Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 341 (2006) 13–18
BBRC
www.elsevier.com/locate/ybbrc

Ghitm is an ortholog of the *Bombyx mori* prothoracic gland-derived receptor (*Pgdr*) that is ubiquitously expressed in mammalian cells and requires an N-terminal signal sequence for expression

Tadashi Yoshida [a], Shinji Nagata [a,b], Hiroshi Kataoka [a,*]

[a] *Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Chiba 277-8562, Japan*
[b] *Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo, Tokyo 113-8657, Japan*

Received 8 December 2005
Available online 5 January 2006

Abstract

In a previous paper, we reported the cloning of a cDNA encoding a putative receptor, *Pgdr*, from the prothoracic gland of the silkworm, *Bombyx mori*. Few studies concerning the orthologous cDNA of *Pgdr* in mammals, a growth hormone-inducible transmembrane protein (*Ghitm*) that encodes a putative receptor, have been performed. Analysis of the distribution of *Ghitm* expression revealed ubiquitous expression in mouse embryo and adult tissues, as well as mammalian cell lines. The pattern of *Ghitm* expression suggested that once *Ghitm* mRNA was expressed in the putative brain region of mouse embryo, *Ghitm*-expressing cells spread ubiquitously throughout all tissues during embryonic development. In addition, Western blot analyses demonstrated that cleavage of the N-terminal portion in GHITM appears to regulate the expression level, suggesting that cleavage is essential for the proper expression of GHITM.

Keywords: *Ghitm*; *Pgdr*; GPCR; Signal peptide

In a previous paper, we reported the molecular cloning of a cDNA encoding a putative heptahelical receptor from the silkworm, *Bombyx mori*, designated as *Pgdr* (Prothoracic Gland-derived Receptor) [1]. *Pgdr* was identified from the prothoracic gland as a novel cDNA candidate encoding the receptor for prothoracicotropic hormone, which is crucial for the regulation of insect molting and metamorphosis [2,3]. A database search of the novel *Pgdr* cDNA sequence in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) showed a number of cDNAs encoding similar sequences. The deposited cDNA sequences have been identified from many animal species belonging to diverse animal phyla, such as fruitfly, nematode, mouse, and human. Of the orthologous cDNAs, *Ghitm* (Growth hormone-inducible transmembrane protein) is the first gene identified from mouse brown adipose tissue as a growth hormone-inducible gene [4]. Three important observations concerning *Ghitm* expression have been reported, so far. Li et al. [4] proposed that *Ghitm* functions in tumorigenesis and in adipose tissues. Nagel et al. [5] revealed that the expression of *Ghitm* mRNA was induced by chemokines in T cells. Zou et al. [6] showed that *Ghitm* mRNA expression was activated in accordance with aging in *Drosophila melanogaster*. These observations, together with the involvement of *Pgdr* in insect molting and metamorphosis, strongly suggest that *Ghitm*/*Pdgr* plays important roles in regulating multiple biological functions, such as GH action, aging, and the immune systems of many different animal phyla.

To commence with a functional analysis of *Ghitm*/*Pgdr* genes in mammals, we here investigated the expression pattern of *Ghitm*/*Pgdr*. Since there have been few reports regarding the expression pattern of *Ghitm* throughout mouse growth and development, we assessed the pattern of *Ghitm* mRNA expression in mouse embryos by in situ

* Corresponding author. Fax: +81 4 7136 3623.
E-mail address: kataoka@k.u-tokyo.ac.jp (H. Kataoka).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.12.141

hybridization and in adult mouse tissues by Northern blot analysis. We also showed that *Ghitm*/*Pgdr* mRNA and its protein are expressed in mammalian cell lines, such as HEK293, COS-7, and CHO. Furthermore, we also report that N-terminal cleavage of GHITM appears to be essential for its proper expression, a finding that was serendipitously observed during the course of experiments designed to monitor the while transient expression of N-terminally and C-terminally tagged-GHITM.

Materials and methods

In situ hybridization. Embryos of BALB/c mice were fixed in 4% paraformaldehyde/PBS solution overnight at 4 °C and then dehydrated by sequentially changing buffers from 25% ethanol/PBST to 100% ethanol. The dehydrated embryos were stored at −20 °C for further use. The embryos were re-hydrated by sequentially changing buffers to PBST. After treatment with proteinase K (2 μg/ml) for 15 min, the re-hydrated embryos were fixed using 0.2% glutaraldehyde and 4% paraformaldehyde and bleached in 6% H_2O_2/PBST. Hybridization was performed in a solution of 50% formamide and 5× SSC (pH 4.5) with 50 μg/ml yeast tRNA, 1% SDS, and 50 μg/ml heparin at 70 °C overnight. After hybridization, embryos were washed three times at 70 °C in 50% formamide/5× SSC (pH 4.5)/1% SDS, three times at 70 °C in 5× SSC (pH 4.5)/0.1% Tween 20, three times at 70 °C in 50% formamide/2× SSC (pH 4.5)/0.1% Tween 20, and finally five times at room temperature in 150 mM NaCl/100 mM Tris–HCl (pH 7.5)/0.1% Tween 20. The embryos were blocked with 1.5% blocking reagent (Roche) in TBST, incubated with anti-DIG antibody conjugated with alkaline phosphatase (Roche), washed five times in TBST, and signals were detected using NBT and BCIP (Promega).

Digoxigenin (DIG) labeled sense and antisense RNA probes were synthesized using the DIG RNA labeling mix (Roche) and SP6 or T7 RNA polymerase (Invitrogen) according to the manufacturer's instructions. The sequence of mouse *Ghitm* shown in Fig. 2B was utilized for preparation of both sense and antisense probes.

Northern hybridization. Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instruction. Mice examined in this study were anesthetized by interperitoneal injection of pentobarbital before dissection. Ten micrograms of the extracted RNAs was loaded in each lane of 1% formaldehyde containing agarose gels. After transferring the electrophoresed RNAs to Hybond N^+ nitrocellulose membranes (Amersham Pharmacia), the blots were hybridized to DIG labeled riboprobes (see in in situ hybridization section) overnight at 68 °C in ULTRAhyb Ultrasensitive hybridization buffer (Ambion). Filters were washed twice at 68 °C in 2× SSC, 0.1% SDS, and then twice at 68 °C in 0.1× SSC, 0.1% SDS. Membranes were incubated with an anti-DIG antibody conjugated to alkaline phosphatase and visualized using CDP-Star (Roche) as a substrate.

Cell culture and transfection. Human embryonic kidney 293 (HEK293) and COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Nacalai Tesque) supplemented with 10% fetal bovine serum (FBS) (JRH Bioscience). Chinese hamster ovary (CHO)-K1 and PC12h cells were grown in F-12 Nutrient Mixture (HAM) (Invitrogen) with 10% FBS and in DMEM with 10% FBS and 5% horse serum (Invitrogen), respectively. All cells were cultured at 37 °C under a humidified atmosphere containing 5% CO_2. PC12h cells were grown on collagen-precoated dishes (Iwaki). Subconfluent cells (60–70%) were transfected with 1 μg of each plasmid using Effectene Transfection Reagents (Qiagen) per 60 mm culture dish.

Immunoblotting. The transiently transfected cells, which were cultured for 48 h, were lysed in lysis buffer (20 mM Tris–HCl (pH 7.5), 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, and 10 mM of 2-mercaptoethanol). The cell lysates were centrifuged at 15,000 rpm for 10 min to remove insoluble debris. Thirty micrograms of samples was separated on a 10% SDS–polyacrylamide gel, which was then transferred onto a PVDF membrane. Signals were detected using an ECL-Advance Kit (Amersham Pharmacia) according to the manufacturer's instructions.

Polyclonal antiserum against a peptide corresponding with the outside of first transmembrane region of GHITM (REYATKTRIRTHRG KTGQELK; This peptide was synthesized as multiple antigen peptide) was raised in rabbit. The following antibodies were applied: monoclonal mouse (clone M2, Sigma) anti-FLAG antibody; monoclonal mouse (clone 9E10, Sigma) anti-myc antibody; HRP-conjugated anti-mouse antibody (DAKO); HRP-conjugated anti-rabbit antibody (Promega).

RNAi experiments. HeLa cells were transfected with siRNAs against human GHITM (sense; 5′-GGC CUC CCC UGU UGU GAA GTT-3′, antisense; 5′-CUU CAC AAC AGG GGA GGC CTT-3′) and lamin A/C (sense; 5′-CUG GAC UUC CAG AAG AAC ATT-3′, antisense; 5′-UGU UCU UCU GGA AGU CCA GTT-3′) using oligofectamine reagents (Invitrogen) according to manufacturer's protocol. Transfected cells were incubated for 48 h and gene translation was analyzed by immunoblotting.

Results

Whole mount in situ hybridization of early mouse embryos

Since *Ghitm* is a growth hormone inducible gene, we examined the expression pattern of *Ghitm* in mouse embryos using whole mount in situ hybridization by virtue of observation of developmental expression. While no *Ghitm* signal was detected in any mouse embryo tissue prior to E9.5 (embryonic day 9.5) (data not shown), *Ghitm* mRNA was expressed in a nerve cord projecting from the prosencephalon to the rhombencephalon of E9.5 mice (Fig. 1A). Increased levels of *Ghitm* expression were observed in the peripheral tissues of embryonic stages following the initiation of embryonic neuron *Ghitm* expression at E9.5 (data of E10.5 mice; Fig. 1A). These data demonstrated that *Ghitm* mRNA expression increased continuously in a whole body pattern throughout mouse embryonic development and was probably initiated by neural striking expression.

Northern blot analysis of Ghitm transcripts in adult mouse

We next performed Northern blot analysis of *Ghitm* transcripts from various adult mouse tissues. As shown in Fig. 1B, two transcripts (1.3 and 2.4 kb), probably a result of alternative splicing, were observed in various tissues as previously reported [4], although slightly different transcriptional levels of the two mRNAs were observed between tissues. In fact, in 5′- and 3′-RACE methodologies, we cloned two different sized cDNAs corresponding to both observed bands (data not shown). Even though two alternatively spliced variants of *Ghitm* were transcribed, sequencing of the substitution of splicing within the 3′-UTR of the *Ghitm* variants indicated that the ORF of the two transcripts was not affected by alternative splicing. *Ghitm* was ubiquitously expressed throughout the body. Relatively high levels of *Ghitm* expression were detected in the brain, heart, liver, kidney, adrenal gland, testis, iBAT, muscle, and stomach, whereas relatively low levels of *Ghitm* expression were observed in the intestine and thymus.

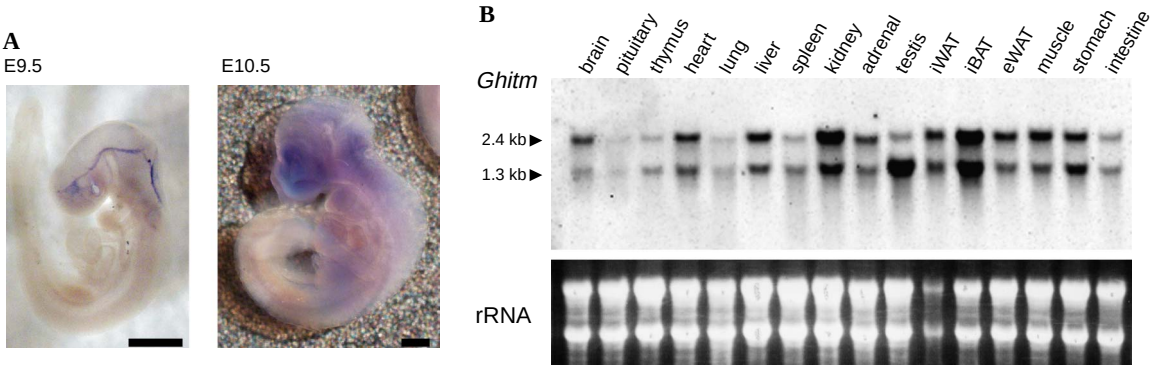

Fig. 1. *Ghitm* mRNA expression in mouse tissues. (A) Expression of *Ghitm* mRNA in the E9.5 and E10.5 mouse embryos, detected by whole mount in situ hybridization. Embryonic stages are indicated at the top of the panel. As control experiments, sense-strand riboprobes of *Ghitm* mRNA were used and no signal was detected (data not shown). Scale bars: 500 μm. (B) Tissue distribution of *Ghitm* mRNAs. Total RNAs were prepared from brain, pituitary, thymus, heart, lung, liver, spleen, kidney, adrenal, testis, iWAT, iBAT, eWAT, muscle, stomach, and intestine of male mice at 10 weeks of age. Ribosomal RNAs (28S and 18S) were used for control.

Ghitm mRNA was highly expressed in many cultured cell lines

Since *Ghitm* was expressed ubiquitously, we investigated whether *Ghitm* mRNA was expressed in commonly used available cell lines. *Ghitm* mRNAs were detected in the various cell lines examined (Fig. 2A); human (HEK293), monkey (COS-7), hamster (CHO), and rat (PC12h). In this Northern blot hybridization analysis, *Ghitm* transcripts were detected using a cRNA probe encoding mouse *Ghitm* because of its high homology with other mammalian *Ghitm* genes (85–95% nucleotide identity). As shown in Fig. 2A, the probe hybridized successfully with the *Ghitm* transcripts derived from all cell lines, and also hybridized to both alternative splicing transcripts (1.3 and 2.4 kb) similar to the data in Northern blot analysis in adult mouse.

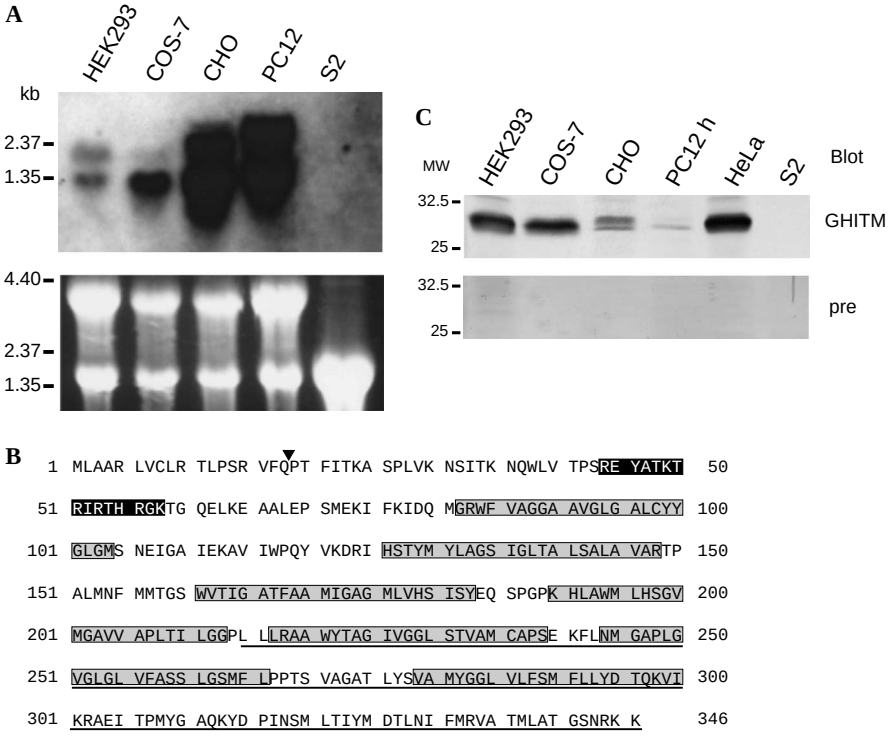

Fig. 2. *Ghitm* gene expression analyses in mammalian cell lines. (A) Northern blot analysis of mammalian *Ghitm* mRNA in cultured cell lines. Total RNAs extracted from these cell lines were blotted and *Ghitm* expression was determined as described in Fig. 1B. As a control, ribosomal RNAs were observed (lower panel). Schneider's S2 cells (derived from *Drosophila* embryonic tissues) were used as a control for non-mammalian cell lines. (B) Amino acid sequence of the mouse *Ghitm* gene. Shaded boxes represent putative transmembrane domains. Peptide sequence used for immunization is shown in a black box. Potential cleavage site determined by SignalP program is indicated with an arrowhead. The site of the cRNA probe used for Northern blotting and in situ hybridizations is underlined. (C) Western blot analysis of mammalian GHITM protein extracted from cultured cell lines. As a control, analyses were performed using pre-immunized serum (''pre'' in the figure).

On the other hand, in *Drosophila* (S2) cells, *Ghitm*/*Pdgr* was not detected with the mouse *Ghitm* probe using our experimental conditions, likely as a result of its low homology (54% nucleotide identity). While differences in hybridization stringency to the mouse *Ghitm* probe prevented direct comparison of the *Ghitm* mRNA expression levels across the cell lines, the *Ghitm* mRNAs appeared to be highly expressed in all the cell lines. The expression of *Ghitm* in these cell lines suggests that the *Ghitm* transcript may be easily manipulated for addressing functional elucidation.

The expression of GHITM proteins in cultured cell lines is controlled by its N-terminal signal sequence

Ghitm expression in the mammalian cell lines allowed us to investigate the functional role of the protein following molecular biology based manipulation of the transcript. To confirm GHITM protein expression in the cell lines, we generated a polyclonal antibody against a synthetic peptide corresponding to an N-terminal portion of the mouse GHITM protein. Immunoblotting analyses using the antibody showed that GHITM was actively expressed in these cell lines. The immunoreactive band detected in the Western blots corresponded to a protein of ~27 kDa, indicating that GHITM protein was translated in the examined cells (Fig. 2C). However, the observed band size of 27 kDa is significantly different from the calculated molecular weight of GHITM (37 kDa). To determine if the immunoreactive band detected in the Western blots is derived from the *Ghitm* gene product, we transfected an expression vector containing FLAG or myc-tagged *Ghitm* cDNA into HeLa cells (Figs. 3A and B). Interestingly, significantly different

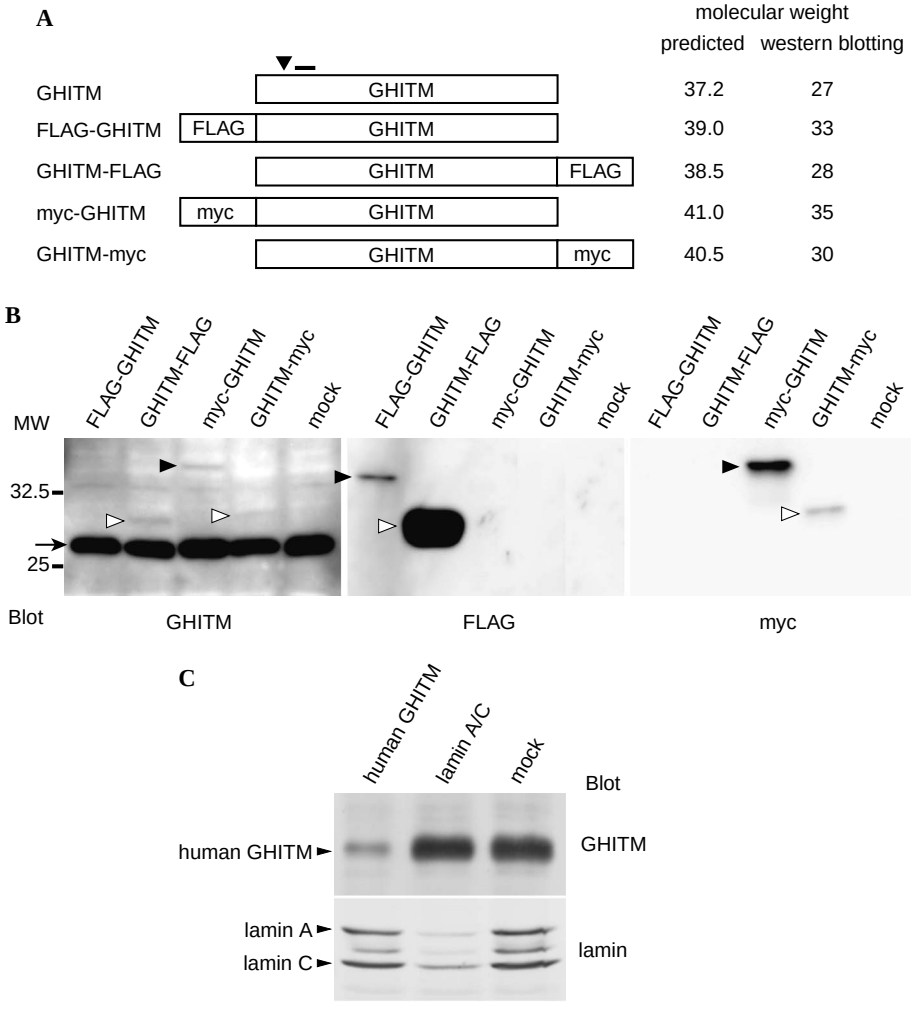

Fig. 3. N-terminal cleavage of mature GHITM protein in mammalian cells. (A) Schematic representation of the mouse Ghitm expression vector constructions. Arrowhead indicates the potential cleavage site. The peptide sequence used to produce the anti-mouse GHITM antibody is indicated as a bar. The predicted molecular weights and actual molecular weights observed in western blot analyses as shown in (C) and the name of each construct are indicated. (B) Western blot analysis of GHITM proteins expressed in HeLa cells. Each expression construct was transfected, incubated for 48 h and the cells were collected. Western blot analysis was performed as described in Fig. 2B. Antibodies used for immunoblotting are indicated below the panels. Individual proteins are indicated with arrow (human GHITM), arrowhead (N-terminally tagged mouse GHITM), or open arrowhead (C-terminally tagged mouse GHITM). FLAG-GHITM expression was below the detection level of the anti-GHITM antibody. (C) Suppression of GHITM expression in HeLa cells. siRNAs corresponding to the human GHITM and lamin A/C were transfected into HeLa cells and gene translation was analyzed by Western blotting. Lamin A/C was used as a positive control of transfection efficiency and as a negative control for siRNA target specificity.

migration patterns were observed in the Western blots depending on the location of the attached tag sequences (Fig. 3B). While the theoretical molecular weights of the N- and C-terminally tagged GHITM proteins were nearly identical, C-terminally tagged GHITM were significantly smaller than N-terminally tagged GHITM (Fig. 3A). This different migration implies that the reduced size of the native GHITM protein may be attributable to an N-terminal truncation. The SignalP server program (http://www.cbs.dtu.dk/services/SignalP/) predicted the presence of a signal peptide at the N-terminus of GHITM (Figs. 2B and 3A). In our results, the N-terminally tagged GHITM proteins were significantly larger in size than the C-terminally tagged GHITM proteins, possibly because the N-terminal tagged peptides, irrespective of the type of tag, blocked the signal peptide cleavage. Interestingly, the different tags resulted in different expression levels, although the sizes of the tagged GHITMs in SDS–PAGE were not different; N-terminally FLAG-tagged GHITM significantly reduced the expression level, whereas an N-terminal myc tag resulted in a higher GHITM expression (Fig. 3B). This result indicates that the type of N-terminal modification affects the expression level of GHITM. These results also suggest that the N-terminal region of GHITM may be important for GHITM expression in the cells.

To further confirm whether this ~27 kDa band was derived from GHITM protein in these cell lines, we transfected siRNAs targeting the endogenously expressing human GHITM protein in HeLa cells. When three different siRNAs were transfected into the HeLa cells, the observed band in the Western blots was reduced 50–80%, the reduced expression is likely the result of the siRNA-induced transcriptional silencing of *Ghitm* (Fig. 3C represents the result of the most effectively reduced expression by siRNA).

Discussion

In this study, we investigated the expression pattern of the mammalian *Ghitm* gene during mouse embryonic development as well as tissue distribution in adult mice. Once *Ghitm* mRNA was expressed in the brain of mouse embryo (E 9.5), *Ghitm* expressing cells spread ubiquitously in almost all tissues through embryonic development. Since *Pgdr* (*Ghitm* ortholog in *Bombyx mori*) was found to be a receptor involved in the postembryonic development of insects, GHITM may regulate the embryonic development in mammals.

We also observed that *Ghitm* gene was expressed in various adult mouse tissues. The ubiquitous expression and high abundance of transcripts indicate the importance of *Ghitm* in the adult, although no biological function has been assigned so far. In Fig. 1B, our observations of *Ghitm* expression in various organs as described below complement the data reported previously by Li et al. [4]. In contrast to the implied *Ghitm* function in lymphocytes, a low expression level was observed in thymus. Li et al. [4] reported strong *Ghitm* expression in iBAT, kidney, muscle, heart, brain, and testis. In addition to their observations, we found strong *Ghitm* expression in the liver, adrenal gland, and stomach, organs that are implicated in energy metabolism. The ubiquitous expression of *Ghitm*, and the distribution of organs with especially high expression level of *Ghitm* in our data, strongly suggests that *Ghitm* plays an important role in regulating energy expenditure, storage, and homeostasis.

Interestingly, the *Ghitm* gene and GHITM protein are expressed in all cultured cells examined so far. We also found that cleavage of an N-terminal signal peptide results in the mature form in vivo. Previously, Li et al. reported the molecular weight of N-terminally His-tagged GHITM protein expressed in L cells to be 42 kDa. Because the predicted size of the GHITM protein is 37 kDa, they speculated that the increased size in the protein may be due to post-translational modifications, such as glycosylation and/or phosphorylation [4]. In contrast to their observation, we detected the GHITM protein as the size of 27 kDa. Furthermore, we showed that GHITM possesses an N-terminal signal sequence, and that N-terminal modifications prevented cleavage of this sequence. A comparison of N- and C-terminal modified GHITM proteins led us to the conclusion that the N-terminal signal sequence of GHITM protein regulates proper expression of the protein.

It is known that about 5–10% GPCRs contain N-terminal signal peptides that are cleaved off by signal peptidases during receptor insertion into the ER membrane [7]. In the case of secretory proteins, cleavable signal peptides are obligatory for their translocation across the ER membrane [8–11]. By contrast, in the case of GPCRs, the roles of these signal peptides vary among receptor types. For example, the signal peptides of the human endothelin B receptor and the human VPAC1 receptor are required for mature receptor translocation across the plasma membrane [12,13]. In the case of the rat corticotropin-releasing factor receptor 1, the signal peptide promotes receptor expression but is not essential for translocation across the plasma membrane [14]. Cleavage of the GHITM signal peptide occurring in the absence of N-terminal tag implies that this signal peptide may be critical for the quality control of the GHITM protein such as translocation and proper function.

Previously, a cDNA sequence extracted from the EST database of the human pituitary tumor, named PTD010, was reported to encode a C-terminally truncated form of GHITM protein [4]. Li et al. suggested that this abnormality in the *Ghitm* gene product in cells leads to tumorigenesis, and that the normal structure of the GHITM protein may be required for the normal growth and/or survival of cells. We did not observe any morphological changes or growth retardation in the *Ghitm* knockdown HeLa cells (data not shown), although GHITM expression in the cells was effectively reduced by RNAi (Fig. 3C). However, these *Ghitm*-impaired cells could offer useful experimental system for analyzing GHITM function in the future, for example, screening of a ligand for GHITM protein.

Previously, the possible associations of *Ghitm* to GH signaling, aging, and immunological function were documented. The important roles of insulin-like growth factor (IGF) and insulin-related signaling pathways in the control of longevity of insects and mammals have been well documented [15,16]. In rodents, several spontaneous or experimentally induced mutations that interfere with GH biosynthesis, GH actions or sensitivity to IGF-I lead to extended longevity [17,18]. In addition, GH, either directly or through GH-induction of IGF-I, has been implicated in lymphocyte development and function [19,20]. We identified relatively strong *Ghitm* expression in some organs involved in energy metabolism, such as the brain, adipose tissue, liver, muscle, adrenal gland, and stomach. In addition, *Ghitm* was strongly expressed in various cultured cell lines. Taking together, these results strongly suggest that *Ghitm* plays an unidentified role in some metabological processes.

The observations in this paper and others lead to the assumption that *Ghitm* genes have important endocrinological roles controlling normal growth in many different animal phyla, although the biological functions of the *Ghitm* family of genes remain to be elucidated. Since a knockout of the *Ghitm* gene in mice might be lethal, we are now trying to make tissue specific knockout mice to facilitate investigation of the molecular behavior of the mouse *Ghitm* gene and to explore GHITM functions in various tissues.

Acknowledgments

We thank members of the H.K. and Kazushige Touhara laboratory for helpful discussion and Joe J. Hull for critical reading of the manuscript. This work was supported by grants to H.K. from the Research for the Future Program of the Japan Society for Promotion of Sciences, Japan.

References

[1] S. Nagata, T. Namiki, R. Ko, H. Kataoka, A. Suzuki, A novel type of receptor cDNA from the prothoracic glands of the silkworm, *Bombyx mori*, Biosci. Bictechnol. Biochem. (in press).

[2] L.I. Gilbert, Q. Song, R. Rybczynski, Control of ecdysteroidogenesis: activation and inhibition of prothoracic gland activity, Invert. Neurosci. 3 (1997) 205–216.

[3] L.I. Gilbert, R. Rybczynski, J.T. Warren, Control and biochemical nature of the ecdysteroidogenic pathway, Annu. Rev. Entomol. 47 (2002) 883–916.

[4] Y. Li, B. Kelder, J.J. Kopchick, Identification, isolation, and cloning of growth hormone (GH)-inducible interscapular brown adipose complementary deoxyribonucleic acid from GH antagonist mice, Endocrinology 142 (2001) 2937–2945.

[5] J.E. Nagel, R.J. Smith, L. Shaw, D. Bertak, V.D. Dixit, E.M. Schaffer, D.D. Taub, Identification of genes differentially expressed in T cells following stimulation with the chemokines CXCL12 and CXCL10, BMC Immunol. 5 (2004) 17.

[6] S. Zou, S. Meadows, L. Sharp, L.Y. Jan, Y.N. Jan, Genome-wide study of aging and oxidative stress response in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. USA 97 (2000) 13726–13731.

[7] E. Wallin, G. von Heijne, Properties of N-terminal tails in G-protein coupled receptors: a statistical study, Protein Eng. 8 (1995) 693–698.

[8] B. Martoglio, R. Graf, B. Dobberstein, Signal peptide fragments of preprolactin and HIV-1 p-gp160 interact with calmodulin, EMBO J. 16 (1997) 6636–6645.

[9] T.A. Rapoport, B. Jungnickel, U. Kutay, Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes, Annu. Rev. Biochem. 65 (1996) 271–303.

[10] R.S. Hegde, V.R. Lingappa, Regulation of protein biogenesis at the endoplasmic reticulum membrane, Trends Cell Biol. 9 (1999) 132–137.

[11] A.E. Johnson, M.A. van Waes, The translocon: a dynamic gateway at the ER membrane, Annu. Rev. Cell Dev. Biol. 15 (1999) 799–842.

[12] R. Kochl, M. Alken, C. Rutz, G. Krause, A. Oksche, W. Rosenthal, R. Schulein, The signal peptide of the G protein-coupled human endothelin B receptor is necessary for translocation of the N-terminal tail across the endoplasmic reticulum membrane, J. Biol. Chem. 277 (2002) 16131–16138.

[13] A. Couvineau, C. Rouyer-Fessard, M. Laburthe, Presence of a N-terminal signal peptide in class II G protein-coupled receptors: crucial role for expression of the human VPAC1 receptor, Regul. Pept. 123 (2004) 181–185.

[14] M. Alken, C. Rutz, R. Kochl, U. Donalies, M. Oueslati, J. Furkert, D. Wietfeld, R. Hermosilla, A. Scholz, M. Beyermann, W. Rosenthal, R. Schulein, The signal peptide of the rat corticotropin-releasing factor receptor 1 promotes receptor expression but is not essential for establishing a functional receptor, Biochem. J. 390 (2005) 455–464.

[15] C.E. Finch, G. Ruvkun, The genetics of aging, Annu. Rev. Genom. Hum. Genet. 2 (2001) 435–462.

[16] L. Guarente, C. Kenyon, Genetic pathways that regulate ageing in model organisms, Nature 408 (2000) 255–262.

[17] A. Bartke, Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging, Endocrinology 146 (2005) 3718–3723.

[18] Z. Laron, Do deficiencies in growth hormone and insulin-like growth factor-1 (IGF-1) shorten or prolong longevity? Mech. Ageing Dev. 126 (2005) 305–307.

[19] L.A. Welniak, R. Sun, W.J. Murphy, The role of growth hormone in T-cell development and reconstitution, J. Leukoc. Biol. 71 (2002) 381–387.

[20] W. Savino, S. Smaniotto, N. Binart, M.C. Postel-Vinay, M. Dardenne, In vivo effects of growth hormone on thymic cells, Ann. N. Y. Acad. Sci. 992 (2003) 179–185.