FEBS Letters 436 (1998) 228–232 FEBS 20896

Different response to inflammation of the multiple mRNAs of rat *N*-acetylglucosaminyltransferase I with variable 5′-untranslated sequences

Takashi Fukada[a], Noriyuki Kioka[a,*], Jun Nishiu[a], Shohei Sakata[a], Hiroshi Sakai[a], Masayasu Yamada[b], Tohru Komano[a]

[a] *Laboratory of Biochemistry, Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
[b] *Laboratory of Animal Reproduction, Division of Animal Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

Received 23 June 1998; revised version received 21 August 1998

Abstract We found that there are at least five subclasses of *N*-acetylglucosaminyltransferase I (GnT-I; EC 2.4.1.101) mRNA with different 5′-untranslated regions in rat brain. These five subclasses were also expressed in many tissues with distinct tissue-specific patterns. Moreover, they were regulated differently in response to acute-phase inflammation. The expression of the most abundant subclass of GnT-I mRNA in rat liver decreased 2.5-fold in response to inflammation, concomitantly with a significant decrease in the total amount of GnT-I mRNA. In contrast, one of the minor subclasses of GnT-I mRNA was induced 10-fold by inflammation.

Key words: Glycosyltransferase; Inflammation; Transcriptional regulation; *N*-Acetylglucosaminyltransferase I

1. Introduction

Among the protein *N*-glycosylation enzymes, *N*-acetylglucosaminyltransferase I (GnT-I; EC 2.4.1.101), which transfers a GlcNAc moiety from UDP-GlcNAc to the oligomannosyl acceptor $Man_5GlcNAc_2$-Asn, initiates the conversion of high-mannose *N*-glycans to hybrid and complex type structures [1]. Since the transfer of GlcNAc by GnT-I is required for the subsequent processing, GnT-I is one of the key enzymes to produce the hybrid and complex *N*-glycans. Although loss of GnT-I activity in cultured cells did not show any apparent effects on the cell growth, GnT-I-deficient transgenic mice die during embryonic development with pleiotropic defects [2–4], suggesting the importance of GnT-I and hybrid and complex *N*-glycans in morphogenesis and development. The regulated expression of GnT-I may control the ratio of complex and hybrid *N*-glycans to high-mannose ones, and thus regulate many biological interactions.

GnT-I cDNAs and/or genomic genes have been isolated from various species, such as rats [5], rabbits [6], mice [7], and humans [8–10] by our and other groups. GnT-I mRNA is regulated in a tissue specific manner in these species. We found that there were two transcripts of GnT-I different in length in rats [5]. The major transcript of GnT-I mRNA was 3.0 kb in most tissues, while the longer 3.3-kb transcript was dominant in the brain. Transcripts different in length have also been observed in mice [7] and humans [10] although their functional significance is unknown.

There are many reports that suggest the involvement of lectin-carbohydrate interactions in immune responses and inflammation. It is especially interesting that IL-1, IL-2 and TNF have lectin activity and bind to high-mannose *N*-glycans [11–13]. The GnT-I substrate, $Man_5GlcNAc_2$-Asn, has been reported to be a good ligand for the lectin activity of IL-2 [13]. Treatment of human monocytes with deoxymannojirimycin, that inhibits Golgi α-mannosidase I and thus increases high-mannose *N*-glycan content, enhances the cytokine activity of IL-1 on these cells [11]. Mannose oligomers which bind to IL-1 and -2 have been reported to have immunosupressive effects in vitro [14]. These findings suggest that the regulation of the high mannose *N*-glycan expression is involved in immune responses and inflammation, although the details are unclear.

We now report that the size difference of rat GnT-I transcripts resulted from the variation of the length of the 5′-untranslated region (UTR), probably due to the alternative usage of transcriptional start sites. We also found at least five classes of GnT-I mRNA expressed with different 5′-UTRs and regulated differently in tissues and in response to inflammation.

2. Materials and methods

2.1. Library construction and screening

Rat brain RNA was isolated by the acid guanidium thiocyanate-phenol-chloroform method [15] and poly(A)$^+$ RNA was purified with the mRNA Purification Kit (Pharmacia). A cDNA library was constructed as previously described [5]. We screened 3×10^6 of plaques with a probe covering the entire coding region of rat liver GnT-I cDNA to obtain 21 positive clones. Nucleotide sequences were determined for 18 of them.

2.2. 5′-RACE

The 5′-distal region of GnT-I cDNA was amplified from rat brain mRNA with a 5′-AmpliFINDER RACE Kit (Clontech) following the manufacturer's instructions. Primer P1 (TCATCACCAAGGGCACTGTCTGAGG), which was complementary to the nucleotides 294–270 of the rat liver GnT-I cDNA [5], was used for cDNA synthesis. The primer P2 (GTCGAATTCCAGCCCACAAAGATGATAGC), complementary to the nucleotides 220–197 with additional bases on its 5′-end for an *Eco*RI site was used for PCR. The products were cloned into the *Eco*RI site of pBluescript (Stratagene).

*Corresponding author. Fax: (81) (75) 753-6104.
E-mail: nkioka@kais.kyoto-u.ac.jp

Abbreviations: GnT-I, *N*-acetylglucosaminyltransferase I; IL, interleukin; TNF, tumor necrosis factor; UTR, untranslated region; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; RT, reverse transcriptase; MMLV, Moloney murine leukemia virus; ORF, open reading frame

The nucleotide sequence data reported in this paper have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB012874 (1AL-3), AB012875 (1AS-3), AB012876 (1B-3), AB012877 (1AS-2-3) and AB012878 (1B-2-3).

PII: S0014-5793(98)01136-3

2.3. Northern hybridization

Total RNA (20 μg) was electrophoresed in formaldehyde-agarose gel and transferred to Hybond N^+ (Amersham). Hybridization was carried out at 42°C with 50% formamide or 60°C without formamide. The entire coding region of the rat GnT-I cDNA was amplified by PCR and used as a probe to detect the total mRNA of GnT-I. The class 1AL-specific probe, which covered the bases in the 5′-UTR between 555 bp and 371 bp upstream from the translational start site, respectively, was amplified by PCR from a cloned 5′-RACE product (see above).

2.4. RT-PCR

cDNA was synthesized from 2 μg of total RNA by MMLV reverse transcriptase with random hexamers and 1/50 of this cDNA was used for PCR. Primers MGF (AAGTGTACTCTCGCCATCCA) and MGR (CAGAGCTCCATAGAGCTTGA) were used for β_2-microglobulin mRNA as an internal control [16,17]. Primers 1AF (GTTGCGCGTTGATCTCC) and R (CAGACTGCTTCTTCAGCATCC) were for GnT-I mRNA including exon 1A, primers 1BF (CATTGCTTGCTAGCAGGAAG) and R for GnT-I mRNA including exon 1B, and primers 2F (TCATCACACTGTGCACATGG) and R for GnT-I mRNA including exon 2. PCR was carried out in 10 μl reaction in the presence of 5 μCi of [α-^{32}P]dATP. Twenty cycles of amplification were made for microglobulin and the exon 1A, 24 cycles for the exon 1B and 2 in Fig. 3. In Fig. 4, 18 cycles of amplification were made for the exon 1A, 21 cycles for the exon 1B, 17 cycles for microglobulin and 14 cycles for angiotensinogen. The products were electrophoresed and analyzed by BAS 2000 image analyzer (Fuji Photo Film). The validity of the PCR quantitation was confirmed by separate experiments shown in Fig. 1.

2.5. Induction of acute-phase response by lipopolysaccharide

Adult female Wistar rats were injected intraperitoneally with 0.6 mg of lipopolysaccharide from *E. coli* O128:B8 (Sigma) dissolved in 6 ml of 0.8% NaCl. Control rats were injected with 6 ml of 0.8% NaCl. Total liver RNA was isolated as described above.

3. Results

3.1. Structures of the alternative forms of rat GnT-I mRNA

To determine the function of the two transcripts different in length, we cloned GnT-I cDNA from rat brain, where the longer transcript of GnT-I is preferentially expressed. Eight-

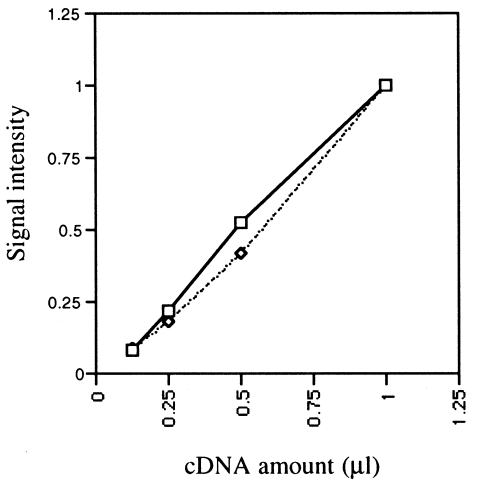

Fig. 1. Confirmation of the validity of RT-PCR quantitation. The class 1A GnT-I (see Fig. 2) and β_2-microglobulin cDNA were amplified from varying amounts of rat liver cDNA on the same conditions as in Fig. 3. Since amplification of the other classes of GnT-I cDNA produced similar results, only the results for these two cDNAs are shown. The signal intensity produced from 1 μl of cDNA was taken as 1. Solid line: β_2-microglobulin; dotted line: class 1A GnT-I.

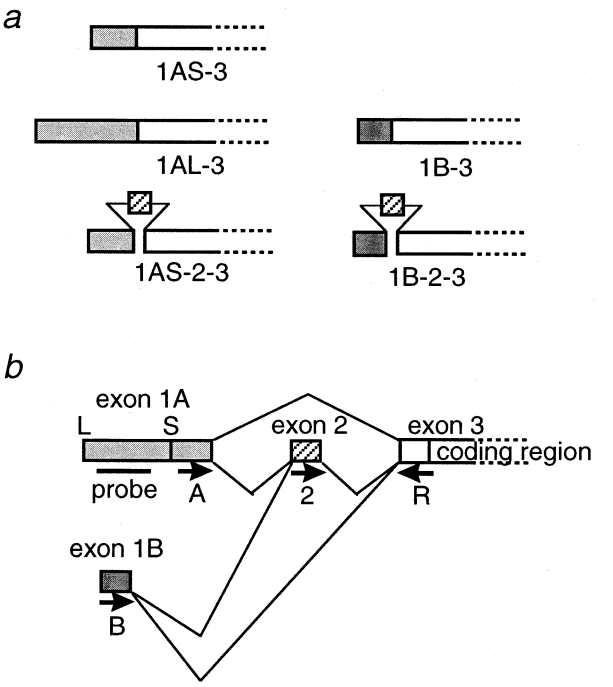

Fig. 2. The 5′-UTR structures of GnT-I mRNA detected by 5′-RACE. a: Schematic structures of the isolated 5′-RACE clones. b: A presumed genome structure of rat GnT-I gene based on the comparison of the five mRNA classes shown in a. It includes three hypothetical transcriptional start sites and an alternatively spliced exon. Arrows indicate the primer arrangements used in RT-PCR shown in Fig. 3. The position of the 1AL-specific probe used in Fig. 4 is also shown.

een clones of GnT-I cDNA were partially sequenced from both ends (data not shown). This preliminary analysis showed that one of the brain clones, later found to belong to the class 1B-3 (see below), had a unique sequence of 31 bases in its 5′-UTR, which was totally different from the corresponding region of the reported liver GnT-I cDNA [5]. Based on these results we hypothesized that the variation of 5′-UTR produces the two transcripts differing in length.

To test this hypothesis, the 5′-UTRs of GnT-I mRNA were isolated from rat brain by 5′-rapid amplification of cDNA ends (5′-RACE), since the 5′-UTR structure of mouse GnT-I mRNA has also been determined by a similar anchored-PCR strategy [18]. The cloned RACE products from rat brain were categorized into five classes (Fig. 2a). Class 1AS-3 consisted of clones which were similar to the liver cDNA. The 5′-UTR of the longest clone in this class was 175 bp and included the whole 5′-UTR of the reported liver cDNA. The class 1AL-3 clones included the whole 5′-UTR of the class 1AS clones and had an additional sequence upstream it. The 5′-UTR of the longest clone of this class was 623 bp. Class 1AS-2-3 was similar to class 1AS-3 but had an additional sequence of 107 bp inserted between the bases −123 and −124 from the start codon of GnT-I. The class 1B-3 clones contained a different sequence from class 1AS-3, 1AL-3 and 1AS-2-3 in the region upstream the base −124, although the sequence downstream the base −123 was the same as class 1AS-3. Class 1B-2-3 was similar to class 1B-3 but the same 107-bp sequence was inserted at the same position as in class 1AS-2-3. A putative genome structure of the rat GnT-I gene

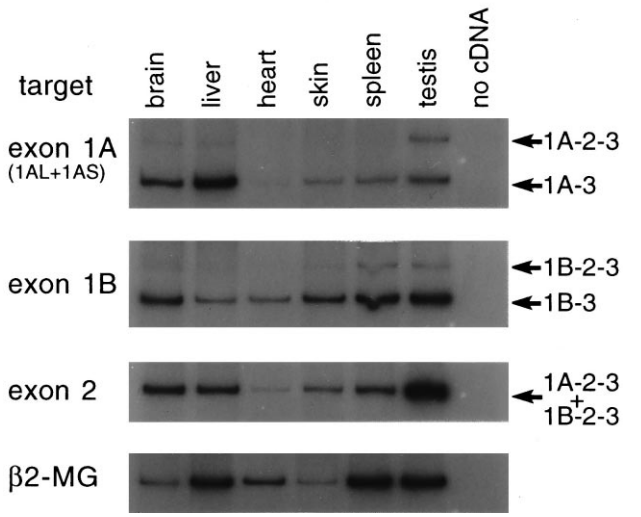

Fig. 3. Expression of GnT-I mRNA subtypes in various tissues. GnT-I mRNA subtypes were amplified from various rat tissues by RT-PCR with the specific primer pairs shown in Fig. 2b; primers A and R for exon 1A, primers B and R for exon 1B, primers 2 and R for exon 2. cDNA synthesis and amplification were performed as described in Section 2. β_2-Microglobulin (β_2-MG) mRNA was used as a control.

was suggested by the comparison of these 5′-UTR structures (Fig. 2b).

3.2. Tissue-specific expression of GnT-I mRNA with the various 5′-UTRs

We determined the expression of the various 5′-UTRs of GnT-I mRNA in rat tissues by RT-PCR. Fig. 3 showed that class 1A-2-3 mRNA was much less abundant than class 1A-3 (i.e. class 1AS-3 plus 1AL-3) in tissues examined. The only exception was found in testis, where the amount of class 1A-2-3 mRNA was comparable to that of class 1A-3. Class 1B-2-3 was much less abundant than class 1B-3 either, even in testis. These data suggested that the transcripts containing exon 2 are much less abundant than those without exon 2 in most tissues, whatever the first exon used. Although we could not determine the exact amount of class 1B-3 mRNA relative to class 1A-3, the analysis of the amplification kinetics (data not shown) suggested that class 1B mRNA was minor compared to class 1A-2-3 at least in the brain. These data suggested that the major 5′-UTR of GnT-I mRNA in rat tissues is either class 1AS-3 or 1AL-3.

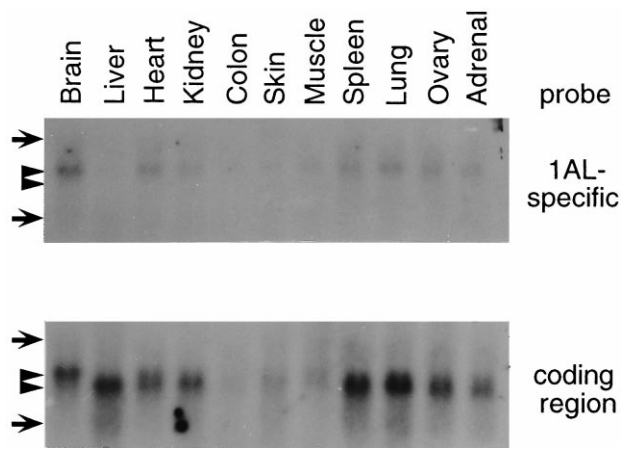

Fig. 4. Detection of the class 1AL GnT-I mRNA from rat tissues by Northern blot hybridization. While the coding region probe detected two mRNA species, 3.3 kb and 3.0 kb (lower panel), the class 1AL-specific probe hybridized only to the 3.3-kb mRNA (upper panel). Note that the 3.3-kb mRNA was most enriched in brain and virtually undetectable in liver. Arrowheads show the positions of 3.3-kb and 3.0-kb GnT-I mRNA on each panel. The arrows indicate the positions of 28 S and 18 S rRNAs.

3.3. Class 1AL-3 mRNA is the 3.3-kb transcript in brain

The difference in length between class 1AS-3 and 1AL-3

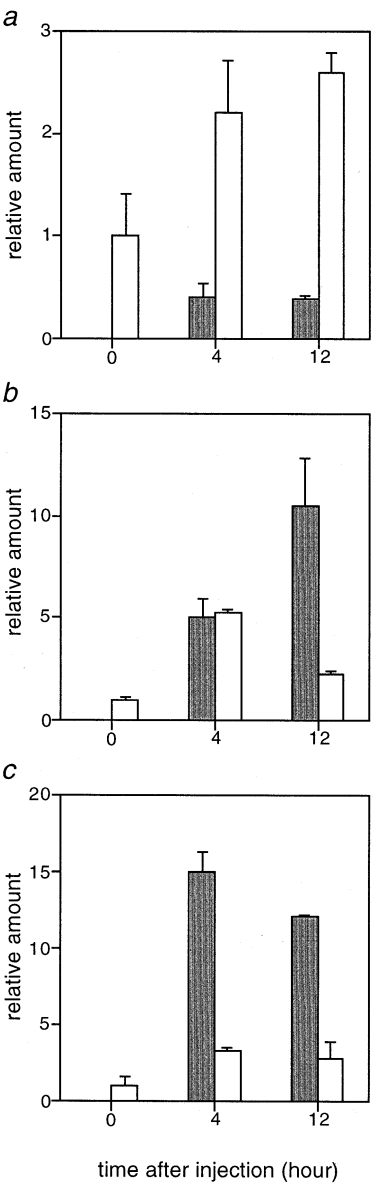

Fig. 5. Acute-phase responses of GnT-I mRNA subclasses in rat liver. Adult female rats were injected intraperitoneally with lipopolysaccharide to induce an acute-phase response. Relative amounts of the class 1A-3 (a), 1B-3 (b), angiotensinogen (c) and β_2-microglobulin (internal control) mRNA in liver were measured by RT-PCR after indicated time. Average of the data from two untreated rats (indicated at 0 h) was taken as 1 on the ordinate. The representative of two independent experiments is shown. Each experiment was done in duplicate and the error bars indicate the standard deviations. Open bars: Control rats; shaded bars: lipopolysaccharide injected rats.

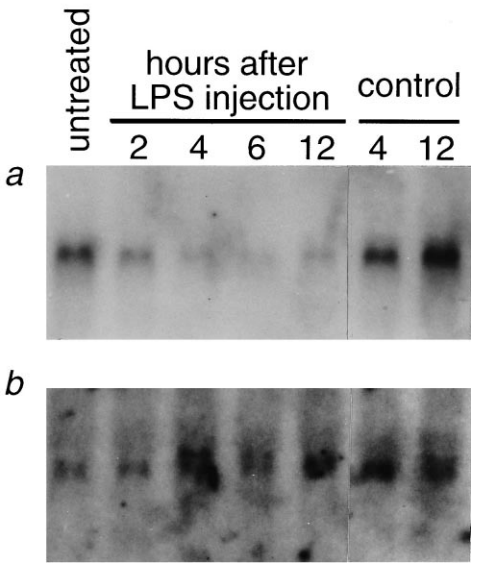

Fig. 6. Transient reduction of the total amount of GnT-I mRNA in liver in response to lipopolysaccharide injection. Twenty micrograms of total liver RNA were analyzed on a Northern blot hybridization, probed with GnT-I coding region (a) or glyceraldehyde-3-phosphate dehydrogenase gene (b) as a control. The representative of two independent experiments is shown.

and estimated amounts of the various 5′-UTRs suggested that the 3.3-kb GnT-I mRNA preferentially expressed in rat brain is class 1AL-3, while the 3.0-kb mRNA expressed in liver is class 1AS-3. We performed Northern blot hybridization analysis with a probe specific to the class 1AL 5′-UTR to examine this assumption (Fig. 4). The 1AL-specific probe hybridized only to the 3.3-kb transcript, and not to the 3.0-kb transcript. 1AL 5′-UTR was expressed strongly in the brain, moderately in the heart, kidneys, spleen, lungs and ovaries, and scarcely in the liver, colon, skin and muscle. This indicated that the expression of class 1AL was roughly proportional to that of the entire GnT-I mRNA (Fig. 4b) except in the brain, liver, and heart. Class 1AL is a major product in the brain and it is scarcely expressed in the liver. The heart showed a moderately high proportion of 1AL mRNA. These results are consistent with the assumption that the 3.3-kb and 3.0-kb mRNA are class 1AL and 1AS, respectively.

3.4. Response of GnT-I mRNAs to inflammation

N-linked glycans have been suggested to play some role in the interaction between lymphocytes [19]. In addition, IL-1 reportedly binds high-mannose type oligosaccharides to generate surface membrane-bound forms [12]. These findings suggest that *N*-glycan structures and GnT-I expression are regulated by some immunological events, such as inflammation. To examine this possibility, we examined the expression of GnT-I mRNAs in rats with acute inflammation induced by the injection of lipopolysaccharide. RT-PCR analysis demonstrated that the acute inflammation caused a 2.5-fold decrease of class 1A mRNA within 4 h and it persisted until 12 h after the injection (Fig. 5). On the other hand, class 1B mRNA increased up to 10-fold after 12 h. Angiotensinogen mRNA, which is known to be induced in liver in acute-phase response by an NF-κB-like factor [20], showed up to 15-fold induction 4 h after the injection and remained at 10∼12-fold induced level until at least 12 h. Northern blot hybridization revealed that the total amount of GnT-I mRNA in liver decreased in response to inflammation (Fig. 6), in agreement with our finding that the majority of GnT-I mRNA in rat liver is class 1AS-3. Hybridization with a class 1A-specific probe confirmed the decrease of this class of GnT-I mRNA (data not shown). We could not detect the class 1B GnT-I mRNA by Northern blot hybridization on the condition we used (data not shown).

4. Discussion

In the present study we showed that the size difference of the previously reported isoforms of rat GnT-I mRNA resulted from the variation of 5′-UTR and there were at least five subclasses of GnT-I mRNA with different 5′-UTRs. These subclasses of GnT-I mRNA were found to be regulated differently in response to inflammation caused by lipopolysaccharide. The total amount of GnT-I mRNA in liver was decreased by the inflammation as a sum of these responses. These observations are the first evidence that suggests the involvement of the GnT-I gene regulation in immune responses and inflammation. Some cytokines, such as IL-1, IL-2 and TNF, were shown to have lectin activity and bind to high-mannose *N*-glycans, including the GnT-I substrate, $Man_5GlcNAc_2$ [11–13]. The lectin activity of IL-2 has been suggested to work in associating the IL-2 receptor β with T-cell receptor (TCR) complex, inducing the IL-2 signal transduction [21]. Furthermore, treatment of human monocytes with deoxymannojirimycin, that increases the high-mannose *N*-glycan content, enhances the cytokine activity of IL-1 on these cells [11]. Altogether, these observations suggest that the regulation of GnT-I expression plays an important role in the inflammatory response, i.e. the decreased expression of the GnT-I would increase the amount of high-mannose *N*-glycan on hepatocytes, enhancing the cytokine activity in acute phase inflammation.

It is also interesting that class 1B mRNA of GnT-I was induced by lipopolysaccharide injection while class 1A-3 mRNA was repressed. Although the induction of class 1B-3 mRNA would not be significant in liver where the majority of GnT-I mRNA was class 1AS-3, this differential regulation suggests that GnT-I expression would rather increase in response to inflammation in some tissues or cells where the transcription of class 1A mRNA is less active. It would allow a variety of tissue-specific responses of GnT-I expression to inflammation.

We found that the difference between the 3.3-kb and 3.0-kb GnT-I mRNAs was in the length of their 5′-UTRs. The 3.3-kb mRNA had an unusually long 5′-UTR (600–630 bp) and contained eight upstream ORFs (uORFs) in the 5′-UTR. This feature suggested the involvement of the translational regulation in the control of GnT-I expression. It is well established that uORFs reduce the translational efficiency of the mRNA in vitro. Furthermore, uORFs in 5′-UTR determined the tissue-specific expression in some cases [22]. Two GnT-I transcripts different in length were also observed in mice [7] and humans ([10], and our unpublished data), suggesting the significance of the 5′-UTR and uORFs in regulating this gene.

Mouse GnT-I has been reported to have two size classes of mRNA similar to rat GnT-I as described above. Yang et al. have determined the 5′-UTR sequences of these mRNAs (*Mgat-1l* and *Mgat-1s*) [18]. These mouse 5′-UTRs were divided by two introns and thus structurally resembled the class

1A-2-3 mRNA of rat GnT-I. The putative second exon of the mouse GnT-I, however, was completely different from the putative exon 2 in the rat, although the other regions were 90% homologous. Moreover, the 22 bases on the 5′-terminal of *Mgat-1s* were not included in *Mgat-1l* and did not have any corresponding sequence in the rat 1AS-3 mRNA. This suggests that the structure of the GnT-I gene in rats may not be identical to that in mice. Another possibility is that *Mgat-1s* was a splicing variant which has not been found, or does not exist, in rats. Analysis of the genomic gene of GnT-I both in rats and mice would be indispensable.

We found at least five subclasses of rat GnT-I mRNA and they responded to inflammation differently. It is interesting to presume that the response of GnT-I expression to inflammation has an important role in immune responses through the cytokine network, by regulating the binding ability of certain cytokines to cell surface *N*-glycans. We need to determine what molecule(s) mediate(s) the regulation of the GnT-I expression and what occurs on the *N*-glycan structures and cytokine networks in response to inflammation.

Acknowledgements: This work was supported in part by grants from the Asahi Glass Foundation and the Inamori Foundation.

References

[1] Kornfeld, R. and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664.
[2] Ioffe, E. and Stanley, P. (1994) Proc. Natl. Acad. Sci. USA 91, 728–732.
[3] Metzler, M., Gertz, A., Sarkar, M., Schachter, H., Schrader, J.W. and Marth, J.D. (1994) EMBO J. 13, 2056–2065.
[4] Campbell, R.M., Metzler, M., Granovsky, M., Dennis, J.W. and Marth, J.D. (1995) Glycobiology 5, 535–543.
[5] Fukada, T., Iida, K., Kioka, N., Sakai, H. and Komano, T. (1994) Biosci. Biotech. Biochem. 58, 200–201.
[6] Sarkar, M., Hull, E., Nishikawa, Y., Simpson, R.J., Moritz, R.L., Dunn, R. and Schachter, H. (1991) Proc. Natl. Acad. Sci. USA 88, 234–238.
[7] Pownall, S., Kozak, C.A., Schappert, K., Sarkar, M., Hull, E., Schachter, H. and Marth, J.D. (1992) Genomics 12, 699–704.
[8] Kumar, R., Yang, J., Larsen, R.D. and Stanley, P. (1990) Proc. Natl. Acad. Sci. USA 87, 9948–9952.
[9] Hull, E., Sarkar, M., Spruijt, M.P.N., Höppener, J.W.M., Dunn, R. and Schachter, H. (1991) Biochem. Biophys. Res. Commun. 176, 608–615.
[10] Yip, B., Chen, S.-H., Mulder, H., Höppener, J.W.M. and Schachter, H. (1997) Biochem. J. 321, 465–474.
[11] Muchmore, A., Decker, J., Shaw, A. and Wingfield, P. (1990) Cancer Res. 50, 6285–6290.
[12] Brody, D.T. and Durum, S.K. (1989) J. Immunol. 143, 1183–1187.
[13] Sherblom, A.P., Sathyamoorthy, N., Decker, J.M. and Muchmore, A.V. (1989) J. Immunol. 143, 939–944.
[14] Muchmore, A.V., Sathyamoorthy, N., Decker, J. and Sherblom, A.P. (1990) J. Leukocyte Biol. 48, 457–464.
[15] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[16] Mauxion, F. and Kress, M. (1987) Nucleic Acids Res. 15, 7638.
[17] Noonan, K.E. et al. (1990) Proc. Natl. Acad. Sci. USA 87, 7160–7164.
[18] Yang, J., Bhaumik, M., Liu, Y. and Stanley, P. (1994) Glycobiology 4, 703–712.
[19] Varki, A. (1993) Glycobiology 3, 97–130.
[20] Ron, D., Brasier, A.R., Wright, K.A., Tate, J.E. and Habener, J.F. (1990) Mol. Cell. Biol. 10, 1023–1032.
[21] Zanetta, J.P., Alonso, C. and Michalski, J.-C. (1996) Biochem. J. 318, 49–53.
[22] Zimmer, A., Zimmer, A.M. and Reynolds, K. (1994) J. Cell Biol. 127, 1111–1119.