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学位論文名	TbGPI16 is an essential component of GPI transamidase in *Trypanosoma brucei* （*Trypanosoma brucei* における GPI transamidase コンポーネント TbGPI16 のノックアウト解析）
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論文内容の要旨

〔 目　　的 〕

Purpose : Glycosylphosphatidylinositol (GPI) is widely used by eukaryotic cell surface proteins for membrane attachment. *De novo* synthesized GPI precursors are attached to proteins posttranslationally by the enzyme complex, GPI transamidase. TbGPI16, a component of the trypanosome transamidase, shares similarity with human PIG-T. It has been shown that PIG-T stabilizes the enzyme complex GPI8, which is the catalytic component responsible for cleavage of GPI-attachment signal sequences, by a disulfide bond. Therefore, we wanted to determine whether TbGPI16 is a functional component of GPI transamidase and forms a disulfide bridge with TbGPI8 by conserved cysteine residues in trypanosomes.

〔 方法ならびに成績 〕

Results and Discussion :

1. *Construction and characterization of the TbGPI16 knockout trypanosome*

To determine whether TbGPI16 is a functional component of GPI transamidase, we knocked out the *TbGPI16* gene by replacement with a NEO or BSD resistance gene in the procyclic form of *T. brucei*. The knockout parasite completely failed to express procyclins on the surface. Transfection with TbGPI16 into these knockout cells restored surface expression to a level similar to that of wild type.

By examination of the GPI biosynthesis in TbGPI16 knockout, we confirmed that GPI precursor biosynthesis was unaffected. This result, taken together with the deficient cell surface procyclin expression, indicates that TbGPI16 is an essential component of GPI transamidase.

2. *Protein complex analysis of TbGPI16 with TbGPI8*

To identify a component covalently linked with TbGPI16, we transfected C-terminally FLAG-GST-tagged

TbGPI16 gene into the TbGPI16 knockout. Transamidase complexes purified by immunoprecipitation with anti-FLAG were analyzed by Western blot using anti-TbGPI8 monoclonal antibody. In this experiment, we demonstrated that TbGPI16 is disulfide linked to TbGPI8. Then, we constructed cysteine-to-serine mutants of TbGPI16. The C239S mutant of TbGPI16 did not form complexes with TbGPI8, indicating that TbGPI16 is disulfide linked to TbGPI8 through cysteine 239 of TbGPI16.

3. *The expression of EP procyclin on the surface of C239S mutant trypanosome*

To examine whether the disulfide linkage is important for the GPI transamidase activity, we determined the abilities of these transfectants to restore the surface expression of EP-procyclin. Wild-type TbGPI16 transfectant restored the surface procyclin expression of this knockout cells, whereas C239S mutants restored it at a significantly lower level.

〔 総　　括 〕

Conclusion : In this study, we showed that TbGPI16 is the orthologue of PIG-T and an essential component of GPI transamidase by creating a TbGPI16 knockout. TbGPI16 forms a disulfide-linked complex with TbGPI8. A cysteine to serine mutant of TbGPI16 was unable to fully restore the surface expression of GPI-anchored proteins upon transfection into the knockout cells. Thus, the disulfide linkage between TbGPI8 and TbGPI16 is important for the full transamidase activity, suggesting its potential role in assisting the proper positioning of these two components and/or stabilization of the complex.

論文審査の結果の要旨

GPIはアフリカ睡眠病を起こすトリパノソーマの増殖及び感染成立において重要である。したがってトリパノソーマGPI生合成系は治療薬開発のよい標的である。本研究では生合成経路の最後のステップである、GPIをタンパク質に結合させるトランスアミデーションのステップに注目し、TbGPI16遺伝子をノックアウトすることでTbGPI16がトランスアミダーゼのコンポーネントとして機能していることを証明することを目的とした。TbGPI16のノックアウト細胞では予想通りタンパクやGPIの生合成は正常であるが、GPIアンカー型タンパク質であるプロサイクリンの細胞表面での発現が消失していた。トリパノソーマのTbGPI16はTbGPI8とジスルフィド結合しておりそのジスルフィド結合はトランスアミダーゼの活性に重要であることを示した。以上の成果は、*TbGPI16*がGPIトランスアミダーゼのコンポーネントの一つとして生理的に機能している事を初めて証明したものであり、学位に値すると考える。