81.7% for GnT-V negative patients ($p<0.01$, Log-rank test). The difference in survival was also significant in patients with the stage II ($p<0.01$, Log-rank test). In conclusion, expression of GnT-V in colon cancer tissue is related to distant metastasis and poor prognosis. Screening of GnT-V expression in colon cancer tissue may provide useful information for the early detection of distant metastasis in postoperative patients.

S6.6

EFFECT OF AZT ON BRANCHING AND SIALYLATION OF N-LINKED OLIGOSACCHARIDES IN CULTURED CELLS

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Recent whole cell work with human erythroleukemia and melanoma cell lines has established that the anti-HIV chemotherapeutic 3'-azido-3'-deoxythymidine (AZT) potently inhibits protein and lipid glycosylation (Steet et. al. (1999) *Glycoconjugate J.* 16(3), 237-245., Yan et. al. (1995) *JBC* 270, 22836-22841). These effects are most likely attributed to the accumulation of AZTMP and its ability to competitively inhibit pyrimidine nucleotide-sugar import into the Golgi membranes. The inhibition of lipid and protein glycosylation, therefore, likely results from the depletion of nucleotide-sugars available for the processing of glycoconjugates by lumenal glycosyltransferases. Earlier studies showed that AZT severely inhibited the synthesis of gangliosides in both K562 erythroleukemia and SK-MEL-30 melanoma cell lines. Here, we report that AZT treatment of K562 and SK-MEL-30 cells results in substantial changes in the branching and sialylation of N-linked oligosaccharides. Lectin analysis of [^{3}H] mannose labeled N-linked glycans from treated and untreated cells reveals a significant decrease in complex tetra- and tri- antennary structures upon AZT treatment and increases in core-fucosylated biantennary and oligomannose structures. In addition, synthesis of longer chain poly-N-acetyllactosamine side chains was markedly inhibited. N-linked glycans from both cell lines exhibited increased affinity for the ß-galactoside-binding lectin RCA-I in the presence of AZT suggesting that the addition of terminal sialic acid is sensitive to the drug. Together, these data suggest that the import of UDP-GlcNAc and CMP-sialic acid are inhibited by AZT treatment. In light of the importance of N-linked glycosylation in the metastatic phenotype of tumor cells, the ability of AZT to alter the branching and sialylation of N-linked oligosaccharides may prove effective as a cancer chemotherapeutic. Furthermore, these changes may contribute to the hematological toxicity accompanying AZT therapy.

S6.7

TRANSCRIPTIONAL REGULATORY MECHANISMS FOR ABERRANT GLYCOSYLATION DEFINING HUMAN TUMOR MALIGNANCY

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The malignant potential of tumors is often correlated with the expression or deletion of specific glycosylation at the tumor cell surface. We investigated regulatory mechanisms of two human glycosyltransferase genes: (i) alpha(1,3)-fucosyltransferase IV (FUTIV); (ii) histo-blood group A/B, as typical examples involved in this phenomenon. alpha(1,3)-fucosyltransferase IV (FucTIV) is responsible for Le^x and myeloglycan-type structures playing an essential role in cell adhesion during embryogenesis and inflammatory response. These structures are also involved in tumor cell adhesive or migratory interactions, and in tumor cell metastasis. The FUTIV minimal promoter region and transcriptional start sites were determined by promoter fusion and 5,-RACE analysis, respectively. An Ets transcription factor consensus binding site (Ets/-350) was found to be necessary for high level promoter activity in myeloid cell lines. Gel shift assays identified the Ets family members Elk-1 and Pu.1 as Ets/-350 binding activities in extracts from U937 and HL60 myeloid cell lines. This site also binds to an as yet unidentified PMA inducible factor. Elk-1 is known to be activated by the ERK, p38 and SAPK/JNK MAPK pathways. Experiments addressing FUTIV induction by specific kinase activators will be presented. Deletion or reduction of A/B expression in tumors has been correlated with tumor malignancy, as documented extensively in many types of human cancer. We are exploring transcriptional down-regulation of A transferase in genetically-comparable A^+ vs. A- cells. Studies are in progress to define the transcription factors involved, and methylation of CpG islands in promoter region.

S6.8

A HOMOGENEOUS CELL-BASED ASSAY TO IDENTIFY N-LINKED CARBOHYDRATE PROCESSING INHIBITORS

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Malignant transformation is accompanied by altered cell surface glycosylation. N-linked oligosaccharides carrying β1-6GlcNAc branches are associated with tumor invasion and metastasis. Therefore, compounds that can enter cells and block biosynthesis of β1-6GlcNAc-branched glycans without overt cytotoxicity are potential anticancer agents. We have developed a homogeneous cell-based assay for detection of such compounds. The method enables identification of agents that block β1-6GlcNAc-branched glycan expression after incubation for 16-20 h with MDAY-D2 tumor cells, thereby protecting the cells from the subsequent addition of leukoagglutinin (L-PHA), a cytotoxic plant lectin. We observed that MDAY-D2 cell number is directly proportional to the level of endogenous alkaline phosphatase activity measured spectrophotometrically in cultures after the addition of substrate. The alkaline phosphatase assay was capable of detecting as few as 1500 cells and showed a broad dynamic range. The method was readily adapted for high-throughput screening as reagent costs are low, and no cell harvesting and washing steps are required. A successful application of this method was achieved with the screen of a 30,000-compound microbial extract library. Under high-throughput operating conditions, the coefficient of variation for controls was found to be 4.2%. The results suggest that measurement of alkaline phosphatase in this cell assay format may be adapted for wider applications in high-throughput screenings for compounds that relieve cells from other growth inhibitors.

S6.9

CHARACTERIZATION OF THE HUMAN CORE 2b β-1,6-N-ACETYLGLUCOSAMINYLTRANSFERASE

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Sequence-based gene discovery has been greatly advanced by bioinformatics technology. A notable outcome of the use of bioinformatics tools is the increase in the number of new glycosyltransferase genes. Moreover, transferase gene families have been formed with most of these newly identified genes. However, homologous gene members have yet to be isolated for a number of transferase genes such as the Core 2 β-1,6-N-acetylglucosaminyltransferase (Core2GnT) gene. Core2GnT activity is found in a number of tissues in the body, but it is not known if this activity is directed by one gene, therefore identification of Core2GnT-like genes would be of particular interest. A search of the Genebank database resulted in the identification of an EST similar to Core 2 GlcNAc-T. Screening of a human intestinal cDNA library produced a full-length cDNA clone (Core 2b GlcNAc-T/C2M) which was shown to be 57% identical /72% similar to Core 2 GlcNAc-T (as also reported by Yeh, J.C. et al and Schwientek, T., et al.). The Core 2b GlcNAc-T cDNA sequence was subcloned into an expression vector and transiently expressed in a mammalian cell line. The expressed protein was isolated and functional assays were performed to determine the acceptor specificity of this enzyme. Interestingly, it was shown that Core 2b GlcNAc-T has specificity to not only a C2 acceptor but to a C4 acceptor as well. The expression pattern of Core 2b GlcNAc-T in human tissues was also explored. Northern blot analysis revealed that Core 2b GlcNAc-T was only expressed in the kidney, small intestine and colon tissues. A detailed analysis showed expression of core2b mRNA throughout gastrointestinal system. Furthermore, it was shown that Core 2b GlcNAc-T mRNA expression is highly up-regulated in human stomach and colon tumors as compared to their matched normal controls. The implications of this increased Core 2b GlcNAc-T expression in colon tumors is currently being assessed. Other on-going studies include the creation of a null Core 2b GlcNAc-T mutation in mice.

S6.10

GD0039 (SWAINSONINE HYDORCHLORIDE) DOSE-RELATED INHIBITION OF L-PHA-BINDING TO MONKEY PERIPHERAL BLOOD LYMPHOCYTES AND TEMPORAL RELATIONSHIP TO GD0039-ELEVATIONS IN ASPARTATE AMINOTRANSFERASE

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L-PHA binds to and is a marker for peripheral blood lymphocyte complex cell surface glycoproteins - products of the N-linked oligosaccharide processing pathway found in the Golgi apparatus. GD0039 inhibits an enzyme in that pathway, Golgi mannosidase II, resulting in the inhibition of L-PHA binding to peripheral blood lymphocytes and to various human and animal tumor cells. Here we report on the relation between the oral dose of GD0039 in the cynomolgus monkey, the degree of inhibition of L-PHA binding to peripheral blood lymphocytes *ex vivo* and the levels of aspartate aminotransferase (AST). We also explored the temporal relation between intermittent intravenous doses of GD0039, the inhibition of L-PHA binding and the levels of AST. In the oral study, sets of 4 males and 4 female monkeys were dosed at GD0039 levels of 0.008, 0.04, 0.2 and 1 mg/kg, twice daily, for 91 days. L-PHA staining of peripheral blood lymphocytes was inhibited in a dose-related manner with over 90% inhibition occurring at the highest dose. AST increased in a dose-related manner. In the intravenous study, groups of one female and one male monkey were dosed with either GD0039 0.075 mg/kg or vehicle control twice daily for 4 cycles of two weeks on and one week off. GD0039 inhibited L-PHA binding to lymphocytes in a cyclical manner reflecting the dosage schedule and there was an inverse relation between the cyclical effects on AST levels and L-PHA binding. The AST effects were reversible with no evidence of liver toxicity. We conclude that the effects of GD0039 on L-PHA binding are dose-related over the dosage range of 0.008 to 1 mg/kg twice daily and are rapidly reversible. The effects of GD0039 on AST are dose and temporally related to the effects on L-PHA.

S7B.1

GLYCOSYLATION MUTANTS IN TRYPANOSOMA CRUZI.

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We have generated mutants of *Trypanosoma cruzi* in order to study the glycosylation in the epimastigote stage of the parasite. Mutagenized cultures (N-methyl-3-nitro-1-nitrosoguanidine/ethane methane sulphonate) were treated with the toxic lectins Concanavalin A (ConA) and Ricin (Ric) and the mutants isolated by selecting cells that resist to killing or agglutination by the lectins. Two mutants resistant to Con A (ConA1 and ConaA2) and one resistant to Ric (Ric1) were obtained and characterized. There was no morphological difference among the mutant cells compared to the wild type. All the mutant cells showed reduced lectin binding as shown by lectin fluorescence microscopy and flow cytometry. However, the ConA resistant cells exhibit different phenotypes as indicated by the differential binding of Wheat Germ Agglutinin (WGA) and Ricin. Blots of SDS-PAGE resolved *T. cruzi* proteins probed with ConA or Ricin showed clearly that many proteins lost their ability to bind the lectins and resembled gels of deglycosylated wild type cells. Taken together those results suggest a defect on the glycosylation pathway in the mutant cells. Confirmation of this point was obtained by comparing the glycosyltransferase activities of wild type cells and mutant cells. The level of beta1-4galactosyltransferase in the Ric mutant cells was reduced more than 20 fold compared to the wild type cells. Regarding the ConA resistant cells, in the mutant ConA1 we have found a reduced level of GlcNAc transferase activity (approx.10 fold) compared to the wild type cells, but no change in enzymatic activity in the ConA2 mutant, thus indicating the defect is in a different step of the glycosylation pathway. Structural analysis of cell surface glycans in both mutant cell lines compared to the wild type should provide evidence of the altered step.

S7B.2

PHENOTYPES OF *CAENORHABDITIS ELEGANS* UDP-GlcNAc:α-3-D-MANNOSIDE β1,2-*N*-ACETYLGLUCOSAMINYLTRANSFERASE I (GnT I) NULL MUTANTS.

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GnT I controls the conversion of oligomannose to hybrid and complex type *N*-glycans. Disruption of the GnT I gene in the mouse results in arrest of embryogenesis. Defects in the synthesis of complex *N*-glycans are associated with several human congenital diseases. To study the role of complex *N*-glycans in the development of multicellular animals, we have cloned and expressed three cDNAs homologous to mammalian GnT I (*gly-12*, *gly-13* and *gly-14*) (Chen SH, Zhou SH, Sarkar M, Spence AM, Schachter H, 1999, *J Biol Chem* **274**: 288-297). Both *gly-12* and *gly-14* encode active enzymes while *gly-13* does not, at least towards the substrates tested. We now report the phenotypes of putative *gly-12, gly-13* and *gly-14* null mutants obtained by UV irradiation in the presence of trimethylpsoralen (TMP). The *gly-12* null mutant has a 1.6 kb deletion in the *gly-12* gene extending from intron 6 to exon 12 (contains the STOP codon). This mutation is a molecular null because the deleted fragment encodes most of the GnT I C-terminal catalytic domain. Mutant animals have no obvious phenotypic defects. They show a normal morphology, move normally and have a normal life span, brood size, generation time and defecation cycle; they are also sensitive to lavamisole, show Dauer formation and SDS resistance and male mutants have a normal tail structure and can mate. The *gly-13* mutation deletes almost the entire gene and homozygous mutant worms are arrested at larval stage L1. The *gly-14* mutation is a 1.7 kb deletion in a region containing highly conserved residues indicating that this mutation is also a molecular null. However, the *gly-14* mutants display wild type phenotypes. A double mutant of *gly-12* and *gly-14* has been created and also shows a wild type phenotype. At present, we are testing whether the *gly-13* mutation can be rescued by a *gly-13* minigene or by *gly-12* and *gly-14* minigenes driven by the *gly-13* promoter. We are also trying to determine the amount of complex *N*-glycans present in these single and double mutants. Supported by the Medical Research Council of Canada.

S7B.3

CLONING AND EXPRESSION OF *DROSOPHILA MELANOGASTER* UDP-GlcNAc:α-3-D-MANNOSIDE β1,2-*N*-ACETYLGLUCOSAMINYLTRANSFERASE I (GnT I).

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A TBLASTN search of the *Drosophila* EST database with human GnT I as probe yielded a clone (GM01211) with 56% identity over 36 C-terminal amino acids. The EST was sequenced and a 550 bp PCR probe was used to screen a *Drosophila* cDNA library in λ-ZAP II. The 2 largest cDNAs from 6 positive phage colonies were isolated and sequenced to reveal a partial 1362 bp ORF lacking a start ATG codon. 5'-RACE showed a 2828 bp cDNA containing a 1368 bp ORF encoding a 456 aa protein with putative N-terminal cytoplasmic (5 aa) and hydrophobic transmembrane (20 aa) domains. The putative catalytic domain of this protein showed 59% aa sequence identity to mammalian GnT I. This cDNA, truncated to remove the N-terminal hydrophobic domain, was expressed in the baculovirus/Sf9 system as a secreted protein containing an N-terminal $(His)_6$ tag. Protein purified by adsorbtion to nickel-NTA beads converted Manα1-6(Manα1-3)Manβ-octyl (M_3-octyl) to Manα1-6(GlcNAcβ1-2Manα1-3)Manβ-octyl with K_m values of ~0.1 and 2 mM for UDP-GlcNAc and M_3-octyl respectively. The temperature optimum was 37 deg C. TBLASTN searches of the Berkeley Drosophila Genome Project (BDGP) database with *Drosophila* cDNA as probe located the GnT I gene in clones BACR03N16 and BACR33D17. This allowed localization of EST clone GM01211 to chromosomal region 57A3-57A4 (chromosome 2R). Comparison of the cDNA and genomic sequences allowed the assignment of seven exons and six introns; all introns showed perfect GT-AG splice site consensus sequences. TBLASTN searches of the *Drosophila* BDGP database with human GnT II as probe revealed a homologous gene (clone BACR07A18) located in chromosomal region 99C6-99E1 (chromosome 3R) and allowed prediction of at least five exons and four introns. A tentative *Drosophila* GnT II cDNA sequence showed a partial 1239 open reading frame lacking a start ATG codon encoding a 413 amino acid protein with a putative N-terminal hydrophobic transmembrane domain. Expression of this cDNA is under way. We thank Dr.Gabrielle Boulianne (Hosp. Sick Children) for the cDNA library and advice, and the Medical Research Council (Canada) for support.

S7B.4

CLONING AND EXPRESSION OF *CAENORHABDITIS ELEGANS* UDP-GlcNAc:α-6-D-MANNOSIDE β1,2-*N*-ACETYLGLUCOSAMINYLTRANSFERASE II (GnT II).

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A TBLASTN search of the *C.elegans* non-redundant database with the human GnT II protein sequence as probe yielded a gene C03E10.4 (Z81458, chromosome V) with 45% identity over 259 amino acid residues. RT-PCR (Marathon cDNA Amplification Kit, Clontech) and an oligo(dT)-primed *C.elegans* cDNA library as template were used to isolate cDNA with a 1461bp ORF encoding a protein (487 aa) with the typical glycosyltransferase type 2 domain structure (N-terminal 9 aa cytoplasmic and 18 aa hydrophobic domains). The ORF contains 6 exons, all with the GT/AG splice site consensus sequence, whereas the entire coding region of mammalian GnT II is on a single exon. GnT II cDNA was subcloned downstream of a heat shock promoter into plasmids pPD49.78 and pPD49.83 previously modified to contain a sequence encoding Myc epitope (1). Recombinant plasmids were injected into wild type N2 worms and transgenic lines carrying the injected DNA on extrachromosomal arrays were established by "roller" phenotype selection. F5 generation young adults were subjected to heat shock at 33°C for 2 h and then at 20°C for another 2 h. The worms were harvested and stored at -70°C. Worms were lysed by sonication and the sonicate was centrifuged; the microsome pellet was resuspended in lysis buffer and protease inhibitor mixture and assayed for enzyme activity. A 50-fold increase in GnT II activity was observed in heat-shocked worms compared to non-shocked controls. A 3kb fragment of GnT II genomic DNA upstream of the ATG start site was subcloned into the pPD11 vector upstream of the *lacZ* reporter gene to examine the spatial pattern of GnT II expression during *C.elegans* development (1). Transgenic worms were examined for β-galactosidase expression. Preliminary results suggest that reporter gene expression is seen in the anterior ganglion, ventral nerve cord and tail ganglion of larvae but is confined to the pharynx in adult worms. We thank the Medical Research Council (Canada) for support. (1) Chen S, Zhou S, Sarkar M, Spence AM and Schachter H. J.Biol.Chem. (1999) **274**: 288-297.

S7B.5

OLIGOMANNOSIDIC N-GYLCANS OF GASTROPODS ARE HIGHLY METHYLATED

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In general there is limited data on the glycosylation of gastropods. Only the N-Glycans of the haemocyanin purified from *Helix pomatia* and *Lymnea stagnalis* have been characterised in detail so far. Skin and viscera glycoproteins of *Arion lusitanicus* and *Arion rufus*, commonly-occuring slugs causing severe damage in European kitchen gardens, contain about one third anionic and two thirds neutral N-glycans. These neutral fractions were analysed by two-dimensional HPLC before and after exo- and endoglycosidase digests, GC-MS and MALDI-TOF. Most of the neutral *Arionidae* N-glycans were identified to be of the oligo-mannosidic type ranging from three to nine mannoses decorated with two or three methyl groups. However, also in viscera and skin preparations of snails with shells (*Arianta arbustorum, Arianta a. alpicola, Fruticicola fruticum, Uriticicola umbrosus*) these structures were detected in remarkable amounts.

S7B.6

DO TWO ARABIDOPSIS O-GLCNAC TRANSFERASE PROTEINS FUNCTION IN A PARTIALLY REDUNDANT MANNER IN GIBBERELLIN SIGNALING?

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O-GlcNAc transferases (OGTs) modify proteins by transferring N-acetylglucosamine to specific serines and/or threonines. Biochemical studies of animal OGT proteins have produced data consistent with the hypothesis that this modification regulates signal transduction cascades. However because of the lack of an OGT mutant, the physiological effect of the loss of OGT activity in animals is not known. Database searches indicate that *OGT* is a single copy gene in animals, but that there are two OGT-like sequences in plants. The first, the arabidopsis *SPINDLY (SPY)* gene was originally identified as a negative regulator of the gibberellin (GA) signal transduction pathway. This is consistant with the hypothesis OGTs regulate signal transduction. A second possible arabidopsis OGT, *SPY2,* has been identified by Genebank searching and has recently been cloned and sequenced. The hypothesis that SPY2 functions in a partially redundant manner to SPY is being tested. Recently, two T-DNA insertional alleles of *SPY2* (*spy2-1*, *spy2-2*) have been identified and homozygotes are being selected. A comparison of the phenotypes of the *spy2* mutants versus wild type and *spy* mutants will be presented. *spy2* mutants will be examined for the loss of O-GlcNAc protein modifications. By characterizing the degree of functional similarity between these two related proteins, more will be learned about the functioning of SPY2, SPY and the role of OGTs in signal transduction.

S7B.7

CHARACTERIZATION OF ARABIDOPSIS SPY AND THE POTENTIAL ROLE OF AN O-GLCNAC TRANSFERASE IN PLANT HORMONE SIGNALING

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Gibberellins (GAs) are involved in most aspects of plant growth and development including germination, elongation growth, flowering, and fruit development. Although the biosynthesis of this tetracyclic diterpene carboxylic acid hormone has been well characterized, relatively little is known about the mechanism of GA action. SPINDLY (SPY) has been identified as a negative regulator of GA signal transduction using molecular genetic approaches in *Arabidopsis thaliana.* SPY shows significant sequence similarity with animal O-GlcNAc transferases (OGTs). Based on this similarity, it is hypothesized that SPY posttranslationaly modifies cytosolic and nuclear proteins by the addition of O-linked N-acetylglucosamine (O-GlcNAc). This suggests that posttranslational modification of proteins by the addition of O-GlcNAc plays a crucial role in plant development. Results of the analysis of O-GlcNAc protein modification in *spy* mutants and *in vitro* assays of SPY's activity will be presented.

S7B.8

FUNCTIONAL ANALYSES OF THE TPR DOMAIN OF SPY.

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Gibberellin (GA) is a plant hormone that regulates many aspects of plant growth and development. Little is known about how plants respond to this hormone. The SPINDLY (SPY) protein has been shown to negatively regulate the GA signal transduction pathway of *Arabidopsis*. Recessive *spy* mutations cause phenotypes consistent with increased GA signaling, including early flowering and germination on paclobutrazol, an inhibitor of GA biosynthesis. SPY contains 10 tetratricopeptide repeats (TPR) at the N-terminus and exhibits similarity to the C-terminus of *O*-GlcNAc transferase (OGT) from *C. elegans* , human and rat, suggesting the involvement of *O*-GlcNAc protein modification in GA signaling. The TPR repeats are found in a variety of proteins, including animal OGT, and are known to mediate protein-protein interactions. Therefore, it is possible that SPY functions by interacting with other proteins through TPR-mediated protein-protein interactions. To gain further insight into the importance of functional domains of SPY, we sequenced fifteen *spy* alleles. The results show that both TPR domain and the OGT domain are important in SPY's proper functioning in GA signal transduction. The phenotypes of plants overexpressing the TPR domain mimick those of *spy* mutant, further indicating that the TPRs of SPY are important. The TPR domain was also used in the yeast two hybrid screening to look for SPY-interacting proteins from arabidopsis.

S7B.9

CLONING AND SUBCELLULAR LOCALIZATION OF THE *LEISHMANIA MAJOR* STT3 HOMOLOG

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Protozoa of the genus *Leishmania* possess a variety of glycoconjugates such as glycosyl-phosphatidylinositols, lipophosphoglycan and N-glycans. The latter are implicated in the parasites virulence as inferred from tunicamycin-resistant *Leishmania* cell lines. To further investigate the role of protein N-glycans in *Leishmania* virulence we initiated the characterization of its oligosaccharyl transferase (OT) which catalyses the transfer of dolichol-bound core oligosaccharides to selected Asn-residues of nascent proteins.
Using degenerated oligonucleotide primers derived from conserved STT3 sequences - a regulatory subunit of the hetero-oligomeric OT-complex - we obtained a specific amplification product from genomic DNA to probe a cosmid library (www.ebi.ac.uk/parasites/leish.html). Positive restriction fragments of isolated cosmids allowed the identification of the *L. major* STT3 homolog. Expression of the identified open reading frame in *Leishmania* promastigotes was confirmed by RT-PCR. Its deduced amino acid sequence shows 21 % identity to yeast and human STT3p. Heterologous expression of LmSTT3 in an conditional lethal stt3 yeast strain did not rescue the phenotype. Thus, structural differences might provide the basis for the development of specific inhibitors.
Expression of LmSTT3::GFP chimera from episomal gene copies lead to a pronounced perinuclear fluorescence which is characteristic for the leishmanial endoplasmic reticulum.

S7B.10

ON EARTH AND IN SPACE: INSECT CELL GLYCOSYLATION PATHWAY. Lokesh Joshi[1], Michael L. Shuler and H.Alan Wood[1], [1]Boyce Thompson Institute and Dept. of Chemical Engineering, Cornell Unversity, Ithaca, NY 14853. Email: <HAW5@CORNELL.EDU>

Investigations were conducted to evaluate the potential for insect cells to produce mammalian-type complex N-linked glycosylation of human proteins with the baculovirus expression vector system. The current paradigm was that insect cells could not perform sialylation. Previous investigations of N-linked glycosylation of secreted human-placental, alkaline phosphatase (SEAP) glycans produced in a variety of insect larvae and cell cultures indicated that 100% of the glycans had only terminal mannose residues, typical of other recombinant glycoproteins produced in insect cell. However, marked alterations in the structures and relative concentrations of N-linked SEAP glycans were observed when different insect cell lines, cell culture media and culture conditions were employed. These changes resulted in a shift from 0% hybrid/complex glycans to 63% hybrid/complex glycans. More importantly, when cells were culture under conditions of simulated microgravity or with the addition of either N-acetylmannosamine or dexamethasone to the culture medium, as much as 20% of the SEAP glycans had terminal sialic acid residues. Approximately 93% of the sialic acid was N-acetylneuraminic acid attached with α2,3 and 2,6 linkages. Accordingly, insect cells do have the entire metabolic pathway to produce therapeutic proteins that require human-type complex N-linked glycosylation.

S7B.11

XYLOGLUCAN BIOSYNTHESIS. Natasha Raikhel, Ahmed Faik, Robyn M. Perrin, Rodrigo Sarria-Millan, Weiqing Zeng, Curtis Wilkerson, and Kenneth Keegstra. MSU-Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

Plant cell walls play a crucial role in plant development, signal transduction, and disease resistance. Although much is known about the structure of various cell wall components, their biosynthesis is largely unknown. Hemicellulose is a heterogeneous group of branched-matrix polysaccharides that bind noncovalently to the surface of the cellulose microfibrils and, therefore, shape the cell wall. Xyloglucan is the principal hemicellulose of higher plants that bind tightly but noncovalently to cellulose microfibrils, cross-linking them into a complex network. Xyloglucan is composed of a beta-1,4-linked glucose backbone decorated with side chains of xylose, galactose, and terminal alpha 1,2-linked fucose. The terminal fucose residues are thought to facilitate interaction between xyloglucan and crystalline cellulose microfibrils. A fucosyltransferase involved in xyloglucan biosynthesis was purified from etiolated pea seedlings. The purified enzyme is specific for defucosylated xyloglucan and does not transfer fucose to other acceptors tested. Fucose is added to the 2-position of galactosyl residues, generating XXFG and XLFG within tamarind seed xyloglucan. Sequence information of peptides derived from the purified protein was used to isolate cDNA clones from both pea and Arabidopsis. The identity of these clones has been confirmed in plants and by expression in heterologous systems. Searches of the Arabidopsis genomic databases led to the identification of the gene encoding xyloglucan fucosyltransferase, as well as genes encoding several additional putative fucosyltransferases. The function of these additional enzymes is currently under investigation. Genomic approaches are also underway to identify additional genes required for xyloglucan biosynthesis.

S7B.12

DOLICHOLS OF UNUSUAL CHAIN LENGHTS ARE USED FOR PROTEIN GLYCOSYLATION IN YEAST

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Dolichylphosphate is an essential intermediate required in protein N- and O-linked glycosylation and in GPI-anchor biosynthesis in the endoplasmic reticulum (ER) of eukaryotic cells. Dolichol and dolichylphosphate are ubiquitous membrane components with a chain length that is species specific. In yeast, dolichols with 14 to 17 isoprene units are predominant, whereas in mammalian cells they are 19 to 22 isoprene units long (1). Polyprenols are synthesized by the sequential addition of isopentenylpyrophosphate moieties to farnesylpyrophosphate. This condensation step is catalyzed by the *cis*-isoprenyltransferase (*cis*-IPTase). Genes encoding *cis*-IPTase activity have been identified in *M. luteus*, *E. coli* and *S. cerevisiae* (2, 3, 4). Yeast cells deleted for the *RER2* locus display a severe growth defect but are still viable, probably due to activity of a homologous locus, *SRT1*. We determined the dolichol and dolichylphosphate content of fast growing revertants of *RER2* deleted cells and of cells overexpressing *SRT1* using HPLC analysis. Dolichols and dolichylphosphates with the unusual chain lenghts of 19 to 22 isoprene residues were found. We showed that these long chain dolichols were used for the biosynthesis of N-glycoprotein. We conclude that yeast contains two *cis*-IPTase activities and that dolichol chain length is determined by the *cis*-IPTase.

References:
1. Rip, J.W., Rupar, C.A., Ravi, K., Carroll, K.K., (1985), *Prog. Lipid Res.*, 24, 269-309
2. Shimizu, N., Koyama, T., Ogura, K., (1999), *J.Biol.Chem.*, 273, 19476-19481
3. Kato, J., Fujisaki, S., Nakajima, K., Nishimura, Y., Sato, M., Nakano, A., (1999), *J.Bacteriol.*, 181, 2733-2738
4. Sato, M., Sato, K., Nishikawa, S., Hirata, A., Kato, J., Nakano, A., (1999), *Mol. Cell. Biol.*, 19, 471-483

S7B.13

Protein Mannosylation in the Yeast Golgi

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In the yeast *Saccharomyces cerevisiae*, N-linked glycans are processed in the Golgi in only one of two ways. The mannosyltransferase Och1p initiates both pathways by transfer of a single α-1,6 linked mannose residue to the core structure. Glycoproteins destined for internal compartments are modified by the addition of a single α-1,2 linked mannose to the residue added by Och1p, followed by a terminating α-1,3 linked mannose. In glycoproteins destined for external compartments core structures are extended to a mannan "outer chain" that consists of a linear backbone of up to 50 α-1,6 linked mannoses with branches containing 2 α-1,2 linked mannoses and a terminating α-1,3 linked mannose. Enzymatic activities catalysing the synthesis of the backbone reside in two distinct protein complexes that both have mannosyltransferase activity *in vitro*. M-Pol 1 (mannan-polymerase 1) contains 2 proteins, Mnn9p and Van1p, acts at an early stage of mannan synthesis and is likely to be involved in the recognition of substrate proteins. M-Pol 2 extends the M-Pol 1 product and contains 4 different proteins in addition to Mnn9p. All the subunits of M-Pol 1 and M-Pol 2 share a type II topology and a catalytic site motif DXD, features of many other unrelated glycosyltransferases. Antisera against Mnn9p and Van1p were used to analyse the stochiometry of the M-Pol1 subunits. M-Pol 1 is a heterodimeric complex that contains only one copy of each Mnn9p and Van1p. M-Pol 2 contains only one copy of Mnn9p. Data on the catalytic activity of M-Pol 1 after mutagenesis of the DXD motif in Van1p and Mnn9p will be presented.

S7B.14

ISOLATION AND CHARACTERIZATION OF DIFFERENT PLANT *N*-ACETYL-GLUCOSAMINYLTRANSFERASE I (*GNTI*) CDNA SEQUENCES, AND GENERATION OF POTATO AND TOBACCO ANTISENSE PLANTS

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We report on the isolation and characterization of full-length cDNA sequences coding for *N*-acetylglucosaminyltransferase I (GnTI) from potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and Arabidopsis (*Arabidopsis thaliana* L. Heynh). The deduced protein sequences show highest homology among the solanaceous species (93% identity between potato and tobacco compared to about 75% with Arabidopsis) but only weak homology with human GnTI (35% identity). In contrast to the corresponding enzymes from animals, all plant GnTI sequences identified contain one putative *N*-glycosylation site which is conserved in potato and tobacco but differs in Arabidopsis. Southern analyses revealed that *GntI* behaves as a single-copy gene. Northern analyses showed that *GntI*-mRNA expression is largely constitutive and elevated in Arabidopsis *cgl* mutants that are deficient in GnTI activity. Upon complementation analysis of *GntI*-expression constructs in the Arabidopsis *cgl* mutant, transgenic potato and tobacco plants with substantial reduction in complex-glycan patterns were obtained. This indicates that expression of heterologous glycoproteins without antigenic properties can be achieved in plant species besides Arabidopsis using antisense technology.

S7B.15

MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF β1,2-XYLOSYL-TRANSFERASE FROM *ARABIDOPSIS THALIANA*

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The transfer of xylose from UDP-xylose to the core β-linked mannose of N-linked oligosaccharides by β1,2-xylosyltransferase (XylT) is a widespread feature of plant glycoproteins which renders them immunogenic and allergenic in man. Here we report the isolation of the *A. thaliana* XylT gene, which contains two introns and encodes a 60.2 kDa protein with a predicted type II transmembrane protein topology typical for Golgi glycosyltransferases. Upon expression of *A. thaliana* XylT cDNA in the baculovirus/insect cell system, a recombinant protein was produced that exhibited XylT activity *in vitro*. Furthermore, the recombinant enzyme displayed XylT activity *in vivo* in the insect cells, as judged by the acquired cross-reaction of cellular glycoproteins with antibodies against the β1,2-xylose epitope. The cloned XylT cDNA as well as the recombinant enzyme are essential tools to study the role of β1,2-xylose in the immunogenicity and allergenicity of plant glycoproteins at the molecular level.

S7B.16

MOLECULAR CLONING OF *N*-ACETYLGLUCOSAMINYLTRANSFERASE II FROM *ARABIDOPSIS THALIANA*

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N-acetylglucosaminyltransferase II (GnTII, EC 2.4.1.143) is a Golgi enzyme involved in the biosynthesis of glycoprotein-bound N-linked oligosaccharides, catalysing an essential step in the conversion of oligomannose-type to complex N-glycans. GnTII activity has been detected in both animals and plants. However, while cDNAs encoding the enzyme have already been cloned from several mammalian sources no GnTII homologue has been cloned from plants so far. Here we report the molecular cloning of an *Arabidopsis thaliana* GnTII cDNA with striking homology to its animal counterparts. The predict domain structure of *A. thaliana* GnTII indicates a type II transmembrane protein topology as it has been established for the mammalian variants of the enzyme. Upon expression of *A. thaliana* GnTII cDNA in the baculovirus/insect cell system, a recombinant protein was produced that exhibited authentic GnTII activity.

S7B.17(L.2)

Plant cell wall biosynthesis: Identification and Characterization of Fucosyltransferase and putative Galactosyltransferases involved in xyloglucan biosynthesis.

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The primary wall of plant cells plays important regulatory roles during cell elongation and intercellular communication. The chemical structure of this organelle is well documented (1), however, the the biosynthesis and deposition of cell wall polysaccharides is still poorly understood. Our effort is concentrated on two glycosyltransferases (Gal- and FucTases) that are involved in decorating the backbone of xyloglucan, a major hemicellulosic polysaccharide in many plant cells.

A xyloglucan-specific fucosyltransferase was purified from a micrososmal fraction derived from etiolated pea seedlings (2). This purified enzyme (PsFT1) was biochemically characterized (3) and peptide sequence information was used to isolate cDNA clone from both pea and Arabidopsis (3). In order to complement our previous study on XG-fucosyltransferase (AtFT1) from *Arabidopsis* (2), the enzyme was expressed, in tobacco cells, that lack this enzyme activity. A tagged version of this enzyme was purified on Ni-column, its product characterized and its biochemical characteristics compared to the pea enzyme.

We have begun a genomic approach toward identification of galactosyltransferases that may be involved in xyloglucan biosynthesis. Sequence comparisons yielded a collection of Arabidopsis genes with sequence similarity to mammalian galactosyltransfrerase genes. The Arabidopsis genes could be clustered into three groups based on the presence of specific motifs in the deduced amino acids sequences. Analysis of Arabidopsis EST databases provided evidence that several of these putative galactosyltransferase genes are expressed.

References:

(1) Carpita and Gibeaut (1993) Plant J., 3, 1-30
(2) Perrin et al., (1999) Science , 284, 1976-1979
(3) Faik et al., (2000) J. Biol. Chem., in press

S8.1

ENZYMATIC MODIFICATION OF RNASE B-ATTACHED N-GLYCANS FOR CONFORMATIONAL STUDIES WITH NMR.

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Carbohydrate moieties of glycoproteins play a major functional role in many vital or pathogenic processes. Many glycostructures have been isolated and investigated so far, but little is known about their three-dimensional structure covalently attached to proteins. NMR is a powerful tool for conformational analysis but for precise conformational analysis studies uniform glycosylation would be required. Bovine RNAse B was purified by affinity chromatography with immobilized Concanavalin A and subsequently was modified enzymatically with GnT-I and β-1,4-GalT, which led to the isolation of a defined glycostructure after affinity chromatography with RCA lectin. Enzymatic steps were monitored by MALDI-TOF spectroscopy and radioassays. A uniform glycan structure was prepared on the mg scale, providing a starting point for NMR investigations concerning conformation and dynamics of glycanchains covalently attached to proteins.

S8.2

Characterization of human mega YAC clone-transformants of a mouse FM3A mutant G258 cell line that is defective in lipid-linked oligosaccharide synthesis.

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The G258 mutant, isolated from the mouse FM3A cell line, is temperature-sensitive (*ts*) for both cell growth and asparagine (Asn)-linked glycosylation due to mutation at a single locus. The biochemical defect in the G258 mutant resides in the formation of lipid-linked oligosaccharide (LLO). The G258 mutant synthesizes the full-sized LLO ($Glc_3Man_9GlcNAc_2$-P-P-Dol) at 33°C but at 39°C, the mutant cells cannot elongate the LLO beyond $Man_3GlcNAc_2$-P-P-Dol. We transfected human genomic DNA fragments into the G258 mutant by the radiation hybrid method. The isolated transformants showed recovery from defects in both *ts* cell growth and Asn-linked glycosylation, and contained a common *Alu*-containing human DNA fragment (1.3 kb). We have cloned the 1.3 kb inter-L1 PCR product from one of the transformants and determined its nucleotide sequence. We used this 1.3 kb fragment as a marker DNA for isolating the human gene that complements the defect of LLO synthesis in the G258 mutant. At first, we designed PCR primers based on the unique sequence in the 1.3 kb fragment and screened a human mega YAC library. We could select YAC clone 923-f-5. The YAC clone 923-f-5 could complement the defect of the G258 mutant by the spheropast fusion method. These results suggest that the human gene that complements the defect in the G258 mutant is localized on human chromosome 13, presumably in the region (1.06 Mb) contained in the YAC clone 923-f-5. This approach may allow us to clone the human gene that complements the defect of LLO synthesis in the G258 mutant. (Supported in part by the Grant-in-Aids from the Ministry of Education, Science, Sports and Culture of Japan, and the Kihara Memorial Foundation)

S8.3

Cloning and characterization of the human mannosyltransferase genes involved in the early assembly of lipid-linked oligosaccharide

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None of the human mannosyltransferase genes involved in the early steps of lipid-linked oligosaccharide synthesis have been analyzed. We have recently cloned the human mannosyltransferase I cDNA as a human homolog of the yeast *ALG1* gene (accession no. AB019038). This gene was designated *Hmat-1*. The *Hmat-1* cDNA encodes a protein of 464 amino acids, similar to the yeast Alg1p with 36% identity. In addition, the *Hmat-1* cDNA complemented the defect of the temperature sensitivity for cell growth in yeast *alg1* mutant. In order to elucidate the human genomic organization of the *Hmat-1* gene, we searched Genbank database and detected a 161 kb genomic clone, 165E7 (accession no. AC007011). From sequence analysis of 165E7, it was revealed that the *Hmat-1* gene consists of at least thirteen exons and contains one TATA and three CAAT boxes. As 165E7 contains exons 1~12 but does not contains further exons, we are screening a human cosmid library to obtain the complete genomic clone of the *Hmat-1* gene.

More recently, we have cloned a human homolog of the yeast *ALG2* gene. This gene is tentatively designated *Hmat-3*. The *Hmat-3* cDNA encodes a predicted protein of 416 amino acids, similar to the yeast Alg2p with 33% identity. Using the *Hmat-3* gene, we searched Genbank database and detected a 197 kb genomic clone, RP11-13B9 (accession no. AL137067). It is derived from human chromosome 9q22.32-31.3, corresponding with our FISH analysis of the *Hmat-3* gene. From sequence analysis of RP11-13B9, it was revealed that the *Hmat-3* gene consists of two exons and contains one TATA and one CAAT boxes. At present, we are testing whether the *Hmat-3* cDNA can complement the yeast *alg2* mutation.

S9.1

β4GAL-T1 EXPRESSION FOLLOWING PROLACTIN STIMULATION OF B-CELLS.

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The effector functions of IgG antibodies are sensitive to changes in galactosylation, which is regulated by β1,4-galactosyltransferase-1 (β4Gal-T1). Prolactin stimulation of the mammary gland is accompanied by increased β4Gal-T1 expression. Both T and B lymphocytes also possess receptors for prolactin. When human B-cells were activated using a combination of anti-IgM, interleukin-2 (IL-2) and prolactin there was a 4.6-fold increase in the level of secreted IgG compared with unstimulated B-cells. The contribution of prolactin to the increased IgG secretion was established by comparing the combined effect of all three activators (anti-IgM, IL-2 and prolactin) with that obtained using only anti-IgM and IL-2. There was then a 2.7-fold increase in IgG secretion above that seen with anti-IgM and IL-2. The levels of terminal galactose on the IgG oligosaccharides remained unchanged following enhanced production of IgG in these experiments. Only a moderate upregulation of β4Gal-T1 mRNA occurred in response to B-cell activation by prolactin. These observations suggest that the correct galactosylation of IgG is maintained during enhanced IgG secretion by activated B-cells without the need for substantial increases in β4Gal-T1 mRNA.

S9.2

SERUM IgG GLYCOSYLATION PATTERN IN AUTOIMMUNE DISEASES.

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IgG consists of two heavy and two light polypeptide chains linked together by disulfide bridges. According to its biological functions the IgG molecule can be divided into the antigen binding fragment (Fab) and the region responsible for the effector functions (Fc). In the Fc region, specifically the two C_2 domains contain conserved glycosylation sites at Asn-297, to which complex biantennary oligosaccharides are attached. Normal serum IgG shows a heterogeneous glycosylation profile that includes neutral and sialylated structures. The neutral ones can be grouped mainly into 3 sets depending on whether they contain 1, 2 or 3 galactose residues (G0, G1 and G2 respectively) in their outer arms. Healthy people show G1>G0 as the characteristic glycosylation pattern. Decreased levels of galactosylation (G0>G1) of serum IgG has bee noted in a restricted range of diseases, in which rheumatic diseases is included. Rheumatoid arthritis (RA) is an autoimmune inflammatory disease that is characterized by the presence of a rheumatoid factor (autoantibodies against the Fc region of IgG) while psoriatic arthritis (PsA) is no associated to autoantibodies. Association between glycosylation changes and disease evolution has been proposed. High levels of hypogalactosylated structures are refereed in patients with RA. This result could explain why immune complexes are abundant in RA. The purpose of the present study was to look at the oligosaccharide profiles of IgG present in RA and PsA patients compared with healthy control. The enzymatic-released oligosaccharides from IgG were labeled with the fluorophore 8-amine 1,3,6 naphthalenetrisulfonic acid, and the oligosaccharide pools were compared using amine bond phase column (NH2-HPLC) under ion-suppression conditions. Although most of the samples corresponding to sick patients showed glycosylation profiles where the G0 structure predominates, we observed normal patterns in clinically arthritic patients. This result suggests the necessity to be extremely careful when using glycosylation pattern as a possible diagnostic marker in RA disease. However PsA seems to better correlates with increased G0 population in serum IgG.

S9.3

GALECTIN-3 IS ONE OF THE ADHESION MOLECULES INVOLVED IN THE EXTRAVASATION OF NEUTROPHILS IN STREPTOCOCCAL PNEUMONIA.

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Recruitment of leukocytes, especially neutrophils, towards inflammatory stimuli and invasive pathogens is one of the most important responses in innate immunity. Neutrophils can remove various pathogens effectively by phagocytosis or by secreting various factors such as defensins and active oxygen metabolites that can kill the pathogens. In general, extravasation of neutrophils across the endothelial barrier is mediated by at least two kinds of adhesion molecules, β_2-integrins and selectins. However, recent studies clearly reveal that this β_2-integrin- and selectin- dependent paradigm does not apply to the diapedesis of neutrophils in streptococcal pneumonia, one of the most lethal infectious respiratory diseases in North America. As tight adhesion of neutrophils to the endothelium (flattening) prior to transendothelial migration is essential, allowing them to withstand the shear stress imparted by blood flow, other adhesion molecules must be involved in the case of streptococcal pneumonia. We have recently obtained results demonstrating that translocation of soluble galectin-3, a macrophage β-galactoside-binding lectin in the affected lung, correlates well with the onset of β_2-integrin-independent extravasation of neutrophils in a pneumonia model *in vivo*. Furthermore, galectin-3 was able to mediate directly the adhesion of neutrophils to endothelial cells *in vitro*, suggesting that galectin-3 may in fact be one of the unidentified adhesion molecules contributing to β_2-integrin-independent extravasation of neutrophils. Galectin-3 has been known to possess immunomodulatory abilities, including the ability to modulate respiratory burst, signal transduction pathways, and IL-1 secretion. The data presented here reveal that galectin-3 also acts as an adhesion factor in leukocyte recruitment.

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S9.4

A NOVEL GLYCOSYLTRANSFERASE AND SULFOTRANSFERASE DIRECT THE EXPRESSION OF A FUNCTIONAL PERIPHERAL NODE ADDRESSIN, THE MECA-79 EPITOPE. Jiunn-Chern Yeh[1], Nobuyoshi Hiraoka[1], Bronislawa Petryniak[2], Jun Nakayama[3], John B. Lowe[2], Jamey D. Marth[4], and Minoru Fukuda[1] . [1]Glycobiology Program, The Burnham Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037, [2]HHMI, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109, [3]Shinshu University Graduate School of Medicine and Central Clinical Laboratories, Matsumoto 390-8621, Japan, [4]HHMI, Department of Medicine, University of California, San Diego, La Jolla, California 92093

Core 2 oligosaccharides have been implicated to be crucial for the synthesis of high affinity carbohydrate ligands for selectins. However, L-selectin-mediated lymphocyte homing and the expression of peripheral node addressins (MECA-79 reactivity) persist after core 2 GnT-leukocyte type is inactivated (1). In the present study, structural analysis of L-selectin carbohydrate ligands in high endothelial venules (HEV) demonstrates that the core 2-based *O*-glycans constitute a minor component in core 2 GnT-leukocyte type knockout mice. This is rather striking since another core 2 GnT, core 2 GnT-mucin type (2) is also present in HEV. Detailed analysis revealed that the majority of *O*-glycans is based on an extended core 1 structure, which has been tentatively determined to be NeuNAcα2→3Galβ1→4[Sulfo6→(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα-R. Using β1,3GnT cloned previously as a probe, we have identified a novel β1,3GnT that forms GlcNAcβ1→3Galβ1→3GalNAcα-R. Together with human L-selectin ligand sulfotransferase (hLSST), the novel β1,3GnT directs the expression of MECA-79 epitope on CHO cells, and 6-sulfo sialyl Lewis X presented on such oligosaccharide structures supports L-selectin-dependent lymphocyte rolling. (Supported by CA71932, CA48737 and CA33000).

1. Ellies L.G., Tsuboi, S., Petryniak, B., Lowe, J. B., Fukuda, M., and Marth, J. D. (1998) *Immunity* 9:881-890
2. Yeh, J.-C., Ong, E. and Fukuda, M. (1999) *J. Biol. Chem.* 274:3215-3221

S10.1

DISSECTION OF THE TWO TRANSFERASE ACTIVITIES OF THE PASTEURELLA HYALURONAN SYNTHASE: TWO ACTIVE SITES EXIST IN ONE POLYPEPTIDE
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Type A *Pasteurella multocida,* the major fowl cholera pathogen, employs a hyaluronan [HA] capsule to avoid host defenses. HA is a linear polysaccharide composed of alternating glucuronic acid (GlcUA) and N-acetyl-glucosamine (GlcNAc) disaccharide repeats. HA is a normal component of the host body, therefore it is not immunogenic. PmHAS (*P. multocida* hyaluronan synthase) is a 972-residue membrane-associated enzyme. PmHAS catalyzes the transfer of both GlcNAc and GlcUA to form the HA polymer; this dual glycosyltransferase activity is rather rare in the field of glycobiology. To define the catalytic and membrane-associated domains, pmHAS mutants were analyzed. Deletion of about 30% of the carboxyl portion of pmHAS resulted in a soluble, active HA synthase suggesting that the carboxyl-terminus is involved in membrane association of the native enzyme but not in catalysis. Within the truncated sequence, there is a duplicated domain containing several motifs that are conserved among many β-glycosyltransferases. Mutagenesis studies were performed on one of the conserved residues in either domain, A1 or A2. All the mutants lost HA polymerizing activity. However, A1 mutants possessed GlcUA-transferase activity, while A2 mutants possessed GlcNAc-transferase activity. The apparent affinities of the mutant enzymes for the precursors were similar to wild-type values. Furthermore, mixing A1 and A2 mutant proteins in the same reaction allowed HA polymerization at levels similar to the wild-type enzyme. These results provide the first direct evidence that pmHAS polypeptide utilizes two separate glycosyltransferase sites.

S10.2

ENZYMOLOGICAL CHARACTERIZATION OF A HYALURONAN SYNTHASE.

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Hyaluronan (HA) is a linear polysaccharide composed of the repeating disaccharide of glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc). HA has essential structural and signaling roles in vertebrates. The enzymes that catalyze the production of HA, HA synthases (HASs), were the first glycosyltransferases found to be capable of catalyzing the addition of two different monosaccharides to a polysaccharide chain. All known HASs utilize UDP-linked sugar precursors. For the eukaryotic HASs, it appears that catalysis takes place inside the cell and then the HA chain is extruded out of the cell. Little else is known about the catalytic mechanism of these enzymes. DG42 (or *xl*HAS1), a HAS from *Xenopus* frog, was expressed in yeast and functional enzyme was isolated from membranes. Studies were first done to determine the kinetics of the HAS activity of DG42. Radiation inactivation was used to determine the number of DG42 monomers contained in the HAS functional unit. Ongoing mutagenesis studies are aimed at determining the amino acid residues important for binding substrate and catalyzing polymerization. A serine residue has been identified that, when changed to other amino acid residues, alters the HA product size. It has yet to be determined whether this is due to a difference in the kinetics of the HAS reaction, a change in the intrinsic regulation of product size, or some combination of the two. Chemical modification is being performed to determine if there are cysteine residues present in or near the substrate-binding sites. There appears to be at least one cysteine that is protected from modification if DG42 is preincubated with substrate before addition of the modifying reagent. Other studies include labeling DG42 with photoactivatible UDP-sugar analogs to aid in determining the substrate-binding sites.

S10.3

ASSOCIATION OF EXT1 AND EXT2 IN HEPARAN SULFATE BIOSYNTHESIS.

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Mutated EXT1 and EXT2 cause the human skeletal disorder hereditary multiple exostoses. They are glycosyltransferases involved in heparan sulfate (HS) chain elongation. However, their overexpression generates an unexpected decrease in overall catalytic activity, indicative of interaction with endogenous HS biosynthetic enzymes. Here, we show that EXT1 and EXT2 associate and by expressing them in Pichia pastoris, which do not synthezise HS, we clearly showed that EXT1 and EXT2 have similar catalytic activities. Remarkably, coexpression of EXT1 and EXT2 lead to a substantial increase in their catalytic activity and also increased N-linked glycosylation indicative of Golgi processing. These observations suggest that the functional unit involved in HS chain elongation is a complex of EXT1 and EXT2 that is localized in the Golgi compartment.

S10.4

MOLECULAR CLONING AND EXPRESSION OF CHONDROITIN 4-SULFOTRANSFERASE

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Chondroitin 4-sulfotransferase (C4ST) catalyzes the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate to position 4 of *N*-acetylgalactosamine residue of chondroitin. The enzyme has been previously purified to apparent homogeneity from the serum free culture medium of rat chondrosarcoma cells. The purified enzyme also catalyzed the sulfation of partially desulfated dermatan sulfate. We have now cloned the cDNA of the mouse C4ST on the basis of the amino acid sequences of peptides obtained from the purified enzyme by protease digestion. This cDNA contains a single open reading frame that predicts a protein composed of 352 amino acid residues. The protein predicts a Type II transmembrane topology. The predicted sequence of the protein contains all of the known amino acid sequence and 4 potential sites for *N*-glycosylation, which corresponds to the observation that the purified C4ST is an *N*-linked glycoprotein. The amino acid sequence of mouse C4ST showed significant sequence homology to HNK-1 sulfotransferase. Comparison of the sequence of mouse C4ST with human HNK-1 sulfotransferase revealed ~29% identity and ~48% similarity at the amino acid level. When the cDNA was introduced in a eukaryotic expression vector and transfected in COS-7 cells, the sulfotransferase activity that catalyzes the transfer of sulfate to position 4 of GalNAc residue of both chondroitin and desulfated dermatan sulfate was overexpressed. Northern blot analysis showed that, among various mouse adult tissues, 5.7 kb message of C4ST was mainly expressed in the brain and kidney.

S11.1

EXPRESSION OF β1,4-GALACTOSYLTRANSFERASE IN THE MOUSE DEVELOPING INTESTINE Dingwei Dai, N. Nanda Nanthkumar, Davis S. Newburg, W. Allan Walker. Combined Program in Pediatric Gastroenterology and Nutrition, Harvard Medical School, Boston, MA. USA

Galactose is the most common carbohydrate present in microvillus membrane glycoconjugates as well as secreted mucin in the small intestine. UDP-Gal:*N*-acetylglucosamine β1,4-galactosyltransferase (GalT) (EC 2.4.1.38) catalyzes the transfer of galactose from GDP-galactose to terminal *N*-acetylglucosamine residues on elongating oligosaccharide chains, which are suggested to be involved in various cellular functions including cell-cell and cell-matrix interactions. In addition to a biosynthetic role for GalT, it has also been found located not only in the Golgi compartment, but also on the cell surface, which is thought to function as a recognition molecule through binding to specific ligands on adjacent cell surface and extracellular matrix, directly related to cell adhesion and migration. Recently it was reported that GalT plays critical roles in the regulation of proliferation and differentiation of epithelial cells after birth. Previously, we showed that GalT was under developmental regulation in rat small intestine. To better understand the regulation of intestinal galactosylation, we have further studied the expression of GalT in different regions of the mouse intestine during postnatal development and the role of cortisone and indigenous microflora on the ontogeny of GalT. The enzyme activity of GalT was assayed in the particulate fraction (105,000 x g pellet) of mucosa, scraped from different regions of mouse intestine during postnatal development. The results showed that low GalT activity was detected throughout the whole immature intestine from the duodenum to the colon, but these activities increased after 3 weeks of postnatal life, rapidly reaching the adult levels. Furthermore, there was a large regional difference in the level of GalT activity, with the lowest activity in the duodenum and the highest activity in the colon. The injection of cortisone precociously induced an increased GalT activity in the whole intestine in 2-week-old suckling mice. This age related changes in GalT activity were less profound in germfree mice than in conventional mice. GalT activity was lower in germfree mice than in conventional mice throughout the whole intestine from the duodenum to the colon at the age of 2 weeks and 4 weeks. These results suggest that intestinal GalT activity is under region-specific, developmental regulation and can be modified by cortisone and that indigenous microflora is one of the environment factors that may contribute to the ontogeny of mouse intestinal GalT during postnatal development. Supported by NIH grants: HD 12437, HD31825 and PO1-DK33506.

S11.2

N-Linked oligosaccharides attached to bovine histone chromosomal proteins are β-*N*-acetylgalactosaminylated

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It is not obvious whether or not mammalian histone chromosomal proteins are N-glycosylated. When we analyzed the partial amino acid sequence of the protein with a molecular weight of 30 K which was derived from lysates of human leukemic cell line and reactive to *Wistaria floribunda* agglutinin (WFA), the sequence showed that it is identical to a part of human histone H1, suggesting that mammalian histone chromosomal proteins are glycosylated. To confirm this observation, commercially available bovine histone H1, H2A, H2B, H3 and H4 proteins were examined by lectin blot analysis using WFA which preferentially interacts with oligosaccharides terminated with β-*N*-acetylgalactosamine in combination with glycosidase treatment. WFA reacted strongly with histone H1, H2A, H2B and H3 proteins but very weakly with histone H4 protein. No lectin-positive bands were obtained upon pretreatment of blots with jack bean β-*N*-acetylhexosaminidase or N-glycanase. The proteins reacted also with Con A and/or leukoagglutinating-phytohem agglutinin (L-PHA), suggesting that they contain bi- to tetra-antennary complex-type oligosaccharides. These results indicate that bovine histone chromosomal proteins contain β-*N*-acetylgalactosaminylated N-linked oligosaccharides.

S11.3

C. ELEGANS CORE 2 N-ACETYLGLUCOSAMINYLTRANSFERASE HOMOLOGUE EXPRESSION PATTERNS: A GFP FUSION SURVEY.

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C. elegans has six Core 2 N-acetylglucosaminyltransferase homologues, *gly*-1, -15, -16, -17, -18 and, *gly*-19. cDNA fragments have been isolated for all of these genes indicating they are transcribed. A survey of the spatial and temporal expression patterns for these genes, using GFP fusion constructs is underway. *gly*-1p::GFP expression was weak and restricted to the seam cells and unidentified cells (neuronal?) in the head. Expression of *gly*-15p::GFP was only seen in the G2 gland cells and showed a sub-cellular localization consistent with the inclusion of a 5'exon of *gly*-15 into the translation product. *gly*-18p::GFP showed a complex expression pattern with expression in the ALA and GLR neurons, VM1 and intestinal muscle cells, seam cells, distal tip cells, coelomocytes and other cells. *gly*-19p::GFP gave a strong signal, restricted to the intestine and anal sphincter. Work is in progress to establish the expression patterns for *gly*-16p::GFP and *gly*–17::GFP. The non-overlapping patterns seen using the fusion constructs for *gly*-18 and 19, suggests that these tandem genes on linkage group I are not regulated as one transcriptional unit, but rather that the intervening 730 bp interval contains a fully functional promoter. The catalytic properties of these gene products are predicted to be Core 2 or I N-acetylglucosaminyltransferases based on sequence homology. Each gene examined has a unique non-overlapping expression pattern. It is therefore possible that the catalytic activities of all six gene products are identical but occur in a distinct set of cells/tissues and thereby influence different aspects of the nematode's phenotype. Null mutations are being isolated for each gene to examine the role of these genes in development and to explore their interactions with substrate and related pathways.

S11.4

FRINGE IS A GLYCOSYLTRANSFERASE THAT MODIFIES THE *O*-LINKED CARBOHYDRATES ON THE EPIDERMAL GROWTH FACTOR-LIKE REPEATS OF NOTCH.
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The Notch proteins are signaling receptors that play key roles in a wide variety of developmental cascades. They become activated upon binding to ligands (*e.g.* Delta, Serrate/Jagged) on adjacent cells. The Fringe proteins are known to modulate the interactions between Notch and its ligands, potentiating the signaling from some ligands (*e.g.* Delta) while inhibiting signaling from others (*e.g.* Serrate). Although the biological activity of Fringe is well established, the mechanism by which it alters Notch function is unknown. Recently, Fringe was shown to share regions of homology with glycosyltransferases, and Notch was shown to bear two unusual forms of glycosylation, *O*-fucose and *O*-glucose, on its tandem epidermal growth factor-like repeats. We have examined the intersection of these two observations and show that three Fringe family members (Lunatic, Manic and *Drosophila* Fringe) display novel glycosyltransferase activity that results in the elongation of these unusual *O*-linked saccharides on Notch. Mutation of a conserved acidic region in *Drosophila* Fringe inactivates its enzymatic activity *in vitro* and its biological activity *in vivo*. These and other results strongly argue that Fringe modulates Notch function through alterations in the *O*-linked saccharide modifications on the Notch protein.

S11.5

ANALYSIS OF Fut9-KNOCK-OUT MOUSE

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The α1,3-fucosyltranferase family comprises six human enzymes. They are named FUT3, 4, 5, 6, 7 and 9. All members catalyze a α1-3 linkage by transferring a Fuc towards the GlcNAc residue of type 2 chain. A phylogenetic tree of the six α1,3-FUTs showed that there are four clusters in the vertebrate *FUT* gene family, the *FUT9* gene subfamily being the first to diverge from the ancestral gene.

Mouse has three functional *α1,3Fut* genes. Fut9-deficient knockout mice have been established and analyzed for Le^x expression. Wild mice expressed abundant Le^x epitopes on proteins in stomach, kidney and leukocytes. These Le^x epitopes on proteins in the three tissues almost disappeared in the KO mice. Brain tissue in wild mice expressed abundant Le^x-carrying glycolipids. KO mice lack Le^x-positive glycolipids in brain. Thus, FUT9 can synthesize the Le^x-epitope both on proteins and glycolipids. FUT9 functions a major role for the Le^x expression *in vivo*, while FUT4 may participate in the Le^x expression when it is enormously expressed in tissue and in the cultured cells. It is now under pathological and physiological investigation of Fut9-KO mouse.

References;
Kudo, T. et al. J. Biol. Chem. 273, 26729-26738, 1998.
Kaneko, M. et al. FEBS Letters, 452, 237-242, 1999.
Nishihara, S. et al. FEBS Letters, 462, 289-294, 1999.

S11.6

FUT9 SYNTHESIZES THE LEWIS X STRUCTURE IN BRIAN

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The expression of Lewis x (Le^x) structure in brain is developmentally regulated, and considered to play a role in cell-cell interaction during neuronal development. In the previous studies we have cloned Fut9 as a candidate for synthesizing the Le^x structure in brain. Mouse has three functional *α1,3Fut* genes, *Fut4*, *Fut7*, and *Fut9*. Fut7 is known to have no activity for the Le^x synthesis. The relative activities of Fut4 and Fut9 for the Le^x synthesis were determined using recombinant enzymes. Fut9 exhibited very strong activity for Le^x synthesis, i.e. more than 10 fold stronger activity than Fut4. We also examined the transcript levels of *Fut4* and *Fut9* in brain and determined which Fut is responsible for the Le^x expression in brain. Both cerebrum and cerebellum in each developmental stage (E17, P0, P7, P30, P100) expressed 10 - 200 times larger amount of *Fut9* transcripts than *Fut4* transcripts. Neuronal cell and glial cell in primary cultures also expressed 10-15 times larger amount of *Fut9* transcripts than *Fut4* transcripts. Moreover, each brain tissue and cell showed unique substrate specificity of Fut 9 for the polylactosamine chain. Tissue staining by anti-Fut9 mAb explained the distribution of Le^x structure. From the above results it was concluded that Fut 9 is the responsible enzyme for Le^x synthesis both on glycoproteins and on glycolipids in brain.
References; Kudo, T. et al. J. Biol. Chem. 273, 26729-26738, 1998. Nishihara, S. et al. FEBS Letters, 462, 289-294, 1999.

S12.1

PROTECTION OF PIG KIDNEY CELLS FROM HUMAN SERUM CYTOTOXICITY BY SYNTHETIC ALPHA-GAL EPITOPE POLYMERS

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Neoglycopolymers of polyacrylamide backbone conjugated with varying densities of Galα1,3Galβ1,4Glc trisaccharide epitopes (α-Gal epitopes) were designed and synthesized. An inhibition ELISA and a flow cytometry assay were established to determine the inhibitory effect of the synthesized polymers against the binding of human natural anti-Gal antibodies to either α-Gal containing glycoproteins or α-Gal antigens on the surface of mammalian cells. In comparison to the α-Gal monomer (Galα1,3Galβ1,4GlcNHAc), the α-Gal polymers dramatically enhanced the inhibition of human anti-Gal antibodies (IgG, IgM, and IgA) binding to mouse laminin or mammalian cells. Increases of 7.8×10^3- and 5.0×10^4 -fold in inhibitory potential of polymer 7C to IgA and IgM (with IC_{50}s of 7.0 and 5.6 nM respectively) were observed over the monomer in inhibition ELISA. Also, the binding enhancement of α-Gal polymers is greater for anti-Gal IgA and IgM than for IgG. Such amplified binding differences among the three anti-Gal isotypes can be utilized to selectively inhibit or remove a particular isotype of anti-Gal antibodies. Flow cytometry assay confirmed that certain α-Gal polymers are effective in inhibition of anti-Gal antibody (in human serum) binding to pig kidney (PK15) cells. Furthermore, α-Gal polymers are demonstrated as better candidates than α-Gal monomer in protecting pig kidney (PK15) cells from the human serum cytotoxicity. Thus, such synthetic carbohydrate polymers may find practical applications in cell xenotransplantations.

S12.2

TEMPERATURE REGULATED DUAL EXPRESSION OF PROKARYOTIC GLUCOSE 1-PHOSPHATE URIDYLYLTRANSFERASE AND BOVINE α1,3-GALACTOSYLTRANSFERASE FROM ESCHERICHIA COLI

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Glucose 1-phosphate uridylyltransferase (GalU) and α1,3-galactosyltransferase (α1,3GT) are the key enzymes involving in biosynthesis of Galα1,3Galβ1,4Glc (α-Gal epitope) from glucose and lactose. We subcloned the previously identified *E. coli galU* gene and bovine *α1,3GT* gene together under the direction of λ PR promoter to construct a recombinant plasmid. Expression in a *E. coli* strain with inactive β-galactosidase avoided hydrolyzing lactose or its analogues which is the acceptor of sugar nucleotides, when the whole cells or rude lysate was utilized for catalysis. The repressor expressed by the build-in λ *cI* gene in the plasmid inhibited the λ PR transcription at 30°C, and the expression reached to higher level at 41°C. However, a comparable amount of recombinant proteins was expressed after incubation at 37°C for overnight, because the promoter would be leaky under the temperature. One copy of promoter was demonstrated to drive the two downstream genes expressing to a level of about 20% each of the total cellular proteins. Since both of the proteins had their own $(His)_6$ tags, Nickel agarose column was used for isolation. Compared with that of GalU, the solubility of α1,3GT was poor, because a large portion of α1,3GT formed inclusion body. The molecular weight of GalU was indicated at 39 kD and α1,3GT at 36 kD by SDS-PAGE. The conformation of GalU and α1,3GT has been determined as tetramer and monomer, respectively, which make it possible to be separated by gel filtration under non-denaturing condition. After purification, about 100 units of GalU and 5 units of α1,3GT were obtained from one liter culture. The whole cells with the dual expression also showed the activities of GalU and α1,3GT.

S12.3

OVEREXPRESSION OF UTP:GLUCOSE 1-PHOSPHATE URIDYLYLTRANSFERASE OF ESCHERICHIA COLI AND ITS APPLICATION IN IN VITRO BIOSYNTHESIS OF GLYCOCONJUCATE

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UTP:glucose 1-phosphate uridylyltransferase (EC 2.7.7.9, GalU), which is also known as UDP-glucose pyrophosphorylase, has been expressed in *E. coli* and characterized. We cloned *galU* gene from the genomic DNA of *E. coli* K-12 strain into a plasmid under T7 hybrid promoter linked to a leader sequence encoding a hexo-histidine containing tag. In host bacteria with T7 polymerase production induced by isopropyl-β-D-thiogalactopyranoside, GalU was over-produced with a large percentage (>90%) of soluble form, and showed as a band at about 39 kD by SDS-PAGE. Nickel-NTA agarose affinity column was used to isolate the histidine tag fused protein, followed by Superdex 200 gel filtration to recognize that the solution conformation of GalU is a tetramer. GalU was further purified using ion exchange chromatography of Mono Q. We generally harvested GalU with yield of around 150 units per liter culture, purity of above 95% and specific activity of 20 units per milligram. The histidine tag would be helpful to keep the high solubility and the specific activity decreased slightly after cleavage of the tag's amino acids with thrombin. To further identify the function, the prepared GalU was added together with other two purified enzymes, UDP-galactose 4-epimerase and α1,3-galactosyltransferase in the *in vitro* synthetic reaction of the trisaccharide, Galα1,3Galβ1,4Glc (α-Gal epitope) started from glucose-1-phosphate and lactose, and the formation of α-Gal was demonstrated by tracking the reaction with [^{14}C]glucose-1-phosphate. This work described a recombinant GalU from *E. coli*, which could be used for the biosynthesis of α-Gal.

S12.4

REFOLDING OF ALPHA (1→3)–GALACTOSYLTRANSFERASE EXPRESSED IN ESCHERICHIA COLI AS INCLUSION BODIES

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Recombinant bovine α(1→3)–Galactosyltransferase (α1-3 GalT, EC 2.4.1.151) has been expressed in Escherichia coli BL21 (DE3) using pET 15b vector which contains an ampicillin-resistant gene and a T7 promoter. Low level of soluble, active enzyme (approximately 8 U/L) was extracted and purified directly from the cell lysate, but a large fraction of recombinant protein was also found in an inactive form as inclusion bodies. To improve the yield of active α1-3 GalT, we studied conditions for correctly refolding the protein from these inclusion bodies. Using two in vitro renaturation methods, dialysis and single step dilution, the enzyme was successfully refolded from the inclusion bodies dissolved in 6M Guanidine Hydrochloride (GuHCl). The effect of process parameters such as protein concentration, redox conditions, temperature, and pH on refolding rate and yield of α1-3 GalT has been systematically investigated. Furthermore, we have evaluated some of the additives that may improve the refolding efficiency of protein. Under the optimal condition, over 50 units of α1-3 GalT can be reproduced from 1 liter of E. coli cell culture.

S12.5

α-Gal Oligosaccharides: Chemistry, Biochemistry and Biomedical Applications.

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Our research laboratory has been focusing on the synthesis and biomedical applications of α-Gal oligosaccharides. α-Gal oligosaccharides are carbohydrate structures bearing a Galα1-3Galβ terminus. This epitope is a glycosidic moiety that is expressed on the surface of cells from most mammalian species excluding humans. The hyperacute rejection in animal-to-human xenotransplantation is mainly due to recognition of human anti-α-Gal antibody to α-Gal epitope on animal cells. Our research programs consist of three parts. (a) The first part includes cloning, expression of α1,3 galactosyltransferase which is responsible for the biosynthesis of the epitope, chemo-enzymatic synthesis of the epitope, conformation analysis of α-Gal oligosaccharides, and polyvalent α-Gal clusters. (b) The second part involves design, synthesis, screening and testing of α-Gal oligosaccharides mimetics through combinatorial approach. (c) The third part of our program deals with the use of α-Gal in immunotherapy. Based on the abundance of anti-α-Gal antibodies IgM and IgG in human blood serum, α-Gal –drug conjugates may enhance the activity of the original drug through anti-Gal mediated immunoresponse *in vivo*. Thus a variety of α-Gal-ligand or α-Gal – drug conjugates have been synthesized and tested. Most of them exhibit enhanced anti-bacterial, or anti-viral, or anti-cancer activities.

S12.6

GLYCOSYLTRANSFERASES INVOLVED IN INNER CORE OLIGOSACCHARIDE MODIFICATION OF *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE.

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The core region of LPS is conceptually divided into the highly conserved inner core and the more variable outer core. The basic inner core structure is further modified in a strain-dependent manner by non-stoichiometric substitutions with residues including rhamnose, galactose, Kdo, phosphate and phosphorylethanolamine. Modification with phosphate residues is required for membrane stability, but the role of the remaining substitutions is unknown. Functions have been assigned to many of the gene products involved in core backbone synthesis, but the role of the *waaS* and *waaZ* gene products have not been determined. Here we show that WaaS and WaaZ are responsible for the transfer of rhamnose and Kdo respectively to KdoII of the inner core. To assign function, non-polar mutants were created in each of the *waaS* and *waaZ* genes in *E. coli* K-12 by gene replacement. Compositional analysis, NMR and mass spectrometry was used to determine the structure of the resultant mutant LPS. Overexpression of WaaZ produces truncated core molecules, suggesting that inner core modifications may dictate the balance of rough and smooth LPS on the cell surface.

S12.7

GLYCOSYLTRANSFERASES REQUIRED FOR ASSEMBLY OF THE R3 CORE OLIGOSACCHARIDE OF *ESCHERICHIA COLI*

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The core oligosaccharide is required for synthesis of a complete lipopolysaccharide molecule and is assembled via membrane-associated glycosyltransferases. We are interested in the molecular basis for the specificity of these enzymes. Bacterial glycosyltransferases are obvious models for structure-function studies, since the diversity of glycoconjugate structures synthesized by bacteria reflects a diversity of glycosyltransferase enzymes with unique specificities. The R3 core type is found in enterohemorrhagic *E. coli* isolates. It is also present in the *E. coli* J5 lineage widely used to study immune responses against the core and in generation of anti-endotoxin monoclonal antibodies. We have characterized the R3 *waa* genetic locus encoding enzymes for core assembly. Two genes, *waaD* and *waaJ* are thought to encode glycosyltransferases involved in completion of the outer core of the R3 core type but their precise specificities were unclear. To address this issue, chromosomal gene knockouts were made in both genes to determine the exact number of sugar residues these enzymes add to the outer core. The enzyme activity of WaaJ was then confirmed *in vitro* using purified WaaJ enzyme and isolated LPS as the acceptor. These studies establish the assembly pathway for the R3 core oligosaccharide.

S12.8

MODULATION OF THE MONO- AND BI- FUNCTIONAL ACTIVITY OF THE *Campylobacter jejuni* Cst-II SIALYLTRANSFERASE: A NOVEL PHASE VARIATION MECHANISM. Michel Gilbert, Marie-France Karwaski, Anna-Maria Cunningham and Warren W. Wakarchuk. Institute for Biological Sciences, NRCC, 100 Sussex Dr., Ottawa, K1A 0R6.

We recently reported the cloning and expression of a bi-functional α-2,3- and α-2,8-sialyltransferase from *Campylobacter jejuni* OH4384. This sialytransferase is involved in the biosynthesis of the lipo-oligosaccharide (LOS) outercore of *C. jejuni* OH4384 and its activity results in the expression of a mimic of the GT1a ganglioside. We cloned and expressed the corresponding *cst-II* gene from four other *C. jejuni* strains, the Penner serotype reference strains O:4, O:10, O:19 and O:41, which are all known to express sialylated LOS outercores. We found that the O:10 and O:41 versions are both bi-functional while the O:4 and O:19 versions are mono-functional and only have the α-2,3-sialyltransferase activity. Residues involved in the α-2,8- activity were readily identified by an alignment of the amino acid sequences of the five versions of Cst-II. Using site-directed mutagenesis we identified the Asn51 as the key residue for the α-2,8- activity. The O:19 version could be converted from a mono-functional sialyltransferase to a bi-functional sialytransferase with the Thr51 to Asn51 mutation. The possibility to modulate the activity of the Cst-II sialyltransferase by changing a single residue suggests that *C. jejuni* could take advantage of this process as a mechanism to modify the structure of the LOS outercore.

S12.9

CHARACTERIZATION OF NOVEL GLYCOSYLTRANSFERASES IMPLIED IN THE BIOSYNTHESIS OF EXOPOLYSACCHARIDES IN LACTIC ACID BACTERIA.

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Extracellular exopolysaccharides (EPS) have a wide array of functions in bacteria and can play role as varied as protection from desiccation or improvement of adherence, pathogenesis or symbiosis. EPS produced in lactic acid bacteria have in particular attracted the attention of the food industry because of their GRAS (generally regarded as safe) status and they can be used as stabilizers or thickeners of yogurt for instance. This has resulted in the elucidation of a large number of varied EPS structures from gram-positive *Streptococcus* and *Lactobacillus* strains. The gene clusters responsible for the EPS production have been identified. They contain *eps* genes which are involved in the EPS repeating unit synthesis, export, polymerization and chain length determination. Some glycosyltransferase (GTF) activities have already been assigned to several *eps* genes from *Streptococcus thermophilus Sfi6* and *Lactobacillus bulgaricus Lfi5* by permeabilized recombinant *E. coli* cells containing these genes and by thin layer chromatography to identify the synthesized products. Since these heterologous expression experiments were not a direct proof of enzyme function, we further investigated ways of overproducing and characterizing them. EpsG from *Sfi6* has now been overexpressed and its enzymatic activity directly determined by capillary electrophoresis. The aim of the work is actually to overexpress and purify the GTF from lactic acid bacteria in order to better characterize them. Further determination of the function of GTF will open the way to carbohydrate engineering.

S12.10

CHARACTERIZATION OF IMINO SUGAR COMPOUNDS FOR INHIBITION OF HUMAN CERAMIDE-SPECIFIC GLUCOSYLTRANSFERASE

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Ceramide glucosyltransferase catalyzes the first glycosylation step of complex glycosphingolipid biosynthesis in mammalian cells. The enzyme activity is inhibited by *N*-alkylated imino sugar compounds, *N*B-DNJ and *N*B-DGJ, both *in vitro* and *in vivo*. Because the inhibition results in the reduction of glycosphingolipids with little toxicity, these compounds have been evaluated in mouse models for lysosomal storage diseases and in clinical trials for Type I Gaucher disease and Fabry disease. To understand the mechanism of inhibition precisely and design more selective inhibitors, we have synthesized a series of structurally related imino sugar compounds. At present, we predict that the structural homology of *N*-alkylated imino sugars to ceramide is the underlying mechanism for inhibition. To aid compound design we have studied the inhibition kinetics using bacterially expressed human ceramide-specific glucosyltransferase.

S12.11

A SOLID-PHASE GLYCOSYLTRANSFERASE ASSAY FOR HIGH-THROUGHPUT SCREENING IN DRUG DISCOVERY RESEARCH

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Glycosyltransferases mediate changes in glycosylation patterns which, in turn, may affect the function of glycoproteins and/or glycolipids and, further downstream, processes of development, differentiation, transformation and cell-cell recognition. Such enzymes, therefore, represent valid targets for drug discovery. We have developed a solid phase glycosyltransferase assay for use in a robotic high-throughput format. Carbohydrate acceptors coupled covalently to polyacrylamide are coated onto 96-well plastic plates. The glycosyltransferase reaction is performed with recombinant enzyme and radiolabeled sugar-nucleotide donor at 37°C, followed by washing, addition of scintillation counting fluid, and measurement of radioactivity using a 96-well β-counter. Glycopolymer construction and coating of the plastic plates, enzyme and substrate concentrations, and linearity with time were optimized using recombinant Core 2 β1-6-N-acetylglucosaminyl-transferase (Core 2 Gn-T). This enzyme catalyzes a rate-limiting reaction for expression of polylactosamine and the selectin ligand sialyl-Lewis^x in O-glycans. A glycopolymer acceptor for β1-6-N-acetylglucosaminyltransferase V was also designed and shown to be effective in the solid-phase assay. In a high-throughput screen (HTS) of a microbial extract library to identify Core 2 Gn-T inhibitors, the coefficient of variance for positive controls was 9.4%. In a controlled, small-scale comparison of the solid-phase assay to a standard, solution-phase (radiometric) method, we observed high concordance for hit validation. Hit extracts are currently being fractionated to identify active molecules for further testing in cell culture and animal models of disease. The solid-phase assay format, which can be adapted for a variety of glycosyltransferase enzymes, allowed a 5-6 fold increase in throughput compared to the corresponding solution-phase assay.

S12.12

A DIRECTED COMBINATORIAL LIBRARY SUCCESSFULLY IDENTIFIES NOVEL GnT INHIBITORS

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Certain complex cell surface oligosaccharides structures have been associated with disease states such as cancer metastasis and inflammation. Inhibitors of selected GlcNAc transferases (eg. GnT-I, GnT-II, GnT-V and core 2 GnT) involved in the biosynthesis of these oligosaccharides structures are targets for therapies against such diseases based on the argument that if expression of these cell surface oligosaccharides are inhibited, the disease can be treated. To generate lead inhibitors against our GnT targets, a 20,358 member directed combinatorial library of uridine terminated peptides was constructed using a solution phase, exponentially branched parallel approach (manual and automated). Defined compounds were generated individually and presented as such in a 96-well format for screening (i.e. one target structure per well). Using commercially available amino acids (AA) and simple carboxylic acids (caps) as building blocks, HBTU activation, both Boc and Fmoc peptide coupling strategies were successfully used to generate targets with the general structure CAP-AA_2-AA_1-5'-*N*-deoxy uridine. Three positions were randomized to provide a significant diversity of compounds and adequately probe the entire UDP-GlcNAc substrate site in the enzyme. High throughput LCMS was used to confirm the identity and assess the purity of target structures in the library. Good target synthesis hit rates and product purity were achieved for final targets prepared on a 12mmole (~10mg) scale. High throughput screening of the library with both radiometric and chemiluminescent assays has identified several selective and structurally similar lead inhibitors for each GnT target (IC_{50} in the 20-100μM range). Activity and selectivity of library hits was confirmed by manual total resynthesis of target structures in active wells. In our efforts to generate SAR data and optimize primary lead structures, small sublibraries of some hits where terminal building blocks have been substituted or omitted have yielded new and interesting inhibitors.

S12.13

GD0039 reduces lung colonization when given after injection of tumor cells.

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Many studies have linked increased levels of L-PHA binding on tumor cells, measuring increased β1,6 branching on carbohydrates, with cancer. In patients with human colorectal cancer, the intensity of L-PHA staining at the time of surgery was shown to be correlated with disease-free and overall survival (Seelentag *et al*, 1999). As part of its ongoing studies into the mechanism of action of compounds that interfere with the formation of these β1,6-branched carbohydrate structures, GlycoDesign Inc. is conducting studies using the lung colonization model in mice. Published data has suggested that pre-treatment of mice with swainsonine, a mannosidase II inhibitor, is required in order to reduce the number of lung colonies formed after injection of the B16F10 melanoma cell line. However, in this study, we show that GD0039, our drug currently in clinical trials, can reduce lung colonization in the same model when administered one day after injection of tumor cells into mice. This observation shows that pre-treatment of mice or of the tumor cells may not be essential. These data demonstrate that GD0039 can interfere with the appearance of tumors after tumor cells have seeded in a target organ.

S12.14

L-PHA binding in peripheral blood lymphocytes as a marker for GD0039 activity

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