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Gss1 protein of the methylotrophic yeast *Pichia pastoris* is involved in glucose sensing, pexophagy and catabolite repression

Andriy S. Polupanov[a,c], Volodymyr Y. Nazarko[a,c], Andriy A. Sibirny[a,b,c,*]

[a] Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, 79005 Lviv, Ukraine
[b] Department of Biotechnology and Microbiology, Rzeszow University, Zelwerowicza 4, Rzeszow 35-601, Poland
[c] Key State Laboratory of Molecular and Cellular Biology, Lviv, Ukraine

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ABSTRACT

In the yeast *Saccharomyces cerevisiae*, the one-at-a-time deletions of either the high-affinity glucose sensor gene *SNF3* or the low-affinity glucose sensor gene *RGT2* only slightly reduced pexophagy; however, deleting both genes greatly reduced pexophagy, evincing interaction beyond the sum of the additive effects, as recently shown. The present study identifies the only *ScSNF3*/*RGT2* ortholog in the methylotrophic yeast *Pichia pastoris* (designated as *PpGSS1*, from GlucoSe Sensor) and describes its roles in autophagic pathways (non-selective and selective). *GSS1* knock-out strain has been constructed. The experiments support the hypothesis that Gss1 plays an important role in autophagic degradation of peroxisomes and glucose catabolite repression in *P. pastoris*.

1. Introduction

Autophagy is a conservative and complex process in eukaryotic cells that provides recycling of intracellular components (e.g. proteins or organelles) and allows the cell to adapt to the environmental changes (such as nitrogen source limitation) (Kelekar, 2005; Schmid et al., 2006). The molecular mechanisms of autophagic degradation of cellular material are under extensive investigations. Non-specific and selective types of autophagy are known (Kiel, 2010; Sibirny, 2011). Pexophagy is a type of selective autophagic degradation of abundant peroxisomes in response to carbon source shift (Farré and Subramani, 2004; Dunn et al., 2005; Monastyrska and Klionsky, 2006). Frequently, pexophagy in yeasts is monitored after the shift of the cells grown in the medium with peroxisome proliferators (oleate, methanol, methylamine) to the medium with glucose or ethanol (Nazarko et al., 2008a,b). Unfortunately, little is known about glucose sensing and signaling under pexophagy induced in glucose medium.

Studies of pexophagy often use the model of baker's yeast *Saccharomyces cerevisiae.* Despite the advantages of methylotrophic yeast *Pichia pastoris*. In baker's yeast, peroxisome proliferation is induced only by oleate. However, both oleate and methanol induce peroxisome proliferation in *P. pastoris*; indeed methanol induces much larger peroxisomes. Additionally, *P. pastoris* possesses two types of peroxisome degradation, macropexophagy and micropexophagy; micropexophagy is induced by glucose in *P. pastoris* (Tuttle and Dunn, 1995). Only macropexophagy is known for baker's yeast.

It was recently shown that simultaneous deletion of two glucose sensors, Snf3 and Rgt2, led to strong pexophagy deficiency in the yeast *S. cerevisiae* (Nazarko et al., 2008a). *S. cerevisiae SNF3* and *RGT2* genes encode for high and low-affinity glucose sensors, respectively. Table 1 describes protein involvement in pexophagy and glucose catabolite repression. Snf3 and Rgt2 have 60% of protein sequence identity and overall are similar to the yeast Hxt (hexose transporters) proteins (Özcan et al., 1996a). Snf3 and Rgt2 possess 12 transmembrane domains (Neigeborn et al., 1986; Özcan et al., 1996a,b). They also contain long C-terminal cytoplasmic tails that play a role in glucose signal transduction (Özcan et al., 1998; Dlugai et al., 2001).

Besides having similar structures, the genes Snf3 and Rg2 have similar functions. Their involvement in glucose signal transduction was identified decades ago. They initiate signaling cascade via interaction with yeast caseine kinase 1(Yck1) and via inactivation of Rgt1, which is a repressor of genes encoding glucose transporters (Neigeborn et al., 1986; Özcan et al., 1996a,b). Recently, complementary roles of ScSnf3 and ScRgt2 were discovered. Defects in ScSnf3 and ScRgt2 have slight effect on glucose-induced pexophagy in oleate-grown cells, whereas simultaneous deletion of

* Corresponding author at: Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, 79005 Lviv, Ukraine. Tel.: +380 322 612 108.
E-mail address: sibirny@yahoo.com (A.A. Sibirny).

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Table 1
List of yeast sensors, transporters and their orthologs with information on their possible role in pexophagy and glucose catabolite repression.

Protein	Organism	Role	Involvement in pexophagy	Involvement in catabolite repression
Gss1	*P. pastoris*	Potential high and low-affinity glucose sensor	Retarded pexophagy [this study]	Impaired glucose catabolite repression [this study]
Snf3	*S. cerevisiae*	High-affinity glucose sensor	Retarded pexophagy [Nazarko et al., 2008a]	Not involved [unpublished data]
Rgt2	*S. cerevisiae*	Low-affinity glucose sensor	Retarded pexophagy [Nazarko et al., 2008a]	Not involved [unpublished data]
Hxs1	*H. polymorpha*	Glucose sensor	Not involved [Stasyk et al., 2008a]	Not involved [Stasyk et al., 2008a]
Gcr1	*H. polymorpha*	Potential glucose transporter	Retarded pexophagy [Stasyk et al., 2004]	Impaired glucose catabolite repression [Stasyk et al., 2004]
Hxt1	*P. pastoris*	Hexose transporter	Not involved [Zhang et al., 2010]	Impaired glucose catabolite repression [Zhang et al., 2010]
Hxt2	*P. pastoris*	Hexose transporter	Not involved [Zhang et al., 2010]	Not involved [Zhang et al., 2010]
Gpr1	*S. cerevisiae*	G-protein coupled receptor involved in cAMP-dependent glucose signaling	Retarded pexophagy [Nazarko et al., 2008a,b]	Not involved [unpublished data]
Gpa2	*S. cerevisiae*	Alpha subunit of the heterotrimeric G protein that interacts with the receptor Gpr1	Retarded pexophagy [Nazarko et al., 2008a,b]	Not involved [unpublished data]
Gpr1	*P. pastoris*	Ortholog of ScGpr1	Not involved [Nazarko et al., 2008a,b]	n/d
Gpa2	*P. pastoris*	Ortholog of ScGpa2	Not involved [Nazarko et al., 2008a,b]	n/d

these genes strongly affected peroxisome degradation in the yeast *S. cerevisiae* (Nazarko et al., 2008a).

In *Hansenula polymorpha*, the *S. cerevisiae* Snf3/Rgt2 ortholog Gcr1 plays an important role in glucose catabolite repression (Stasyk et al., 2004). Mutants lacking Gcr1 are able to grow on methanol in the presence of toxic glucose analog 2-deoxy-D-glucose; they showed constitutive synthesis of methanol oxidase and the presence of peroxisomes in glucose medium without methanol (Stasyk et al., 2004). It was demonstrated that deficiency in *GCR1* retarded but did not block pexophagy upon adaptation of methanol-grown cells to glucose. However, that the role of Gcr1 membrane protein remains opaque. It was suggested that Gcr1 acts as glucose sensor but its role as high affinity glucose transporter may be plausible because Gcr1 protein lacks long cytoplasmic tail, which appears in the *S. cerevisiae* Snf3 and Rgt2 sensor proteins (Stasyk et al., 2004).

A hexose transporter homologue gene, *HXS1* (*He*X*ose S*ensor), was identified and shown to be involved in transcriptional regulation in response to hexoses (Stasyk et al., 2008a). The Hxs1 gene's protein sequence is most similar to the *S. cerevisiae* transporter-like glucose sensors, Snf3 and Rgt2. Interestingly, Hxs1 deficiency has moderate effect on glucose utilization and growth, much less than the effect of *Sc*Snf3 and *Sc*Rgt2 deficiencies. However, despite of high homology to the hexose transporters, the overexpression of heterologous *HXS1* in *S. cerevisiae hxt null* mutant did not restore growth on glucose, suggesting its non-transporting function. The glucose sensor Hxs1 was involved in neither glucose catabolite repression nor glucose-induced pexophagy. A third identified glucose sensor gene *HXT1* encodes functional hexose transporter (Stasyk et al., 2008a).

Two hexose transporters were recently identified in the yeast *P. pastoris*, Hxt1 and Hxt2, which are transcriptionally regulated by glucose. Deletion of *PpHXT1* but not *PpHXT2*, led to the expression of alcohol oxidase in glucose medium due to glucose catabolite repression impairment. However, mutant lacking *Pp*Hxt1 was normal in both respects, glucose utilization and peroxisome degradation (Zhang et al., 2010).

To study the role of glucose sensors in the autophagic pathways, we have found that knock out mutants defective in orthologs of *S. cerevisiae GPR1* and *GPA2* genes are involved in cAMP-dependent glucose signaling. It was shown that in contrast to *S. cerevisiae*, the Δ*gpr1* and Δ*gpa2* mutants of *P. pastoris* do not have any defects in pexophagy (Nazarko et al., 2008a,b). The role of *P. pastoris* alternative glucose sensing system (homologous to *S. cerevisiae* Snf3/Rgt2 sensing system) in autophagy was not studied so far. In this paper, we describe *P. pastoris* Gss1 protein, the ortholog of *S. cerevisiae* Snf3/Rgt2 and *H. polymorpha* Gcr1 and Hxs1 sensors, and its role in pexophagy. It was found that Gss1 protein plays a role of glucose sensor and is involved in pexophagy and glucose catabolite repression in this yeast.

2. Materials and methods

2.1. Strains, plasmids, and transformation

The strains used in this study are listed in Table 2.

2.2. GSS1 deletion cassette construction

Flanking regions of *GSS1* ORF were amplified with PCR using the primers AB1 and AB2 (upstream region, 987 bp), AB3 and AB4 (downstream region, 616 bp), digested with restriction enzymes and cloned into pOS5 vector (Stasyk et al., 1999) carrying *ScARG4* as a selectable marker to create pAB2. Vector was designed for deletion of full ORF of gene. Plasmid carrying *GSS1* deletion cassette was verified with PCR and restriction enzyme analysis. To obtain deletion mutants GS200 wild-type strain of *P. pastoris* was transformed with this deletion cassette (PCR amplificated). The transformants were selected on glucose minimal medium without arginine. The knock-out strains were selected among the transformants by PCR and Southen blot (Supplementary Fig. S2).

2.3. DNA procedures

Standard DNA techniques were carried out essentially accordingly to previously described (Sambrook et al., 1989). Transformation of *P. pastoris* was performed by electroporation and Li-acetate method (Toby and Golemis, 2001).

2.4. Media and growth conditions

All synthetic media contained 50 mM potassium phosphate buffer (PiB) pH 7.5. Synthetic minimal medium (SMD) contained 0.17% yeast nitrogen base without amino acids and ammonium sulfate (YNB), 2% glucose, 0.5% ammonium sulfate, and 40–50 mg/l of auxotrophic amino acids if needed.