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研究課題：Clarification of a novel degradation pathway for O-Man glycans in S. cerevisiae
研究報告：

(a) Abstract:

There are two types of protein glycosylations known to occur in budding yeast, *Saccharomyces cerevisiae*; *N*-glycosylation on Asn residues and *O*-glycosylation (*O*-mannose glycans) on Ser/Thr residues. They play multiple roles in various cellular contexts, and their biosynthetic pathway has been well characterized. In sharp contrast, we know little about the catabolic pathway for these glycans. For the last decade we have gained knowledge on the catabolic pathways for *N*-glycans and their precursor (dolichol-linked oligosaccharides) in budding yeast, while virtually nothing was known for the degradation of *O*-mannose glycans.

We recently discovered that, when yeast is grown in media with mannose as a sole carbon source (mannose-media), novel free, unconjugated glycans were formed. Upon detailed structural characterization, it was found that they are **free, unconjugated glycans structurally identical to *O*-mannose glycans ("free *O*-glycans")**. With these preliminary data in hand, in this proposal we aim at clarifying the molecular mechanism by which this free *O*-glycans is generated.

(b) Objective:

We tried to gain insight into the formation mechanism of free *O*-glycans in *Saccharomyces cerevisiae*.

(c) Method:

We utilized various yeast mutants to characterize how these free *O*-mannose glycans are generated.

(d) Results:

In terms of the mechanism how these free *O*-mannose glycans are generated, we initially speculated that these "free" *O*-mannose glycans may be generated either by (1) release of *O*-mannose glycans from a putative de-*O*-mannosylating enzyme so-called "endo-*O*-mannosidase (EOMase) (Fig.), or alternatively (2) glycans are elongated by biosynthetic enzymes for *O*-mannose glycans on free Mannose in cells. To validate this hypothesis, we carried out experiments using various genetic mutants, and it was found that, in mutants having defect in the first attachment of *O*-mannose glycans, significant reduction of the amount of free *O*-glycans were observed.

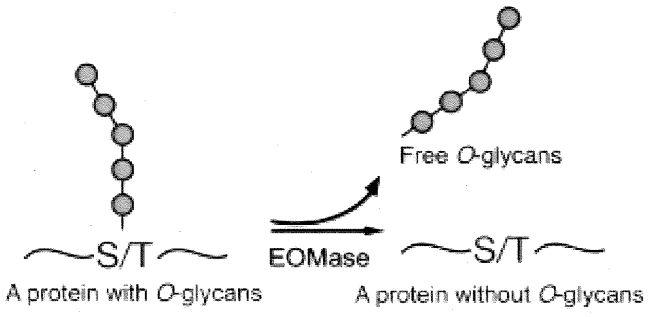

Fig. Release of free *O*-glycans by EOMase.

SAMPLE OF THE ABSTRACT

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Moreover, we did not see the elongation of polymannoses on mannose-derivatives when they are included in the culture media. Based on these results, we favored the former possibility, and the occurrence of novel de-*O*-mannosylating enzyme (EOMase) was strongly suggested.

As we see the occurrence of free *O*-mannose glycans only when yeasts were cultured in mannose-media, we hypothesized that they are under the regulation of glucose-repression (i.e. gene expression is suppressed when glucose is available from the media). Accordingly, we tested various defective mutants for the glucose-repression, and found that a deletion of *cyc8*, a transcription factor involved in glucose-repression, resulted in vast increase of the amount of free *O*-glycans in mannose-media. Moreover, *cyc8* mutants exhibited unusual sensitivity toward mannose-media, implying that Cyc8 may be involved in the controlled expression of a putative EOMase gene, and *cyc8*-deletion mutant may cause constitutive activation of EOMase, leading to cell wall-defect and sensitivity toward mannose-media. Indeed, *cyc8* mutants also exhibited a sensitivity towards cell wall-disturbing reagents. Taken all results together, it was hypothesized that the expression of EOMase gene was strictly regulated and its overexpression may cause the unwanted de-*O*-mannosylation of cell wall proteins, leading to the cell wall defect.

We are currently trying to develop an *in vitro* assay system for EOMase. Once assay method is established, we aim at purifying EOMase from yeast extract. We will then identify the EOMase and will clarify its physiological function by in-depth phenotypic analyses of its gene-deletion mutant (in case that the gene was not essential), or its temperature-sensitive or a tetracycline-controlled transcriptional suppression (tet-off) mutant (in case that the gene was essential).

(e) Discussion:

In this study we could identify the novel catabolic event for *O*-mannose glycans in budding yeast. EOMase has not been identified in any organisms, and therefore this study serves as a first step towards clarifying the novel catabolic pathway. Since *O*-mannose glycans are conserved between yeast to human, it would be interesting to see how well this catabolic pathway is conserved in eukaryotes. As *O*-manose glycans are implicated in the genetic disorders (muscular dystrophies) in human, the identification of EOMase gene may become a valuable tool for structural analysis of human *O*-mannose glycans.

(f) Publication:

Hiroto Hirayama, Tsugiyo Matsuda, Yae Tsuchiya, Ritsuko Oka, Junichi Seino, Chengcheng Huang, Kazuki Nakajima, Yoichi Noda, Yuichi Shichino, Shintaro Iwasaki, and Tadashi Suzuki (2019) "Free glycans derived from *O*-mannosylated glycoproteins suggest the presence of an *O*-glycoprotein degradation pathway in yeast", **The *Journal of Biological Chemistry*, 294,** 15900-15911. (doi: 10.1074/jbc.RA119.009491) **(Selected as "Editors' Picks").**