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Functional analysis of novel glycosyltransferases toward the remodeling of the fission yeast glycans

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Research aims

The glycans from the fission yeast *Schizosaccharomyces pombe* contain large amounts of α-linked D-galactose (Gal) in addition to α-linked D-mannose (Man) unlike other well-studied yeasts or human. Alpha galactosylation, which is one of the significant characteristics of *S. pombe*, can be a primary hindrance to the development of therapeutic glycoprotein production system in this yeast, because α-galactosylation can cause the immune response in human body. To overcome this problem, the complete elimination of α-galactosylation is required. Previously we succeeded in eliminating α-linked Gal residues by disruption of a *gms1*$^+$ gene, which encodes Golgi localized UDP-Gal transporter. However UDP-Gal transporter activity is required for β1,4-galactosylation processes that produce the biantennary complex oligosaccharides in a subsequent humanization step in *S. pombe*. As an alternative approach, we constructed a septuple α-galactosyltransferase-related gene disruptant (7GalTΔ) and then analyzed its glycan structures. The glycan structural analysis of the 7GalTΔ mutant revealed that it still had α1,3-linked Gal residues, indicating the presence of at least one more additional unidentified α1,3-galactosyltransferase[1]. In this study, we aimed to identify the novel α1,3-galactosyltransferase(s) and to construct the *S. pombe* α-galactosylation null mutant by multiple α-galactosyltransferase gene disruptions.

Methods

The fission yeast strains were cultivated in YES medium at 30°C and harvested in early stationary phase. Cell surface glycoproteins were extracted by autoclaving at 121°C for 90 min in citrate buffer followed by precipitation with methanol. The precipitates were dissolved in hot water, dialyzed and lyophilized. Oligosaccharides were liberated from glycoproteins by hydrazinolysis, followed by *N*-acetylation. The reducing ends of the liberated oligosaccharides were pyridylaminated (PA) as described[2]. The PA-oligosaccharides were analyzed by size-fractionation HPLC.

Results

In order to identify undiscovered α1,3-galactosyltransferase(s), we searched for glycosyltransferase genes in the *S. pombe* genome sequence. Among the predicted glycosyltransferase genes, three genes (designated *otg1*$^+$, *otg2*$^+$ and *otg3*$^+$ for alpha one, three-galactosyltransferase, respectively) with unknown functions were identified. We disrupted the three genes from the 7GalTΔ mutant to construct a strain lacking 10 presumptive α-galactosyltransferase genes (10GalTΔ). The PA-glycans were prepared from glycoproteins from wild-type, *gms1*Δ, 7GalTΔ and 10GalTΔ strains, and were analyzed by size-fractionation HPLC. The chromatogram of the 10GalTΔ strain was quite similar to that of the *gms1*Δ strain. In the *otg1*Δ*otg2*Δ*otg3*Δ triple disruption strain, the peaks corresponding to tetra-saccharides containing an α1,3-Gal residue were absent, but peaks corresponding to di- and tri-saccharides containing an α1,2-Gal residue were still present (Fig. 1).

To assess enzymatic activity of Otg proteins, they were expressed in 10GalTΔ cells. Solubilized membrane preparations from 10GalTΔ cells in which Otg proteins expressed were used as the enzyme source (Fig. 2). Assays carried out with UDP-Gal as donor substrate revealed that the Otg2 protein had Gal transfer activity toward a $Man_9GlcNAc_2$-PA and Manα1,2-Manα1,2-Man-PA. In addition, the Otg3 protein exhibited Gal transfer activity toward the $Man_9GlcNAc_2$-PA. Generation of an α1,3-linkage was confirmed by HPLC analysis, α-galactosidase digestion analysis, ^{1}H

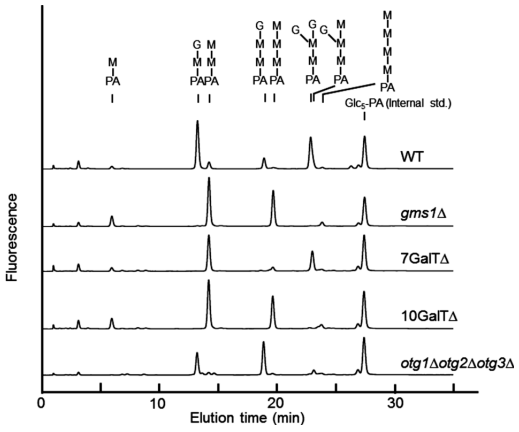

Fig. 1. Size-fractionation HPLC of *O*-linked glycans from 10GalTΔ strain.

The chromatograms show *O*-linked oligosaccharides from each fission yeast strain. The vertical bars indicate the elution positions of the standard Man-PA and previously identified peaks in wild-type strain. In the schematic structures shown, the vertical and the diagonal bars between the letters indicate α1,2- and α1,3-linkages, respectively. Abbreviations: G, galactose; M, mannose; PA, pyridylamino.

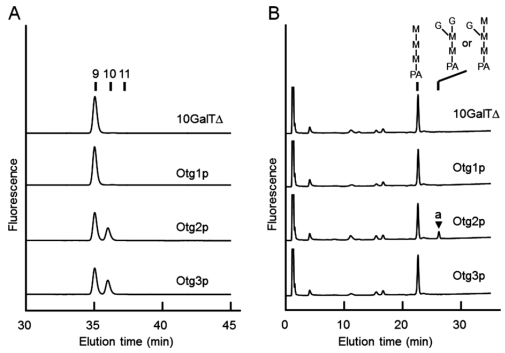

Fig. 2. *In vitro* enzymatic assay of Otg proteins.

The size-fractionation HPLC profiles of the reaction mixtures using the acceptor substrate $Man_9GlcNAc_2$-PA (A), Manα1,2Manα1,2Man-PA (B) and solubilized enzyme extracts from 10GalTΔ expressing Otg proteins are shown.

NMR spectroscopy and LC-MS/MS analysis. These results indicate that Otg2p and Otg3p are involved in α1,3-galactosylation of *S. pombe* oligosaccharides.

Conclusion

We have shown for the first time that the Otg proteins are glycosyltransferases with the ability to form α1,3-linkages between Gal and α-Man residues. Complete elimination of α-galactosylation in *S. pombe* was achieved by multiple deletions of newly found *otg*$^+$ genes. Moreover, the 10GalTΔ mutant constructed in the present study is likely to be valuable with respect to subsequent β-galactosylation, because α-galactosylation was absent, and simultaneously the Golgi-localized UDP-Gal transporter was still present.

References

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3) Ohashi, T., Fujiyama, K., and Takegawa, K. (2012) Identification of novel α1,3-galactosyltransferase and elimination of α-galactose-containing glycans by disruption of multiple α-galactosyltransferase genes in *Schizosaccharomyces pombe*. *J. Biol. Chem.* **287**: 38866–38875.