Study on interorganellar sterol transport in Saccharomyces cerevisiae

その他のタイトル	酵母におけるオルガネラ間ステロール輸送機構に関する研究
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論文の内容の要旨

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論文題目　**Study on interorganellar sterol transport in *Saccharomyces cerevisiae***
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Sterols are primary components of eukaryotic biological membranes. Sterols are included in the plasma membrane and organelles at distinct ratios, and have been considered to play important roles in the structures and functions of the membranes. Sterols are synthesized in the endoplasmic reticulum (ER) and transported to the plasma membrane and other organelles. Sterols are transported between membranes through vesicle transport-dependent and -independent manners. It has been proposed that lipid transfer proteins (LTPs), which extract lipids from membranes and insert them into other membranes, and membrane contact sites (MCSs), where two membranes are retained in close proximity, are involved in non-vesicular sterol transport. However, the molecular mechanism of intermembrane sterol transport remains elusive. This is largely due to the unavailability of a simple and quantitative method to evaluate intracellular sterol transport. In this study, systems to evaluate transport of sterol synthesized in the ER to mitochondria and to vacuole were constructed in the yeast *Saccharomyces cerevisiae*, and the mechanism of intermembrane sterol transport was investigated.

1. Construction of a system to study sterol transport from the ER to mitochondria in *S. cerevisiae*

Mitochondrial membranes contain sterols, and sterol synthesis has been shown to be critical for mitochondria morphogenesis in *S. cerevisiae*. However, the mechanism of sterol transport from the ER to mitochondria is unknown. Here, a system was designed to assay sterol transport from the ER to mitochondria to elucidate the mechanism of interorganellar sterol transport. In this system, a recombinant sterol acyltransferase SatA of *Aeromonas salmonicida* fused with a mitochondrial targeting signal and a membrane spanning domain of yeast Pet100 and EGFP (mito-SatA-EGFP) was expressed in a mutant defective in the intrinsic sterol acyltransferases Are1 and Are2 in the ER. It is expected that sterol transport to the mitochondria is evaluated by measuring sterol esterification in the

mitochondria. By microscopic observation and fractionation of cell extracts, it was suggested that mito-SatA-EGFP localized to the mitochondria. When the *are1*Δ*are2*Δ strain expressing mito-SatA-EGFP was incubated in the presence of [^{3}H]methionine, radiolabeled sterol was converted to steryl ester by mito-SatA-EGFP in the *are1*Δ*are2*Δ strain[1]. In addition, when the ER membrane containing radiolabeled sterols were incubated with purified mitochondria containing mito-SatA-EGFP *in vitro*, sterol esterification was observed in a time-dependent manner. These results indicate that this system is applicable to the elucidation of the mechanism of the sterol transport from the ER to mitochondria.

2. Role of yeast oxysterol-binding protein homologs in sterol transport from the ER to mitochondria

Oxysterol-binding protein (OSBP)-related proteins (ORPs), including OSBP homologs (Osh1-7) of *S. cerevisiae*, are candidates of LTPs that transport sterols, but it has also been proposed that ORPs function as lipid sensors or regulatory proteins in various cellular processes but not as LTPs. None of *OSH* genes is required for growth, but the deletion of all seven *OSH* genes causes cell lethality, indicating that the ORPs share an essential function, which is still unknown. To investigate the involvement of Osh proteins in sterol transport from the ER to mitochondria, sterol transport was examined *in vitro* using membranes and cytosolic fractions prepared from the strains having genetic backgrounds that bear the wild-type *OSH4* or the temperature-sensitive allele *osh4-1* along with the deletions of the other six *OSH* genes (*osh*Δ *OSH4* and *osh*Δ *osh4-1*) with mito-SatA-EGFP. At permissive temperature (25°C), lower sterol esterification was observed in the presence or absence of the cytosolic fraction of *osh*Δ *OSH4* or *osh*Δ *osh4-1* strain, compared with that in the presence of the cytosolic fraction of wild-type strain. At non-permissive temperature (37°C), sterol esterification was lower in the presence of the *osh*Δ *OSH4* cytosolic fraction than in the presence of the wild-type cytosolic fraction. In addition, the sterol esterification was significantly lower in the reaction containing the *osh*Δ *osh4-1* cytosolic fraction than that with the *osh*Δ *OSH4* cytosolic fraction. Addition of the purified recombinant His6-tagged Osh4 protein, instead of the cytosolic fraction, facilitated sterol esterification in the *in vitro* sterol transport reaction. Significant difference was not observed in the ergosterol contents in the mitochondrial fractions prepared from the *osh*Δ *OSH4* and *osh*Δ *osh4-1* strains cultivated at 25°C. In contrast, the ergosterol content in the mitochondrial fraction of *osh*Δ *osh4-1* strain cultured at 37°C was significantly lower than that of the *osh*Δ *OSH4* strain cultured under the same condition. These results suggest that Osh4 mediates sterol transport from the ER to mitochondria. In addition, purified His6-tagged Osh5, Osh6, or Osh7 and the cytosolic fraction

prepared from the strain that has *OSH1*, *OSH2*, or *OSH3* expressed under the native promoter with deletions of the other *OSH* genes also facilitated sterol esterification in the *in vitro* sterol transport reaction from the ER to mitochondria. These results suggest that Osh proteins share an ability to mediate sterol transport between membranes.

It has been proposed that Osh4 and Osh6/7 transport sterols and phosphatidylserine (PS) to the Golgi and plasma membrane, respectively, through counter-exchange for phosphatidylinositol 4-phosphate (PI4P). To investigate the involvement of PI4P in sterol transport from the ER to mitochondria, *in vitro* sterol transport from the ER to mitochondria was examined using Osh4 mutant proteins Osh4^{R236E,K242E,K243E} and Osh4^{H143A,H144A}, which have been shown to be defective in the binding to PI4P, but not to sterols. These mutant proteins facilitated sterol esterification to comparable extents to the wild-type Osh4, raising the possibility that PI4P is not involved in sterol transport from the ER to mitochondria. *S. cerevisiae* has three genes, *PIK1*, *STT4*, and *LSB6*, encoding PI-4-kinases. *In vitro* or *in vivo* sterol transport analysis using the temperature-sensitive *pik1-83* mutant strain and the deletion mutant of *LSB6* suggested that Pik1 and Lsb6 are dispensable for sterol transport from the ER to mitochondria. In contrast, sterol esterification by mito-SatA-EGFP in the temperature-sensitive *stt4-4* mutant at restrictive temperature was slightly lower than that in the wild-type strain. In addition, the deletion mutant of *SAC1* encoding phosphoinositide phosphatase had a tendency to exhibit higher sterol esterification by mito-SatA-EGFP than the wild-type strain. These results raise the possibility that Stt4 and Sac1 are directly or indirectly involved in sterol transport from the ER to mitochondria.

3. Roles of other lipid transfer proteins and membrane contact sites in sterol transport from the ER to mitochondria

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) proteins are LTPs, which have been suggested to bind and transport lipids. *S. cerevisiae* has six ER membrane proteins with STRAT-like domains, one of which, Ltc1/Lam6, localizes in the ER-mitochondria contact and was proposed to mediate sterol transport. To investigate the involvement of START-domain proteins in sterol transport from the ER to mitochondria, sterol esterification by mito-SatA-EGFP was examined in the mutant deleted for *LTC1* or its paralog *LAM5*. Sterol esterification was slightly lower in the *ltc1*Δ strain, but not in the *lam5*Δ strain, than the wild-type strain in 15 min incubation, raising the possibility that Ltc1 is involved in sterol transport from the ER to mitochondria.

Mitochondria form contacts with the ER by the ER-mitochondria encounter structure (ERMES) complex and the ER-membrane protein complex (EMC), and with the vacuole (vacuole and

mitochondria patch, vCLAMP) marked by Vps39 and Ypt7. To investigate the involvement of the MCSs in sterol transport from the ER to mitochondria, sterol esterification by mito-SatA-EGFP was measured in the deletion mutants of the genes required for formation of these MCSs. Sterol esterification was lower in the deletion mutant of genes encoding ERMES components and *VPS39*. In addition, ergosterol content in the mitochondrial fraction of the *vps39*Δ strain was lower than that of the wild-type strain. These results suggest that the contact site between the ER and mitochondria and that between the vacuole and mitochondria are involved in sterol transport from the ER to mitochondria.

4. Construction of a system to evaluate sterol transport from the ER to vacuole in *S. cerevisiae*

Sterol is also included in the vacuolar membrane and plays an important role in vacuole fusion. However, the transport mechanism of sterol synthesized in the ER to vacuole is unknown. Here, a system was designed to assay sterol transport from the ER to vacuole to elucidate the mechanism of sterol transport. In this system, sterol acyltransferase SatA fused with vacuolar protein Nyv1 and EGFP (vac-SatA-EGFP) was expressed in a mutant defective in the intrinsic sterol acyltransferases Are1 and Are2 in the ER. By fluorescent microscopic observation, vac-SatA-EGFP was found to be localized to the vacuole. When the *are1*Δ*are2*Δ strain expressing vac-SatA-EGFP was incubated with [^{3}H]methionine, ^{3}H-labeled steryl ester was detected. There results suggest that sterol transport from the ER to vacuole can be evaluated with this system.

Conclusion

Systems to evaluate transport of sterol synthesized in the ER to mitochondria and to vacuole were constructed and the mechanisms were investigated. Osh proteins were shown to mediate sterol transport from the ER to mitochondria and a model in which all Osh proteins share a function to transport sterols between membranes was proposed. In addition, involvement of the ER-mitochondria and vacuole-mitochondria contact sites in the sterol transport was also suggested. The system to assess sterol transfer by determining sterol esterification by a recombinant sterol acyltransferase will be applicable to the study of sterol transport between various membranes.

Reference

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