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N-Acetylglucosaminyltransferase I Activity in Bovine Ovarian Follicular Fluids from Dominant and Atretic Follicles

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Abstract: The activity of α3-D-mannoside-β-1,2-*N*-acetylglucosaminyltransferase I (GnT I; EC 2.4.1.101), which catalyzes the first step in the conversion of oligomannose to complex or hybrid *N*-glycans of glycoproteins, was detected in bovine follicular fluid (bFF). The GnT I activity in bFF had a pH optimum of 5.8 and an absolute requirement for either Co^{2+}, Mn^{2+}, or Mg^{2+}, the activity being stimulated by these cations in the above order. The apparent K_m value for α1-3α1-6 mannopentaose of GnT I in bFF was 2.17 mM. The substrate specificity for the GnT I activity decreased in the following order: α1-3α1-6 mannopentaose > α1-3α1-6 mannotriose > α1-3 mannobiose. The GnT I activity in bFF from large atretic follicles was significantly higher than in that from large dominant follicles. Moreover there was no significant difference between the GnT I activities in bFF from dominant follicles collected before and after surge of luteinizing hormone (LH surge). These data suggest that the GnT I activity in bFF may reflect functional changes in the microenvironment which lead to follicular atresia.

Key words: *N*-acetylglucosaminyltransferase I, dominant follicle, atretic follicle, follicular fluid, bovine

Development of a follicular wave in monovular species is a complex process in which a single follicle continues to grow and develop while the rest of the follicles cease growing and regress. The beginning of the difference in growth rates between the two largest follicles is termed 'follicle deviation'[1] and in cattle, that deviation occurs when the largest follicle reaches a mean diameter of 8.5 mm.[2] The process of follicle selection is under systemic regulation by follicle-stimulating hormone (FSH) and LH as well as local regulation by factors that modulate the actions of gonadotropins.[3] The selected follicle continues to grow as a dominant follicle, whereas the other follicles undergo atresia.[4] Other factors such as follicle growth inhibitory factors may act independently of gonadotropins in follicle selection;[5] however, their possible action has not as yet been convincingly demonstrated.

In reproductive physiology, follicular atresia is a key phenomenon in follicle selection, which determines the species-specific number of newborn. The apoptosis that was first reported by Kerr *et al*.[6] is a determinant in follicular atresia.[7] It has been reported that sugar chain moieties of glycoconjugates on the cell surface are involved in apoptotic cell death.[8,9] Since complex *N*-glycans are the most heterogeneous of the *N*-glycans of glycoproteins,[10] these glycans may be involved in apoptotic cell death[11] as well as in various biological phenomena as typified by congenital disorders of glycosylation type IIa.[12,13] The conversion of oligomannose *N*-glycans to complex or hybrid *N*-glycans with antenna structures is catalyzed by *N*-acetylglucosaminyltransferases (GnT I to VI), α-1,6-fucosyltransferase and α-mannosidases. The initial step in this process is catalyzed by GnT I;[10] this suggests that high expression of GnT I activity may be involved in apoptotic cell death through the conversion of oligomannose *N*-glycans to complex or hybrid *N*-glycans of a key glycoprotein.

Glycosyltransferases catalyze the transfer of a sugar moiety from a sugar nucleotide as a donor to an acceptor. Those which are type II membrane proteins are generally localized in the Golgi apparatus[14] and it has been reported that some of these enzymes are released through cleavage by an endogenous protease or proteases, and are then secreted from the cell.[15] Indeed, many glycosyltransferases have been detected as soluble forms in extracellular fluids such as testicular and epididymal fluids,[16] uterine and oviductal fluids[17] as well as in serum,[18,19] colostrum,[20] amniotic fluid and milk.[21] It has been documented, in addition, that proteolytic cleavage and secretion of glycosyltransferases into extracellular fluids are affected in various pathological conditions such as malignancy and inflammation[15,17,18] as well as the progress of the estrous cycle.[17] These findings suggest that the activities of soluble glycosyltransferases may have significant physiological roles, including apoptotic cell death, in some mammalian tissues.

In view of the above, we examined the soluble GnT I activity in the bovine follicular fluid (bFF) from large

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atretic follicles as well as from large dominant follicles.

MATERIALS AND METHODS

Materials. The following manno oligosaccharides were purchased from Funakoshi Co., Tokyo, Japan; α1-3 mannobiose [Man(α1-3)Man], α1-6mannobiose [Man(α1-6)Man], α1-3α1-6mannotriose [Man(α1-6)[Man(α1-3)] Man] and α1-3α1-6mannopentaose [Man(α1-6)[Man(α1-3)]Man(α1-6)[Man(α1-3)]Man]. Adenosine-5′-triphosphate (disodium salt) (ATP), D-*N*-acetylglucosamine (D-GlcNAc) and uridine 5′-diphospho-*N*-acetylglucosamine (disodium salt) (UDP-GlcNAc) were obtained from Sigma Co., St Louis, USA. UDP-[6-^{3}H]-GlcNAc (16 Ci/ mmol) was from PerkinElmer, Inc., MA, USA. Bio-Rad AG1-X8 ion exchange resin (Cl^- form, 200–400 mesh) was purchased from Bio Rad Laboratories, Hercules, USA.

Preparation of follicular fluid. The ovaries from Holstein cows were collected at a local slaughterhouse within 10–20 min after death and transported to the laboratory on ice. The stage of the estrous cycle was defined by microscopic observation of the ovaries.[22] The bFF were aspirated from antral follicles using a disposable syringe with a 20-guage needle. Aspirated bFF was centrifuged at 1000× g for 5 min to remove granulosa cells, then the weight of bFF was measured. The diameter (mm; D) of follicle was estimated using the following equation: $D = 14.077 \times W^{0.311}$, where W represents the weight (g) of bFF. This equation was obtained from a preliminary experiment in which the correlation between the weight of bFF and follicular diameter was examined (Murasawa *et al.*, personal communication). The bFF from follicles whose diameter was over 8 mm were not pooled but were investigated individually; the bFF from small follicles, whose diameter was 2–6 mm, were pooled. All bFF samples were centrifuged to remove follicular debris and stored at −30°C prior to hormone or GnT I assay. All bFF samples were classified on the basis of their estradiol-17β (E_2) / progesterone (P_4) ratio. This was calculated from the E_2 and P_4 concentrations in bFF. The bFF from a dominant follicle was defined as having a E_2/P_4 ratio$>$1 while that from an atretic follicle had a E_2/P_4 ratio $<$ 1.

Preparation of follicular fluids from collected follicles in vivo. Holstein cows were kept under the normal management program of the Center for Field Science of the university and fed with corn silage, hay and concentrate, with permanent free access to water. Experimental procedures complied with the Guide for Care and Use of Agriculture Animals of Obihiro University. To induce luteolysis all cows received 500 μg of a prostaglandin F2α (PGF 2α) analogue (cloprostenol [estrumate]; Sumitomo Pharm. Co., Osaka, Japan) at the middle stage of the estrous cycle, and were assigned randomly as follows: Group 1) Follicular aspiration was performed at a luteal phase. A new follicular wave and ovulation of the preovulatory follicle was induced by a GnRH analogue (Fertirelin acetate 100 μg; [Conceral]; Takeda Pharm. Co., Osaka, Japan) injected 48 h after PGF2α. A dominant follicle was aspirated 7 days after GnRH injection, Group 2) Follicular aspiration was performed at follicular phase before LH surge. A dominant follicle was aspirated 42 h after an injection of PGF2α, Group 3) Follicular aspiration was performed at the follicular phase after LH surge. GnRH injected 48 h after PGF2α to induce endogenous LH surge. A dominant follicle was aspirated 24 h after GnRH injection. The day of follicular aspiration was designated as Day 0.

The dominant follicle of each cow was aspirated by transvaginal ultrasound-guided aspiration. For ultrasound guidance of the aspiration needle, an ultrasound scanner (SSD-5500, Aloka Co., Tokyo, Japan) equipped with a 7.5 MHz transvaginal convex transducer (UST-M15-21079, Aloka Co.) attached to an 18G stainless steel needle guide was used. When no dominant follicle could be identified under ultrasound, all follicles with diameters greater than 8 mm were aspirated. All bFF were brought to the laboratory on ice, and then centrifuged to remove follicular debris. The bFF samples were kept separately at −30°C until hormone or GnT I assay.

Estradiol-17β and progesterone determination. E_2 and P_4 concentrations in each of the follicular fluids were determined by double-antibody enzyme immunoassays (EIA) using 96-well ELISA plates (Corning Glass Works, Corning, NY), as described previously.[23] Steroid assays were performed after diethyl ether extractions, follicular fluid being diluted 50 or 500-fold to permit measurement of E_2 concentrations in EIA within the optimal range of the standard curve.[24] The standard curve for P4 ranged from 0.05 to 50 ng/mL, and the effective dose (ED_{50}) of the assay was 1.1 ng/mL.[25] The intra- and interassay coefficients of variation (CVs) were 4.7 and 6.5%, respectively, and the recovery rate for P_4 (1 ng) added to 1 mL samples was 95%. The standard curve for E_2 ranged from 2 to 2000 pg/mL, and the ED_{50} of the assay was 72 pg/mL. The intra- and interassay CVs were 6.8 and 8.8%, respectively. The recovery rate of E_2 was 75%.

Determination of GnT I activity of follicular fluid. GnT I activity was assayed by measuring the incorporation of [^{3}H]-GlcNAc into the substrate mannopentaose, as described previously for GnT I in rat testicular and epididymal fluid.[16]

The incubation mixture (100 μL) consisted of 50 mM sodium cacodylate buffer (pH 5.8), 20 mM $MnCl_2$, 2 mM ATP, 200 mM D-GlcNAc, 1.5 mM UDP-GlcNAc containing UDP-[^{3}H]-GlcNAc (10 kBq/ 100 μL incubation mixture), 6.7 mM α1-3α1-6 mannopentaose and 10 μL of follicular fluid. Parallel incubations without an acceptor were performed as well. After incubation for 4 h at 37°C, the mixture was cooled in ice to stop the enzyme reaction and immediately passed through 4 mL of Bio-Rad AG1-X8 ion exchange resin (Cl^- form, 200–400 mesh), contained in a Pasteur pipette, to remove residual UDP-[^{3}H]-GlcNAc. Immediately after the elution, the incorporation of [^{3}H]-GlcNAc was determined by liquid scintillation counting using a Packard Tri-Carbo scintillation counter.

The concentration of protein in bFF was determined by the Lowry method[26] using bovine serum albumin as the standard.

Statistical analysis. Although the activities of GnT I were usually expressed with the amount of radio activity of the reaction products, the values for the GnT I activities were converted into relative ratios on the basis of the

GnT I activity of dominant follicular fluid to simplify Figs. 6 and 7. The data are presented as mean and standard error of the mean (SEM). The significance of differences in GnT I activity of examined follicular fluid was assessed by the student t-test. Differences were considered significant at $p<0.01$.

RESULTS

The GnT I activity was characterized using bFF from dominant follicles.

Optimum pH.

Preliminary experiments with 50 mM sodium cacodylate buffer (pH 7.0) showed that bFF from both dominant and atretic follicles had significant GnT I activity, catalyzing the transfer of GlcNAc from UDP-GlcNAc to α1-3α1-6 mannopentaose. The GnT I activity of both fluids was optimal at pH 5.8. The data on Fig. 1 were obtained with the bFF from dominant follicles. The activities increased beyond pH 7.0 with sodium cacodylate buffer. The assay was performed with another buffer, *i.e*. Tris-HCl buffer to clarify if this increase is observed in the experiment using it, too. In subsequent experiments all measurements were done at the optimum pH. The GnT I activity increased linearly in proportion to the amount of these fluids present in the reaction mixture and to the reaction times (data not shown).

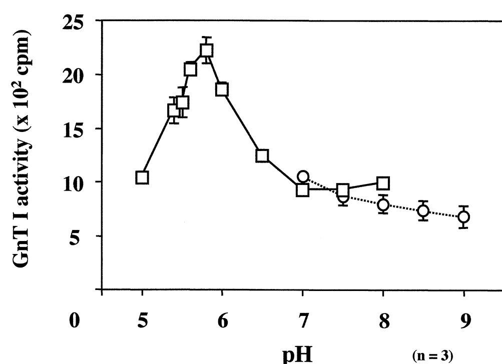

Fig. 1. Effect of pH on N-acetylglucosaminyltransferase I (GnT I) activity in bovine follicular fluid (bFF) from dominant follicles.

The GnT I activity was estimated as described in METHODS. The solid line and square (—□—) indicate the expression of GnT I activity in sodium cacodylate buffer, and the dotted line and circle (---○---) indicate the expression of the GnT I activity in Tris-HCl (50 mM, pH 7–9). Numbers in parenthesis indicate the repetitions of the experiment.

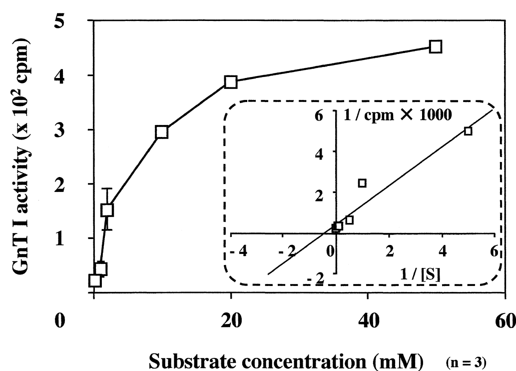

Fig. 2. Effect of substrate (α1-3α1-6 mannopentaose) concentration on GnT I activity in bFF, measured at the optimum pH of 5.8 (see Fig. 1).

Inset shows the Lineweaver-Burk plot. Numbers in parenthesis indicate the repetitions of the experiment.

Protein concentration in both dominant and atretic follicular fluids; specific activity.

No significant differences in protein concentration between the bFF from dominant and atretic follicles or between bFF obtained before or after LH surge *in vivo* (4.3 +/− 0.1%) were detected. The highest specific GnT I activity was in the pooled bFF collected from atretic follicles whose diameter was 2 to 6 mm (21.1 +/− 1.7 nM products/h/mg protein of bFF), while the lowest was in bFF from a dominant follicle (12.2 +/− 2.0 nM products/h/mg protein of bFF). The characterization of the GnT I activity in bFF was performed using pooled bFF from dominant follicles.

K_m values and substrate specificities.

The effects of changes in substrate concentration on GnT I activity are shown in Fig. 2. The apparent Km value for α1-3α1-6 mannopentaose, calculated form Lineweaver-Burk plots, was 2.17 mM.

Four different manno oligosaccharides, all tested at 6.7 mM, were examined as possible acceptors for the GnT I in bFF. Their effectiveness was in the order: α1-3α1-6 mannopentaose$>$$\alpha$1-3$\alpha$1-6 mannotriose$>$$\alpha$1-3 mannobiose. No activity was detected using α1-6 mannobiose (Fig. 3).

Effect of bivalent cations.

The GnT I activity in bFF had an absolute requirement for either Mn^{2+} or another bivalent cation of which Co^{2+} was the most effective, followed by Mn^{2+} and Mg^{2+} in that order (Fig. 4). The optimum concentration of Mn^{2+} was 50 mM (Fig. 5). No GnT I activity could be demonstrated in either fluid when Zn^{2+}, Cd^{2+} or Ca^{2+} was used instead of Mn^{2+}.

Comparison of GnT I activities in bFF from dominant and atretic follicles.

The bFF from atretic follicles had higher GnT I activities than the bFF from dominant follicles, particularly in the case of the large follicles (Fig. 6). However, neither

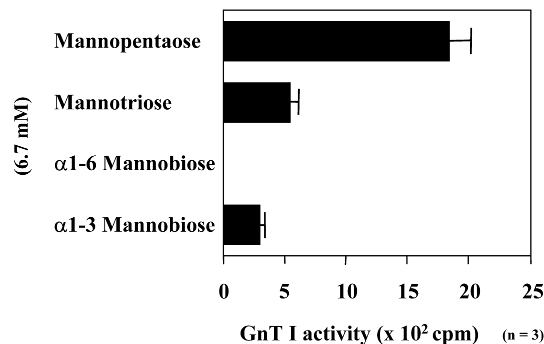

Fig. 3. Substrate specificity of GnT I activity in bFF.

Conditions were as in Fig. 2, except that the substrate was varied. Each concentration of substrate was 6.7 mM. Numbers in parenthesis indicate the repetitions of the experiment.

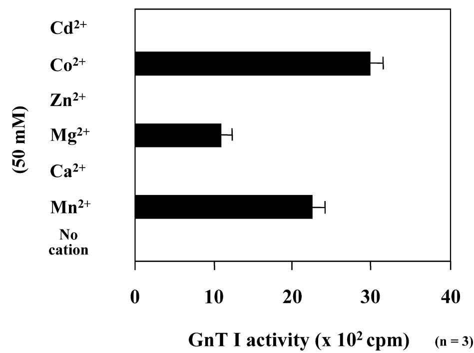

Fig. 4. Effects of various bivalent cations on GnT I activity of bFF.

Conditions were as in Fig. 2, except that the bivalent cation was varied. The values were measured with 50 mM of each cation. Numbers in parenthesis indicate the repetitions of the experiment.

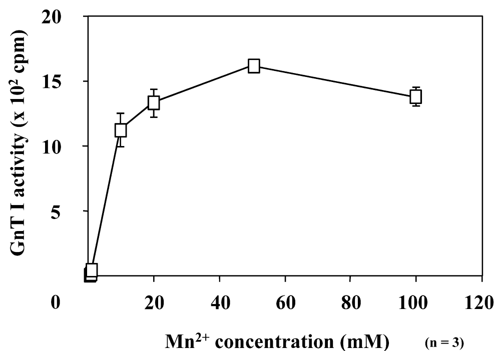

Fig. 5. Effect of Mn^{2+} concentration on GnT I activity in bFF.

Conditions were as in Fig. 2, except that the Mn^{2+} concentration was varied. Numbers in parenthesis indicate the repetitions of the repetitions of the experiment.

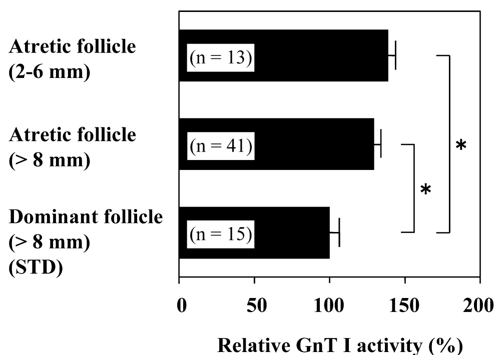

Fig. 6. Relative GnT I activities in bFF from small and large atretic follicles, and the activity in bFF from a dominant follicle.

The activity in the bFF from the dominant follicle is defined as being 100%. The results are expressed as means +/−SEM. The letters (*) above the bars denote a significance difference at $p<$ 0.01. Conditions were as in Fig. 2, except that the 50 mM Co^{2+} was used instead of Mn^{2+}. Numbers in parenthesis indicate the repetitions of the experiment.

the bFF from dominant nor the bFF from atretic follicles showed significant differences of GnT I activity with respect to follicular size (data not shown). By the student t-test, no significant differences of the GnT I activities were observed among the following three follicular fluids: dominant follicles at middle luteal phase (diameter: 16.9 +/− 1.5 mm), before LH surge (14.4 +/− 2.8), and after LH surge (21.5 +/− 2.2) (Fig. 7). But the differ-

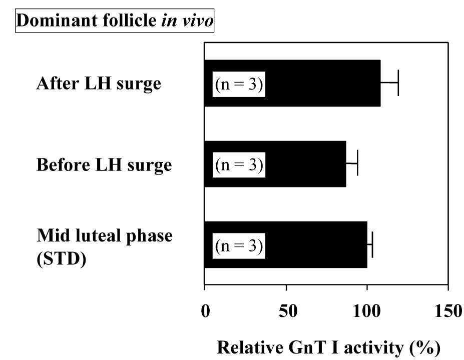

Fig. 7. Relative GnT I activities in bFF collected from large dominant follicles at middle luteal phase, before and after LH surge *in vivo*.

The results are represented as in Fig. 6, except that the values are expressed relative to the activity in large dominant follicles, which is used as the standard (STD), whose bFF had been aspirated *in vivo* at middle luteal phase, and there are no letters above the bars. Conditions were as in Fig. 6. Numbers in parenthesis indicate the repetitions of the experiment.

ence of the GnT I activities in the bFF from dominant follicle at the these phases may show that this enzyme activity decreased before LH surge and then increased after LH surge, again.

DISCUSSION

This is the first report indicating that GnT I activities are present in mammalian follicular fluid. The properties of this enzyme activity, such as optimum pH and requirement for bivalent cations, were similar to those for the GnT I activities of rat liver,[27] rabbit liver[28] as well as rat testicular fluid[16] showing that this enzyme is GnT I, but were different from those for rat epididymal fluid[16] as shown in Table 1. The enzyme has the substrate specificities for α1-3α1-6 mannopentaose, α1-3α1-6 mannotriose, and α1-3 mannobiose, but not for α1-6 mannobiose; this is typical substrate specificity for GnT I.

Glycosyltransferases are generally localized in the Golgi apparatus and require sugar nucleotides for the glycosylation.[14] Since the presence of sugar nucleotides in follicular fluid has not as yet been demonstrated, it is still unclear whether carbohydrate modifications of glycoconjugates by soluble glycosyltransferases occur on the granulosa cell surface or on soluble peptides in the follicular fluid during follicular development. In addition one cannot exclude the possibility that glycosylation by soluble glycosyltransferases occurs in the extracellular fluid since sugar nucleotides as well as soluble glycosyltransferase activities have been detected in the milk or colostrum of some mammalian species.[29,30] Furthermore, Lazarowski *et al*.[31] reported the release of UDP-glucose from human astrocytoma, bronchial epithelial cells, and rat glioma cells into the extracellular environment. These observations suggest that glycosylation may occur in the extracellular fluid. If UDP-GlcNAc were detected in bFF, this would suggest that carbohydrate modification of glycoconjugates on the granulosa cell surface or on the soluble peptides by this GnT I activity occurs in follicular fluid during follicular development.

The GnT I activity in bFF from an atretic follicle was

Table 1. GnT I activity of bovine follicular fluid; comparison with those of male genital tract fluids and liver of rat and liver of rabbit.

Enzyme source	Optimun pH	K_m value (mM)*2	Effect of bivalent cation	Effective Mn^{2+} conc. (mM)	Specific activity*5
Bovine follicular fluid*1	5.8	2.17	$Co^{2+}>Mn^{2+}>Mg^{2+}$*3	50	0.20
Rat testicular fluid	6.0	0.57	$Co^{2+}>Mn^{2+}>Mg^{2+}>Ca^{2+}$*3	100	0.039
Rat cauda epididymal fluid	7.0	0.38	$Mn^{2+}>Ca^{2+}$*3	50〜*4	0.044
Rat liver[27]	5.5	0.4	$Co^{2+}>Mn^{2+}>Cd^{2+}>Mg^{2+}>Ca^{2+}>Zn^{2+}$	20〜100	0.18*6
Rabbit liver[28]	5.6	0.25	$Mn^{2+}>Co^{2+}>Mg^{2+}>Cd^{2+}>Ca^{2+}$	70	0.31

*1Collected from dominant follicles. *2α1-3α1-6 mannopentaose was used as a substrate for GnT I activities in both bovine follicular fluid and rat male genital tract fluids, while heptasaccharide peptide was used as a substrate for that in rat and rabbit liver. *3Zn^{2+} and Cd^{2+} had no effect on GnT I activity. *4At Mn^{2+} concentration above 50 mM the cauda epididymal fluid became insoluble. *5nmol of products amount/min/mg of protein. *6Enzyme activity of tissue homogenates.

significantly higher than that in bFF from a dominant follicle (Fig. 6). However, there was no follicular size-dependence on the intensity of the GnT I activity in the follicular fluid (data not shown). Additionally, the GnT I activity in bFF from a dominant follicle had no susceptibility to a LH surge (Fig. 7), the conclusive signal inducing ovulation. This suggests that LH surge does not affect the conversion of oligomannose to complex or hybrid *N*-glycans of glycoproteins in the mature follicles. The present data rather point to the possibility that the conversion of *N*-glycans from oligomannose to complex type occurs on the glycoconjugates of the atretic follicle. Kimura *et al*.[32,33] reported that sugar chain modification of some glycoconjugates of granulosa cells may be involved in granulosa cell apoptosis during follicular atresia in the pig, and they also suggested that complex *N*-glycans are involved in granulosa cell apoptosis.[34] These observations support the contention that glycosyl conversion by the GnT I activity detected in this study might be involved in the granulosa cell apoptosis during follicular atresia. Therefore the difference between the GnT I activity in fluids from dominant and atretic follicles may be caused by the expression of the GnT I enzyme protein in granulosa cells; this remains to be elucidated.

Recent studies show that glycosyltransferases have biological functions other than glycoconjugate biosynthesis. For example, it has been reported that the GnT V enzyme induces the release of fibroblast growth factor-2 from heparin sulfate proteoglycan in human umbilical vein endothelial cells, leading to angiogenesis.[35] Similarly it could be suggested that the high expression of GnT I activity in the atretic follicle might be involved in granulosa cell apoptosis, even in the absence of UDP-GlcNAc from the bFF. The relationship between the conversion of oligomannose to complex *N*-glycans of glycoconjugates on the granulosa cell surface by soluble GnT I in follicular fluid from atretic follicle and granulosa cell apoptotic cell death during follicular atresia needs to be clarified. To this end it may be necessary, from the results of experiments which co-cultured granulosa cells in which GnT I is over-expressed with exogenous UDP-GlcNAc, to determine the facilitatory effect of the soluble GnT I activity in the extracellular fluid on the induction of apoptosis.

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REFERENCES

1) O.J. Ginther, K. Kot, L.J. Kulick and M.C. Wiltbank: Emergence and deviation of follicles during the development of follicular waves in cattle. *Theriogenology*, **48**, 75–87 (1997).
2) O.J. Ginther, M.C. Wiltbank, P.M. Fricke, J.R. Gibbons and K. Kot: Selection of the dominant follicle in cattle. *Biol. Reprod*., **55**, 1187–1194 (1996).
3) O.J. Ginther, M.A. Reg, D.R. Bergfelt, F.X. Donadeu, M.C and K. Kot: Follicle selection in monovular species. *Biol. Reprod*., **65**, 638–647 (2001).
4) G.B. Marion, H.T. Gier and J.B. Choudary: Micromorphology of the bovine ovarian follicular system. *J. Anim. Sci*., **27**, 451–465 (1968).
5) D.G. Armstrong and R. Webb: Ovarian follicular dominance: the role of intraovarian growth factors and novel protein. *Rev. Reprod*., **2**, 139–146 (1997).
6) J.F. Kerr, A.H. Wyllie and A.R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer*, **26**, 239–257 (1972).
7) F.M. Hughes Jr. and W.C. Gorospe: Biochemical identification of apoptosis (programmed cell death) in granulosa cells: evidence for a potential mechanism underlying follicular atresia. *Endocrinology*, **129**, 2415–2422 (1991).
8) V. Bazil, J. Brandt, A. Tsukamoto and R. Hoffman: Apoptosis of human hematopoietic progenitor cells induced by crosslinking of surface CD43, the major sialoglycoprotein of leukocytes. *Blood*, **86**, 502–511 (1995).
9) S. Akamatsu, S. Yazawa, K. Zenita, H. Matsumoto, T. Tachikawa and R. Kannagi: Elevation of an α(1,3)fucosyltransferase activity correlated with apoptosis in the human colon adenocarcinoma cell line, HT-29. *Glycoconj. J*., **13**, 1021–1029 (1996).
10) A. Kobata: Structures and functions of the sugar chains of glycoproteins. *Eur. J. Biochem*., **209**, 483–501 (1992).
11) M. Demetriou, M. Granovsky, S. Quaggin and J.W. Dennis: Negative regulation of T-cell activation and autoimmunity by Mgat5 *N*-glycosylation. *Nature*, **409**, 733–739 (2001).
12) T.W. Rademacher, R.B. Parekh and R.A. Dwek: Glycobiology. *Annu. Rev. Biochem*., **57**, 785–838 (1988).
13) H. Schachter: Congenital disorders involving defective *N*-glycosylation of proteins. *Cell Mol. Life Sci*., **58**, 1085–1104 (2001).
14) J.C. Paulson and K.J. Colley: Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. *J. Biol. Chem*., **264**, 17615–17618 (1989).
15) S. Kitazume, Y. Tachida, R. Oka, K. Shinohara, T.C. Saido and Y. Hashimoto: Alzheimer's β-secretase, β-site amyloid precursor protein-cleaving enzyme, is responsible for cleavage secretion of a Golgi-resident sialyltransferase. *Proc. Natl. Acad. Sci*., **98**, 13554–13559 (2001).
16) H. Nozaki, T. Matsuzawa, T. Nakamura, I. Arai and T.

Urashima: Are there two forms of β2-*N*-acetylglucosaminyltransferase I in rat testicular and epididymal fluids? *Biochim. Biophys. Acta*, **1649**, 140-145 (2003).

17) D.R. Tulsiani, C.A. Chayko, M.C. Orgebin-Crist and Y. Araki: Temporal sugar glycosyltransferases activities in the genital tract of the hamster during the estrous cycle. *Biol. Reprod*., **54**, 1032–1037 (1996).

18) H.A. Kaplan, B.M. Woloski, M. Hellman and J.C. Jamieson: Studies on the effect of inflammation on rat liver and serum sialyltransferase. Evidence that inflammation causes release of Gal β1 leads to 4GlcNAc α2 leads to 5 sialyltransferase from liver. *J. Biol. Chem*., **258**, 11505–11509 (1983).

19) A. Sarnesto, T. Kohlin, J. Thurin and M. Blaszczyk-Thurin: Purification of H gene-encoded β-galactoside α 1-2 fucosyltransferase form human serum. *J. Biol. Chem*., **265**, 15067–15075 (1990).

20) A. Elhammer and S. Kornfeld: Purification and characterization of UDP-*N*-acetylglucosamine: polypeptide *N*-acetylgalactosaminyltransferase from bovine colostrums and murine lymphoma BW5147 cells. *J. Biol. Chem*., **261**, 5249–5255 (1986).

21) A.C. gerber, I. Kozdrowski, S.R. Wyss and E.G. Berger: The charge heterogeneity of soluble human galactosyltransferase isolated from milk, amniotic fluid and malignant ascites. *Eur. J. Biochem*., **93**, 453–460 (1979).

22) J.J. Ireland, R.L. Murphee and P.B. Coulson: Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. *J. Dairy Sci*., **63**, 155–160 (1980).

23) T.J. Acosta, A. Miyamoto, T. Ozawa, M.P. Wijayagunawardane and K. Sato: Local release of steroid hormones, prostaglandin E_2, and endothelin-1 from bovine mature follicles *in vitro*: effects of luteinizing hormone, endothelin-1, and cytokines. *Biol. Reprod*., **59**, 437–443 (1998).

24) T.J. Acosta, T. Ozawa, S. Kobayashi, K. Hayashi, M. Ohtani, W.D. Kraetzl, K. Sato, D. Schams and A. Miyamoto: Preovulatory changes in the local release of vasoactive peptides, prostaglandin F (2α), and steroid hormones from bovine mature follicles *in vivo. Biol. Reprod*., **63**, 1253–1261 (2000).

25) A. Miyamoto, K. Okuda, F.J. Schweigert and D. Schams: Effects of basic fibroblast growth factor, transforming growth factor-β and nerve growth factor on the secretory function of the bovine corpus luteum *in vitro. J. Endocrinol*., **135**, 103–114 (1992).

26) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem*., **193**, 265–275 (1951).

27) G. Moller, F. Reck, H. Paulsen, K.J. Kaur, M. Sarkar, H. Schachter and I. Brockhausen: Control of glycoprotein synthesis: Substrate specificity of rat liver UDP-GlcNAc: Man α 3R β2-*N*-acetylglucosamintyltransferase I using synthetic substrate analogues. *Glycoconj. J*., **9**, 180–190 (1992).

28) Y. Nishikawa, W. Pegg, H. Paulsen and H. Schachter: Control of glycoprotein synthesis. Purification and characterization of rabbit liver UDP-*N*-acetylglucosaminyltransferase: α-3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I. *J. Biol. Chem*., **263**, 8270–8281 (1988).

29) A. Gil and F. Sanchez-Medina: Acid-soluble nucleotides of cow's, goat's and sheep's milks, at different stages of lactation. *J. Dairy Res*., **48**, 35–44 (1981).

30) A. Gil and F. Sanchez-Medina: Acid-soluble nucleotides of human milk at different stages of lactation. *J. Dairy Res*., **49**, 301–307 (1982).

31) E.R. Lazarowski, D.A. Shea, R.C. Boucher and T.K. Harden: Release of cellular UDP-glucose as a potential extracellular signaling molecules. *Mol. Pharmacol*., **63**, 1190-1197 (2003).

32) Y. Kimura, N. Manabe, H, Y. Imai, A. Myoumoto and H. Miyamoto: Histochemical examination of cellular carbohydrate chains which change during ovarian follicular atresia in the porcine ovary. *J. Jpn. Emb. Trans. Soc*., **19**, 101–110 (1997).

33) Y. Kimura, N. Manabe, H. Matsushita, C. Tajima, A. Myoumoto and H. Miyamoto: Examination of granulosa cell glycoconjugates which change during follicular atresia in the pig ovary. *J. Reprod. Dev*., **44**, 35–44 (1998).

34) Y. Kimura, N. Manabe, S. Nishihara, H. Matsushita, C. Tajima, S. Wada and H. Miyamoto: Up-regulation of the α2,6-sialyltransferase messenger ribonucleic acid increases glycoconjugates containing α2,6-linked sialic residues in granulosa cells during follicular atresia of porcine ovaries. *Biol. Reprod*., **60**, 1475–1482 (1999).

35) T. Saito, E. Miyoshi, K. Sasaki, N. Nakano, H. Eguchi, K. Honke and N. Taniguchi: A secreted type of β1,6-*N*-acetylglucosaminyltransferase V (GnT V) induces tumor angiogenesis without mediation of glycosylation: a novel function of GnT-V distinct from the original glycosyltransferases activity. *J. Biol. Chem*., **277**, 17002–17008 (2002).

ウシ優性卵胞と閉鎖卵胞の卵胞液中における *N*-アセチルグルコサミニルトランスフェラーゼ I 活性の解析

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ウシ優性卵胞と閉鎖卵胞の両卵胞液（bFF）中にβ2-*N*-アセチルグルコサミニルトランスフェラーゼI（GnT I）活性を検出した．本酵素活性は至適pHを5.8に示し，二価金属イオン絶対依存性（Co^{2+}＋>Mn^{2+}>Mg^{2+}）と基質特異性（α1-3α1-6マンノペンタオース>α1-3α1-6マンノトリオース>α1-3マンノビオース）を有していた．α1-3α1-6マンノペンタオースに対するK_m値は2.17 mMであった．閉鎖卵胞のbFF中GnT I活性は優性卵胞のbFF中のそれより顕著に高かった．一方，*t*-検定解析により，優性卵胞のbFF中のGnT I活性はLHサージの前後で有意な変化を示さなかった．以上の結果から，顕著に亢進した閉鎖卵胞のbFF中GnT I活性は卵胞の閉鎖化に関与している可能性が推察された．