MINIREVIEW
(Article for special issue on Galectins)

Trends in Glycoscience and Glycotechnology
Vol. 30 No. 172 (January–May 2018) pp. SE51–SE65
doi: 10.4052/tigg.1734.1SE

Human Milk Oligosaccharides as Essential Tools for Basic and Application Studies on Galectins

Tadasu Urashima[1]; Jun Hirabayashi[2]; Sachiko Sato[3]; and Akira Kobata[4]

[1]Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan
[2]Biotechnology Research Institute for Drug Discovery (BRD), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan
[3]Glycobiology and Bioimaging laboratory, Research Centre for Infectious Diseases, Faculty of Medicine, Laval University, Quebec city, Canada
[4]Noguchi Institution, Tokyo, Japan
FAX: +81–155–49–5577, E-mail: urashima@obihiro.ac.jp

(Received on October 24, 2017, accepted on November 8, 2017)

Key Words: *human milk oligosaccharides, HMOs, milk oligosaccharides, galectins, HMOs dataset, HMO core structures, HMO biosynthesis, galectin binding specificity*

Abstract

It is now recognized that human milk oligosaccharides (HMOs) can function both as prebiotics and as decoy receptors that inhibit the attachment of pathogenic microorganisms to the colonic mucosa. They can also act as immune modulators and as colonic maturation stimulators in breast-fed infants. These functions could be mediated by biological interaction between a variety of HMOs and lectins including galectins, selectins and siglecs. There are more than 100 HMOs; they have structural units such as H type 1: Fucα1-2Galβ1-3GlcNAc, Lewis a: Galβ1-3(Fucα1-4)GlcNAc, Lewis b: Fucα1-2Galβ1-3(Fucα1-4)GlcNAc, Lewis x: Galβ1-4(Fucα1-3)GlcNAc, sialyl Lewis a: Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc, and sialyl Lewis x: Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc. It can be expected that these units may be utilized as tools for studies on the sugar-binding specificities of lectins including galectins, monoclonal antibodies, virus capsid proteins and bacterial toxins. This mini-review presents the dataset of comprehensive HMO structures, including recently clarified ones, in tabular form, for its utilization in such studies, including those of carbohydrate-binding specificity of galectins. In addition, this review introduces recent *in vivo* and clinical studies, which may be relevant to the biological functions and future utilization of HMOs.

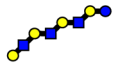
A. Introduction

Human milk contains around 60 g/L of lactose (Galβ1-4Glc), as well as 12–13 g/L of a variety of milk oligosaccharides in mature milk and 22–24 g/L in colostrum. Whereas human milk oligosaccharides (HMOs) comprise several monosaccharide components, such as glucose (Glc), *N*-acetylglucosamine (GlcNAc), galactose (Gal), fucose (Fuc) and *N*-acetylneuraminic acid (Neu5Ac), they always have a lactose unit at their reducing ends. Although by 2009 the structures of about 115 HMOs had been characterized and clarified based on 13 core series (1, 2), a comprehensive database, including large novel structures, had not been described.

When breast-fed infants consume their mother's milk, the majority of the HMOs are not digested and absorbed within the small intestine and therefore reach the infant's colon. It has been suggested from *in vitro* studies that the HMOs function as prebiotics and decoy receptors, inhibiting the attachment of certain types of pathogenic microorganisms to the colonic mucosa, and as modulators of colonic epithelial maturation (1, 3–5). It has been also shown that some strains of *Bifidobacterium bifidum*, *B. longum* ssp. *infantis*, and *B. breve* are able to grow in medium containing HMOs as the only carbon source (6–8). It has been also clarified that *B. bifidum* is characterized by a unique metabolic pathway in which HMOs are digested by several extracellular and intracellular glycosidases (7). Notably, low concentrations of some HMOs are detected in plasma from venous blood after the consumption of breast milk (9, 10). Thus, it appears that a minor part of HMOs is absorbed within the small intestine and enters the circulation. It is possible that those HMOs have immune modulation effects such as anti-inflammation, as suggested by *in vitro* studies using blood leukocytes (11), platelets and endothelial cells (12). It can be assumed that the recognition of those HMOs by some endogenous lectins including galectins, selectins and siglecs is related to those biological activities of HMOs. Therefore, studies on the interaction between HMOs and lectins may generate useful information relating to the utilization of HMOs in therapy, such as immunomodulation and inhibition of the establishment of infection. Notably, as some HMOs have been recently produced on an industrial scale (13), these studies combined with advances in the industrial production of HMOs may in near future open up the possibility of the utilization of HMOs for the treatment and/or prevention of human diseases.

HMOs consist of more than 100 individual molecules. They have been so far utilized as tools for studies on the sugar binding

Table 1. The 19 core structures of HMOs by symbolic representations.

	Name	Structures	CFG format	Number of recognition units
I.	Lactose	Gal(β1-4)Glc		1
II	Lacto-*N*-tetraose	Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc		2
III.	Lacto-*N*-neotetraose	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc		2
IV.	Lacto-*N*-novopentaose I	Gal(β1-4)GlcNAc(β1-6) Gal(β1-3) Gal(β1-4)Glc		2*
V.	Lacto-*N*-hexaose	Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)Glc		2
VI.	Lacto-*N*-neohexaose	Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc		2
VII.	*para*-Lacto-*N*-hexaose	Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc		3
VIII.	*para*-Lacto-*N*-neohexaose	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc		3
IX.	Lacto-*N*-octaose	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)Glc		3
X.	Lacto-*N*-neooctaose	Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc		3
XI.	*iso*-Lacto-*N*-octaose	Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)Glc		3
XII.	*iso*-Lacto-*N*-neooctaose	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc		3
XIII.	*novo*-Lacto-*N*-neooctaose	Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc		3
XIV.	*para*-Lacto-*N*-octaose	Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc		4
XV.	*para*-Lacto-*N*-neooctaose	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc		4
XVI.	Lacto-*N*-decaose	Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)Glc		3
XVII.	Lacto-*N*-neodecaose	Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)Glc		3
XVIII.	*iso*-Lacto-*N*-decaose	Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc		3
XIX.	*novo*-Lacto-*N*-decaose	Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc		4

CFG symbols are used to express individual monosaccharides with different colors, whereas different glycosidic linkages are shown by different bond angles in a clockwise format; *i.e.*, 1-2 linkage (6:00 O'clock), 1-3 (7:30), 1-4 (9:00), and 1-6 (10:30). On the other hand, α and β anomers are represented by *thin* and *thick* lines, respectively. In the right 'glycotope' column, disaccharide units, to which galectins are expected to bind or not in theory, are shown in *green* and *red* colors, respectively. *The number includes Galβ1-3 structure in the β1-3 branch in addition to LacNAc unit in the β1-6 branch.

specificities of several lectins, monoclonal antibodies or virus capsid proteins (14–21). In this mini-review, we present a comprehensive dataset, in tabular form, of the chemical structures of HMOs that have been characterized to date, in order to provide a HMO database as a reference for future research on the sugar-binding specificities, inhibitors and biological functions of galectins.

B. Previous and Newly Proposed Core Structures of HMOs

Structural studies of HMOs began during the 1950's, and by 2009 about 115 structures had been determined by several groups. Based on the characterized HMOs, a core series of 13 structures, was proposed (shown in black in Table 1). The 115 HMOs are synthesized by substitution of the 13 core structures with α1-2 linked Fuc at Galβ1-3/4 residues, with α1-3/4 linked Fuc at GlcNAc, with α1-3 linked Fuc at Glc at the reducing terminal, with α2-3/6 linked Neu5Ac at Galβ1-3/4 residues, and with α2-6 linked Neu5Ac at GlcNAc. In addition, trace levels of the oligosaccharides containing sulfate have been found.

Since 2010 the approaches for structural characterization of HMOs have been dramatically improved by the development of techniques involving a combination of exoglycosidase digestion with microchip-based high-performance liquid chromatography with a polar graphite carbon column, along with matrix-assisted-laser desorption/ionization-time-of-flight mass spectrometry, and multistep mass spectrometry. Owing to this advance, 47 more structures have been identified, yielding a total of 162 HMO structures to date (see Table 2). In this mini-review, we propose that the previous 13 core series can be expanded to 19, which include a new series consisting of *iso*-lacto-*N*-neooctaose, *novo*-lacto-*N*-neooctaose, *para*-lacto-*N*-neooctaose, *iso*-lacto-*N*-decaose, and *novo*-lacto-*N*-decaose (shown in *red* in Table 1). Lacto-*N*-novopentaose I is also included in Table 1, while this is not a new core unit and is relatively minor in HMOs. In fact, FS-novoLNP I (#**159** in Table 2) is a sole HMO whose core is lacto-*N*-novopentaose I. The proposed nomenclatures of novel HMOs are also shown in Table 2.

C. Possible Biosynthetic Pathway of HMO Core Structures

Based on the recent report by Kobata (2) and the 162 HMOs structures described above, it is possible to suggest biosynthetic pathways of HMOs as shown in Fig. 1. Here, only 4 common enzymes are used throughout the syntheses: iGnT (1-3 extension), IGnT (1-6 branching), β3Gal-T (type 1 termination), β4Gal-T (type 2 formation). Hereby, 18 core structures of HMOs can be synthesized in a systematic manner. However, an issue remains to be elucidated with respect to the substrate specificity of IGnT (β1-6GlcNAc transferase); *i.e.*, whether this enzyme can transfer GlcNAc even after completion of type 2 LacNAc extension (dashed arrows in Fig. 1). GlcNAcβ1-3Lac is a reported substrate for IGnT to form a branched tetrasaccharide, GlcNAc β1-3(GlcNAcβ1-6)Lac, but it is possible that IGnT can substantially transfer GlcNAc to the type 2 LacNAc-extended structure. In other words, other β1,6*N*-acetylglucosaminyltransferases, but not IGnT may transfer GlcNAc to this type 2 unit. Indeed, Prudden *et al.* (77) synthesized Galβ1-4GlcNAcβ1-3(GlcNAcβ1-6)Lac using *N*-acetyllactosaminide β1,6-*N*-acetylglucosaminyltransferase (GCNT2) with LN*n*T as an acceptor and UDP-GlcNAc as a donor. This knowledge indicates that different glycosyltransferases can potentially be employed for the biochemical synthesis of a series of HMOs that can be used in industry.

D. Structural Features of HMOs

The following structural units are present in the 162 known HMOs:

H type 1 (H 1): Fucα1-2Galβ1-3GlcNAc, Lewis x (Le^x): Gal β1-4(Fucα1-3)GlcNAc, sialyl Lewis x (SLe^x): Neu5Acα2-3Gal1-4(Fucα1-3)GlcNAc, Lewis a (Le^a): Galβ1-3(Fucα1-4)GlcNAc, sialyl Lewis a (SLe^a): Neu5Acα2-3Galβ1-3(Fucα1-4) GlcNAc, Lewis b (Le^b): Fucα1-2Galβ1-3(Fucα1-4)GlcNAc, and Lewis y (Le^y): Fucα1-2Galβ1-4(Fucα1-3)GlcNAc. In addition to the above, 2′-FL (**#1** in Table 2) represents a structural unit of H type 2 (H 2): Fucα1-2Galβ1-4Glc(NAc).

The 162 HMOs are not always detected in the milk of every donor. The most HMOs are found in the milk of 80% of the donors, denoted secretor (Le^{a-b+}), while oligosaccharides containing non-reducing α1-2 linked Fuc including 2′-FL: Fucα1-2Galβ1-4Glc, LNFP I: Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc, and LNDFH I: Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, which are predominant in secretor milk, are not found in the milk of 15% donors, denoted non-secretor (Le^{a+b-}) (78). This is due to absence of the FUT2 enzyme in non-secretor donors. Furthermore, oligosaccharides containing α1-4 linked Fuc, such as LNFP II, Galβ1-3(Fuc α1-4)GlcNAcβ1-3Galβ1-4Glc and LNDFH I, are not found in the milk of 5% donors, denoted Lewis negative (Le^{a-b-}); this is due to absence of the FUT3 enzyme in Lewis-negative donors.

E. Quantitative Aspects of HMOs

The HMOs listed in Table 2 are not found in equal concentrations. The 13 representative neutral HMOs are 2′-FL (#**1** in Table 2), 3-FL (#**2**), DF-L(LDFT) (#**7**), LNT (#**8**), LN*n*T (#**9**), LNFP I (#**10**), LNFP II (#**11**), LNFP III (#**12**), LNFP V (#**13**), LNDFH I (#**14**), LNDFH II (#**15**), LNH (#**17**), LN*n*H (#**18**), and the 8 rep-

Table 2. Chemical structures of HMOs and architecture of recognition glycotopes for galectins.

No.	Abbreviations	Structures	Glycotope	Ref
1	2'-FL			22
2	3-FL			23
3	β3'-GL			24
4	β4'-GL			25
5	β6'-GL			26
6	LNTri II			27
7	DF-L(LDFT)			28
8	LNT			29
9	LN*n*T			30
10	LNFP I			31
11	LNFP II			32
12	LNFP III			33
13	LNFP V			34
14	LNDFH I			35
15	LNDFH II			36
16	LNDFH III			24
17	LNH			37
18	LN*n*H			38
19	F-LNH I			39
20	F-LNH II			40
21	F-LNH III			41
22	F-LN*n*H II			42
23	F-LN*n*H I			38
24	F-*para*-LNH I			43
25	F-*para*-LNH II			44
26	F-*para*-LN*n*H			42
27	DF-LNH II			37
28	DF-LNH I			39
29	DF-LN*n*H			45
30	DF-*para*-LNH			46
31	DF-*para*-LNH II			42
32	DF-*para*-LNH III			42
33	DF-*para*-LN*n*H			46
34	TF-LNH I			47
35	TF-LNH II			41
36	TF-*para*-LNH I			48
37	TF-*para*-LNH II			44
38	TF-*para*-LN*n*H			44
39	*iso*-LNO			42
40	*novo*-LN*n*O			42
41	F-LNO I			49
42	F-LNO II			41
43	F-LNO III			41
44	F-LN*n*O			50
45	F-LN*n*O II			42
46	F-*iso*-LNO			51
47	F-*iso*-LN*n*O I			42
48	F-*novo*-LN*n*O			42
49	F-*para*-LNO			42
50	DF-*iso*-LN*n*O			42

Table 2. Continued.

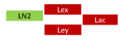
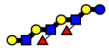
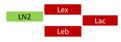
No.	Abbreviations	Structures	Glycotope	Ref
51	DF-LNO I			50
52	DF-LNO II			50
53	DF-LNO III			42
54	DF-LN*n*O I			50
55	DF-LN*n*O II			50
56	DF-LN*n*O III			42
57	DF-*iso*-LNO I			52
58	DF-*iso*-LNO II			52
59	DF-*iso*-LNO III			42
60	DF-*iso*-LNO IV			42
61	DF-*iso*-LNO V			42
62	DF-*iso*-LNO VI			42
63	DF-*iso*-LNO VII			42
64	DF-*para*-LN*n*O			42
65	TF-LNO I			50
66	TF-LNO II			42
67	TF-LN*n*O			47
68	TF-*iso*-LNO I			52
69	TF-*iso*-LNO II			51
70	TF-*iso*-LNO III			42
71	TF-*iso*-LNO IV			42
72	TF-*iso*-LN*n*O			42
73	Tetra-F-*iso*-LNO			45
74	Tetra-F-*para*-LNO			45
75	Penta-F-*iso*-LNO			45
76	LND			53
77	F-LND I			53
78	F-LND II			15
79	DF-LND I			54
80	DF-LND II			54
81	DF-LND III			54
82	DF-LND IV			54
83	DF-LND V			54
84	DF-LND VI			54

Table 2. Continued.

No.	Abbreviations	Structures	Glycotope	Ref
85	-			54
86	-			54
87	TriF-LND I			54
88	TriF-LND II			54
89	TriF-LND III			54
90	TriF-LND IV			54
91	TriF-LND V			41
92	TriF-LND VI			41
93	TriF-LND VII			41
94	-			54
95	-			54
96	TetraF-LND I			54
97	TetraF-LND II			54
98	TetraF-LND III			54
99	-			54
100	F-LN*n*D I			54
101	F-LN*n*D II			54
102	DF-LN*n*D			54
103	*iso*-LND			42
104	DF-*novo*-LND			42
105	-			55
106	3'-SL			56
107	6'-SL			57
108	F-SL			58
109	LST a			59
110	LST b			59
111	LST c			59
112	LST d			58
113	F-LST a			58
114	F-LST b			60
115	F-LST c			61
116	S-LNH I			37
117	S-LNH II			62
118	S-LN*n*H I			38
119	S-LN*n*H II			63
120	S-*para*-LNnH			64
121	FS-LNH			65
122	FS-LNH I			66
123	FS-LNH II			66

Table 2. Continued.

No.	Abbreviations	Structures	Glycotope	Ref
124	FS-LNH III			66
125	FS-LNH IV			67
126	FS-LN*n*H I			58
127	FS-LN*n*H II			38
128	FS-*para*-LN*n*H I			64
129	FS-*para*-LN*n*H II			64
130	DFS-LNH I			66
131	DFS-LNH III			59
132	DFS-LNH IV			59
133	DFS-LN*n*H			66
134	S-LNO			64
135	FS-LNO I			68
136	FS-LNO II			64
137	FS-*iso*-LNO			69
138	DFS-*iso*-LNO I			69
139	DFS-*iso*-LNO II			69
140	DFS-LNO I			69
141	DFS-LNO II			64
142	DFS-LNO III			64
143	TFS-LNO			64
144	TFS-*iso*-LNO			68
145	DS-LNT			70
146	FDS-LNT I			71
147	FDS-LNT II			72
148	DS-LNH I			71
149	DS-LNH II			71
150	DS-LN*n*H			66
151	FDS-LNH I			73
152	FDS-LNH II			73
153	FDS-LNH III			67
154	FDS-LN*n*H			73
155	TS-LNH			74
156	SLN*n*D			64
157	-			75
158	-			75
159	FS-*novo*-LNP I			66
160	DF-*para*-LNH sulfate I			76
161	DF-*para*-LNH sulfate II			76
162	TF-*para*-LNH sulfate			76

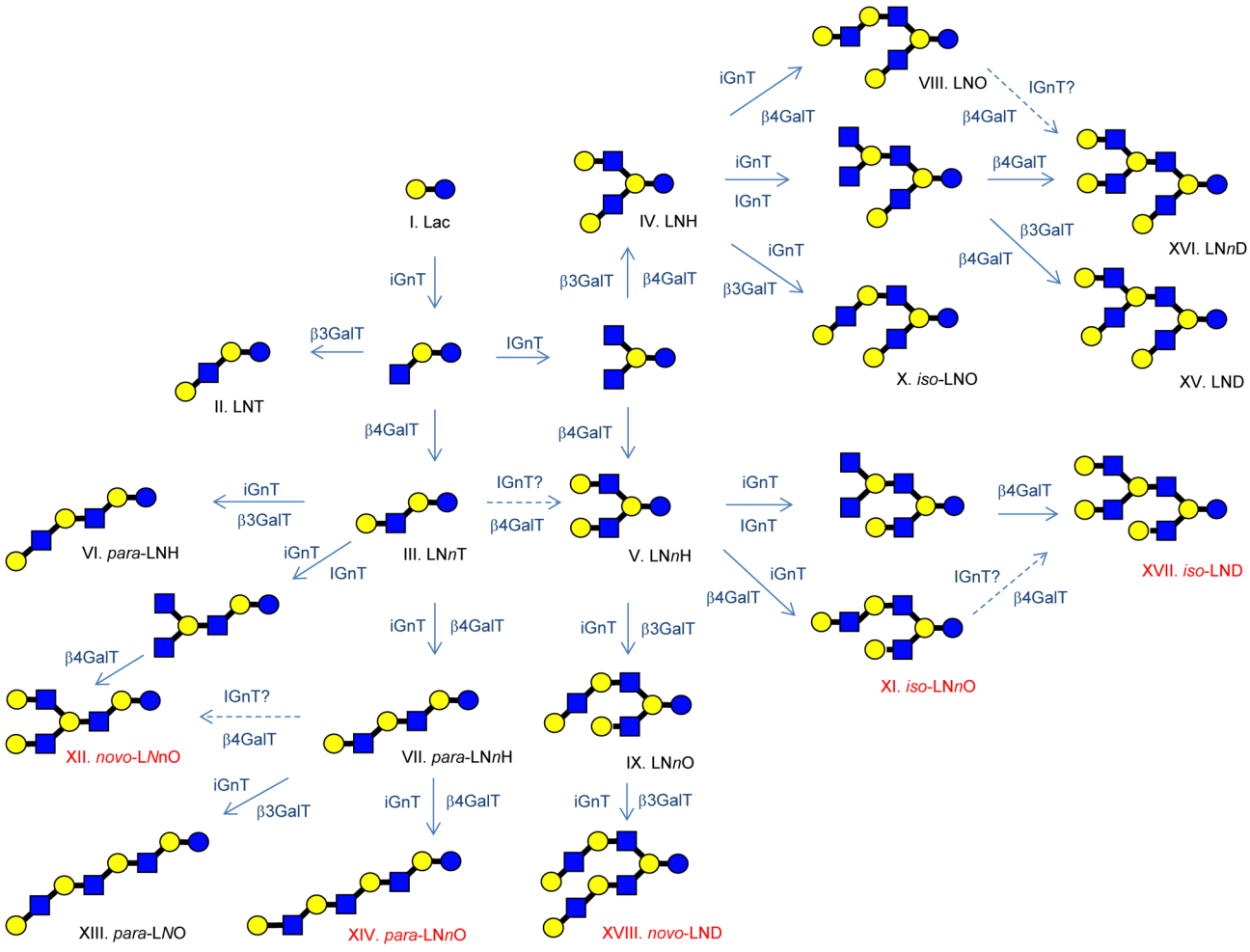


Fig. 1. The possible biosynthetic pathway of 18 core units of HMOs. The biosynthetic pathway of lacto-*N*-novopentaose I is not included.

resentative acidic HMOs are 3′-SL (#**106**), 6′-SL (#**107**), LST a (#**109**), LST b (#**110**), LST c (#**111**), F-LST a (#**113**), F-LST b (#**114**), DS-LNT (#**145**). The concentrations of these representative neutral and acidic HMOs in the milk of secretor, non-secretor and Lewis-negative donors, at several lactation stages (78, 79) have been quantified by high-performance, high-pH-anion-exchange chromatography – pulse-amperometry detection, and high-performance liquid chromatography or capillary electrophoresis, after labeling. The data for the concentration of each HMO, however, differ considerably between different publications, and each value appears to be dependent on the quantitative method used. Further studies are required to establish the consensus values. We refer to the excellent review by Thurl *et al.* (80) concerning these values.

The most significant feature of HMOs is the predominance of type 1 oligosaccharides, which contain Galβ1-3GlcNAc, over type 2 saccharides containing Galβ1-4GlcNAc. The milks of non-human species either contain only type 2 saccharides as in the milks of cow, goat, sheep, pig and horse, or else the type 2 predominates over the type 1 as in some apes (79, 81, 82). In human milk, LNT and LNFP I, the representative type 1 HMOs, are found at concentrations that are three to four times greater than those of the type 2, *i.e.*, LN*n*T and LNFP III.

F. Galectins Bind to Milk Oligosaccharides of Human and Other Mammals

Recently, the group led by Cummings reported that HMOs are possible ligands for endogenous human lectins, such as galectins and DC-SIGN (19, 20). They conducted comprehensive interaction analysis using a glycan microarray platform of the Consortium for Functional Glycomics (CFG) of USA; *i.e.*, interaction analysis between 247 HMOs (162 were completely characterized, while the remaining 85 are not, but included for the analysis) and human galectin-1, 3, 4, 7, 8, and 9. It provides a powerful approach to obtain broad information about basic properties of lectin-binding specificity.

As described by Iwaki and Hirabayashi (18) and by others

Table 3. K_d values (μM) reported for human galectins and HMOs.

No.	Abbreviations	Structures	Glycotope	hGal-1 (C2S)	hGal-3C	hGal-7	hGal-9N
0	Lactose		Lac	1200 (0.27)	260 (0.069)	670 (0.33)	850 (0.073)
1	2'-FL		H1	-	180 (0.10)	880 (0.25)	440 (0.14)
8	LNT		LN1 Lac	370 (0.86)	23 (0.78)	120 (1.8)	61 (1.0)
9	LN*n*T		LN2 Lac	320 (1)	18 (1)	220 (1)	62 (1)
10	LNFP I		H1 Lac	360 (0.89)	13 (0.72)	110 (2.0)	47 (1.3)
11	LNFP II		Lea Lac	-	20 (0.90)	850 (0.36)	130 (0.48)
12	LNFP III		Lex Lac	-	37 (0.49)	640 (0.26)	57 (1.1)
14	LNDFH I		Leb Lac	-	32 (0.56)	1200 (0.18)	39 (1.6)
15	LNDFH II		Lea Lex	-	-	-	-
18	LN*n*H		LN2 LN2 Lac	91 (4.0)	12 (1.5)	120 (1.8)	100 (0.62)
17	LNH		LN2 LN1 Lac	110 (3.3)	12 (1.5)	48 (4.6)	52 (1.2)
106	3'-SL		Neu5Ac Lac	-	560 (0.032)	2000 (0.11)	-
107	6'-SL		Neu5Ac Lac	-	-	-	-
108	F-SL(F-SLn)		Neu5Ac Lex	-	-	-	-

Data are from Hirabayashi *et al.* (17) for FAC. *Note* that FAC measurement was performed at 20°C and that FAC data were obtained using PA-oligosaccharides, which have open ring structure at the reducing ends. Data for #**108** (Neu5Acα2-3Gal(Fucα1-4)Glc; sialylLe^x) is actually that for Neu5Acα2-3Gal(Fucα1-4)GlcNAc in the previous FAC analysis but included for reference. Values in parentheses are relative affinities compared with LN*n*T (underlined). Dash (-) denotes not detectable.

over many years, galectins show evolutionarily conserved sugar-binding specificity beyond biological (*e.g.*, human, nematode, fungi) and molecular species (galectin-1–12). *Firstly*, galectins recognize a β-galactoside disaccharide as a recognition unit, represented by lactose and *N*-acetyllactosamine (Galβ1-4GlcNAc). *Secondly*, they require three OH groups essential for recognition; *i.e.*, 4-OH and 6-OH of a non-reducing terminal βGal, and 3-OH of reducing terminal Glc, GlcNAc or GalNAc, in which side chains of hydrophilic residues (*e.g.*, His, Asn, Arg and Glu) located in the evolutionarily conserved carbohydrate-recognition domain (CRD) are essential for forming a hydrogen bond network. However, in case of binding to a type 1 lactosamine structure (Galβ1-3GlcNAc), the third recognition (3-OH of GlcNAc) is replaced by 4-OH of the same GlcNAc (for details, *see* Fig. 4 in the chapter by Iwaki and Hirabayashi).

From the viewpoint described above, consensus rules for galectin recognition to HMOs can be derived: in other words, galectins cannot bind to HMOs if any of the following 3 modifications occur to the recognition units, either lactose or LacNAc.

1) When the 6-OH of a non-reducing terminal βGal is sialylated by the action of ST6 to form the Siaα2-6Gal linkage.
2) When the 6-OH of a non-reducing terminal βGal is substituted with GlcNAc for branching by the action of IGnT.
3) When a Lewis structure is formed by fucosylation to 3-OH of a reducing terminal Glc or GlcNAc (in case of type 1 lactosamine, fucosylation to 4-OH instead).

In any event, modification of the 4-OH of a non-reducing terminal βGal is not permitted.

Other modifications do not necessarily harm galectin binding, and some rather enhance the binding to some galectins. They include fucosylation of 2-OH of a non-reducing terminal βGal, as well as various modifications of 3-OH of a non-reducing terminal βGal (*e.g.*, α2-3Sia, α/β1-3Gal, α1-3GalNAc, and LacNAc extension *via* β1-3 linkage). These rules work well for galectins not only human but also of other organisms.

It is possible to predict which HMOs can be recognized by galectins, since the above described recognition rules are consistent, and thus applicable to any complex HMO. Table 2 summariz-

es a list of structures of HMOs with their symbolic representations, in which CFG symbols are used to express individual monosaccharides with different colors. Further, in the figure different glycosidic linkages are represented by different bond angles in a clockwise format (1-2 linkage; 6:00 O'clock, 1-3; 7:30, 1-4; 9:00, and 1-6; 10:30), while α and β anomers are represented by *thin* and *thick* lines, respectively. In the right 'glycotope' column, disaccharide units, to which galectins can either bind or not bind in theory, are shown in *green* and *red* colors, respectively.

Unfortunately, there are only few studies which have undertaken K_a ($1/K_d$) determinations for galectins and HMOs, and the number of HMOs is around 10, with the largest molecules being LNH and LN*n*H. However, from Table 3, it is evident that the above prediction is totally consistent in the light of experimental data determined by frontal affinity chromatography (FAC), a quantitative method originally developed by Kasai *et al.* (83; also *see* the chapter by Iwaki and Hirabayashi). Recently, Shams-Ud-Doha *et al.* (84) reported a new method to determine K_d values between HMOs and galectins, based on ion mobility/electrospray ionization-mass spectrometry. While the reported K_d values significantly differ from those in Table 3, possibly due to differences in experimental conditions, they basically agree in that HMOs for which no binding is expected actually showed no binding to galectins. They include HMOs consisting of 'all red' (no-binding) recognition units, such as LNDFH II (#**15** in Table 3) and 6′-SL (#**107**), and F-SL (#**108**).

There are many useful milk oligosaccharides other than those of human origin (82). Supplementary Table 1 lists a series of milk oligosaccharides of non-human origins. They include oligosaccharides containing xeno-antigenic epitopes, αGal and *N*-glycolylneuraminic acid (Neu5Gc). Other interesting examples include Gal β1-3 oligomers extended from lactose, which have been found in the tammar wallaby and related marsupial mammals. Intriguingly, the latter structure, *i.e.*, (Galβ1-3)$_n$-modification is common to the surface glycan of a pathogenic protozoan, *Leishmania major*, possibly involved in the recognition by galectin-3 and 9, as described by one of the authors of this chapter (85; *see* next section for detail).

G. Potential Use of Milk Oligosaccharides for the Study of Carbohydrate-binding Specificity of Galectins

Many laboratories have been using HMOs to determine the carbohydrate-binding specificity of lectins. Especially, in the case of galectins, HMOs have been extensively utilized for more than 30 years to determine the detailed carbohydrate-binding specificity since galectins shows preferential binding for β-galactosides, which are dominant structures of HMOs (17, 21, 86–89). The extensive database on the binding preference of galectins for each HMO has contributed both to our understanding on the biological roles of galectins and to the pharmaceutical development of specific inhibitors for galectins. For example, the finding that α2-6 sialyl modification of the galactose residue reduces the affinity of galectin-1 for the *N*-acetyllactosamine structure is now linked to the immunological role of galectin-1 in T cell regulation (90). It has been known that galectins also bind to ABO(H) blood groups (17, 19, 21, 87–89, 91), and many microorganisms express HMO-like structures. A recent study suggests that galectin-4 and −8 bind and kill ABO blood group antigen-expressing *Escherichia coli*, while galectin-3 binding does not affect the viability (92). A species of the protozoan *Leishmania, L. major*, expresses unique β-galactosides, (Galβ1-3)$_n$ on its surface. While this structure is not found in HMOs, milk from the tammar wallaby contains lactose modified with (Galβ1-3)$_n$ (82). By using the tammar wallaby's (Galβ1-3)$_n$-lactose, it was shown that galectin-3 and −9 recognize *L. major* through binding to this unique glycotope (85, 93). Interestingly, this binding specificity feature appears to be extended to a galectin in the insect host, the sand fly *Phlebotomus paratasi* (94). It is suggested that this binding to (Galβ1-3)$_n$ is critical for the survival of the parasite in the vector fly (95).

Recently, Prudden *et al.* (77) succeeded in the synthesis as many as 60 HMOs, including asymmetrical multiantennary structures, using an enzymatic strategy with a number of human glycosyltransferases. It is expected that their 'synthetic' HMOs will be available for detailed studies on the carbohydrate binding specificity of each galectin.

H. Potential Utilization of Milk Oligosaccharide for the Development of Drugs for Inhibition of Galectins

Milk oligosaccharides from both human and non-human origins contribute to biomedicine in providing useful information about galectin-glycan ligand interactions. Notably, the oligosaccharides are synthesized according to consistent rules, resulting in as many as 19 core structures which have both branching and extension, whose binding features are considerably different among galectin species as well as their individual CRDs. Moreover, milk oligosaccharides including HMOs are rich in 3′-OH modification with sulfate, sialic acids (αNeu5Ac and αNeu5Gc), blood group epitopes (αGal/αGalNAc), for which some galectins show distinct preference. To verify the specific binding by galectins, non-inhibitory saccharides are also available with 4-OH and 6-OH modifications of βGal.

To date, several examples of galectin inhibitors are available and their clinical applications are being studied, including galectin-3 inhibitors for the treatment of lung fibrosis. Here, inhibi-

tor design is firstly based on selection of βGal as a frame structure; *e.g.*, thiodigalactoside (96, 97) and *N*-acetyllactosamine backbones (98). Chemical modification of OH groups to enhance the affinity for a specific galectin molecule is another critical factor in design of the synthesis (for comprehensive review, *see* the chapter by Dernavit *et al.*). Such galectin inhibitors are also useful as agonists for studies on the histochemical localization of endogenous galectins (99).

Not only galectins, but also other lectins can be targets of 'glycomimetics' in drug chemistry, as represented by the successful development of rivipansell (GMI-1070), a pan-selectin inhibitor developed by a US company, GlycoMimetics (100); this has now entered a phase 3 clinical trial for the cure of vaso-occlusive crisis in sickle cell-derived anemia (https://sicklecellanemianews.com/2017/05/11/phase-3-trial-recruiting-to-test-rivipansel-for-vaso-occlusive-crisis-in-sickle-cell-disease/). Notably, it took more than a quarter century for this to be achieved. In view of the fact that there is a great variety of milk oligosaccharides with a diversity of structural parameters affecting galectins affinity and selectivity, libraries of milk oligosaccharides of various species including humans, reliable interaction data as well, would be extremely valuable. At present, however, reported K_d values are available for only about a dozen milk oligosaccharides (Table 2). Though comprehensive data obtained by glycan microarray are available in the world-wide activity of CFG (19), they are not necessarily consistent in terms of K_d. Establishment of a systematic preparation of a large scale of milk oligosaccharides is urgently required for both basic and application studies.

I. Recent Studies of the Functions of HMOs

It has been suggested that HMOs, as well as bovine and caprine milk oligosaccharides, have several biological functions; for example, they act as prebiotics and may have the potential of preventing infection, modulating immunity and to be involved in intestinal maturation. It is also reported that HMOs could prevent necrotizing enterocolitis and activate the brain-gut axis. We refer to a number of reviews related to the biological functions of HMOs (1, 3–5, 7). This mini-review introduces only a few recent topics concerning *in vivo* and clinical studies on the functions of HMOs.

Tarr *et al.* (101) reported on an *in vivo* study describing the diminishing of stressor-induced anxiety-like behavior in mice when they were administrated a laboratory diet containing 3′-SL and 6′-SL.

Recently, large scale preparation of HMOs, including 2′-FL and LN*n*T, has been successful and their food safety has been studied using *in vivo* experiments with model animals (102–104). In parallel, clinical study with bottle-fed infants consuming formula containing 2′-FL and LN*n*T have begun to establish that this incorporation is safe and has no negative effects on the subjects.

In one of these clinical trials, Goehring *et al.* (105) determined the concentrations of inflammatory cytokines, such as interleukin(IL)-1α, IL-1β, IL-6 and tumor necrosis factor-α, in venous plasma, comparing breast-fed infants with infants who were bottle-fed with three different types of infant formulas: control formula containing only 2.4 g/L of galacto-oligosaccharides (GOS) and no 2′-FL, experimental formula 1 containing 2.2 g/L GOS and 0.2 g/L 2′-FL, and experimental formula 2 containing 1.4 g/L GOS and 1 g/L 2′-FL. The results showed that the concentrations of those inflammatory cytokines in plasma were significantly lower in breast-fed infants and in those receiving experimental formulas 1 and 2, which had been supplemented with 2′-FL, than in the control group of bottle-fed infants. There was no difference between breast-fed infants and experimental formula 1 or 2-fed infants with respect to inflammatory cytokines, confirming the biological significance of milk 2′-FL. Indeed, their recent study suggests the potential of HMO 2′-FL for the treatment of necrotizing enterocolitis (106).

Puccio *et al.* (107) evaluated the effects of infant formula supplemented with two HMOs, 2′-FL and LN*n*T, on infant growth tolerance and morbidity. The authors compared the difference in infant weight between the baseline visit and the visit at age 4 months, other anthropometric measures (weight, length, body mass index and head circumference), digestive tolerance (flatulence, spitting-up, and vomiting), stool characteristics (stool consistency and frequency), behavior patterns (restlessness, colic and nighttime awakenings), formula intake and morbidity (parent-reported lower respiratory tract infections and concomitant medications) between Test and Control groups. The results showed that there were no differences with respect to anthropometric index between the two groups, while the supplemented formula improved stool characteristics, behavior patterns and the frequency of medical treatments. This study suggests that 2′-FL and LN*n*T may have health benefits for bottle-fed infants.

J. Concluding Remarks

By chemical synthesis, chemo-enzymatic synthesis or whole-cell microbial biotransformations with recombinant *E. coli* strains, gram scale productions have been successful for a few HMOs including 2′-FL, LN*n*T, LNT, 3′-SL and 6′-SL, some of which have been produced on an industrial scale by a number of companies including Glycom A/S (Denmark), Elicityl (France), Jennewein Biotechnologies (Germany), Glycosynth LLC (USA), Friesland Campina (The Netherlands). Many studies on the functions of HMOs and of other milk oligosaccharides, including those of cows and goats, have been performed *in vitro* using epithelial cells.

Recently *in vivo* studies using model animals, and human clinical studies, have also begun. This advance has been made possible by the successful large-scale preparation of 2′-FL and LN*n*T, but only a few HMOs are available at this stage.

It can be predicted that in the near future a variety of HMOs will be utilized as tools to clarify the sugar binding specificities of lectins including galectins, virus capsid proteins, monoclonal antibodies and bacterial toxins, as such information is critical for our understanding of the various biological phenomena controlled by glycans. Not only natural HMOs separated from human milk but also synthetic HMOs should be available as tools for these studies (77). We emphasize, in addition, that future studies designed to clarify the glycosyltransferases involved in the biosynthesis of HMOs in lactating mammary epithelial cells, and relevant studies relating to the human genome, will be highly advantageous for the applied field as well as basic science.

Acknowledgments

We thank Dr. Michael Messer of the University of Sydney for editing English. This work was supported by a Grant-in-Aid for Scientific Research ©, grant No. 16K08003, from the Japan Society for the Promotion of Science, Sports and Culture (Japan).

References

1. Urashima, T., Kitaoka, M., Terabayashi, T., Fukuda, K., Ohnishi, M., and Kobata, A. (2011) in Oligosaccharides: Source, Properties and Applications (Gorden, N.S. ed.), pp. 1–58, Nova Science Publishers, Inc, New York.
2. Kobata, A. (2016) in Prebiotics and Probiotics in Human Milk (McGuire, Mi., McGuire Ma., and Bode, B. eds.), pp. 17–44, Academic Press, Amsterdam.
3. Bode, L. (2012) *Glycobiology* **22**, 1147–1162.
4. Urashima, T., Asakuma, S., Kitaoka, M., and Messer, M. (2011) in Encyclopedia of Dairy Science, Second Edition (Fuquay, J.W., P.F. Fox, P.F. and McSweeney, P.L.H. eds.), vol. 3, pp.241–273, Amsterdam, Academic Press.
5. Donovan, S. M., Wang, M., Li, M., Friedberg, I., Schwartz, S. L., and Chapkin, R. S. (2012) *Adv. Nutr.* **3**, 450S–455S.
6. Asakuma, S., Hatakeyama, E., Urashima, T., Yoshida, E., Katayama, T., Yamamoto, Y., Kumagai, H., Ashida, H., Hirose, J., and Kitaoka, M. (2011) *J. Biol. Chem.* **286**, 34583–34592.
7. Kitaoka, M. (2012) *Adv. Nutr.* **3**, 422S–429S.
8. Garrido, D., Barille, D., and Mills, D. A. (2012) *Adv. Nutr.* **3**, 415S–421S.
9. Goehring, K. C., Kennedy, A. D., Prieto, P. A., and Buck, R. H. (2014) *PLoS One* **9**, e101692.
10. Ruhaak, L. R., Stroble, C., Underwood, M. A., and Lebrilla, C. B. (2014) *Anal. Bioanal. Chem.* **406**, 5775–5784.
11. Bode, R., Rudloff, S., Kunz, C., Strobel, S., and Klein, N. (2004) *J. Leukoc. Biol.* **76**, 820–826.
12. Schumacher, G., Bendas, G., Stahl, B., and Beermann, C. (2006) *Nutr.* **22**, 620–627.
13. Sprenger, G. A., Baumgartner, F., and Salbermann, C. (2017) *J. Biotechnol.* **258**, 79–91.
14. Shang, J., Piskarev, V. E., Xia, M., Huang, P., Jiang, X., Likhosherstov, L. M., Novikova, O. S., Newburg, D. S., and Ratner, D. M. (2013) *Glycobiology* **23**, 1491–1498.
15. Yu, Y., Mishra, S., Song, X., Lasanajak, Y., Bradley, K. C., Tappert, M. M., Air, G. M., Steinhauer, D. A., Halder, S., Cotmore, S., Tattersall, P., Agbandje-McKenna, M., Cumming, R. D., and Smith, D. F. (2012) *J. Biol. Chem.* **287**, 44784–44799.
16. Yu, Y., Lasanajak, Y., Song, X., Hu, L., Ramani, S., Mickum, M. L., Ashline, D. J., Prasad, B. V. V., Estes, M. K., Reinhold, V. N., Cummings, R. D., and Smith, D. F. (2014) *Mol. Cell. Proteomics* **13**, 2944–2960.
17. Hirabayashi, J., Hashidate, J. T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Urashima, T., Oka, T., Futai, M., Muller, W. E. G., Yagi, F., and Kasai, K. (2002) *Biochim. Biophys. Acta* **1572**, 232–254.
18. Iwaki, J., Tateno, H., Nishi, N., Minamisawa, T., Nakamura-Tsuruta, S., Itakura, Y., Kominamo, J., Urashima, T., Nakamura, T., and Hirabayashi, J. (2011) *Biochim. Biophys. Acta* **1810**, 643–651.
19. Noll, A. J., Gourdine, J. P., Yu, Y., Lasanajak, Y., Smith, D. F., and Cummings, R. D. (2016) *Glycobiology* **26**, 655–669.
20. Noll, A. J., Yu, Y., Lasanajak, Y., Duska-McEwen, G., Buck, R. H., Smith, D. F., and Cummings, R. D. (2016) *Biochem. J.* **473**, 1343–1353.
21. Sato, S., and Hughes, R. C. (1992) *J. Biol. Chem.* **267**, 6983–6990.
22. Kuhn, R., Baer, H. H., and Gauhe, A. (1956) *Chem. Ber.* **89**, 2513.
23. Montreuil, J. (1956) *C. R. Acad. Sci.* **242**, 192–193.
24. Donald, A. S. R., and Feeney, J. (1988) *Carbohydr. Res.* **178**, 79–91.
25. Sugawara, M., and Idota, T. (1995) Proceedings of the Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry. Sapporo, Japan, p.132.
26. Yamashita, K., and Kobata, A. (1974) *Arch. Biochem. Biophys.* **161**, 164–170.
27. Dabrowski, U., Egge, H., and Dabrowski, J. (1983) *Arch. Biochem. Biophys.* **224**, 254–260.
28. Kuhn, R., and Gauhe, A. (1958) *Justus Liebigs Ann. Chem.* **611**, 249–252.
29. Kuhn, R., and Baer, H. H. (1956) *Chem. Ber.* **89**, 504–511.
30. Kuhn, R., and Gauhe, A. (1962) *Chem. Ber.* **95**, 518–522.
31. Kuhn, R., Baer, H. H., and Gauhe, A. (1956) *Chem. Ber.* **89**, 2514–2523.
32. Kuhn, R., Baer, H. H., and Gauhe, A. (1958) *Chem. Ber.* **91**, 364–374.
33. Kobata, A., and Ginsburg, V. (1969) *J. Biol. Chem.* **244**, 5496–5502.
34. Ginsburg, V., Zopf, D. A., Yamashita, K., and Kobata, A. (1976) *Arch. Biochem. Biophys.* **175**, 565–568.

35. Kuhn, R., Baer, H. H., and Gauhe, A. (1958) *Justus Liebigs Ann. Chem.* **611**, 249–253.
36. Kuhn, R., and Gauhe, A. (1960) *Chem. Ber.* **93**, 647–651.
37. Kobata, A., and Ginsburg, V. (1972) *J. Biol. Chem.* **247**, 1525–1529.
38. Kobata, A., and Ginsburg, V. (1972) *Arch. Biochem. Biophys.* **150**, 273–281.
39. Yamashita, K., Tachibana, Y., and Kobata, A. (1977) *Arch. Biochem. Biophys.* **182**, 546–555.
40. Dua, V. K., Goso, K., Dube, V. E., and Bush, C. A. (1985) *J. Chromatogr. A* **328**, 259–269.
41. Wu, S., Tao, N., German, J. B., Grimm, R., and Lebrilla, C. B. (2010) *J. Proteome Res.* **9**, 4138–4151.
42. Ashline, D. J., Yu, Y., Lasanajak, Y., Song, X., Hu, L., Ramani, S., Prasad, V., Estes, M. K., Cummings, R. D., Smith, D. F., and Reinhold, V. N. (2014) *Mol. Cell. Proteomics* **13**, 2961–2974.
43. Sabharwal, H., Nilsson, B., Grönberg, G., Chester, M. A., Dakour, J., Sjöblad, S., and Lundblad, A. (1988) *Arch. Biochem. Biophys.* **265**, 390–406.
44. Bruntz, R., Dabrowski, U., Dabrowski, J., Ebersold, A., Peter-Katalinic, J., and Egge, H. (1988) *Biol. Chem. Hoppe Seyler* **369**, 257–273.
45. Haeuw-Fièvre, S., Wieruszeski, J. M., Plancke, Y., and Strecker, G. (1993) *Eur. J. Biochem.* **215**, 361–371.
46. Yamashita, K., Tachibana, Y., and Kobata, A. (1977) *J. Biol. Chem.* **252**, 5408–5411.
47. Strecker, G., Fièvre, J. M., Wieruszeski, J. M., Michalski, J. C., and Montreuil, J. (1992) *Carbohydr. Res.* **226**, 1–14.
48. Strecker, G., Wieruszeski, J. M., Michalski, J. C., and Montreuil, J. (1988) *Glycoconj. J.* **5**, 385–396.
49. Yamashita, K., Tachibana, Y., and Kobata, A. (1976) *Biochemistry* **15**, 3950–3955.
50. Tachibana, Y., Yamashita, K., and Kobata, A. (1978) *Arch. Biochem. Biophys.* **188**, 83–89.
51. Kogelberg, H., Piskarev, V. E., Zhang, V., Lawson, A. M., and Chai, W. (2004) *Eur. J. Biochem.* **271**, 1172–1186.
52. Strecker, G., Wieruszeski, J. M., Michalski, J. C., and Montreuil, J. (1989) *Glycoconj. J.* **6**, 169–182.
53. Chai, W., Piskarev, V. E., Zhang, Y., Lawson, A. M., and Kogelberg, H. (2005) *Arch. Biochem. Biophys.* **434**, 116–127.
54. Amano, J., Osanai, M., Orita, T., Sugahara, D., and Osumi, K. (2009) *Glycobiology* **19**, 601–614.
55. Blank, D., Geyer, H., Maass, K., Yamashita, K., and Geyer, R. (2012) *Anal. Biochem.* **421**, 680–690.
56. Kuhn, R., and Brossmer, R. (1959) *Chem. Ber.* **93**, 647–651.
57. Kuhn, R. (1959) *Naturwissenschaften* **46**, 43–50.
58. Grönberg, G., Lipniunas, P., Lundgren, T., Erlansson, K., Lindh, F., and Nilsson, B. (1989) *Carbohydr. Res.* **191**, 261–278.
59. Kuhn, R., and Gauhe, A. (1965) *Chem. Ber.* **98**, 395–413.
60. Wieruszeski, J. M., Chekkor, A., Bouquelot, S., Montreuil, J., Strecker, G., Peter-Katalinic, J., and Egge, H. (1985) *Carbohydr. Res.* **137**, 127–133.
61. Smith, D. F., Prieto, P. A., McCrumb, D. K., and Wang, W. C. (1987) *J. Biol. Chem.* **262**, 12040–12047.
62. Wang, W. T., Lundgren, T., Lindh, F., Nilsson, B., Grönberg, G., Brown, J. P., Mentzer-Dibert, H., and Zopf, D. (1992) *Arch. Biochem. Biophys.* **292**, 433–441.
63. Tarrago, M. T., Tucker, K. H., van Halbeek, H., and Smith, D. F. (1988) *Arch. Biochem. Biophys.* **267**, 353–362.
64. Wu, S., Grimm, R., German, J. B., and Lebrilla, C. B. (2011) *J. Proteome Res.* **10**, 856–868.
65. Yamashita, K., Tachibana, Y., Takasaki, S., and Kobata, A. (1977) *Arch. Biochem. Biophys.* **182**, 546–555.
66. Grönberg, G., Lipniunas, P., Lundgren, T., Lindh, F., and Nilsson, B. (1992) *Arch. Biochem. Biophys.* **296**, 597–610.
67. Kitagawa, H., Nakada, H., Kurosaka, A., Hiraiwa, N., Numata, Y., Fukui, S., Funakoshi, I., Kawasaki, T., Yamashina, I., Shimada, I., and Inagaki, F. (1989) *Biochemistry* **28**, 8891–8897.
68. Kitagawa, H., Nakada, H., Fukui, S., Funakoshi, I., Kawasaki, T., Yamashina, I., Tate, S., and Inagaki, F. (1993) *J. Biochem.* **114**, 504–508.
69. Kitagawa, H., Nakada, H., Fukui, S., Funakoshi, I., Kawasaki, T., Yamashina, I., Tate, S., and Inagaki, F. (1991) *Biochemistry* **30**, 2869–2876.
70. Grimmonprez, L., and Montreuil, J. (1968) *Bull. Soc. Chim. Biol. (Paris)* **50**, 843–855.
71. Kitagawa, H., Takaoka, M., Nakada, H., Fukui, S., Funakoshi, I., Kawasaki, T., Tate, S., Inagaki, F., and Yamashina, I. (1991) *J. Biochem.* **110**, 598–604.
72. Grönberg, G., Lipniunas, P., Lundgren, T., Lindh, F., and Nilsson, B. (1990) *Arch. Biochem. Biophys.* **278**, 297–311.
73. Yamashita, K., Tachibana, Y., and Kobata, A. (1976) *Arch. Biochem. Biophys.* **174**, 582–591.
74. Fièvre, S., Wieruszeski, J. M., Michalski, J. C., Lemoine, J., Montreuil, J., and Strecker, G. (1991) *Biochem. Biophys. Res. Commun.* **177**, 720–725.
75. Kitagawa, H., Nakada, H., Numata, T., Kurosaka, A., Fukui, S., Funakoshi, I., Kawasaki, T., Shimada, I., Inagaki, F., and Yamashina, I. (1990) *J. Biol. Chem.* **265**, 4859–4862.
76. Guerardel, Y., Morelle, W., Plancke, Y., Lemoine, J., and Strecker, J. (1999) *Carbohydr. Res.* **320**, 230–238.
77. Prudden, A. R., Liu, L., Capicciotti, C. J., Wolfert, M. A., Wang, S., Gao, Z., Meng, L., Moremen, K. W., and Boons, G. J. (2017) *Proc. Natl. Acad. Sci. U.S.A.* **114**, 6954–6959.
78. Kobata, A. (2010) *Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci.* **86**, 1–17.
79. Urashima, T., Asakuma, S., Leo, F., Fukuda, K., Messer, M., and Oftedal, O. T. (2012) *Adv. Nutr.* **3**, 473S–482S.
80. Thurl, S., Munzert, M., Boehm, G., and Stahl, B. (2017) *Nutr. Rev.* DOI: 10.1093/nutrit/nux044
81. Urashima, T., Taufik, E., Fukuda, K., and Asakuma, S. (2013) *Biosci. Biotechnol. Biochem.* **77**, 455–466.
82. Urashima, T., Messer, M., and Oftedal, O. T. (2016) in Prebiotics and Probiotics in Human Milk (McGuire, Mi., McGuire, Ma. and Bode, L. eds.) pp. 45–139, Academic Press, London, San Diego, Cambridge, Oxford.
83. Kasai, K., Oda, Y., Nishikata, M., and Ishii, S. (1986) *J. Chromatogr. A* **376**, 33–47.
84. Shams-Ud-Doha, K., Kitova, E. N., Kitov, P. I., St-Pierre, Y., and Klassen, J. S. (2017) *Anal. Chem.* **89**, 4914–4921.
85. Pelletier, I., Hashidate, T., Urashima, T., Nishi, N., Nakamura, T., Futai, M., Arata, Y., Kasai, K., Hirashima, M., Hirabayashi, J., and Sato, S. (2003) *J. Biol. Chem.* **278**, 22223–22230.
86. de Waard, A., Hickman, S., and Kornfeld, S. (1976) *J. Biol. Chem.* **251**, 7581–7587.
87. Leffler, H., and Barondes, S. H. (1986) *J. Biol. Chem.* **261**, 10119–10126.

88. Sparrow, C. P., Leffler, H., and Barondes, S. H. (1987) *J. Biol. Chem.* **262**, 7383–7390.
89. Stowell, S. R., Arthur, C. M., Mehta, P., Slanina, K. A., Blixt, O., Leffler, H., Smith, D. F., and Cummings, R. D. (2008) *J. Biol. Chem.* **283**, 10109–10123.
90. Toscano, M. A., Bianco, G. A., Ilarregui, J. M., Croci, D. O., Correale, J., Hernandez, J. D., Zwirner, N. W., Poirier, F., Riley, E. M., Baum, L. G., and Rabinovich, G. A. (2007) *Nat. Immunol.* **8**, 825–834.
91. Stowell, S. R., Cho, M., Feasley, C. L., Arthur, C. M., Song, X., Colucci, J. K., Karmakar, S., Mehta, P., Dias-Baruffi, M., McEver, R. P., and Cummings, R. D. (2009) *J. Biol. Chem.* **284**, 4989–4999.
92. Stowell, S. R., Arthur, C. M., Dias-Baruffi, M., Rodrigues, L. C., Gourdine, J. P., Heimburg-Molinaro, J., Ju, T., Molinaro, R. J., Rivera-Marrero, C., Xia, B., Smith, D. F., and Cummings, R. D. (2010) *Nat. Med.* **16**, 295–301.
93. Pelletier, I., and Sato, S. (2002) *J. Biol. Chem.* **277**, 17663–17670.
94. Dobson, D. E., Kamhawi, S., Lawyer, P., Turco, S. J., Beverly, S. M., and Sacks, D. L. (2010) *PLoS Pathog.* **6**, e1001185.
95. Kamhawi, S., Ramalho-Ortigao, M., Pham, V. M., Kumar, S., Lawyer, P. G., Turco, S. J., Barillas-Mury, C., Sacks, D. L., and Valenzuela, J. G. (2004) *Cell* **119**, 329–341.
96. Salomonsson, E., Carisson, M. C., Osla, V., Hendus-Altenburger, R., Kahi-Knutson, B., Oberg, C. T., Sundin, A., Nilsson, R., Nordberg-Karisson, E., Nilsson, U. J., Karisson, A., Rini, J. M., and Leffler, H. (2010) *J. Biol. Chem.* **285**, 35079–35091.
97. Rajput, V. K., MacKinnon, A., Mandal, S., Collins, P., Blanchard, H., Leffler, H., Sethi, T., Schambye, H., Mukhopadhyay, B., and Nilsson, U. J. (2016) *J. Med. Chem.* **59**, 8141–8147.
98. Atmanene, C., Ronin, C., Téletchéa, S., Gautier, F. M., Djedaïni-Pilard, F., Ciesielski, F., Vivat, V., and Grandjean, C. (2017) *Biochem. Biophys. Res. Commun.* **489**, 281–286.
99. Roy, R., Cao, Y., Kaltner, H., Kottari, N., Shiao, T. C., Belkhadem, K., André, S., Manning, J. C., Murphy, P. V., and Gabius, H. J. (2017) *Histochem. Cell Biol.* **147**, 285–301.
100. Magnani, J. L. (2004) *Arch. Biochem. Biophys.* **426**, 122–131.
101. Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., and Balley, M. T. (2015) *Brain Behav. Immun.* **50**, 166–117.
102. Charbonneau, M. R., O'Donnell, D., Blanton, L. V., Lebrilla, C., Mills, D. A., and Gordon, J. I. (2016) *Cell* **164**, 859–871.
103. Coulet, M., Phothirath, P., Constable, A., Marsden, E., and Shilter, B. (2013) *Food Chem. Toxicol.* **62**, 528–537.
104. Coulet, M., Phothirath, P., Allais, L., and Schilter, B. (2014) *Regul. Toxicol. Pharmacol.* **68**, 59–69.
105. Goehring, K. C., Marriage, B. J., Oliver, J. S., Wilder, J. A., Barrett, E. G., and Buck, R. H. (2016) *J. Nutr.* **146**, 2559–2566.
106. Good, M., Sodhi, C. P., Yamaguchi, Y., Jia, H., Lu, P., Fulton, W. B., Martin, L. Y., Prindle, T., Nino, D. F., Zhou, Q., Ma, C., Ozolek, J. A., Buck, R. H., Goehring, K. C., and Hackam, D. J. (2016) *Br. J. Nutr.* **116**, 1175–1187.
107. Puccio, G., Alliet, P., Cajozzo, C., Janssens, E., Corsello, G., Sprenger, N., Wernimont, S., Egli, D., Gosoniu, L., and Steenhout, P. (2017) *J. Pediatr. Gastroenterol. Nutr.* **64**, 624–631.

Information of the Authors

Tadasu Urashima graduated from Tokyo University of Agriculture and Technology in 1980 and received his PhD from Tohoku University, Graduate School of Agriculture in 1986. He started his professional carrier in 1986 as an Assistant Professor at Obihiro University of Agriculture and Veterinary Medicine, and has been a Professor since 2003. His main research interests are in evolutional and physiological aspects of milk oligosaccharides in human and a variety of other mammalian species.

Jun Hirabayashi Born in Feb 28, 1958 in Tochigi prefecture in Japan. Education: Bachelor of Science, Tohoku University (Mar., 1980), Master of Science (chemistry), Tohoku University Graduate School (Mar., 1982). Professional Carrier: Assistant professor, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa, Japan (Apr., 1982), Lecturer (Apr., 1989), PhD in science (chemistry), Tohoku University (Dec., 1989); Team leader, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan (Nov., 2002); Visiting professor, Kagawa University, Kagawa, Japan (Sep., 2003); Deputy director of Research Center for Medical Glycoscience, AIST (Dec., 2006), Prime senior researcher, AIST (Apr., 2012). Main research fields: Biochemistry, Glycoscience, Glycotechnology, Lectin Engineering, evolutionary chemistry.
Councilor, The Japanese Society of Carbohydrate Research (JSCR)
Director, Japan Consortium for Glycoscience and Glycotechnology (JCGG)

Sachiko Sato graduated from Faculty of Pharmaceutical Science, Chiba University. After working in Nippon Hoffman-la-Roche Research Centre in Kamakura, she joined as postgraduate student in the laboratory of Dr. Akira Kobata, the Institute of Medical Science, the University of Tokyo, Japan in 1987. She also worked in the laboratory of Dr. R. Colin Hughes, MRC: National Institute for Medical Research in London, UK, where she first encountered an interesting cytosolic mammalian lectin, now called galectin-3. She obtained her Ph.D. from the University of Tokyo in 1994. As postdoctoral fellow in the laboratory of Dr. Ron Kopito, Stanford University, she was involved in the work on cystic fibrosis that established the precedent that a genetic disease can be remediated by a chemical mean for the first time. This concept has been elaborated as “corrector” treatment for cystic fibrosis. After her post-doc training in Research Center for Infectious Diseases, Faculty of Medicine, Laval University, Quebec, Canada, she became principal investigator of the laboratory of glycobiology in this centre in 1999 and is full professor in the Department of Microbiology and Immunology, Faculty of Medicine, Laval University. She is also director of the Bioimaging platform since 2003. While her research interest has always been to elucidate the role of galectins in host-pathogen interactions, the interest of her laboratory recently has been extended to the study of the therapeutic potential of galectins for the treatment of muscular dystrophies and the development of a new single-cell lineage tracking system for glycobiology.

Akira Kobata graduated from Faculty of Pharmaceutical Science, School of Medicine, the University of Tokyo in 1956 and received his Ph.D. in Biochemistry in 1962. In 1967, he joined the Section of Biochemistry, Laboratory of Biochemical Pharmacology, NIAMD (Section Chief, Victor Ginsburg), NIH. During that time, he elucidated the whole biosynthetic pathway of ABH and Lewis antigenic determinants. In 1971, he moved to Kobe University as Professor of the Department of Biochemistry, School of Medicine. There, he developed a series of sensitive methods to investigate the structures of the N-linked sugar chains. On moving to the University of Tokyo as the Professor and Chairman, Department of Biochemistry, Institute of Medical Science in 1982, he expanded his research to the structures, metabolism, functions, and pathology of the N-linked sugar chains. He served as the Director of Institute of Medical Science from 1990 to 1992 and became Professor Emeritus of the University of Tokyo in 1993. He was awarded numerous prizes, including Science and Technology Prize from Toray Science Foundation (1985), Claude S. Hudson Award of American Chemical Society (1992), PSJ Award from Pharmaceutical Society of Japan (1992), and the Japan Academy Prize (1992). He was a Fogarty Scholar-in-Residence from 1985 to 1987, Auckland Foundation Visiting Professor in 1988. In 1993, he was appointed as the Director of Tokyo Metropolitan Institute of Gerontology, where he developed a new glycobiology area in the field of aging research. From 2000, he has been the Director Emeritus of Tokyo Metropolitan Institute of Gerontology. Currently, he is the scientific advisor of the Noguchi Institute, a non-profit institution established for the study of carbohydrate chemistry in Japan.