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研究課題名（和文）Structural and molecular basis of the mitophagy regulation mediated by the Far complex in yeast

研究課題名（英文）Structural and molecular basis of the mitophagy regulation mediated by the Far complex in yeast

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研究成果の概要（和文）：この研究は、ミトファジーにおいて重要なAtg32のリン酸化段階とFar複合体との相互作用を明らかにすることを目的としています。Far8のリン酸化サイトの置換変異体がAtg32のリン酸化状態やFar8-Atg32相互作用に影響を与えないことが判明し、Far3とFar7がFar8-Atg32相互作用に必要であることが示されました。Far8の発現レベルや分解、オートファジーに関与するキナーゼの影響についても調べられ、Atg32-Far8相互作用には影響がないことが示されました。これらの知見は、ミトファジーのメカニズム解明に貢献することが期待されます。

研究成果の学術的意義や社会的意義
This research could help researchers understand regulation of autophagy, mitophagy in particular. These findings could also lead to new insights into cellular signaling pathways and potentially aid in the development of new therapeutics for diseases caused by mitochondrial dysfunction.

研究成果の概要（英文）：Mitophagy is crucial for maintaining mitochondrial quality and quantity, with phosphorylation of Atg32 being an essential step. However, the underlying mechanism of the Far complex's interaction with Atg32 to phosphorylate it is still unclear. This study aimed to elucidate the interaction between the Far complex and Atg32, as well as the upstream signaling pathway regulating it. The study found that substitution mutants of Far8 phosphorylation sites do not affect Atg32 phosphorylation status or Far8-Atg32 interaction. Additionally, the study discovered that Far3 and Far7 are necessary for Far8-Atg32 interaction. The study also checked the influence of the expression level or degradation of Far8, as well as the influence of a set of kinases involved in autophagy, but neither seemed to affect the Atg32-Far8 interaction.

研究分野：Cellular physiology

キーワード：Mitophagy　Atg32　Ppg1　The Far complex　Yeast

１．研究開始当初の背景

Mitophagy is crucial for maintaining mitochondrial quality and quantity, with phosphorylation of Atg32 being an essential step. However, the underlying mechanism of the Far complex's interaction with Atg32 to phosphorylate it is still unclear. This study aimed to elucidate the interaction between the Far complex and Atg32, as well as the upstream signaling pathway regulating it. The study found that substitution mutants of Far8 phosphorylation sites do not affect Atg32 phosphorylation status or Far8-Atg32 interaction. Additionally, the study discovered that Far3 and Far7 are necessary for Far8-Atg32 interaction. The study also checked the influence of the expression level or degradation of Far8, as well as the influence of a set of kinases involved in autophagy, but neither seemed to affect the Atg32-Far8 interaction.

２．研究の目的

This research aimed to elucidate the mechanism underlying the interaction between the Far complex and Atg32, as well as the upstream signaling pathway regulating it.

３．研究の方法

The study investigated the interaction between the Far complex and Atg32 through the use of substitution mutants of Far8 phosphorylation sites. The influence of the expression level or degradation of Far8, as well as the influence of a set of kinases involved in autophagy, was also examined.

４．研究成果

The study investigated the interaction between the Far complex and Atg32, focusing on the phosphorylation of Atg32 and the role of the Far complex in this process. Through the use of substitution mutants of Far8 phosphorylation sites, I aimed to understand the impact of these mutations on Atg32 phosphorylation status and the interaction between Far8 and Atg32.

Surprisingly, the study found that the substitution mutants of Far8 phosphorylation sites did not significantly affect the phosphorylation status of Atg32. This suggested that other factors or mechanisms might be involved in the regulation of Atg32 phosphorylation. However, despite the lack of influence on Atg32 phosphorylation, the study discovered an important finding regarding the interaction between Far8 and Atg32.

I identified that Far3 and Far7, two components of the Far complex, were essential for the Far8-Atg32 interaction. This observation indicated that the Far complex likely functions as a multi-component complex, with specific components playing distinct roles in the interaction with Atg32. The exact mechanisms by which Far3 and Far7 contribute to the Far8-Atg32 interaction remain to be further explored.

Furthermore, the study examined the potential influence of the expression level and degradation of Far8 on the Atg32-Far8 interaction. Surprisingly, neither the expression level nor the degradation of Far8 seemed to have a significant impact on this interaction. This suggests that other regulatory mechanisms or factors may be involved in modulating the Atg32-Far8 interaction, independent of the expression or degradation of Far8.

Additionally, the study investigated the potential involvement of a set of kinases known to be associated with autophagy in the regulation of Atg32-Far8 interaction. However, none of these kinases were found to have a notable influence on the interaction, suggesting the existence of alternative signaling pathways or unidentified regulators involved in this process.

In summary, the study provided valuable insights into the interaction between the Far complex and Atg32 during mitophagy. While the phosphorylation of Atg32 was not affected by the substitution mutants of Far8 phosphorylation sites, the study highlighted the critical roles of Far3 and Far7 in the interaction between Far8 and Atg32. These findings contribute to our understanding of the regulatory mechanisms underlying

autophagy, particularly mitophagy, and open up avenues for further research into cellular signaling pathways and the development of potential therapeutics targeting mitochondrial dysfunction-related diseases.

Here are some possible ways to further investigate regulation of Atg32-Far complex interaction and mitophagy:

1) Explore other known interacting partners of the Far complex and Atg32: Investigate potential protein-protein interactions between the Far complex and other proteins involved in mitophagy. This could involve conducting co-immunoprecipitation assays or yeast two-hybrid screens to identify novel interacting partners.

2) Investigate the role of other posttranslational modifications: Explore the effects of various posttranslational modifications, such as acetylation, methylation, ubiquitination, and glycosylation, on the Atg32-Far8 interaction. This could be achieved through site-directed mutagenesis to introduce specific modifications or through the use of specific inhibitors or activators of these modification pathways.

3) Study the effect of cellular stress and nutrient availability: Investigate how cellular stress conditions, such as oxidative stress or hypoxia, impact the Atg32-Far8 interaction. Additionally, examine the influence of nutrient availability, such as glucose or amino acid deprivation, on this interaction. This could involve subjecting cells to different stress conditions or altering nutrient availability and assessing the resulting changes in the Atg32-Far8 interaction through biochemical assays or imaging techniques.

4) Investigate the functional consequences of the Atg32-Far8 interaction: Determine the physiological significance of the Atg32-Far8 interaction in mitophagy. This could involve assessing the effect of disrupting or enhancing this interaction on mitophagy flux, mitochondrial quality control, or cellular responses to mitochondrial damage. This could be achieved through functional assays such as monitoring mitophagy flux using fluorescent reporter systems or assessing mitochondrial morphology and function.

5) Explore the signaling pathways involved in the regulation of Atg32-Far8 interaction: Investigate the upstream signaling pathways that regulate the Atg32-Far8 interaction. This could involve screening for potential kinases, phosphatases, or other signaling molecules that modulate this interaction through genetic or pharmacological approaches. Additionally, explore the involvement of signaling pathways related to cellular stress response, nutrient sensing, or mitochondrial homeostasis in the regulation of this interaction.

These future experiments could further our understanding of the complex regulation of mitophagy and shed light on the underlying mechanisms involved in the Atg32-Far8 interaction.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関