Characterization of two different types of UDP-glucose/-galactose 4-epimerase involved in galactosylation in fission yeast

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Schizosaccharomyces species are currently the only known organisms with two types of genes encoding UDP-glucose/-galactose 4-epimerase, *uge1*⁺ and *gal10*⁺. A strain deleted for *uge1*⁺ exhibited a severe galactosylation defect and a decrease in activity and in UDP-galactose content when grown in glucose-rich medium (2 % glucose), indicating that Uge1p is a major UDP-glucose/-galactose 4-epimerase under these growth conditions. In contrast, *gal10*⁺ was efficiently expressed and involved in galactosylation of cell-surface proteins in low-glucose medium (0.1 % glucose and 2 % glycerol), but not in galactose-containing medium. In a *uge1Δgal10Δ* strain, the galactosylation defect was suppressed and UDP-galactose content restored to wild-type levels in galactose-containing medium. Disruption of *gal7*⁺, encoding galactose-1-phosphate uridylyltransferase, in the *uge1Δgal10Δ* strain reversed suppression of the galactosylation defect and reduced levels of UDP-galactose, indicating that galactose is transported from the medium to the cytosol and is converted into UDP-galactose via galactose 1-phosphate by Gal7p in *Sch. pombe*.

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INTRODUCTION

Glycoproteins in the fission yeast *Schizosaccharomyces pombe* contain a large amount of galactose in addition to both N- and O-linked mannan (Manners & Meyer, 1977; Moreno *et al.*, 1985; Ikeda *et al.*, 2009; Ohashi *et al.*, 2009), indicating that *Sch. pombe* is equipped with mechanisms for glycoprotein galactosylation like animal cells. Research has demonstrated that the outer chain structure of galactomannan has an α -1,2-linked galactose or pyruvylated galactose attached to a poly- α -1,6-linked mannose backbone (Gemmell & Trimble, 1998), and that the pyruvylated galactose epitope bears remarkable structural resemblance to the N-acetylneuraminic acid-linked galactose epitope found in mammalian cells (Gemmell & Trimble, 1996). These galactose residues are enzymically modified by galactosyltransferases using UDP-galactose as substrate (Andreishcheva *et al.*, 2004; Chappell *et al.*, 1994; Yoko-o *et al.*, 1998). UDP-galactose is transported to the Golgi lumen from the cytosol by the Golgi-localized UDP-galactose transporter (Gms1p) (Tabuchi *et al.*, 1997; Tanaka & Takegawa, 2001). In *Sch. pombe*, the precise manner in which UDP-galactose is synthesized in the

cytosol has been unclear. In the cytosol, the key enzyme for UDP-galactose synthesis is UDP-galactose/-glucose 4-epimerase, which catalyses the interconversion of UDP-galactose and UDP-D-glucose. Interestingly, *Schizosaccharomyces* species have two types of UDP-glucose/-galactose 4-epimerase, while other organisms have only one. It is not known why two types of epimerase are needed and how they are regulated in *Sch. pombe*.

In *Saccharomyces cerevisiae*, Gal10p is the sole UDP-glucose/-galactose 4-epimerase, which consists of a UDP-glucose/-galactose 4-epimerase domain and a galactose mutarotase domain (Majumdar *et al.*, 2004; Scott & Timson, 2007; Thoden & Holden, 2005), and which catalyses mutarotation of α -galactose to β -galactose. Expression of the *GAL10* gene is regulated by Gal4p, a DNA-binding transcription factor required for activation of the *GAL* genes in cells growing on galactose as sole carbon source (De Robichon-Szulmajster, 1958; Platt & Reece, 1998; Lohr *et al.*, 1995). In contrast to *Sch. pombe*, UDP-galactose is not used as a glycosylation substrate by glycosyltransferases because secreted glycoproteins are mannosylated in *Sac. cerevisiae*. Therefore, in *Sac. cerevisiae*, UDP-galactose and UDP-glucose/-galactose 4-epimerase function solely in galactose metabolism.

Abbreviations: HRP-PNA, horseradish peroxidase-conjugated peanut lectin.

The metabolism of galactose in most organisms occurs via the Leloir pathway (Frey, 1996; Holden *et al.*, 2003), comprising galactokinase (EC 2.7.1.6), galactose-1-phosphate uridylyltransferase (EC 2.7.7.12) and UDP-glucose/-galactose 4-epimerase (EC 5.1.3.2) (see Fig. 7b). In the first step of this pathway, cytosolic α -D-galactose is phosphorylated by ATP-dependent galactokinase to yield galactose 1-phosphate (Gal-1-P). Next, Gal-1-P uridylyltransferase transfers UMP from UDP-glucose (UDP-Glc) to Gal-1-P via a two-step ping-pong mechanism, releasing glucose 1-phosphate (Glc-1P) and forming UDP-galactose. Finally, NAD⁺-dependent UDP-galactose-4-epimerase converts UDP-galactose to UDP-Glc. The glucose 1-phosphate is used in glycolysis and the UDP-galactose is utilized in mammals for galactosylation of glycoproteins.

In *Sch. pombe*, the metabolism of galactose is poorly understood because cells are not able to grow on galactose as a sole carbon source. This phenotype makes it difficult to analyse the Leloir pathway, despite the fact that genes encoding galactokinase, Gal-1-P uridylyltransferase and UDP-glucose/-galactose epimerase are present in the *Sch. pombe* genome.

In the present study, we describe the role of two types of UDP-galactose/-glucose 4-epimerase in *Sch. pombe*, Uge1p and Gal10p.

METHODS

Strains, medium and genetic methods. The wild-type *Sch. pombe* strain ARC039 (*h⁻ leu1-32 ura4-C190T*) was used. The *gms1Δ* strain was constructed as described by Tabuchi *et al.* (1997). Standard rich medium (YES) and synthetic minimal medium (MM) for growing *Sch. pombe* were used as described by Moreno *et al.* (1991). *Sch. pombe* cells were transformed by the lithium acetate method or by electroporation (Morita & Takegawa, 2004; Okazaki *et al.*, 1990; Suga & Hatakeyama, 2001; Suga *et al.*, 2000). *Escherichia coli* XL1-blue (Stratagene) was used for all cloning procedures. Standard genetic methods have been described (Alfa *et al.*, 1993).

Gene disruptions. The *uge1⁺* locus (SPBC365.14) was disrupted in the wild-type *Sch. pombe* strain by replacing an internal *uge1⁺* gene fragment with the *Sch. pombe ura4⁺* gene. To amplify the *uge1⁺* gene by PCR, the following oligonucleotides were used: sense, 5'-GTAGTCGCATGAAGCTGGTG-3', and antisense, 5'-ACCTAA-TCGATAGCTCGAGCGC-3'. A 1.97 kbp fragment was recovered and ligated into pGEM-T EASY vector (Promega). A *Hind*III site within the cloned *uge1⁺* open reading frame was digested and a 1.6 kbp *ura4⁺* gene was inserted. To disrupt the *gal10⁺* locus (SPBPB2B2.12C), the following oligonucleotides were used: sense, 5'-CATCTGTGCTTTATTCTTCTGTGCCCC-3', and antisense, 5'-GAACAAAAGATGCTTAATCGGTGGG-3'. A *Hind*III site within the cloned *gal10⁺* open reading frame was digested and the *ura4⁺* gene was inserted. To disrupt the *gal7⁺* locus (SPBPB2B2.10c), the following oligonucleotides were used: sense, 5'-GTACGATCC-AACATGTTACCTCTGCCCTGG-3', and antisense, 5'-GGAGCTT-GGTGAAGACCCATTGAGTAGGGG-3'. A *Hind*III site within the cloned *gal7⁺* open reading frame was digested and the *ura4⁺* gene was inserted. Linearized DNA fragments carrying the disrupted *uge1⁺*, *gal10⁺* and *gal7⁺* genes, respectively, were used to transform the wild-type haploid ARC039 strain, and *ura4⁺* transformants were selected. To confirm that the corresponding individual genes had

been disrupted, *ura4⁺* transformants were analysed by Southern blotting and PCR to verify correct integration of the deletion constructs.

Lectin staining. Horseradish peroxidase-conjugated peanut lectin (HRP-PNA) was used to detect galactose residues on the cell surface as described previously (Takegawa *et al.*, 1996). Briefly, cells were grown to stationary phase in YES or MM at 30 °C. Harvested cells were washed twice with distilled water and then resuspended in distilled water. Cells were fractured using glass beads to yield a crude protein extract. Extracted protein was spotted onto filter paper and dried. The filters were then washed twice in 10 mM Tris/HCl buffer (pH 7.4), 0.15 M NaCl and 0.05 % Tween 20 at room temperature for 5 min, followed by addition of HRP-PNA at room temperature and incubation for 1 h with continuous shaking. The filter paper was incubated in 0.03 % 3,3'-diaminobenzidine and 0.003 % H₂O₂ in PBS until spots were visible.

Acid phosphatase staining. Glycosylation of acid phosphatase was analysed as described by Huang & Snider (1995), with minor modifications. Briefly, cells were grown in 5 ml MM medium to mid-exponential phase at 30 °C. To induce acid phosphatase, cells were centrifuged, washed and resuspended in 5 ml phosphate-free MM medium, and incubated for 12 h at 30 °C. Cells were then collected by centrifugation, washed once in 62.5 mM Tris/HCl (pH 6.8), and suspended in 240 μ l lysis buffer (62.5 mM Tris/HCl pH 6.8, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 0.1 mM dithiothreitol and 10 % glycerol, pH 6.8). A cell lysate was prepared using 0.5 mm glass beads in a Mini BeadBeater (BioSpec Products). The lysate was recovered and centrifuged at 15 000 g for 10 min at 4 °C, and the supernatant was loaded onto a 6 % native polyacrylamide gel. Electrophoresis and staining of acid phosphatase activity were performed as described by Schweingruber *et al.* (1986).

Northern-blot analysis. Total RNA from cultures with an OD₆₀₀ of 0.8–1.0 was extracted by the glass bead method described above (Sambrook *et al.*, 1989). An aliquot (20 μ g) of total RNA was separated on a 1 % (w/v) agarose gel containing 20 % formaldehyde. Subsequently, RNA was blotted onto Hybond-N membranes in 10 × SSC buffer (1.5 M NaCl, 0.15 M sodium citrate, pH 7.0) and hybridized with gene-specific probes. The probes were obtained by PCR using the following primers. 5'-GGTGTACTTTGGTGA-GAGCGACTGAGGAC-3' and 5'-CAGCCTAGTAATATACGCG-GTAGAAGCC-3' for *leu1⁺* (constitutive endogenous control), 5'-CGGAGGTGCTCATCCCTCTGGTGAAGTCCG-3' and 5'-TTACTT-ATATGTCTTGGTATGGTGCAGGTC-3' for *uge1⁺*, and 5'-AAT-AATTAAGACACGACTAACGTCTATTT-3' and 5'-TTAATAGT-ATTCTCCACTATTGTACGATGC-3' for *gal10⁺*. These primers amplified 502 bp probes, which were subsequently labelled using the AlkPhos Direct Labelling and Detection system (GE Healthcare).

Assay of epimerase activity. Cells were cultured to the exponential phase in MM. A sample (100 OD₆₀₀ units) of cells was resuspended in 600 μ l ice-cold-buffer consisting of 0.1 M glycine/KOH (pH 8.7) and 2 mM EDTA. To lead to glucose repression of the cells, the cells were collected by centrifugation, and incubated for 12 h in MM medium supplemented with 0.1 % glucose (and 2 % galactose) as the carbon source. Epimerase was extracted by the glass beads method from the resuspended cells. Epimerase activity was measured by a two-step assay (Brahma & Bhattacharyya, 2004). Briefly, extracted epimerase (4 μ l) and 0.5 mM UDP-galactose (1 μ l) were mixed with 75 μ l ice-cold buffer. The mixture was then incubated for 30 min at 30 °C and boiled for 10 min, after which ice-cold-buffer containing 18 mM NAD⁺ and 12 milliunits UDP-glucose dehydrogenase (Sigma) was added. A₃₄₀ values were measured immediately for half of the sample volume (zero time) and for the other half volume after 30 min at 30 °C (30 min). One unit of UDP-galactose 4-epimerase catalyses the

conversion of 1 μ mol UDP-galactose to UDP-glucose, and thus the formation of 2 μ mol NADPH, per minute. Each nanomole of UDP-galactose converted to UDP-glucose corresponds to an A_{340} change of 0.02.

Analysis of UDP-galactose levels. Samples for analysis of UDP-galactose were prepared as described by Huang & Snider (1995). The UDP-galactose level was measured with the Amplex Red Galactose/Galactose Oxidase Assay kit (Invitrogen) according to the manufacturer's instructions.

Plasmid constructs. For overexpression of *gal10*⁺, the cDNA was amplified by PCR using the following primers: 5'-GTTTTCATATGGCGGTTCAAGACGAATACA-3' (including a *Nde* I site) and 5'-GTTTGGATCCTTAATAGTATTCTCCACTA-3' (including a *Bam*HI site). Derivatives of the thiamine-repressible expression vectors pREP1 and pREP41 were used to overexpress *gal10*⁺ (Nakamura *et al.*, 2001).

RESULTS

Sch. pombe has two types of UDP-glucose/-galactose 4-epimerase

Proteins that shared significant homology with human GALE and with *Sac. cerevisiae* GAL10 were identified through BLAST searches of the *Sch. pombe* genome and were designated *uge1*⁺ (SPBC365.14c) and *gal10*⁺ (SPBPB2B2.12c), respectively (Fig. 1, Table 1). The Uge1 protein was characterized by the presence of only an epimerase domain. GALE and *Sch. pombe* Uge1p are similar in size (348 and 355 aa, respectively) and share more than 50 % amino acid sequence identity (Fig. 1). On the other hand, the Gal10 protein was characterized by the presence of a mutarotase domain fused to the epimerase domain, which were also similar in size (699 and 713 aa, respectively) and also shared more than 50 % amino acid sequence identity with Sc Gal10p (Fig. 1). Table 1 shows UDP-glucose/-galactose epimerase homologues from various organisms. It is interesting to note that only *Schizosaccharomyces* species have two types of UDP-glucose/-galactose 4-epimerase, while other yeast species have only the Sc Gal10 type (epimerase and mutarotase domains). This might be related to the fact that unlike

Fig. 1. Domain characterization in two types of UDP-glucose/-galactose 4-epimerase: domain structures of the *Sch. pombe* Uge1 (Sp Uge1p), *Sch. pombe* Gal10 (Sp Gal10p), *Sac. cerevisiae* Gal10 (Sc Gal10p), and human GALE (GALE) proteins. Boxes representing domains with significant amino acid sequence similarity are filled in with the same pattern.

other yeast species, *Schizosaccharomyces* species secrete galactomannoproteins and possess galactosylation mechanisms mediated by galactosyltransferases, and have UDP-galactose transporters (Chappell *et al.*, 1994; Ohashi *et al.*, 2009; Tabuchi *et al.*, 1997; Umeda *et al.*, 2000). Therefore, like mammalian cells, *Schizosaccharomyces* species must produce UDP-galactose constitutively from UDP-glucose via UDP-glucose/-galactose 4-epimerase as a substrate for galactosyltransferases and UDP-galactose transporters.

A *uge1* Δ strain exhibits a severe galactosylation defect in rich medium

In order to determine which UDP-glucose/-galactose epimerases are functional in *Sch. pombe*, we constructed *uge1*⁺ and *gal10*⁺ gene disruptants. To confirm the extent of the glycosylation defect, we examined acid phosphatase as a representative highly glycosylated glycoprotein (Dibenedetto & Cozzani, 1975), whose migration on native gel electrophoresis depends on the size and number of *N*-linked glycans (Schweingruber *et al.*, 1986; Tanaka *et al.*, 2001). The analysis revealed that acid phosphatase prepared from the *uge1* Δ strain migrated faster than that from wild-type strains, but similarly to that from a *gms1* Δ strain lacking the UDP-galactose transporter and which is known to contain secreted glycoproteins lacking galactose (Fig. 2a) (Tanaka *et al.*, 2001). Acid phosphatase from the *gal10* Δ strain had almost the same mobility as that from the wild-type strain, while that from the *uge1* Δ *gal10* Δ disruptant migrated similarly to acid phosphatase from the *gms1* Δ strain. This result indicates that the oligosaccharide content is reduced in acid phosphatase prepared from the *uge1* Δ strain. To analyse the effect of cell-surface galactosylation, galactose-specific HRP-PNA staining was used to detect quantitative differences in the galactosylation of cell-surface proteins. Like the *gms1* Δ mutant, neither the *uge1* Δ strain nor the *uge1* Δ *gal10* Δ strain reacted with PNA (Fig. 2b). In addition, the *uge1* Δ and the *uge1* Δ *gal10* Δ strains exhibited sensitivity to hygromycin B (Fig. 2c), which is an aminoglycoside antibiotic used for isolating glycosylation mutants (Dean, 1995; Takegawa *et al.*, 1996; Yoko-o *et al.*, 2001). These results suggest that Uge1p is the predominant enzyme involved in galactosylation of glycoproteins in *Sch. pombe*.

gal10⁺ is efficiently expressed in low-glucose medium but not in galactose-containing medium

To test UDP-glucose/-galactose epimerase activity in the disruptants, the two-step assay was used (Brahma & Bhattacharyya, 2004) (Fig. 3a). In glucose-rich medium (2 % glucose), the *uge1* Δ and the *uge1* Δ *gal10* Δ double mutant had about 20 % activity compared to that of wild-type. On the other hand, epimerase activity of the *gal10* Δ mutant showed little change. These results indicate that Uge1p is responsible for most epimerase activity in rich medium containing 2 % glucose.

Table 1. UDP-glucose/-galactose 4-epimerase homologues from various organisms

Organism	Name of homologue (and accession number)	
	Epimerase domain only	Epimerase + mutarotase domain
<i>Saccharomyces cerevisiae</i>	–	Gal10 (NP_009575)
<i>Vanderwaltozyma polyspora</i> DSM 70294	–	Hypothetical protein (XP_001647142)
<i>Lachancea thermotolerans</i> CBS 6340	–	KLTH0A01958p (XP_002551545)
<i>Zygosaccharomyces rouxii</i> CBS 732	–	Hypothetical protein (XP_002494552)
<i>Kluyveromyces lactis</i> NRRL Y-1140	–	GALX_KLULA (XP_455462)
<i>Candida tropicalis</i> MYA-3404	–	GAL10 bifunctional protein (XP_002550320)
<i>Candida dubliniensis</i> CD36	–	GAL10 bifunctional protein (XP_002416873)
<i>Candida albicans</i> SC5314	–	Gal10 (XP_713766)
<i>Debaryomyces hansenii</i> CBS767	–	Hypothetical protein (XP_457787)
<i>Pichia guilliermondii</i> ATCC 6260	–	Hypothetical protein (XP_001482100)
<i>Pichia stipitis</i> CBS 6054	–	Gal10 (XP_001383357)
<i>Lodderomyces elongisporus</i> NRRL YB-4239	–	GAL10 bifunctional protein (XP_001526820)
<i>Schizosaccharomyces pombe</i>	Uge1 (NP_596043)	Gal10 (NP_596858)
<i>Schizosaccharomyces japonicus</i> yFS275	Hypothetical protein (XP_002173024)	Hypothetical protein (XP_002174949)
<i>Schizosaccharomyces octosporus</i> yFS286	Hypothetical protein (SOCG_04717.2)	Hypothetical protein (SOCG_02640.2)
<i>Yarrowia lipolytica</i> CLIB122	–	YALI0E26829p (XP_504440)
<i>Botryotinia fuckeliana</i> B05.10	Hypothetical protein (XP_001550259)	–
<i>Sclerotinia sclerotiorum</i> 1980	Gal10 (XP_001590586)	–
<i>Aspergillus niger</i>	Hypothetical protein (XP_001401007)	–
<i>Aspergillus terreus</i> NIH2624	UDP-glucose 4-epimerase (XP_001216252)	–
<i>Aspergillus fumigatus</i> Af293	UDP-glucose 4-epimerase (XP_753568)	–
<i>Aspergillus oryzae</i> RIB40	Hypothetical protein (XP_001827449)	–
<i>Talaromyces stipitatus</i> ATCC 10500	UDP-glucose 4-epimerase (XP_002479094)	–
<i>Magnaporthe grisea</i> 70-15	Hypothetical protein (XP_362429)	–
<i>Penicillium marneffeii</i> ATCC 18224	UDP-glucose 4-epimerase (XP_002146810)	–
<i>Aspergillus clavatus</i> NRRL 1	UDP-glucose 4-epimerase (XP_001274365)	–
<i>Penicillium chrysogenum</i> Wisconsin 54-1255	Pc21g10370 (XP_002568072)	–
<i>Aspergillus nidulans</i> FGSC A4	Hypothetical protein (XP_662331)	–
<i>Neurospora crassa</i> OR74A	GAL10 bifunctional protein (XP_957519)	–
<i>Clostridium cellulolyticum</i> H10	UDP-glucose 4-epimerase (YP_002504732)	–
<i>Arthrobacter aureus</i> TC1	UDP-glucose 4-epimerase (YP_948505)	–
<i>Bacillus subtilis</i> subsp. <i>subtilis</i> strain 168	UDP-glucose 4-epimerase (NP_391765)	–
<i>Escherichia coli</i> ATCC 8739	UDP-glucose 4-epimerase (ACA78806)	–
<i>Streptococcus gordonii</i> strain Challis substrain CH1	GalE-1 (YP_001450301)	–
<i>Dictyostelium discoideum</i> AX4	UDP-glucose 4-epimerase (XP_643834)	–
<i>Arabidopsis thaliana</i>	UGE1 (NP_172738)	–
<i>Oryza sativa</i>	Os09g0323000 (NP_001062869)	–
<i>Rattus norvegicus</i>	GALE (NP_542961)	–
<i>Mus musculus</i>	GALE (NP_848476)	–
<i>Homo sapiens</i>	GALE (NP_001008217)	–

In *Sac. cerevisiae*, transcription of *GAL10* is regulated by the Gal4p transcription factor, a positive regulator of gene expression for galactose-induced genes such as *GAL1*, *GAL2*, *GAL7*, *GAL10* and *MEL1*, which play important roles in galactose metabolism (Hashimoto *et al.*, 1983; Keegan *et al.*, 1986; Lohr *et al.*, 1995). Gal4p recognizes a 17 bp sequence (5'-CGGRNNRCYNYNCNCCG-3') in the upstream activating sequence (UAS_{GAL}) of these genes (Lohr *et al.*, 1995; Fedor & Kornberg, 1989). Interestingly, the *Sch. pombe* genome lacks a *gal4*⁺ homologue, and the upstream sequences of *uge1*⁺ and *gal10*⁺ also lack a typical UAS_{GAL} as far as we can ascertain. Therefore, we checked

the mRNA levels of *uge1*⁺ and *gal10*⁺ in cells growing in galactose-containing medium (Fig. 3b). Northern analysis indicated the presence of *uge1*⁺ mRNA in cells growing in 2 % glucose medium, but no increase was observed in cells grown in medium containing 2 % glucose and 2 % galactose. These results indicate that *uge1*⁺ was expressed constitutively and was not induced by galactose. On the other hand, *gal10*⁺ mRNA was expressed weakly regardless of the presence of galactose. These findings indicate that *uge1*⁺ encodes a major UDP-glucose/-galactose epimerase in cells growing in rich medium containing 2 % glucose.

Fig. 2. Characterization of the glycosylation defect and growth phenotype of *uge1Δ* and *gal10Δ* strains. (a) Lysates from cells induced to express acid phosphatase were subjected to gel electrophoresis and stained for acid phosphatase activity. Wild-type (WT) and each disruptant are shown. (b) The dot-spot staining assay of cell lysates indicates agglutination with HRP-PNA. (c) Cells were streaked onto YES plates containing 25 μg hygromycin B ml⁻¹ and incubated at 30 °C for 3 days.

In most organisms, glucose plays important regulatory roles in the expression of many genes, including those encoding proteins that function in respiration and glycolysis, utilization of alternative carbon sources, glucose transporters, and others (Johnston, 1999; Rolland *et al.*, 2001, 2002). In *Sch. pombe*, however, these control mechanisms have not been analysed in detail (Tanaka *et al.*, 1998; Hoffman, 2005; Kig *et al.*, 2005), especially for enzymes needed for utilization of galactose as an alternative carbon source. Interestingly, *Sch. pombe* is not able to use galactose as a sole carbon source. Therefore, we tested whether *uge1⁺* mRNA and *gal10⁺* mRNA were induced in

Fig. 3. Enzymic activity of UDP-glucose/-galactose 4-epimerase and expression level of *uge1⁺* and *gal10⁺*. (a) UDP-glucose/-galactose 4-epimerase activity was observed in each disruptant. Wild-type and mutant strains were cultured in MM containing 2% glucose or 0.1% glucose and 2% galactose as carbon sources. UDP-glucose 4-epimerase was extracted from the cultures and activity was monitored by measuring A_{340} . Values are means $\pm$ SEM, $n=3$. (b) Northern blot analysis of *uge1⁺* and *gal10⁺* mRNA. Total RNA was extracted from the wild-type cells grown in glucose-containing (2% glucose) medium, or in medium containing both 2% glucose and 2% galactose, or under glucose-repressing (0.1% glucose, 2% galactose) conditions. Each RNA sample (20 μg) was separated on agarose gel in the presence of formamide, blotted onto a nylon membrane and hybridized to *uge1⁺*, *gal10⁺* and *leu1⁺* probes.

glucose-repressed (0.1% glucose, 2% galactose) medium. *uge1⁺* mRNA was detected at the same level as in cells growing in 2% glucose medium, indicating that *uge1⁺* mRNA is expressed constitutively and is not induced as a consequence of glucose repression (Fig. 3b). *gal10⁺* mRNA was detected at the same level as *uge1⁺* mRNA in cells grown in 0.1% glucose, 2% galactose medium, indicating that *gal10⁺* is induced during glucose repression (Fig. 3b). We next tested whether the UDP-glucose/-galactose epimerase activity in the *uge1Δ* strain was elevated in cells grown in the 0.1% glucose/2% galactose medium (Fig. 3a). The *uge1Δ* strain grown in 0.1% glucose, 2% galactose medium showed a rise of UDP-glucose/-galactose epimer-

ase activity while no detectable increase in activity was observed in the *uge1Δgal10Δ* strain. These results indicate that *gal10⁺* is efficiently expressed in cells grown in 0.1 % glucose medium and Gal10p has UDP-glucose/-galactose epimerase activity in *Sch. pombe*.

Under glucose-repressing conditions, Gal10p is involved in galactosylation of cell-surface proteins

We demonstrated that the *uge1Δ* and the *uge1Δgal10Δ* strains were not reactive with HRP-PNA and were sensitive to hygromycin B (Fig. 2b, c) due to insufficient galactosylation of secreted cell-surface glycoproteins. To verify the role of Gal10p in galactosylation, we confirmed suppression of the galactosylation defect in the *uge1Δ* strain under glucose-repressing conditions (Fig. 4). The hygromycin B sensitivity of the *uge1Δ* strain was overcome under preculture conditions in YES (0.1 % Glc, 2 % glycerol) preculture medium, but not in YES (2 % Glc) preculture medium (Fig. 4a). Further, the HRP-PNA staining showed that galactosylation of the cell surface of the *uge1Δ* mutant was restored in the YES (0.1 % Glc, 2 % glycerol) medium (Fig. 4b). These results indicate that Gal10p can replace loss of Uge1p in the *uge1Δ* mutant in YES (0.1 % Glc, 2 %

glycerol) medium, which is consistent with the activity (Fig. 3a) and expression (Fig. 3b) data.

In complementary studies, we next investigated the effect of *gal10⁺* overexpression on galactosylation in the *uge1Δ* strain (Fig. 5) using the pREP1 plasmid containing the thiamine-repressible wild-type promoter (Maundrell, 1990). The induced level is about $80 \times$ greater than the repressed level. pREP41 has a $6 \times$ lower induced level and a $15 \times$ lower repressed level than the wild-type promoter (Basi *et al.*, 1993; Maundrell, 1993; Forsburg & Rhind, 2006). As expected, HRP-PNA staining showed that galactosylation of the cell surface of the *uge1Δ* strain was restored in an expression-dependent manner (Fig. 5a), as was the amount of acid phosphatase-associated oligosaccharides (Fig. 5b).

Fig. 4. Growth and galactosylation phenotypes of *uge1Δ* and *gal10Δ* strains in glucose-repressing medium. (a) Wild-type and each disruptant were pre-cultured to exponential phase in YES medium containing 2 % glucose or 0.1 % glucose and 2 % glycerol. Cells were then streaked onto YES plates (0.1 % glucose, 2 % glycerol and 20 μg hygromycin B ml⁻¹), and incubated for 3 days at 30 °C. (b) The dot-spot HRP-PNA staining assay. Wild-type and each disruptant were cultured to exponential phase in YES medium containing 2 % glucose or 0.1 % glucose and 2 % glycerol. Each cell lysate was stained with HRP-PNA.

Fig. 5. Phenotype of the *uge1Δ* strain overexpressing *gal10⁺* under the *nmt* promoters. Each strain was incubated in MM containing 2 % glucose for 16 h. The *uge1Δ* strain overexpressed *gal10⁺* either under the pREP1 or pREP41 *nmt* promoters. Cell lysates of each strain were stained with HRP-PNA (a), and subjected to gel electrophoresis followed by staining for acid phosphatase activity (b).

Gal7p is associated with UDP-galactose biosynthesis in *Sch. pombe*

As documented above, *Sch. pombe* cannot grow on galactose-containing medium with galactose as sole carbon source. This phenotype has greatly complicated analysis of the Leloir pathway in this yeast. Therefore, we wanted to determine whether galactose was transported from the medium to the cytosol and whether it was converted into UDP-galactose via Gal-1-P. The *uge1Δgal10Δ* strain is useful for assessing this pathway in *Sch. pombe*. If galactosylation of the cell surface is recovered on galactose-containing medium in the *uge1Δgal10Δ* strain, this indicates that the galactose residues in the glycoproteins were synthesized using UDP-galactose formed from Gal-1-P by Gal-1-P uridylyltransferase. Fig. 6(a) shows that HRP-PNA staining of the *uge1Δ* and *uge1Δgal10Δ* strains was indeed restored in the galactose-containing medium.

In *Sch. pombe*, a search for homologue(s) of *Sac. cerevisiae* GAL7, which encodes Gal-1-P uridylyltransferase, identified SPBPB2B2.10c, designated *gal7⁺* in this study. The predicted amino acid sequences of the *Sc* GAL7 and *gal7⁺*

gene products share 63 % identity. To examine whether *gal7⁺* is involved in the recovery of HRP-PNA staining, we constructed a *uge1Δgal10Δgal7Δ* triple disruptant (Fig. 6a). HRP-PNA staining was not observed in either the *uge1Δgal10Δgal7Δ* strain or the *uge1Δgal10Δgms1Δ* triple disruptant in galactose-containing medium. These results indicate that galactose is transported into the cytosol from the medium and is converted into UDP-galactose via Gal-1-P by Gal7p in *Sch. pombe*.

We next determined the UDP-galactose content of the cytosol in these strains (Fig. 6b). All the disruptants except *gal10Δ* had reduced levels of cytosolic UDP-galactose when grown in 2 % glucose medium. After incubation in medium containing 1 % galactose, the *uge1Δ* and *uge1Δgal10Δ* strains had increased amounts of cytosolic UDP-galactose. In contrast, increased UDP-galactose content in the *uge1Δgal10Δgal7Δ* strain was not detected in cells grown in the 1 % galactose-containing medium. These results indicate that Gal7p is involved in UDP-galactose biosynthesis in *Sch. pombe*.

DISCUSSION

Glycoproteins in *Sch. pombe* contain a large amount of galactose in addition to both N-linked and O-linked mannan (Manners & Meyer, 1977; Moreno *et al.*, 1985; Ikeda *et al.*, 2009; Ohashi *et al.*, 2009). The galactose residues are modified by galactosyltransferases using UDP-galactose as substrate (Andreishcheva *et al.*, 2004; Chappell *et al.*, 1994; Yoko-o *et al.*, 1998). UDP-galactose is transported to the Golgi lumen from the cytosol by a Golgi-localized UDP-galactose transporter (Gms1p) (Tabuchi *et al.*, 1997; Tanaka & Takegawa, 2001). In *Sch. pombe*, biosynthesis of cytosolic UDP-galactose is poorly characterized. UDP-galactose/glucose 4-epimerase is the key enzyme involved in UDP-galactose synthesis in the cytosol, where it catalyses the interconversion of UDP-galactose and UDP-D-glucose. Interestingly, *Schizosaccharomyces* species have two types of UDP-glucose/-galactose 4-epimerase homologues, although other organisms have only one kind. It is not known why two types of epimerase are needed and how they are regulated in *Sch. pombe*.

Here we show (1) that *Sch. pombe* has two genes encoding functional epimerases (named *uge1⁺* and *gal10⁺*); (2) that Uge1p is a major UDP-glucose/-galactose epimerase expressed constitutively in cells grown in glucose-rich medium (2 % glucose, Fig. 7a); (3) that *gal10⁺* is efficiently expressed and involved in galactosylation of cell surface proteins in low-glucose medium (0.1 % glucose, Fig. 7a), but not in galactose-containing medium; and (4) that galactose is transported into the cytosol and is converted into UDP-galactose via Gal-1-P by Gal7p in *Sch. pombe* (Fig. 7b).

In this study, we shed new light on the Leloir pathway from the standpoint of UDP-galactose synthesis in *Sch. pombe* (Fig. 7). It is interesting to note that *Schizosaccharomyces*

Fig. 6. Galactosylation phenotype and UDP-galactose content in cells grown in galactose-containing medium. (a) Cells were incubated in MM containing 2 % glucose (Glc+, Gal−), or 1 % glucose and 1 % galactose (Glc+, Gal+). Cell lysates of each strain were stained with HRP-PNA. (b) UDP-galactose was extracted from strains cultured to exponential phase in MM containing 2 % glucose (2 % Glc MM) or 1 % glucose and 1 % galactose (1 % Glc, 1 % Gal MM). UDP-galactose content was monitored by measuring A_{340} . Values are means $\pm$ SEM, $n=3$.

Fig. 7. Proposed model for UDP-galactose synthesis in *Sch. pombe*. (a) UDP-galactose synthesis in glucose medium. Uge1p is constitutively expressed in 2% glucose medium (Uge1 > Gal10) and Gal10p is induced to the same level as Uge1p (Uge1 ≅ Gal10) in 0.1% glucose medium. UDP-galactose is transported by the Gms1p UDP-Gal transporter into the Golgi lumen, where it is utilized for galactosylation of glycoproteins. (b) UDP-galactose synthesis in the *uge1Δgal10Δ* strain in galactose-containing medium. Galactose is transported into the cytosol from the medium by unidentified transporters and is converted into UDP-galactose via galactose-1-phosphate by Gal7p.

species are the only organisms known to have two types of UDP-glucose/-galactose 4-epimerase (Figs 1 and 7, Table 1). To our knowledge, other yeast species only have the Sc Gal10 type of epimerase, which has both epimerase and mutarotase domains. A key difference between these yeast species and

Sch. pombe is that *Sch. pombe* can modify galactose for use in synthesizing glycoproteins, while other yeasts modify mannose for the same purpose (Gemmell & Trimble, 1999). This might suggest that the Uge1 type of epimerase with only an epimerase domain could have evolved for

efficient UDP-galactose production for galactosylation of glycoproteins in *Sch. pombe*. We previously reported that disruption of the *gms1*⁺ gene led to a complete loss of protein galactosylation, due to a defect in transport of UDP-galactose from the cytosol to the lumen of the Golgi apparatus as a substrate for galactosyltransferase (Fig. 7) (Tabuchi *et al.*, 1997; Tanaka *et al.*, 2001). The *gms1Δ* strain exhibited aberrant cell morphology and increased sensitivity to digestion with β -glucanase and to various drugs, such as hygromycin B, sodium orthovanadate and calcofluor white. Further, the *gms1Δ* strain was incapable of sexual conjugation during nutritional starvation and of non-sexual flocculation (Tanaka *et al.*, 1999, 2001). The *uge1Δ* strain also had a severe galactosylation defect and a decrease in epimerase activity and UDP-galactose content when grown in glucose-rich medium (2% glucose) (Figs 2, 3 and 6b), indicating that Uge1p is a major UDP-glucose/-galactose epimerase expressed constitutively in 2% glucose medium (Fig. 7a).

In *Sac. cerevisiae*, all the galactose-inducible genes (*GAL1*, *GAL10*, *GAL7* and *GAL2*) are coordinately regulated at the level of transcription in response to galactose by Gal4p, Gal80p and Gal3p (De Robichon-Szulmajster, 1958; Lohr *et al.*, 1995; Platt & Reece, 1998). In the absence of galactose, binding of Gal80p to Gal4p effectively limits active transcription of the *GAL* genes. In the presence of galactose, Gal3p–Gal80p interactions are triggered in the cytoplasm, resulting in redistribution of Gal80p from the nucleus to the cytoplasm, thereby relieving inhibition of Gal4p and resulting in *GAL* gene expression (Peng & Hopper, 2002). However, the *Sch. pombe* genome has no homologues of Gal4p, Gal80p or Gal3p. Further, the promoter sequences of *uge1*⁺, *gal10*⁺ and *gal7*⁺ also lack typical Gal4p-binding sequences [UAS_{GAL}: 5'-CGGRNN-RCYNYNCNCCG-3' (Fedor & Kornberg, 1989; Lohr *et al.*, 1995)]. In this study, we found that *gal10*⁺ is efficiently expressed and involved in galactosylation of cell-surface proteins in cells grown in low (0.1%) glucose medium, but not in galactose-containing medium (Figs 3b, 4 and 7a). We previously reported that the *inv1*⁺ gene that encodes invertase is repressed in the presence of glucose (Tanaka *et al.*, 1998). The transcription of *inv1*⁺ is regulated by Scr1p, which is required for glucose repression of *inv1*⁺ (Tanaka *et al.*, 1998) and *fbp1*⁺ (Neely & Hoffman, 2000). Similarly, Scr1p may have repressed the transcription of *gal10*⁺ in 2% glucose medium.

Sch. pombe is not able to grow on galactose-containing medium with galactose as sole carbon source. This phenotype makes it difficult to analyse the Leloir pathway in *Sch. pombe*, despite the presence of the three relevant enzymes, galactokinase, Gal-1-P uridyltransferase and UDP-glucose/-galactose epimerase. To address this problem, we used a *uge1Δgal10Δ* strain. Recovery of galactosylation in the *uge1Δgal10Δ* strain indicated that UDP-galactose was produced by sequential reaction of galactokinase and Gal7 without UDP-glucose/-galactose epimerase (Figs 6 and 7b). These results also suggest that *Sch.*

pombe is able to transport galactose from the medium into the cytosol by unidentified transporters. In *Sac. cerevisiae*, Gal2p (Tschopp *et al.*, 1986), which is an integral plasma membrane protein predicted to contain 12 transmembrane domains (Nehlin *et al.*, 1989) is able to transport galactose. *GAL2* is also regulated at the level of transcription in response to galactose by Gal4p (De Robichon-Szulmajster, 1958; Lohr *et al.*, 1995; Platt & Reece, 1998), and loss of Gal2p's permease activity renders cells unable to utilize galactose as a sole carbon source (Douglas & Condie, 1954). In contrast, a *GAL2* homologue was not found in the *Sch. pombe* genome. Multiple genes that encode hexose transporters (Ght1 to Ght6) have been identified in *Sch. pombe* (Heiland *et al.*, 2000). These transporters are involved in glucose, fructose and gluconate transport from the extracellular milieu into the cytosol (Heiland *et al.*, 2000). However, a galactose transporter has not yet been identified (Fig. 7b). Using the *uge1Δgal10Δ* strain should help reveal not only the function of galactokinase and Gal7p, but also the galactose transport machinery in *Sch. pombe*. *Schizosaccharomyces japonicus* and *Sch. octosporus* also lack genes involved in regulation of the Leloir pathway, suggesting that the features of galactose metabolism identified in *Sch. pombe* may also apply in other *Schizosaccharomyces* species.

We were not able to analyse the mutarotase domain of Gal10p in this study. Gal10p was efficiently expressed under low (0.1%) glucose conditions, suggesting that mutarotase activity might be elevated under these conditions. Further studies will be necessary to determine how galactokinase and Gal7p are regulated under various growth conditions in *Sch. pombe*.

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REFERENCES

- Alfa, C., Fantes, P., Hyams, J., McLoed, M. & Warbrick, E. (1993). *Experiments with Fission Yeast: a Laboratory Course Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Andreishcheva, E. N., Kunkel, J. P., Gemmill, T. R. & Trimble, R. B. (2004). Five genes involved in biosynthesis of the pyruvylated Gal β 1,3-epitope in *Schizosaccharomyces pombe* N-linked glycans. *J Biol Chem* **279**, 35644–35655.
- Basi, G., Schmid, E. & Maundrell, K. (1993). TATA box mutations in the *Schizosaccharomyces pombe nmt1* promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. *Gene* **123**, 131–136.
- Brahma, A. & Bhattacharyya, D. (2004). UDP-galactose 4-epimerase from *Kluyveromyces fragilis*. Evidence for independent mutarotation site. *Eur J Biochem* **271**, 58–68.
- Chappell, T. G., Hajibagheri, M. A., Ayscough, K., Pierce, M. & Warren, G. (1994). Localization of an α -1,2 galactosyltransferase

- activity to the Golgi apparatus of *Schizosaccharomyces pombe*. *Mol Biol Cell* 5, 519–528.
- Dean, N. (1995). Yeast glycosylation mutants are sensitive to aminoglycosides. *Proc Natl Acad Sci U S A* 92, 1287–1291.
- De Robichon-Szulmajster, H. (1958). Induction of enzymes of the galactose pathway in mutants of *Saccharomyces cerevisiae*. *Science* 127, 28–29.
- Dibenedetto, G. & Cozzani, I. (1975). Nonspecific acid phosphatase from *Schizosaccharomyces pombe*. Purification and physical chemical properties. *Biochemistry* 14, 2847–2852.
- Douglas, H. C. & Condie, F. (1954). The genetic control of galactose utilization in *Saccharomyces*. *J Bacteriol* 68, 662–670.
- Fedor, M. J. & Kornberg, R. D. (1989). Upstream activation sequence-dependent alteration of chromatin structure and transcription activation of the yeast *GAL1–GAL10* genes. *Mol Cell Biol* 9, 1721–1732.
- Forsburg, S. L. & Rhind, N. (2006). Basic methods for fission yeast. *Yeast* 23, 173–183.
- Frey, P. A. (1996). The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose. *FASEB J* 10, 461–470.
- Gemmill, T. R. & Trimble, R. B. (1996). *Schizosaccharomyces pombe* produces novel pyruvate-containing N-linked oligosaccharides. *J Biol Chem* 271, 25945–25949.
- Gemmill, T. R. & Trimble, R. B. (1998). All pyruvylated galactose in *Schizosaccharomyces pombe* N-glycans is present in the terminal disaccharide, 4,6-O-[(R)-(1-carboxyethylidene)]-Gal β 1,3Gal α 1. *Glycobiology* 8, 1087–1095.
- Gemmill, T. R. & Trimble, R. B. (1999). Overview of N- and O-linked oligosaccharide structures found in various yeast species. *Biochim Biophys Acta* 1426, 227–237.
- Hashimoto, H., Kikuchi, Y., Nogi, Y. & Fukasawa, T. (1983). Regulation of expression of the galactose gene cluster in *Saccharomyces cerevisiae*. Isolation and characterization of the regulatory gene *GAL4*. *Mol Gen Genet* 191, 31–38.
- Heiland, S., Radovanovic, N., Hofer, M., Winderickx, J. & Lichtenberg, H. (2000). Multiple hexose transporters of *Schizosaccharomyces pombe*. *J Bacteriol* 182, 2153–2162.
- Hoffman, C. S. (2005). Except in every detail: comparing and contrasting G-protein signaling in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Eukaryot Cell* 4, 495–503.
- Holden, H. M., Rayment, I. & Thoden, J. B. (2003). Structure and function of enzymes of the Leloir pathway for galactose metabolism. *J Biol Chem* 278, 43885–43888.
- Huang, K. M. & Snider, M. D. (1995). Isolation of protein glycosylation mutants in the fission yeast *Schizosaccharomyces pombe*. *Mol Biol Cell* 6, 485–496.
- Ikeda, Y., Ohashi, T., Tanaka, N. & Takegawa, K. (2009). Identification and characterization of a gene required for α 1,2-mannose extension in the O-linked glycan synthesis pathway in *Schizosaccharomyces pombe*. *FEMS Yeast Res* 9, 115–125.
- Johnston, M. (1999). Feasting, fasting and fermenting. Glucose sensing in yeast and other cells. *Trends Genet* 15, 29–33.
- Keegan, L., Gill, G. & Ptashne, M. (1986). Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein. *Science* 231, 699–704.
- Kig, C., Turkel, S. & Temizkan, G. (2005). Isolation and characterization of glucose derepressed invertase mutants from *Schizosaccharomyces pombe*. *Biosci Biotechnol Biochem* 69, 2475–2478.
- Lohr, D., Venkov, P. & Zlatanova, J. (1995). Transcriptional regulation in the yeast *GAL* gene family: a complex genetic network. *FASEB J* 9, 777–787.
- Majumdar, S., Ghatak, J., Mukherji, S., Bhattacharjee, H. & Bhaduri, A. (2004). UDPgalactose 4-epimerase from *Saccharomyces cerevisiae*. A bifunctional enzyme with aldose 1-epimerase activity. *Eur J Biochem* 271, 753–759.
- Manners, D. J. & Meyer, M. T. (1977). The molecular structures of some glucans from the cell walls of *Schizosaccharomyces pombe*. *Carbohydr Res* 57, 189–203.
- Maundrell, K. (1990). *nmt1* of fission yeast. A highly transcribed gene completely repressed by thiamine. *J Biol Chem* 265, 10857–10864.
- Maundrell, K. (1993). Thiamine-repressible expression vectors pREP and pRIP for fission yeast. *Gene* 123, 127–130.
- Moreno, S., Ruiz, T., Sanchez, Y., Villanueva, J. R. & Rodriguez, L. (1985). Subcellular localization and glycoprotein nature of the invertase from the fission yeast *Schizosaccharomyces pombe*. *Arch Microbiol* 142, 370–374.
- Moreno, S., Klar, A. & Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol* 194, 795–823.
- Morita, T. & Takegawa, K. (2004). A simple and efficient procedure for transformation of *Schizosaccharomyces pombe*. *Yeast* 21, 613–617.
- Nakamura, T., Nakamura-Kubo, M., Hirata, A. & Shimoda, C. (2001). The *Schizosaccharomyces pombe spo3⁺* gene is required for assembly of the forespore membrane and genetically interacts with *psy1⁺*-encoding syntaxin-like protein. *Mol Biol Cell* 12, 3955–3972.
- Neely, L. A. & Hoffman, C. S. (2000). Protein kinase A and mitogen-activated protein kinase pathways antagonistically regulate fission yeast *fbp1* transcription by employing different modes of action at two upstream activation sites. *Mol Cell Biol* 20, 6426–6434.
- Nehlin, J. O., Carlberg, M. & Ronne, H. (1989). Yeast galactose permease is related to yeast and mammalian glucose transporters. *Gene* 85, 313–319.
- Ohashi, T., Ikeda, Y., Tanaka, N., Nakakita, S., Natsuka, S., Giga-Hama, Y. & Takegawa, K. (2009). The *och1* mutant of *Schizosaccharomyces pombe* produces galactosylated core structures of N-linked oligosaccharides. *Biosci Biotechnol Biochem* 73, 407–414.
- Okazaki, K., Okazaki, N., Kume, K., Jinno, S., Tanaka, K. & Okayama, H. (1990). High-frequency transformation method and library transducing vectors for cloning mammalian cDNAs by trans-complementation of *Schizosaccharomyces pombe*. *Nucleic Acids Res* 18, 6485–6489.
- Peng, G. & Hopper, J. E. (2002). Gene activation by interaction of an inhibitor with a cytoplasmic signaling protein. *Proc Natl Acad Sci U S A* 99, 8548–8553.
- Platt, A. & Reece, R. J. (1998). The yeast galactose genetic switch is mediated by the formation of a Gal4p-Gal80p-Gal3p complex. *EMBO J* 17, 4086–4091.
- Rolland, F., Winderickx, J. & Thevelein, J. M. (2001). Glucose-sensing mechanisms in eukaryotic cells. *Trends Biochem Sci* 26, 310–317.
- Rolland, F., Winderickx, J. & Thevelein, J. M. (2002). Glucose-sensing and -signalling mechanisms in yeast. *FEMS Yeast Res* 2, 183–201.
- Sambrook, J., Fritsch, E. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schweingruber, M. E., Fluri, R., Maundrell, K., Schweingruber, A. M. & Dumermuth, E. (1986). Identification and characterization of thiamin repressible acid phosphatase in yeast. *J Biol Chem* 261, 15877–15882.
- Scott, A. & Timson, D. J. (2007). Characterization of the *Saccharomyces cerevisiae* galactose mutarotase/UDP-galactose 4-epimerase protein, Gal10p. *FEMS Yeast Res* 7, 366–371.

- Suga, M. & Hatakeyama, T. (2001).** High efficiency transformation of *Schizosaccharomyces pombe* pretreated with thiol compounds by electroporation. *Yeast* **18**, 1015–1021.
- Suga, M., Isobe, M. & Hatakeyama, T. (2000).** Cryopreservation of competent intact yeast cells for efficient electroporation. *Yeast* **16**, 889–896.
- Tabuchi, M., Tanaka, N., Iwahara, S. & Takegawa, K. (1997).** The *Schizosaccharomyces pombe* *gms1*⁺ gene encodes an UDP-galactose transporter homologue required for protein galactosylation. *Biochem Biophys Res Commun* **232**, 121–125.
- Takegawa, K., Tanaka, N., Tabuchi, M. & Iwahara, S. (1996).** Isolation and characterization of a glycosylation mutant from *Schizosaccharomyces pombe*. *Biosci Biotechnol Biochem* **60**, 1156–1159.
- Tanaka, N. & Takegawa, K. (2001).** Functional characterization of Gms1p/UDP-galactose transporter in *Schizosaccharomyces pombe*. *Yeast* **18**, 745–757.
- Tanaka, N., Ohuchi, N., Mukai, Y., Osaka, Y., Ohtani, Y., Tabuchi, M., Bhuiyan, M. S., Fukui, H., Harashima, S. & Takegawa, K. (1998).** Isolation and characterization of an invertase and its repressor genes from *Schizosaccharomyces pombe*. *Biochem Biophys Res Commun* **245**, 246–253.
- Tanaka, N., Awai, A., Bhuiyan, M. S., Fujita, K., Fukui, H. & Takegawa, K. (1999).** Cell surface galactosylation is essential for nonsexual flocculation in *Schizosaccharomyces pombe*. *J Bacteriol* **181**, 1356–1359.
- Tanaka, N., Konomi, M., Osumi, M. & Takegawa, K. (2001).** Characterization of a *Schizosaccharomyces pombe* mutant deficient in UDP-galactose transport activity. *Yeast* **18**, 903–914.
- Thoden, J. B. & Holden, H. M. (2005).** The molecular architecture of galactose mutarotase/UDP-galactose 4-epimerase from *Saccharomyces cerevisiae*. *J Biol Chem* **280**, 21900–21907.
- Tschopp, J. F., Emr, S. D., Field, C. & Schekman, R. (1986).** *GAL2* codes for a membrane-bound subunit of the galactose permease in *Saccharomyces cerevisiae*. *J Bacteriol* **166**, 313–318.
- Umeda, K., Yoko-o, T., Nakayama, K., Suzuki, T. & Jigami, Y. (2000).** *Schizosaccharomyces pombe* *gmd3*⁺/*alg11*⁺ is a functional homologue of *Saccharomyces cerevisiae* *ALG11* which is involved in *N*-linked oligosaccharide synthesis. *Yeast* **16**, 1261–1271.
- Yoko-o, T., Roy, S. K. & Jigami, Y. (1998).** Differences in in vivo acceptor specificity of two galactosyltransferases, the *gmh3*⁺ and *gma12*⁺ gene products from *Schizosaccharomyces pombe*. *Eur J Biochem* **257**, 630–637.
- Yoko-o, T., Tsukahara, K., Watanabe, T., Hata-Sugi, N., Yoshimatsu, K., Nagasu, T. & Jigami, Y. (2001).** *Schizosaccharomyces pombe* *och1*⁺ encodes α -1,6-mannosyltransferase that is involved in outer chain elongation of *N*-linked oligosaccharides. *FEBS Lett* **489**, 75–80.

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