Trace Nutrients Research 33 : 13－20 (2016)

Original Article

Relationship between the glycosphingolipids and phospholipids synthesis and the mycelial growth in *Neurospora crassa*

Ryuta TOBE[§], Yasushi TANI[§], Miwa KATAOKA[§], Yasunori YAMASHITA, and Hisaaki MIHARA[†]
Department of Biotechnology, College of Life Sciences, Ritsumeikan University[*]

Summary

Fungi contain several types of glycosphingolipids (GSLs) in their cell membranes, which play important roles in various biological processes. Recently, novel fungal neogala-series GSLs were identified in some fungal species, including *Neurospora crassa*. GSLs have also been implicated in mycelial synthesis. However, little is known about the physiological functions, metabolic regulation, and biosynthetic pathway of GSLs. In this study, we demonstrated that several *N. crassa* mutant strains that had a defect in a gene putatively involved in GSL biosynthesis were deficient in mycelial growth in a temperature-dependent manner. In addition, results of the component analysis of phospholipids and GSLs from these strains suggested a novel relationship among phospholipids, GSLs, and mycelial growth.

Abbreviations: CL, cardiolipin; FNG-GSLs, fungal neogala-series glycosphingolipids; Gal-Cer, β-galactosylceramide; GCLs, glycosphingolipids; Glc-Cer, β-glucosylceramide; GIPCs, glycosyl-inositol phosphoceramide; IPCs, inositol phosphoceramide; MIPCs, mannosyl-inositol phosphoceramide; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

Introduction

Glycosphingolipids (GSLs), which consist of a hydrophilic carbohydrate chain of variable length and structure linked to a hydrophobic ceramide (*N*-acylsphingosine) moiety, are essential membrane lipids of eukaryotic organisms[1, 2]. Among GSLs, β-glucosylceramide (Glc-Cer) is commonly found in plants, fungi, and animals[3], whereas β-galactosylceramide (Gal-Cer) is present only in fungi and animals[3, 4]. On the other hand, inositol-containing glycosphingolipids are restricted to plants and fungi[4]. In mammals, Glc-Cer-type GSLs play pivotal roles in signaling, development, and immunological responses[1, 2], are mainly distributed in neural tissues, and play an important role in maintaining the structure, function, and stability of myelin[5, 6].

In fungi, four types of GSLs have been known to date. Two of them are the main neutral GSLs, Glc-Cer and Gal-Cer, which are essentially similar to those found in mammals. Glc-Cer functions during fungal growth and dimorphism, and is involved in lipid raft formation and virulence[7–9], whereas little is known about the function of Gal-Cer in fungi. The third type of GSLs, acidic glycosylinositol phosphoceramides (GIPCs), contains a phytosphingoid base constituting the core structure of inositol phosphoceramide (IPC), along with the addition of mannose, galactosamine, galactose, and/or xylose[10, 11]. In general, GIPCs are essential membrane components required for fungal growth[12, 13]. In addition to the above well-known GSLs, the fourth type is neutral fungal neogala-series GSLs (FNG-GSLs), which contain a characteristic fungal neogala-series core structure, Galβ1–6Galβ1-Cer, with a phytosphingoid base in the ceramide moiety, and has been identified in several fungal species including *Mucor hiemalis*, *Rhizopus microsporus*, *Rhizomucor pusillus*, *Absidia corymbifera*[14], and *Hirsutella rhossiliensis*[15].

FNG-GSLs have received considerable attention as a potential target for new antifungal agents[16]. Though FNG-GSLs are proposed to substitute for GIPCs in certain species of GIPC-lacking fungi[14, 15], their exact roles and their synthetic pathway remain to be elucidated. *Neurospora crassa* has been widely investigated as a model organism,

* Address : 1–1–1 Nojihigashi, Kusatsu, Shiga 525–8577, Japan
§ These authors contributed equally to this work.
† To whom correspondence should be addressed.
Tel: +81–77–561–2732; Fax: +81–77–561–2659; E-mail: mihara@fc.ritsumei.ac.jp

and its genomic sequence analysis was completed in 2003[17]. However, little is known about genes and proteins involved in GSL metabolism. In this study, we investigated the physiological functions and the putative biosynthetic pathway of GSLs in *N. crassa* by using bioinformatics data and gene-deletion mutants. Furthermore, the analysis of phospholipids and GSLs from the mutant strains by thin-layer chromatography (TLC) suggested that phospholipids and GSLs might be correlated with the mycelial growth of *N. crassa*.

Materials and Methods

Materials

Silica gel 60 thin-layer chromatography (TLC) plates were purchased from Merck (Darmstadt, Germany). Phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and cardiolipin (CL) were purchased from Avanti Polar Lipids Inc. (Alabama, USA). All other chemicals were purchased from Wako Pure Chemical Co. (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan).

Fungal strains and culture

A wild-type strain of *N. crassa* (DSM1257) was obtained from the German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ, Braunschweig, Germany). Mutant strains of *N. crassa* (FGSC13263, FGSC11790, FGSC16215, FGSC21512, FGSC11881, FGSC22132, and FGSC15707) were provided by the Fungal Genetics Stock Center (Manhattan, Kansas City, USA)[18, 19]. *N. crassa* was cultured in Vogel's medium[20] at normal (25℃ and 30℃) and at a high (40℃) temperature for 2 days. The cultured hyphae were washed twice with distilled water, collected by vacuum filtration, and stored at −80℃ before use.

Measurement of hyphae diameter

The spore suspension was diluted in water and incubated at 30℃ for 13 hr. The grown hyphae were observed by microscopy (Olympus BX50), and the diameters of hyphae were measured.

Extraction and purification of glycosphingolipids (GSLs)

Fungal strains were cultured in Vogel's medium, washed, and freeze-dried. GSLs were extracted by using the Bligh-Dyer method[21]. Briefly, dried mycelia (100 mg) were suspended with 5.7 ml of chloroform-methanol-water (5:10:4) and mixed by vortexing for 30 sec. After incubation at room temperature (RT) for 2 min, 4.5 ml chloroform and 1.5 mL of distilled water were added and mixed. The mixture was centrifuged at 1,500 rpm at RT for 15 min, and the lower organic solvent layer was obtained as total lipid fraction. The fraction was dried again and subjected to mild alkaline hydrolysis in methanol-1 M potassium hydroxide (95:5) at 37℃ for 12 hr. The hydrolysate was acidified to pH 1.0 with 12 M HCl and incubated at RT for 1 hr. After addition of chloroform-methanol-water (100:11:24), followed by mixing and centrifugation at 1,500 rpm at RT for 15 min, the lower layer was obtained as the GSL fraction.

Thin-layer chromatography (TLC) analysis

For the analysis of phospholipids, the total lipid fractions were subjected onto TLC and separated with chloroform-acetone-methanol-acetate-water (5:2:1:1:0.5). Detection of phosphorus moieties was carried out using the Harness reagent[22]. Each spot was determined by comparison with authentic reagent (PA, PC, PE, PS, and CL). For the analysis of glycolipids, GSL fractions were subjected onto TLC, and were separated with chloroform-methanol (95:5) and chloroform-methanol-water (60:35:8). Sugar moieties were detected using an orcinol-H_2SO_4 reagent[23].

Results and Discussion

Candidate genes involved in GSL metabolism in *N. crassa*

To predict the genes involved in GSL metabolism in *N. crassa,* we used bioinformatics data from public gene databases, including the KEGG pathway database (www.genome.jp/kegg/pathway.html). Based on the annotation of each gene/protein in the database, putative biochemical functions were assigned to the candidate genes/proteins. In this study, we focused on the following seven genes/proteins, which might be involved in GSL metabolism in *N. crassa*: acyl-CoA-dependent ceramide synthase (NCU00008), glycosylhydrolase family 35-1 (NCU00642), aureobasidin-resistance protein (NCU02282), dihydroceramidase (NCU02969), glucosylceramidase (NCU04395), sphinganine C4-monooxygenase protein SUR2 (NCU06465), and sphingolipid δ-4 desaturase (NCU08927). *N. crassa* mutant strains (FGSC13263, FGSC11790, FGSC16215, FGSC21512, FGSC11881, FGSC22132, and FGSC15707) with one of these genes knocked out were used for further analyses (Table 1).

Temperature-dependent inhibition of mycelial growth

The mycelial fungus *N. crassa* grows well at 25–30℃,

Table 1 Mutant strains and their mycelial growth at 25℃ and 40℃.

Strain	Gene deleted	Gene annotation*	Mycelia**	
			25℃	40℃
DSM1257	None (Wild-type)	−	+	+
FGSC13263	*NCU00008*	Acyl-CoA-dependent ceramide synthase	−	+
FGSC11790	*NCU00642*	Glycosylhydrolase family 35-1	−	+
FGSC16215	*NCU02282*	Aureobasidin-resistance protein	+	+
FGSC21512	*NCU02969*	Dihydroceramidase	+	+
FGSC11881	*NCU04395*	Glucosylceramidase	−	+
FGSC22132	*NCU06465*	Sphinganine C4-monooxygenase protein	+	+
FGSC15707	*NCU08927*	Sphingolipid δ-4 desaturase	−	−

*Gene annotations are from the KEGG database.
**The strains were cultured in Vogel's liquid medium for 2 days. Plus (+) and minus (−) signs indicate a normal mycelial growth phenotype and an impaired mycelial growth phenotype, respectively.

but shows various heat shock responses under heat-stress conditions (> 40℃)[24]. Previous studies suggested that ceramide synthesis is related to stress-induced cell death[24] and that coordinated modulation of GSL, phospholipid, and sterol content is involved in regulation of fluid properties of plasma-membrane during temperature acclimatization in *N. crassa*[25]. To investigate the effect of deletion in each of the 7 candidate genes of *N. crassa* on the growth phenotype, the wild-type and mutant strains were grown in Vogel's liquid medium at normal (25℃ and 30℃) and at a high (40℃) temperature respectively for 2 days. The mycelial growth in FGSC16215 (*ΔNCU02282*), FGSC21512 (*ΔNCU02969*), and FGSC22132 (*ΔNCU06465*) mutant strains showed no significant changes at both the temperatures as compared with that of the wild-type. On the other hand, the mycelial growth of FGSC13263 (*ΔNCU00008*), FGSC11790 (*ΔNCU00642*), FGSC11881 (*ΔNCU04395*), and FGSC15707 (*ΔNCU08927*) mutant strains was strongly inhibited at both 25℃ and 30℃ (Fig. 1A). Light microscopic analysis of these 4 mutant strains grown at 25℃ showed a unicellular somatic growth of these mutant cells without formation of the hyphae (Fig. 1B). There were no differences in the unicellular growth rate of the 4 mutants (data not shown). However, the inhibition of mycelial growth in FGSC13263, FGSC11790, and FGSC11881 strains was completely suppressed at 40℃ (Fig. 1A), whereas FGSC15707 exhibited impaired mycelial growth at 40℃ (data not shown).

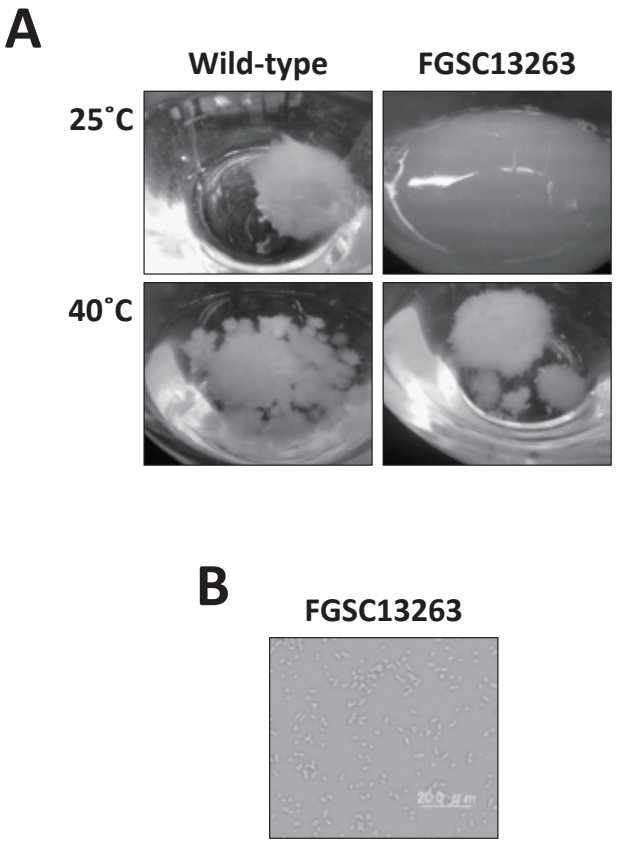

Fig. 1 Growth phenotypes of the wild-type and FGSC13263 mutant strains. (A) The strains were cultured in Vogel's liquid medium at 25℃ or 40℃ for 2 days. The result of the FGSC13263 mutant is shown as representative of those of FGSC11790, FGSC11881, and FGSC15707 mutant strains, which also showed the same phenotype as FGSC13263 at 25℃. (B) The image shows light microscopic analysis of the FGSC13263 strain (scale bar = 20.0 μm).

Impaired mycelial growth was correlated with FNG-GSL synthesis and phospholipid composition

Filamentous fungi grow by apical extension of their hyphal tip cells. Fungal hyphal growth requires complex mechanisms including Spitzenkörper, polarisome, endo- and exocytosis, and sterol-rich plasma membrane domains[26, 27]. The polarization of sterol- and sphingolipid-enriched domains (lipid rafts) as well as phospholipid asymmetry has also been linked to morphogenesis and biological activity in diverse cell types[28, 29]. Therefore, we

analyzed GSLs of the 7 mutant strains to examine if those mutations affect the membrane composition of the cells. TLC analysis of GSL fractions isolated from the wild-type and mutant strains grown in Vogel's liquid medium at 25℃ or 40℃ for 2 days demonstrated that no GSLs were detected in FGSC13263, FGSC11790, FGSC11881, and FGSC15707 when grown at 25℃ (Fig. 2A). The impaired GSL production was suppressed when the mutant strains, except FGSC15707, were grown at 40℃. FGSC15707 did not produce GSLs at 25℃ and at 40℃. Alternatively, FGSC16215, FGSC21512, and FGSC22132 strains, which formed mycelia in the liquid medium irrespective of the growth temperature, produced GSLs at levels similar to that produced by the wild-type strain at both 25℃ and 40℃. Therefore, the impaired mycelial growth in FGSC13263, FGSC11790, FGSC11881, and FGSC15707 strains could be attributed to a defect in the GSL production.

We also analyzed total lipid fractions from the 7 mutant strains to examine changes in phospholipids composition in the cell membrane. When grown at 25℃, there was no significant change in the phospholipid compositions in the mycelial cells of FGSC16215, FGSC21512, and FGSC22132 mutant strains as compared with that of the wild-type strain (Fig. 2B). In contrast, phospholipid compositions in the unicellularly grown FGSC13263, FGSC11790, FGSC11881, and FGSC15707 mutant strains showed marked differences from those of the mycelia of the wild-type strain (Fig. 2B and Table 2). The mycelial growth-impaired strains frequently had an increased amount of phosphatidylethanolamine (PE) and a decreased amount of phosphatidic acid (PA) as compared with the wild-type strain. When grown at 40℃, there were slight, but not significant differences in the phospholipid compositions among all the strains except FGSC15707. These data suggest that phospholipid composition is associated with mycelial growth phenotype and GSL synthesis in *N. crassa*.

Mycelial growth on a solid agar medium

We tested if cultivation on a solid agar medium can affect mycelial growth in the mutant strains, FGSC13263, FGSC11790, FGSC11881, and FGSC15707. The wild-type and the mutant strains were seeded on Vogel's agar plate and incubated at 30℃. Unlike the results of the liquid cultivation experiment (Fig. 1), mycelial growth was observed in FGSC13263, FGSC11790, FGSC11881, and FGSC15707 mutant strains as well as in the wild-type strain (data not shown). Light microscopic analysis revealed that the diameters of the hyphae of FGSC13263, FGSC11881, and FGSC15707 strains were smaller than those of the wild-type strain (Fig. 3). TLC analysis of the mycelia of these 4 mutant strains harvested from the solid agar medium

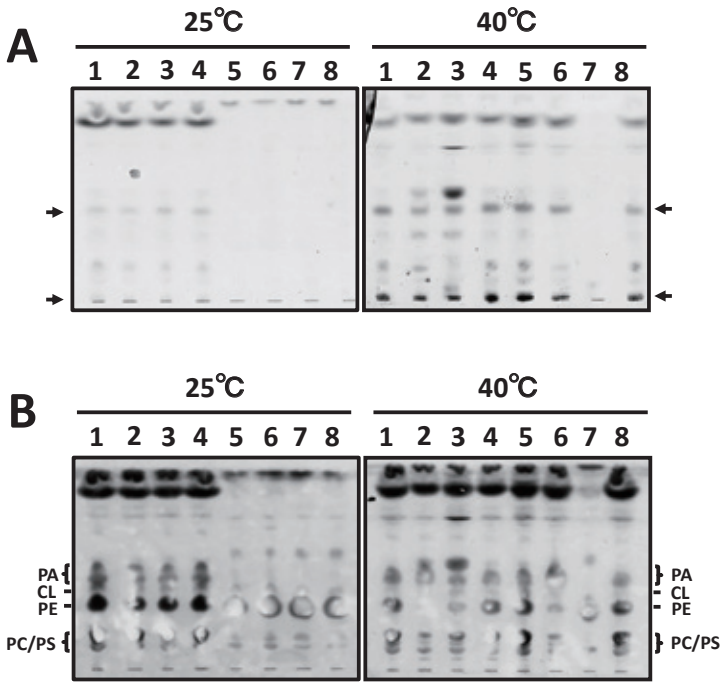

Fig. 2 TLC analysis of GSLs and phospholipids obtained from the wild-type and the mutant strains. The strains were cultured in Vogel's medium at 25℃ or 40℃ for 2 days, and GSLs (A) and phospholipids (B) were extracted and analyzed by TLC as described in *Materials and Methods*. Lane 1, wild-type; lane 2, FGSC16215; lane 3, FGSC22132; lane 4, FGSC21512; lane 5, FGSC11790; lane 6, FGSC13263; lane 7, FGSC15707; and lane 8, FGSC11881. Arrows indicate the position of GSLs (A), and PA, CL, PE, and PC/PS (B).

Table 2 Changes in phospholipid composition in the mutant strains.

Strain (FGSC)	Mycelia*		GSLs**		PA**		CL**		PE**		PC/PS**	
	25℃	40℃	25℃	40℃	25℃	40℃	25℃	40℃	25℃	40℃	25℃	40℃
13263	−	+	−	+/−	−	+/−	+/−	+/−	+	−	−	−
11790	−	+	−	+/−	−	+/−	+/−	+/−	+	+/−	−	+/−
11881	−	+	−	+/−	−	+/−	+/−	+/−	+	+/−	−	+/−
15707	−	−	−	−	−	−	+/−	+/−	+	+	−	−

*The strains were cultured in Vogel's liquid medium at 25℃ or 40℃ for 2 days. Plus (+) and minus (−) signs indicate a normal mycelial growth phenotype and an impaired mycelial growth phenotype, respectively.

**(−), (+), and (+/−) indicate a decrease, an increase, and no significant change, respectively, in the amount of the corresponding lipid component as compared to those of the wild-type strain. PA, CL, PE, PC, and PS represent phosphatidic acid, cardiolipin, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine, respectively.

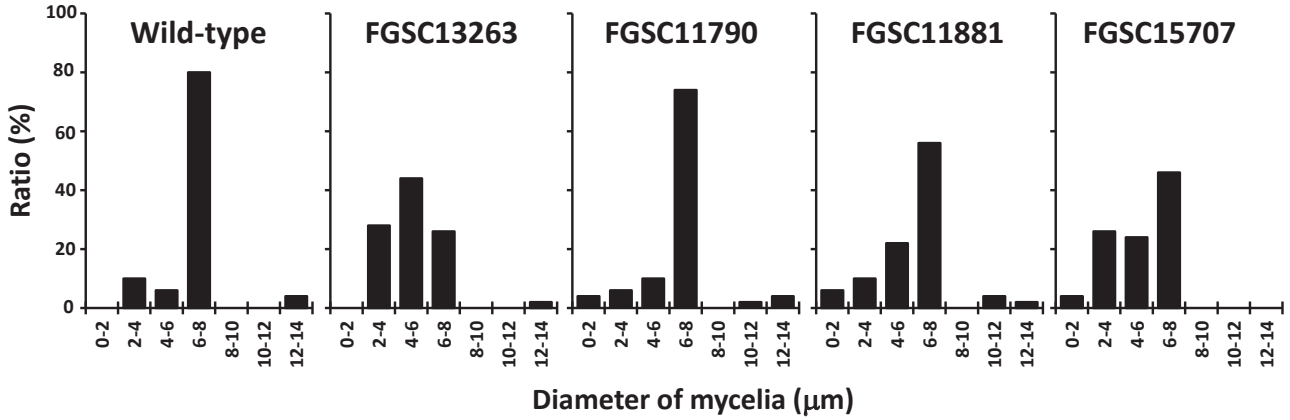

Fig. 3 Diameters of the mycelia of *N. crassa* wild-type and FGSC13263, FGSC11790, FGSC11881, and FGSC15707 mutant strains. Each strain was cultured on Vogel's agar plate at 30℃ for 2 days. The diameters of 50 mycelia were measured by using microscopy and their ratios (%) are shown.

showed that the GSL and phospholipids profiles were similar to those with the wild-type strain (data not shown).

Possible implication for the mechanism of mycelial growth

In this study, we found that FGSC13263, FGSC11790, FGSC11881, and FGSC15707 strains, when cultivated at 25–30℃, exhibited impaired mycelial growth phenotype that was associated with the significant loss of GSLs and PA, along with the increased levels of PE in the membrane fraction.

FGSC13263 is deficient in *NCU00008*, which is similar to the acyl-CoA-dependent ceramide synthase genes, known as *lag1* and *lac1* of *Saccharomyces cerevisiae*[30] and *Candida albicans*[31]. Lag1p and Lac1p have a redundant function in *S. cerevisiae*. In case of *C. albicans*, its enzymes are functionally distinct: only the lack of Lag1p causes severe defects in the growth and hyphal morphogenesis of *C. albicans*[31]. *N crassa* also contains *NCU02468*, which is a paralog of *NCU00008*, where the amino acid sequence identity between the gene products is only 21%. The NCU00008 protein is more similar to Lag1p, while NCU02468 is relatively close to Lac1p. Because FGSC13263 formed mycelia at 40℃, but not at 25℃, *NCU02468* might play a role in ceramide synthesis at a high temperature (40℃).

NCU00642, which is deleted in FGSC11790, codes for a putative *β*-galactosidase belonging to glycoside hydrolase family 35 (GH35)[32]. The function of fungal enzymes belonging to GH35 is largely unknown. However, a study on mammalian enzyme suggested that it is potentially involved in the conversion of lactosylceramide to glucosylceramide[33]. In addition to *NCU00642*, there are 3 paralogous genes in the genome of *N. crassa*. Therefore, our data suggested that any of them could compensate for the loss of *NCU00642* function in mycelial growth at a high temperature.

Among the four genes, only *NCU04395* has been previously cloned and characterized as endo-1,6-*β*-D-glucanase gene (*neg-1*) in *N. crassa* IFO-6068[34, 35]. A mutant strain with base changes in *neg-1* showed no apparent phenotypic changes under normal growth condition, but exhibited

a marked inhibition of the hyphal growth in the presence of Congo-red, SDS, and cetyltrimethyl ammonium bromide, which affected the structural integrity of the fungal cell walls or membranes[34]. In contrast, no difference was observed between the growth of the wild-type and mutant strains when the cells were treated with neutral detergent, Tween 80, or grown under high osmotic stress in 0.4–1.0 M NaCl or 0.8–1.0 M sorbitol[34]. These results suggest that *neg-1* affects the cell wall *β*-glucan structure, which is consistent with our results that showed impaired mycelial growth phenotype in FGSC11881. The loss of *NCU04395* may be complemented by a heat-inducible gene that has function similar to that of *NCU04395* under high-temperature conditions.

Interestingly, the *NCU08927*-deficient mutant did not form mycelia at 25℃ and 40℃. *NCU08927* is annotated as a sphingolipid *δ*-4 desaturase gene, which encodes an integral membrane protein required for sphingosine biosynthesis[36]. This enzyme converts D-erythro-sphinganine to D-erythro-sphingosine and *δ*4-desaturated sphingolipids, which provide an early signal that triggers their entry into both meiotic and spermatid differentiation pathways during *Drosophila* spermatogenesis[36]. In many eukaryotic organisms, sphingolipids desaturated at the *δ*4-position serve as important signaling molecules for cellular proliferation, differentiation, and motion[37], as well as cell cycle arrest and apoptosis[38]. Therefore, the lack of NCU08927 may be critical for the life cycle processes, including mycelial growth of fungi.

In view of the importance of GSLs in a wide variety of cellular process, identification of novel genes involved in the metabolism of those lipids may provide new insights into function of GSLs in filamentous fungi.

Acknowledgements

This work was supported by the Program for the Third-Phase R-GIRO Research (to H. M.) from the Ritsumeikan Global Innovation Research Organization, Ritsumeikan University, by a Grant-in-Aid for Scientific Research (B) 16H04913 (to H. M.) from JSPS, and by a Grant-in-Aid for Young Scientists (B) 16K18691 (to R. T.) from JSPS.

References

1) Wennekes T, van den Berg RJ, Boot RG, van der Marel GA, Overkleeft HS, Aerts JM (2009) Glycosphingolipids—nature, function, and pharmacological modulation. Angew Chem Int Ed Engl 48: 8848–8869.
2) Pontier SM, Schweisguth F (2012) Glycosphingolipids in signaling and development: from liposomes to model organisms. Dev Dyn 241: 92–106.
3) Sabourdy F, Kedjouar B, Sorli SC, Colie S, Milhas D, Salma Y, Levade T (2008) Functions of sphingolipid metabolism in mammals—lessons from genetic defects. Biochim Biophys Acta 1781: 145–183.
4) Warnecke D, Heinz E (2003) Recently discovered functions of glucosylceramides in plants and fungi. Cell Mol Life Sci 60: 919–941.
5) Uemura S, Go S, Shishido F, Inokuchi J (2014) Expression machinery of GM4: the excess amounts of GM3/GM4S synthase (ST3GAL5) are necessary for GM4 synthesis in mammalian cells. Glycoconj J 31: 101–108.
6) Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, Suzuki K, Popko B (1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell 86: 209–219.
7) Rodrigues ML, Travassos LR, Miranda KR, Franzen AJ, Rozental S, de Souza W, Alviano CS, Barreto-Bergter E (2000) Human antibodies against a purified glucosylceramide from *Cryptococcus neoformans* inhibit cell budding and fungal growth. Infect Immun 68: 7049–7060.
8) Nimrichter L, Cerqueira MD, Leitao EA, Miranda K, Nakayasu ES, Almeida SR, Almeida IC, Alviano CS, Barreto-Bergter E, Rodrigues ML (2005) Structure, cellular distribution, antigenicity, and biological functions of *Fonsecaea pedrosoi* ceramide monohexosides. Infect Immun 73: 7860–7868.
9) Rhome R, Singh A, Kechichian T, Drago M, Morace G, Luberto C, Del Poeta M (2011) Surface localization of glucosylceramide during *Cryptococcus neoformans* infection allows targeting as a potential antifungal. PLoS One 6: e15572.
10) Aimanianda V, Clavaud C, Simenel C, Fontaine T, Delepierre M, Latge JP (2009) Cell wall *β*-1,6-glucan of *Saccharomyces cerevisiae*: structural characterization and *in situ* synthesis. J Biol Chem 284: 13401–13412.
11) Takahashi HK, Toledo MS, Suzuki E, Tagliari L, Straus AH (2009) Current relevance of fungal and trypanosomatid glycolipids and sphingolipids: studies defining structures conspicuously absent in mammals. An Acad Bras Cienc 81: 477–488.
12) Cheng J, Park TS, Fischl AS, Ye XS (2001) Cell cycle progression and cell polarity require sphingolipid biosynthesis in *Aspergillus nidulans*. Mol Cell

Biol 21: 6198–6209.
13) Hu W, Sillaots S, Lemieux S, Davison J, Kauffman S, Breton A, Linteau A, Xin C, Bowman J, Becker J, Jiang B, Roemer T (2007) Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. PLoS Pathog 3: e24.
14) Aoki K, Uchiyama R, Yamauchi S, Katayama T, Itonori S, Sugita M, Hada N, Yamada-Hada J, Takeda T, Kumagai H, Yamamoto K (2004) Newly discovered neutral glycosphingolipids in aureobasidin A-resistant zygomycetes: Identification of a novel family of Gala-series glycolipids with core Galα1–6Galβ1–6Galβ sequences. J Biol Chem 279: 32028–32034.
15) Tani Y, Funatsu T, Ashida H, Ito M, Itonori S, Sugita M, Yamamoto K (2010) Novel neogala-series glycosphingolipids with terminal mannose and glucose residues from *Hirsutella rhossiliensis*, an aureobasidin A-resistant ascomycete fungus. Glycobiology 20: 433–441.
16) Nimrichter L, Rodrigues ML (2011) Fungal glucosylceramides: from structural components to biologically active targets of new antimicrobials. Front Microbiol 2: 212.
17) Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang S, Nielsen CB, Butler J, Endrizzi M, Qui D, Ianakiev P, Bell-Pedersen D, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stange-Thomann N, Barrett R, Gnerre S, Kamal M, Kamvysselis M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metzenberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li W, Pratt RJ, Osmani SA, DeSouza CP, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seiler S, Dunlap J, Radford A, Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebbole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422: 859–868.
18) McCluskey K, Wiest A, Plamann M (2010) The Fungal Genetics Stock Center: a repository for 50 years of fungal genetics research. J Biosci 35: 119–126.
19) Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, Weiss RL, Borkovich KA, Dunlap JC (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. Proc Natl Acad Sci USA 103: 10352–10357.
20) Vogle HJ (1956) A convenient growth medium for *Neurospora*. Microbial Genet Bull 13: 42–43.
21) Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917.
22) Gentner PR, Bauer M, Dieterich I (1981) Thin-layer chromatography of phospholipids. Separation of major phospholipid classes of milk without previous isolation from total lipid extracts. J Chromatogr 206: 200–204.
23) Bruckner J (1955) Estimation of monosaccharides by the orcinol-sulphuric acid reaction. Biochem J 60: 200–205.
24) Plesofsky NS, Levery SB, Castle SA, Brambl R (2008) Stress-induced cell death is mediated by ceramide synthesis in *Neurospora crassa*. Eukaryot Cell 7: 2147–2159.
25) Aaronson LR, Martin CE (1983) Temperature-induced modifications of glycosphingolipids in plasma membranes of *Neurospora crassa*. Biochim Biophys Acta 735: 252–258.
26) Steinberg G (2007) Hyphal growth: a tale of motors, lipids, and the Spitzenkorper. Eukaryot Cell 6: 351–360.
27) Harris SD (2008) Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems. Mycologia 100: 823–832.
28) Oura T, Kajiwara S (2010) *Candida albicans* sphingolipid C9-methyltransferase is involved in hyphal elongation. Microbiology 156: 1234–1243.
29) Martin SW, Konopka JB (2004) Lipid raft polarization contributes to hyphal growth in *Candida albicans*. Eukaryot Cell 3: 675–684.
30) Teufel A, Maass T, Galle PR, Malik N (2009) The longevity assurance homologue of yeast *lag1* (Lass) gene family. Int J Mol Med 23: 135–140.
31) Cheon SA, Bal J, Song Y, Hwang HM, Kim AR, Kang WK, Kang HA, Hannibal-Bach HK, Knudsen J, Ejsing CS, Kim JY (2012) Distinct roles of two ceramide synthases, CaLag1p and CaLac1p, in the morphogenesis of *Candida albicans*. Mol Microbiol 83: 728–745.
32) Tanthanuch W, Chantarangsee M, Maneesan J, Ketudat-Cairns J (2008) Genomic and expression analysis of glycosyl hydrolase family 35 genes from rice (*Oryza sativa* L.). BMC Plant Biol 8: 84.

33) Zschoche A, Furst W, Schwarzmann G, Sanhoff K (1994) Hydrolysis of lactosylceramide by human galactosylceramidase and GM1-β-galactosidase in a detergent-free system and its stimulation by sphingolipid activator proteins, sap-B and sap-C. Activator proteins stimulate lactosylceramide hydrolysis. Eur J Biochem 222: 83–90.
34) Oyama S, Inoue H, Yamagata Y, Nakajima T, Abe K (2006) Functional analysis of an endo-1,6-β-D-glucanase gene (*neg-1*) from *Neurospora crassa*. Biosci Biotechnol Biochem 70: 1773–1775.
35) Oyama S, Yamagata Y, Abe K, Nakajima T (2002) Cloning and expression of an endo-1,6β-D-glucanase gene (*neg1*) from *Neurospora crassa*. Biosci Biotechnol Biochem 66: 1378–1381.
36) Ternes P, Franke S, Zahringer U, Sperling P, Heinz E (2002) Identification and characterization of a sphingolipid δ4-desaturase family. J Biol Chem 277: 25512–25518.
37) Pyne S, Pyne NJ (2000) Sphingosine 1-phosphate signalling in mammalian cells. Biochem J 349: 385–402.
38) Mathias S, Pena LA, Kolesnick RN (1998) Signal transduction of stress via ceramide. Biochem J 335: 465–480.