Elucidation of the mechanism underlying capsular polysaccharide biosynthesis in *Cryptococcus neoformans*

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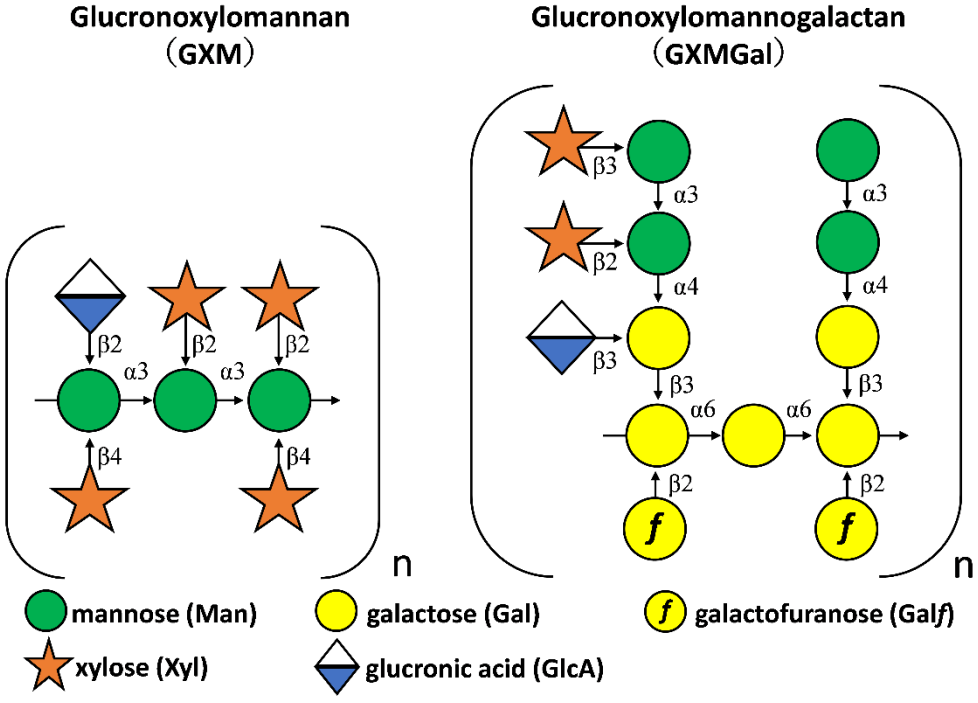
Research objective

Cryptococcus neoformans, a yeast belonging to the division Basidiomycota, is the primary etiological agent of cryptococcosis. This pathogen synthesizes a thick capsular polysaccharide on the surface layer of its cell wall, which is considered the main virulence factor contributing to the pathogenicity of the organism as it aids in evasion of the immune system of the host. The capsule is composed of two polysaccharides, namely glucuronoxylomannan and glucuronoxylomannogalactan; however, the mechanism underlying their biosynthesis remains ambiguous. Therefore, the objective of this study was to identify the glycosyltransferases responsible for capsule biosynthesis in *C. neoformans*.



Structures of GXM and GXMGal

Methods

In *C. neoformans*, disruption of the GDP-mannose (Man), UDP-xylose (Xyl), UDP-glucuronic acid (GlcA), and UDP-galactose (Gal) transporter genes results in aberrations in capsule size[1,2,3]. This led to the hypothesis that glycosyltransferases involved in capsular

production might exist among the proteins localized in the Golgi apparatus. The candidate proteins were expressed as recombinant proteins using a bacterial expression system, and their glycosyltransferase activities were evaluated. Furthermore, an analysis was conducted to examine the impact of disrupting candidate genes on capsular synthesis in *C. neoformans*.

Results

The candidate proteins that were effectively expressed in a soluble form by the bacterial expression system were assayed for activity with varying combinations of 4-methylumbelliferylated sugar (4MU) and sugar-nucleotides. Consequently, among these proteins, two were discovered to generate reaction products when exposed to the combination of 4MU-β-Gal and GDP-Man. These proteins were designated Cryptococcal β-Galactoside Mannosyltransferase 1 (Cgm1) and Cgm2, owing to their shared homology of approximately 40%. The reaction product of Cgm1 was prepared and purified via high-performance liquid chromatography, and its structure was analyzed using ^{1}H-NMR and methylation gas chromatography-mass spectrometry (GC-MS). The Cgm1 products structurally resembled 4MU-β-Gal with the C1-position of α-Man attached to the C4-position, indicating that Cgm1 is a β-Gal α-(1→4)-Man transferase. Subsequently, *cgm1* and *cgm2* disruptants of *C. neoformans* H99 were constructed using the CRISPR/Cas9 system and their phenotypes were assessed. The *cgm1* disruptant strain exhibited a minor discrepancy in growth from the wild-type strain at 30°C; nonetheless, growth was substantially delayed at 37°C. Conversely, the *cgm2* disruptant strain displayed no discernible phenotype. As some acapsular mutants are known to be temperature-sensitive at 37°C[3, 4], we hypothesized that Cgm1 might be involved in capsule biosynthesis. Based on the results of Cgm1 enzyme activity analysis, Cgm1 was predicted to be involved in the biosynthesis of the galactomannan side chain of GXMGal. GXMGal exhibits a weak interaction with GXM, and in the GXM-deficient strain, it diffuses into the culture supernatant, facilitating purification. Therefore, a double disruptant of *cgm1* and *cap59* was engineered, which was an α-(1→3)-Man transferase gene presumed to be involved in the biosynthesis of GXM. A comparative analysis of the structure of GXMGal using ^{13}C-NMR and methylation GC-MS revealed that the *cgm1* disruptant strain-generated GXMGal appeared to have lost or eliminated the galactomannan side chain. These results indicate that Cgm1 is a β-Gal α-(1→4)-Man transferase involved in the biosynthesis of the galactomannan side chain of GXMGal.

Conclusion

The study findings established Cgm1 and Cgm2 as novel β-Gal α-(1→4)-Man transferases, among which Cgm1 was found to be involved in the biosynthesis of GXMGal in *C. neoformans*. Considering the absence of Cgm1 homologs in mammals, including humans, and

the exhibition of a temperature-sensitive phenotype in the *cgm1*-disrupted strain at 37°C, Cgm1 may be considered an effective potential target for the development of novel antifungal agents to combat cryptococcosis.

References

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