博士（生命科学）　陸　　吉　順

学位論文題名

Study of the multi-glycosyltransferase complex involved in initiation of lipid-linked oligosaccharide biosynthesis in endoplasmic reticulum

（小胞体リン脂質オリゴ糖（LLO）前駆体生合成の開始を司る糖転移酵素複合体に関する研究）

学位論文内容の要旨

N-Glycosylation is an essential posttranslational modification of proteins occurring in eukaryotes. *N*-Glycosylation begins with the multistep biosynthesis of a highly conserved lipid-linked oligosaccharide (LLO), which is extended by a series of membrane-associated Alg (asparagine-linked glycosylation) glycosyltransferases to produce $Glc_3Man_9GlcNAc_2$-PP-dolichol in the endoplasmic reticulum (ER). Defects in LLO biosynthesis lead to diseases known as type I congenital disorders of glycosylation (CDG I), underscoring its biological and clinical importance. The first two steps of LLO biosynthesis are catalyzed sequentially by a functional multienzyme complex comprised of the Alg7 GlcNAc phosphotransferase and the heterodimeric Alg13/Alg14 UDP-GlcNAc transferase on the cytosolic face of the ER. In the Alg13/14 glycosyltransferase, Alg14 works as transmembrane protein that recruits cytosolic Alg13 protein to the ER membrane through interaction between their C-termini. However, the molecular mechanism of complex formation among Alg7, Alg13, and Alg14 still remains unclear.

Unlike the other ER glycosyltransferases involved in LLO assembly, both Alg7 and Alg13/Alg14 display significant sequence similarity to bacterial proteins involved in the early stages of peptidoglycan synthesis. Alg7, which catalyzes the initial step of LLO biosynthesis, has long been recognized as a member of conserved family of UDP-HexNAc-1-P transferases that include the bacterial MraY glycosyltransferase that catalyzes the formation of the first lipid-linked intermediate of peptidoglycan in *Escherichia coli*. The second step in LLO synthesis is catalyzed by Alg13/14 glycosyltransferase. Similarly, the bacterial homologue of Alg13/14 glycosyltransferase,

MurG, catalyzes the second membrane-associated step of peptidoglycan synthesis. Alg13 is homologous to C-terminal catalytic domain of MurG, while Alg14 is homologous to the N-terminal domain of MurG that contains the membrane association domain. Although the membrane association mechanism of MurG has been well-defined, how the eukaryotic Alg14 protein associates with the membrane still remains obscure.

Bioinformatic analysis revealed that eukaryotic Alg14 contains an evolved N-terminal region that is missing in its bacterial orthologue MurG. Here, I found that this N-terminal region of *Saccharomyces cerevisiae* Alg14 could localize its GFP-fusion to the ER membrane. Deletion of this region caused no significant phenotype at permissive temperature, while a severe growth defect was observed when the temperature was raised up to 38.5 °C. Over-expression of Alg7 could partially complement the temperature sensitive phenotype, suggesting a functional interaction between N-terminal region of Alg14 and Alg7. Coimmunoprecipitation provided the direct evidence for their interaction. Moreover, it was shown that Alg14 lacking the N-terminal region still remained on the ER membrane through a nonperipheral association, indicating the existence of another membrane-binding site. Further mutational studies guided by the 3D structural analysis of Alg14 identified a conserved α-helix involved in the second membrane association site that contributes to an integral interaction and protein stability.

Based on the structural analyses of Alg14, a model was proposed for how the Alg7/13/14 multienzyme complex is formed, in which Alg14 acts as a central scaffold that is anchored tightly to the ER surface through two different binding sites and recruits two catalytic partners, Alg7 and Alg13, to form the multiglycosyltransferase complex that initiates *N*-glycosylation.

The data here clearly suggest that the mechanism of membrane association is different between eukaryotes and prokaryotes, since MurG lacks the additional N-terminal region found in Alg14. Considering the fact that MurG is an essential bacterial glycosyltransferase found in all organisms that synthesize peptidoglycan and is a target for the design of new antibiotics, from a practical viewpoint, my data provide new insights that may impact the design of specific antibiotics that target the interaction of MurG with the bacterial membrane.

学位論文審査の要旨

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博士学位論文審査等の結果について（報告）

N-Glycosylation is an essential posttranslational modification of proteins occurring in eukaryotes. *N*-Glycosylation begins with the multistep biosynthesis of a highly conserved lipid-linked oligosaccharide (LLO), which is extended by a series of membrane-associated Alg (asparagine-linked glycosylation) glycosyltransferases to produce $Glc_3Man_9GlcNAc_2$-PP-dolichol in the endoplasmic reticulum (ER). Defects in LLO biosynthesis lead to diseases known as type I congenital disorders of glycosylation (CDG I), underscoring its biological and clinical importance. The first two steps of LLO biosynthesis are catalyzed sequentially by a functional multienzyme complex comprised of the Alg7 GlcNAc phosphotransferase and the heterodimeric Alg13/Alg14 UDP-GlcNAc transferase on the cytosolic face of the ER. In the Alg13/14 glycosyltransferase, Alg14 works as transmembrane protein that recruits cytosolic Alg13 protein to the ER membrane through interaction between their C-termini. However, the molecular mechanism of complex formation among Alg7, Alg13, and Alg14 still remains unclear.

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synthesis is catalyzed by Alg13/14 glycosyltransferase. Similarly, the bacterial homologue of Alg13/14 glycosyltransferase, MurG, catalyzes the second membrane-associated step of peptidoglycan synthesis. Alg13 is homologous to C-terminal catalytic domain of MurG, while Alg14 is homologous to the N-terminal domain of MurG that contains the membrane association domain. Although the membrane association mechanism of MurG has been well-defined, how the eukaryotic Alg14 protein associates with the membrane still remains obscure.

Bioinformatic analysis revealed that eukaryotic Alg14 contains an evolved N-terminal region that is missing in its bacterial orthologue MurG. Here, the author found that this N-terminal region of *Saccharomyces cerevisiae* Alg14 could localize its GFP-fusion to the ER membrane. Deletion of this region caused no significant phenotype at permissive temperature, while a severe growth defect was observed when the temperature was raised up to 38.5 °C. Over-expression of Alg7 could partially complement the temperature sensitive phenotype, suggesting a functional interaction between N-terminal region of Alg14 and Alg7. Co-immunoprecipitation provided the direct evidence for their interaction. Moreover, it was shown that Alg14 lacking the N-terminal region still remained on the ER membrane through a nonperipheral association, indicating the existence of another membrane-binding site. Further mutational studies guided by the 3D structural analysis of Alg14 identified a conserved α-helix involved in the second membrane association site that contributes to an integral interaction and protein stability.

In conclusion, based on the structural analyses of Alg14, the author proposed a model for how the Alg7/13/14 multienzyme complex is formed, in which Alg14 acts as a central scaffold that is anchored tightly to the ER surface through two different binding sites and recruits two catalytic partners, Alg7 and Alg13, to form the multi-glycosyltransferase complex that initiates *N*-glycosylation. The data clearly suggest that the mechanism of the membrane association of Alg13/14 glycosyltransferase and MurG is different between eukaryotes and prokaryotes, since MurG lacks the additional N-terminal region found in Alg14. Considering the fact that MurG is an essential bacterial glycosyltransferase found in all prokaryotes that synthesize peptidoglycan and is a target for the design of new antibiotics, from a practical viewpoint, these results provide new insights that may impact the design of specific antibiotics that target the interaction of MurG with the bacterial membrane. Therefore, we acknowledge that the author is qualified to be granted the Doctorate of Life Science from Hokkaido University.