博士論文（要約）

Study on interorganellar sterol transport in *Saccharomyces cerevisiae*
(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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Table of contents
List of abbreviation

A list of abbreviations

ABC	ATP-binding cassette
ACAT	acyl coenzyme A: cholesterol acyltransferase
ATCC	American Type Culture Collection
BSA	bovine serum albumin
CPY	carboxypeptidase Y
DHE	dehydroergosterol
EDTA	ethylenediamine-*N,N,N',N'*-tetraacetic acid
EMC	endoplasmic reticulum-membrane protein complex
ERAD	endoplasmic reticulum-associated degradation
ERMES	endoplasmic reticulum-mitochondria encounter structure
EUROSCARF	EUROPEAN SACCAROMYCES CEREVISIAE ARCHIVES FOR FUNCTIONAL ANALYSIS
FFAT	two phenylalanines in acidic tract
GOLD domain	Golgi dynamics domain
HOPS	homotypic fusion and vacuole protein sorting
IPTG	isopropyl-β-D-1-thiogalactopyranoside
LDL	low density lipoprotein
LTP	lipid transfer protein
MBOAT	membrane-bound *O*-acyltransferase
MCS	membrane contact site
MLN	metastatic lymph node
MOM	mitochondrial outer membrane
NBD	7-nitrobenz-2-oxa-1,3-diazol-4-yl
NBRP	National BioResource Project
NPC	Niemann Pick C
NVJ	nucleus-vacuole junction
ORD	OSBP-related domain
ORP	OSBP-related protein
OSBP	oxysterol-binding protein
PBS	phosphate buffered saline
PC	phosphatidylcholine

PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PH domain	pleckstrin-homology domain
PI	phosphatidylinositol
PI4P	phosphatidylinositol-4-phosphate
$PI(4,5)P_2$	phosphatidylinositol-4,5-biphosphate
PIP	phosphoinositide
PMN	piecemeal microautophagy of the nucleus
PS	phosphatidylserine
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SLiCE	Seamless Ligation Cloning Extract
SE	steryl ester
SMP	synaptotagmin-like, mitochondrial and lipid-binding protein
SNARE	soluble N-ethylmaleimide-sensitive factor attached protein receptor
StAR	steroidogenic acute regulatory protein
START	StAR-related lipid transfer
TBS	tris buffered saline
TCA	trichloroacetic acid
TLC	thin layer chromatography
TMD	transmembrane domain
TOM	translocase of the outer mitochondrial membrane
VAP	vesicle-associated membrane protein-associated protein
vCLAMP	vacuole and mitochondria patch

Introduction

Sterols are one of the primary and essential components of biological membranes of eukaryotes. While cholesterol is the major sterol in mammals, sitosterol and stigmasterol are major in plants and ergosterol is major in fungi, including yeasts (Fig. 0-1). Sterols are implicated in various cellular functions, including regulation of membrane permeability and fluidity (1), the formation of membrane microdomain for cell signaling, secretory transport, and cytoskeletal organization (2). Sterols are present in the plasma membrane and most organelle membranes at specific ratios (3,4).

0-1 Biosynthesis and incorporation of ergosterol and its esterification in budding yeast *Saccharomyces cerevisiae*

Cells of the budding yeast *Saccharomyces cerevisiae* obtain sterols via two routes: either through biosynthesis of ergosterol or by uptake of sterols from the surrounding environment. Sterol uptake takes place in yeast under anaerobic conditions or under aerobic conditions when sterol synthesis is compromised with heme deficiency (5). Yeast cells also uptake sterols by the mutation *upc2-1*, a specific allele of *UPC2* encoding a transcription factor that activates genes involved in the sterol synthesis and uptake (6). ATP-binding cassette (ABC) transporters Aus1 and Pdr11 mediate sterol uptake (7). Sterols have been shown to be transported by non-vesicular transport mechanism(s) from the plasma membrane (PM) to the ER (8).

Sterols are synthesized under aerobic conditions in the organelle ER. There are more than 20 distinct reactions for sterol synthesis (Fig 0-2) (1). Farnesyl pyrophosphate is synthesized from acetyl-CoA in the mevalonate pathway. Farnesyl pyrophosphate can be used not only for the synthesis of sterol, but also for the synthesis of many other important metabolites. Therefore, deletion of genes involved in the mevalonate pathway is lethal. In the ergosterol biosynthetic pathway, farnesyl pyrophosphate is converted to the ergosterol through 11 reactions. Formation of

squalene to zymosterol in the ergosterol biosynthetic pathway is essential for growth, but synthesis of fecosterol to ergosterol is not essential for cell survival. Most of the proteins responsible for ergosterol biosynthesis in yeast are localized in the ER, but Erg1, Erg6, and Erg7 have been found in both the ER and lipid droplet (LD) (9).

The excess sterols are esterified to steryl ester (SE) and stored at LDs. The esterification of sterols in yeast occurs at the ER and the sterol acyltransferases, Are1 and Are2, are responsible for this process (10,11). Are2 is the major yeast SE synthase with a preference to esterify ergosterol, whereas Are1 is in preference to esterify sterol precursors (10). The simultaneous deletion of *ARE1* and *ARE2* results in the lack of SE formation, but does not affect the viability of yeast cells although free ergosterol is accumulated (12). The transport of SE from the ER to LDs is considered to be mediated in a non-vesicular manner (13,14), but the mechanism is still unknown.

SE hydrolases Tgl1, Yeh1 and Yeh2 contribute to SE hydrolysis. Yeh1 and Tgl1 localize to LD, whereas Yeh2 exists in the PM. Both SE formation and turnover are important for maintenance of sterol homeostasis, however the regulatory link between SE formation and hydrolysis needs further study (15,16).

0-2 Sterol transport

The sterol contents of the PM and organelles are quite different. The highest concentration of ergosterol is found in the PM and in the secretory vesicles (4). In contrast, ergosterol contents of the ER, mitochondria, vacuole and other organelles are relatively low (Table 0-1). In the mammalian cells, cholesterol represents less than 5% of total lipids in the ER and up to 40% in the *trans*-Golgi and the PM (3,17,18).

Sterols synthesized in the ER are transported to other organelles. Inter-membrane sterol transport is carried out by vesicular and non-vesicular trafficking mechanism. Transport of sterol between the ER and the PM is mostly independent of vesicular trafficking (19,20). Sterol is insoluble in aqueous phase, and its spontaneous desorption and free diffusion between membranes is time-consuming (21), and therefore its non-vesicular transport needs the assistance by other molecules. A fraction of the ER comes to close proximity to other organelles and form zones to

facilitate metabolites exchange, which is known as membrane contact sites (MCSs). Lipid transfer proteins (LTPs) contain core lipid-binding pockets and mobile “lid” domains that protect the bound lipids in the pockets from the aqueous environment (22). MCSs and LTPs have been proposed to assist sterol transport between membranes.

0-3 Lipid metabolism and transfer by lipid transfer proteins (LTPs)

Oxysterol-binding protein (OSBP)-related proteins (ORPs) are widely conserved in eukaryotes (22). ORPs all contain a lipid-binding domain named OSBP-related domain (ORD). OSBP was originally identified as a protein that binds to oxysterol, but some ORPs have been shown to bind sterols, including cholesterol (23,24). Due to their ability to bind sterols, roles of ORPs have been considered to be mediate sterol transport between membranes.

One intriguing feature of ORPs is that multiple paralogs of ORP genes are encoded in eukaryotic genomes (25). While twelve ORP genes, *OSBP* and *ORP1-ORP11*, are found in human genome, *S. cerevisiae* has seven OSBP-homolog genes, *OSH1-OSH7*. In *S. cerevisiae*, none of the seven *OSH* genes is essential for growth, but eliminating all Osh proteins is lethal (26). All yeast Osh proteins are likely to be soluble proteins, but the localization of each one is quite different. Osh1 was suggested to localize to nucleus-vacuole junction (NVJ). Osh2, Osh3 and Osh6 were suggested to localize near the ER-PM contacts. Osh4 was diffused in the cytosol and localized near the *trans*-Golgi (27). It was suggested that all Osh proteins contributed to cellular sterol distribution (28). The *in vitro* assays showed that Osh4 transported sterol between two membranes (27,29). Osh4 has been suggested to mediate sterol transport from the PM to ER (24). However, other group reported that Osh4 did not work as a sterol transporter and that the sterol transport between the PM and the ER was independent of Osh4 (30). In addition, Osh6 and Osh7 were suggested to play a role in phosphatidylserine (PS) transport rather than sterol transport (31,32). The deletion of *OSH6* and *OSH7* reduced PS accumulation at the PM (31).

In human cells, ORPs have also been proposed to be involved in the sterol

transport. ORP5 and ORP8 have transmembrane domains (TMDs) and are targeted to the ER (33). ORP5, but not ORP8, interacted with Niemann Pick C (NPC) 1 to mediate the exit of cholesterol from endosomes/lysosomes. Moreover, ORP5 could transport sterol *in vitro* at a similar efficiency to Osh4 (33). ORP5 and ORP8 were also suggested to form tethers between the ER and PM to facilitate the transport of PS from the ER to the PM. The overexpression of ORP5 increased the PM-localized PS (34). ORP1S and ORP2 facilitated cholesterol transport from the PM to LD. Knockdown of both ORP1S and ORP2 caused sterol transport defect from the PM to the ER and LDs (35). The overexpression of ORP2 also stimulates newly synthesized cholesterol transport out of ER (36). OSBP and ORP9L can bind cholesterol and transport it between liposomes *in vitro* (23).

On the other hand, roles of ORPs as lipid sensors or regulators in various cellular processes have also been proposed. ORPs have been reported to function as lipid sensors or regulators in exocytosis (37), plasma membrane sterol organization (30), ceramide transport (38,39), phosphoinositide metabolism (40), TORC1 signaling and nitrogen sensing (41), phospholipid synthesis (42), extracellular signal-regulated kinase (ERK) signaling (43), STAT3 signaling (44), and regulation of the ABC transporter (45). Therefore, common molecular functions of ORPs have remained unclear.

Another family of LTPs has also been reported to mediate sterol transport between organelles. The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain was suggested to bind lipids, including sterol (46). There are 15 START proteins in mammalian cells, STARD1-STARD15. STARD4 could transport sterol rapidly between liposomes (47), and was suggested to transport cholesterol from the late secretory pathway to the ER (46-48). Depletion of STARD4 resulted in an increase in cholesterol retention at the PM and a decrease in acyl coenzyme A:cholesterol acyltransferase (ACAT)-mediated esterification in the ER (48), while its overexpression increased sterol transport to the endocytic recycling compartment and to the ER (47). STARD3 (also known as MLN64: metastatic lymph node 64) has been proposed to be implicated in mitochondrial cholesterol import (49,50). The depletion of STARD3 markedly reduced the transport of low density lipoprotein (LDL)-derived cholesterol to mitochondria (49). Six proteins containing START-like domains have been identified in yeast *S. cerevisiae* (51). Lam1/Ysp1,

Sip3, Ysp2/Ltc4 and Lam4/Ltc3 localize to the ER-PM contact sites, and deletion of one of the genes encoding the first three proteins reduced the PM-to-ER sterol flux by 30-50% (51). Two other proteins, Lam5/Ltc2 and Lam6/Ltc1, localize to the ER-mitochondria and ER-vacuole contact sites. These START-like domain proteins have a transmembrane domain at their C-termini and the cytosolic domain of Ltc1 has been shown to transport sterol between membranes (52).

0-4 Lipid exchange at membrane contact sites (MCSs)

In the yeast *S. cerevisiae*, MCSs have been identified between the ER and PM (53,54), the ER and Golgi (55), the ER and LD (56), the ER and mitochondria (57-59), the nucleus and vacuole (60,61), and mitochondria and vacuole (62,63). The ER membrane vesicle-associated membrane protein-associated proteins (VAPs), Scs2 and Scs22, are proposed to be involved in the formation of the MCS between the ER and PM. It was reported that Osh3, one of ORPs, localizes to the PM-ER contacts by association with ER membrane through binding to VAPs with two phenylalanines in acidic tract (FFAT) motifs and association with PM through binding to phosphoinositides (PIPs) with pleckstrin-homology (PH) domains. The ORP domain (ORD) of Osh3 activates PI phosphatase Sac1 in the ER to hydrolyze phosphatidylinositol-4-phosphate (PI4P). Thus, Osh3 might regulate PIP metabolism at ER-PM MCS (40).

The yeast tricalbins, Tcb1, Tcb2 and Tcb3, and Ist2, related to mammalian TMEM16 ion channels, were suggested to be involved in the formation of the ER-PM MCS with Scs2 and Scs22. The simultaneous deletion of six genes, *SCS2*, *SCS22*, tricalbin genes, and *IST2*, caused the separation of ER from PM and accumulation of cytoplasmic ER (54). Whether tricalbins and Ist2 facilitate lipid transport between the ER and PM is still unknown.

ER tethers mitochondria by the ER-mitochondria encounter structure (ERMES) complex in *S. cerevisiae*. ER integral membrane protein Mmm1 (mitochondrial morphology maintenance), mitochondrial protein Mdm10 (mitochondrial distribution and morphology) and Mdm34, and cytosolic protein Mdm12 constitute the ERMES complex (58). Whether ERMES complex facilitates sterol exchange is unknown.

Another complex named conserved ER membrane protein complex (EMC) forming the MCS between the ER and mitochondria has been found. Deletion of EMC components reduced PS transfer from the ER to mitochondria (59). Whether Emc proteins assist sterol transport between the ER and mitochondria is needed to be evaluated.

The vacuole membrane protein Vac8 and the nuclear membrane protein Nvj1 constitute the NVJ (60). Recently, new proteins have been found to form NVJ independent of tether Nvj1. Mdm1 is an ER anchored protein and associates with vacuolar membrane. Mdm1's paralogue Nvj3 also localizes to the ER-vacuole MCSs (61). Nvj2 is another ER membrane protein that localizes to ER-vacuole MCSs, however, was not required for NVJ formation (54). Whether these proteins assist lipid transport in NVJ is still unclear. Nvj2 also tethers the ER and medial-Golgi in *S. cerevisiae* and facilitates non-vesicular of ceramide transport between these compartments (55).

The vacuolar homotypic fusion and vacuole protein sorting (HOPS) tethering complex subunit Vps39/Vam6 and the Rab GTPase Ypt7 are the components of vacuole and mitochondria patch, vCLAMP. Simultaneous deletion of genes encoding vCLAMP and ERMES components caused lethality and a defect in phospholipid transfer (62,63). Whether vCLAMP and ERMES influence sterol transport is still unknown.

0-5 This study

In this study, the budding yeast *S. cerevisiae* was used to study sterol transport from the ER to mitochondria and vacuole.

In the chapter 1, *in vivo* and *in vitro* systems using exogenous sterol acyltransferase were constructed to evaluate sterol transport from the ER to mitochondria.

In the chapter 2, the *in vitro* systems constructed in the chapter 1 were used to analyze the involvement of Osh proteins in the sterol transport from the ER to mitochondria. In addition, involvement of PI4P in sterol transport from the ER to mitochondria was also examined.

In the chapter 3, roles of other LTPs and MCSs for sterol transport from the ER to mitochondria was investigated.

In the chapter 4, *in vivo* system to evaluate sterol transport from the ER to vacuole was constructed using exogenous sterol acyltransferase.

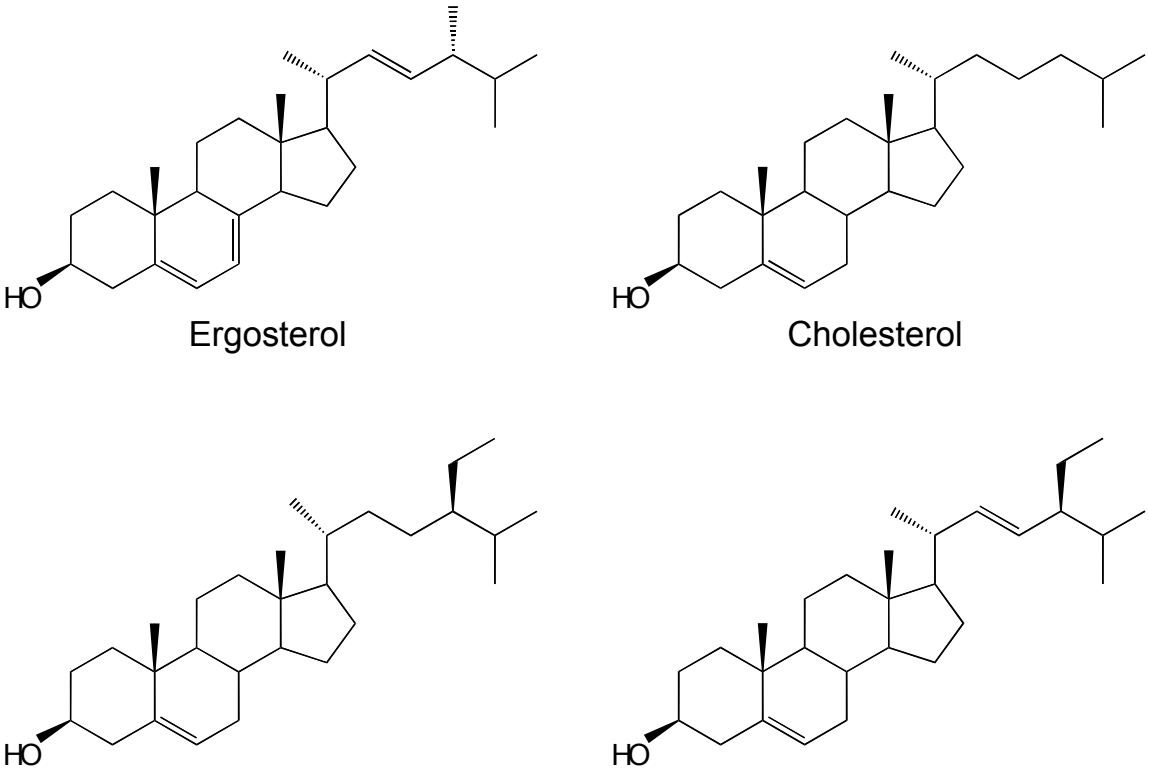

Fig. 0-1 The structures of major sterols in fungi, mammals, and plants

The structures of ergosterol in fungi, cholesterol in mammals, and sitosterol and stigmasterol in plants are shown.

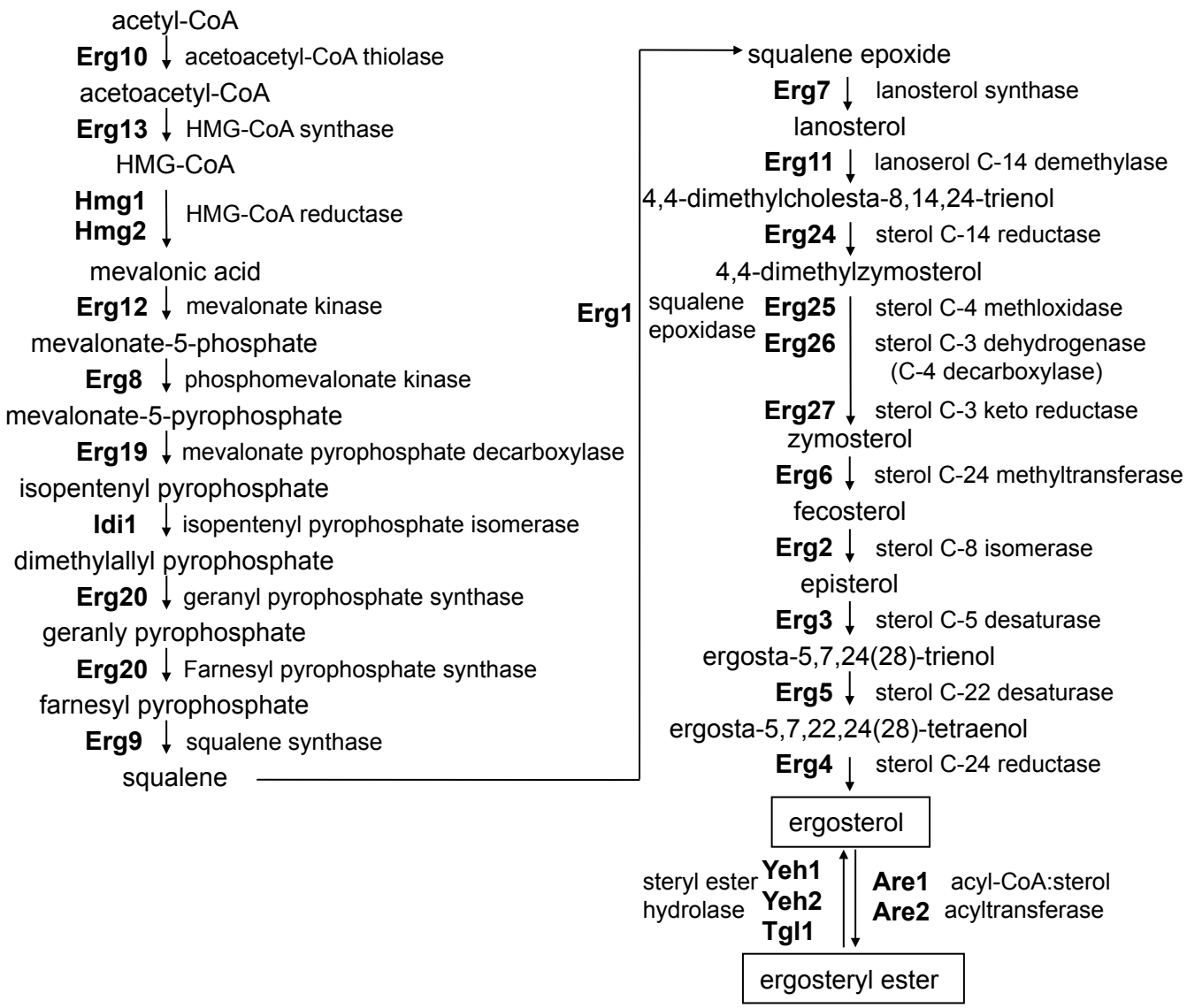

Fig. 0-2 Ergosterol biosynthesis in *Saccharomyces cerevisiae*

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Chapter 1 Construction of systems to study sterol transport from the ER to mitochondria in *Saccharomyces cerevisiae*

1-1 Introduction

Sterols are included in the mitochondrial inner and outer membranes, although their relative contents in *Saccharomyces cerevisiae* are somehow different from those in mammals. In *S. cerevisiae*, the ergosterol content of mitochondrial outer membrane (6 μg ergosterol per mg of organellar protein) is less than that of inner membrane (25 μg ergosterol per mg of organellar protein) (64). In contrast, in mammals, the cholesterol content of mitochondrial outer membrane (40 μg cholesterol per mg of organellar protein) is higher than that of inner membrane (less than 10 μg cholesterol per mg of organellar protein) (65).

Sterols play important roles in the biogenesis and maintenance of mitochondrial membranes (65,66), and ergosterol synthesis is crucial for mitochondrial morphogenesis in the yeast *S. cerevisiae* (67). Depletion of Erg1, Erg7, Erg8, Erg10, Erg12, Erg13, Erg25, Erg26, Erg27, Mvd1, or Ncp1, which are required for the biosynthesis of ergosterol in *S. cerevisiae*, caused clumped and swollen mitochondria (67). In addition, cholesterol is transported from the endoplasmic reticulum (ER) to mitochondria, and used for steroid hormone synthesis in mammals (65). However, the mechanisms underlying the sterol transport from the ER to mitochondria remain unclear.

Several experimental approaches have been used to investigate sterol transport between membranes *in vitro* and *in vivo*. Fluorescence-labeled sterol and radiolabeled sterol have been used for the analysis of the transport of sterol. Dehydroergosterol (DHE), a naturally fluorescent sterol, has been used to visualize sterol transport in living cells (30,68). ^{14}C-cholesterol is also used to probe sterol transport pathway in living cells (24). However, the fluorescence-labeled and radiolabeled sterols are introduced into the cells from the medium, and can be used only for the study of sterol transport from the PM to intracellular organelles, but not for the transport between

intracellular organelles. In mammalian cells, cholesterol is converted to pregnenolone at the mitochondrial inner membrane. The production of pregnenolone is used to evaluate cholesterol transport to the mitochondria (69). However, the system can just be used to observe sterol transport to mitochondria, but not to other organelles. Liposomes containing DHE or radiolabeled sterol have been used to study the sterol transport *in vitro* (24,27,29). However, liposomes are artificial membranes, and it is not clear whether the sterol transport between liposomes reflect the intermembrane sterol transport in living cells.

The lack of knowledge about the mechanism of the sterol transport from the ER to mitochondria is largely due to the unavailability of simple and quantitative method to evaluate sterol transport between these organelles. Here, *in vivo* and *in vitro* systems to evaluate sterol transport from the ER to mitochondria were constructed by expressing a mitochondrially targeted recombinant glycerophospholipid:cholesterol acyltransferase in a yeast mutant that is defective in endogenous acyl-CoA:sterol acyltransferases in the ER.

The "Materials and Methods", "Results", and "Discussion" were deleted. As the contents of this chapter are anticipated to be published in a paper in a scholarly journal, they cannot be published online. The paper is scheduled to be published within 1 year.

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

2-1 Introduction

Oxysterol-binding protein (OSBP) is a member of a family of sterol-binding proteins (43). Human has 12 oxysterol-binding protein (OSBP)-related protein (ORP) genes, and splicing generates 16 different protein products (22). *S. cerevisiae* has 7 ORP genes called *OSH1-7* (26). OSBP and ORPs, including the yeast homologues, are candidates of LTP that transport sterol between organelles (26). ORPs all contain the core lipid binding ORD and the conserved motif EQVSHHPP, a specific binding motif for the head group of phosphatidylinositol-4-phosphate (PI4P) ligand (22). A fraction of ORPs contains additional motifs and domains. In *S. cerevisiae*, Osh1-3 have PH domain involved in binding to phosphoinositide and FFAT motif involved in binding to the ER membrane protein Scs2, a yeast ortholog of VAPs. In addition, Osh1-2 contain ankyrin repeat motif and Osh3 contains Golgi dynamics (GOLD) domain (Fig. 2-1). The functions of ankyrin repeat motif and GOLD are unknown. In *S. cerevisiae*, none of the seven *OSH* genes is required for cell viability, but the deletion of all seven *OSH* genes causes cell lethality, indicating that the ORPs in *S. cerevisiae* have an overlapping essential function, which remains unclear (26).

Osh4/Kes1 is the most well-characterized ORP in yeast. It has been shown that Osh4 facilitates cholesterol and ergosterol transfer between membranes *in vitro* (24). The crystal structure of Osh4 was solved and it was shown that Osh4 is built around a central antiparallel β-sheet of 19 strands that form a near-complete β-barrel. A tunnel runs through the center of the barrel. Residues 1-29 form a lid that covers the entrance of the tunnel. One sterol molecule binds to Osh4 within the central tunnel of the β-barrel. In addition, the Osh4 without sterol binding exposes potential phospholipid-binding sites, which are suggested to bind phospholipid on membranes and perform sterol exchange (84). It is unclear whether other Osh proteins have abilities to transport sterol. Osh6 and Osh7 have been shown to mediate transport of

phosphatidylserine (PS) from the ER to mitochondria (31).

It has been suggested that PI4P gradient facilitates ORP-mediated lipids transport. In *S. cerevisiae*, PI4P is generated from the phosphatidylinositol (PI) at the *trans*-Golgi by Pik1 (85) and at the PM by Stt4, and is degraded into PI at the ER by Sac1 (86). Therefore, PI4P is enriched at the *trans*-Golgi and PM, but is absent from or much less in the ER. The phosphorylation of PI into PI4P at the *trans*-Golgi and PM and the degradation of PI4P into PI at the ER maintain the PI4P gradient (83). Osh4 was suggested to transport sterol from the ER and exchange it with PI4P at the *trans*-Golgi, and then transfer PI4P back to the ER, where PI4P is dephosphorylated to PI by Sac1 (Fig. 2-2) (87). Osh6 and Osh7 are considered to exchange PS with PI4P at the PM, and then transfer PI4P to the ER (32). In human cells, OSBP and ORP5/8 are also suggested to transfer sterol or PS from the ER to the *trans*-Golgi or the PM, respectively, and exchange the lipid with PIP (34,88,89) (Fig. 2-2). However, other group reported that phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$], but not PI4P, was exchanged with PS in the transport between the ER and the PM (89). Whether other ORPs also function as the PIP/sterol or PIP/phospholipid exchanger should be further validated.

On the contrary, it has also been proposed that Osh proteins regulate membrane sterol organization and intracellular sterol distribution, but are not required for sterol transfer between the ER and PM in yeast (28,30). In addition, ORPs have been proposed to function as lipid sensors or regulators in various cellular processes as described in chapter 0. Thus, functions of Osh proteins and ORPs remain to be clarified.

Here, the involvement of Osh proteins and PI4P in sterol transport from the ER to mitochondria was investigated by the system using mito-SatA-EGFP established in chapter 1.

The “Materials and Methods”, “Results”, and “Discussion” were deleted. As the contents of this chapter are anticipated to be published in a paper in a scholarly journal, they cannot be published online. The paper is scheduled to be published within 1 year.

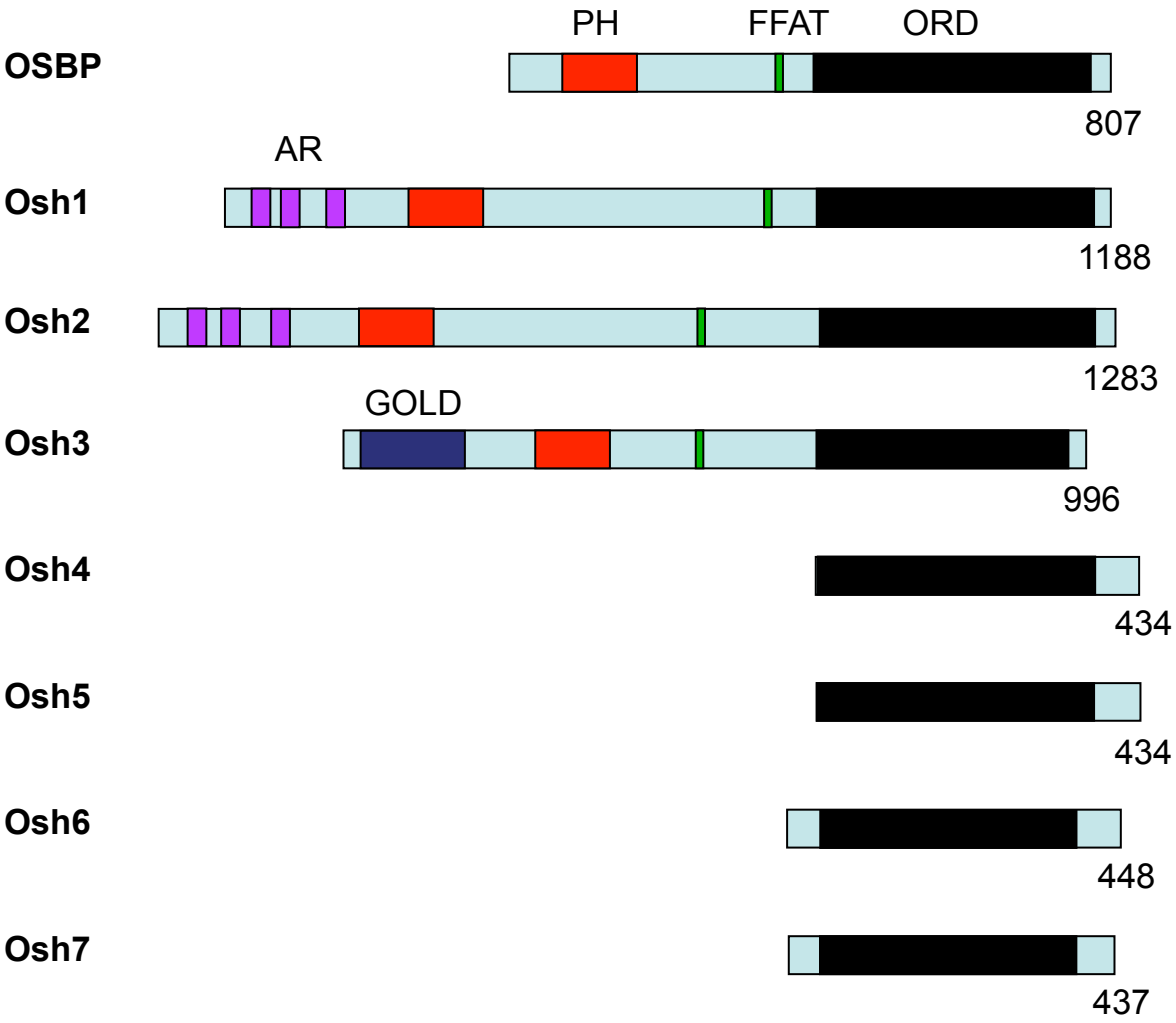

Fig. 2-1 Structures of oxysterol-binding protein homologs, Osh1–Osh7, in *S. cerevisiae* and human OSBP

ORD: Oxysterol binding protein-related protein domain.
PH domain: pleckstrin-homology domain
FFAT: two phenylalanines in acidic tract
AR: Ankyrin repeat
GOLD domain: Golgi dynamics domain

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

3-1 Introduction

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain protein is one of the LTPs families, which was suggested to bind and transfer lipids in mammalian cells (46,47). In yeast *S. cerevisiae*, there are six START-like proteins, Ysp1, Ysp2, Sip3, Lam4, Lam5/Ltc2, and Lam6/Ltc1. These proteins have a transmembrane domain at their C-termini. Ysp1, Ysp2, Sip3, and Lam4 have been shown to localize at ER-PM contact sites and were proposed to transport of sterol between these membranes (51). In contrast, Lam6/Ltc1 and its paralog Lam5/Ltc2 localize at ER-mitochondria contact sites. The cytosolic domain of Lam6/Ltc1 was shown to transport sterol between liposomes *in vitro* (52). Based on these results, it was proposed that Lam6/Ltc1 is involved in sterol transport, but the exact roles of Lam6/Ltc1 and Lam5/Ltc2 in sterol transport from the ER to mitochondria remain to be determined.

It has been reported that mitochondria form contacts with the ER by the ER-mitochondria encounter structure (ERMES) complex and with the vacuole (vacuole and mitochondria patch, vCLAMP) marked by Vps39 and Ypt7 (Fig. 3-1) (58,62). It was reported that the ERMES complexes facilitate phospholipid exchange at the ER-mitochondria interface and Gem1 regulate the number and size of the ERMES complex (58,105). However, it was also reported that the ERMES complex and Gem1 do not directly affect phospholipid transport (106). Thus, the function of the ERMES complex in lipid transport is still under debate. vCLAMP has been reported to facilitate phospholipid transport between vacuole and mitochondria (62,63). Yeast cells can grow in the absence of ERMES or vCLAMP, but simultaneous elimination of both contact sites caused lethality, suggesting that these complexes share a common function, possibly in the lipid transport. In addition, the ER-membrane protein complex (EMC) has been identified and shown to be involved

in the contacts formation between the ER and mitochondria. EMC is composed of Emc1-Emc6 in the ER. The simultaneous lack of the EMC and ERMES complexes also caused cell lethality, suggesting that these complexes share an overlapping function, possibly in the lipid transport (59). Despite these studies, roles of these MCSs in intermembrane transport of lipid, particularly in that of sterols, remain elusive.

Here, the involvement of Lam6/Lam5, ERMES complex, EMC complex, and vCLAMP in sterol transport from the ER to mitochondria was examined with a system using mito-SatA established in chapter 1.

The "Materials and Methods", "Results", and "Discussion" were deleted. As the contents of this chapter are anticipated to be published in a paper in a scholarly journal, they cannot be published online. The paper is scheduled to be published within 5 years

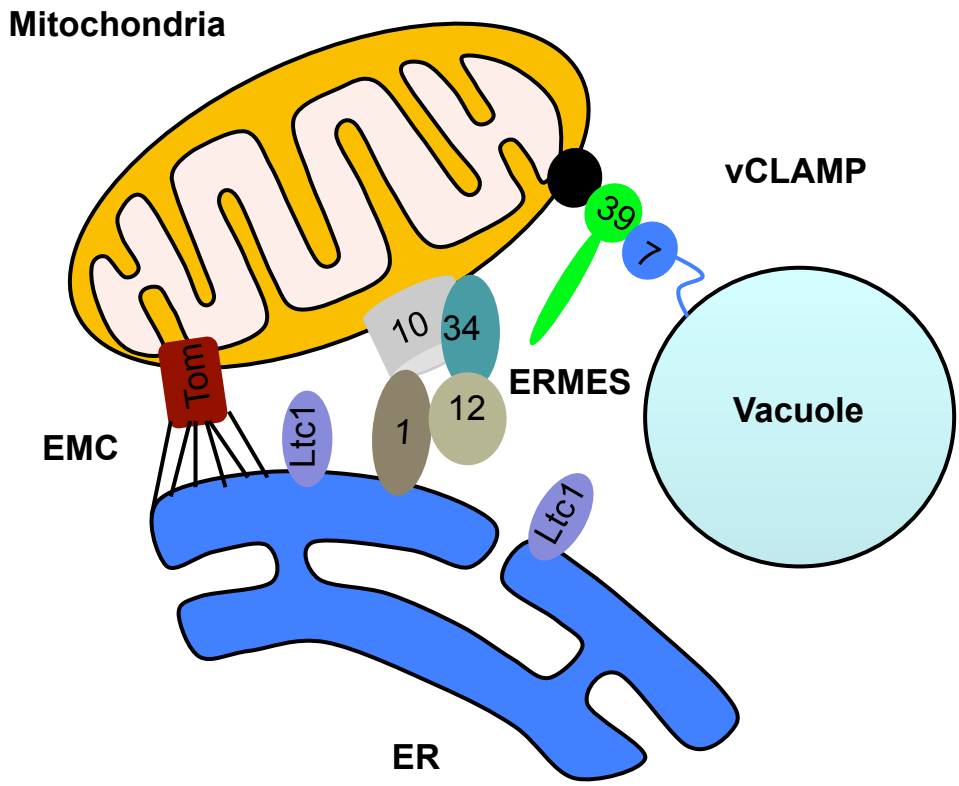

Fig. 3-1 Schematic model of roles of the membrane contact sites between mitochondria and ER or vacuole
1: Mmm1; 10: Mdm10; 12: Mdm12; 34: Mdm34; 7: Ypt7; and 39: Vps39

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

4-1 Introduction

In budding yeast *S. cerevisiae*, ergosterol is required for the Sec18/ATP-dependent priming step of homotypic vacuole fusion (114). The addition of antifungal antibiotic natamycin, which interacts with ergosterol in the plasma membrane, inhibited vacuole fusion at the priming phase (115). Fusion activity of vacuole was decreased in *erg5*Δ cells (114), and depletion of *ERG3*, *ERG5*, *ERG6*, *ERG24* and *ERG28*, involved in ergosterol biosynthesis, caused striking vacuole fragmentation (109). On the other hand, the overexpression of *ERG6* elevated sterol levels on the vacuole membrane and enhanced their homotypic fusion (116). It was also shown that sterols on the vacuole form domains in response to nutrient deprivation, changes in the pH of the growth medium, and other stresses (117). These results indicate that sterols play important roles in the structure and functions of the vacuole in *S. cerevisiae*. However, the mechanism of transport of sterol synthesized in the ER to vacuole is still unknown.

Here, a system was designed to evaluate sterol transport from the ER to vacuole by constructing a yeast strain that lacks endogenous sterol acyltransferases in the ER but has a bacterial sterol acyltransferase in the vacuole.

The "Materials and Methods", "Results", and "Discussion" were deleted. As the contents of this chapter are anticipated to be published in a paper in a scholarly journal, they cannot be published online. The paper is scheduled to be published within 5 years.

Conclusion

Sterols are primary and essential components of biological membranes in eukaryotic cells. To understand how biological membranes are organized and maintained, it is important to elucidate the mechanisms of synthesis, transport, and metabolism of membrane lipids. However, little is known about transport of lipids from their synthetic compartment to other compartments. In this study, the mechanisms of sterol transport from the ER to mitochondria and vacuole in yeast *S. cerevisiae* were investigated.

The conclusion of each chapter was deleted. As the contents of these chapters are anticipated to be published in papers in scholarly journals, they cannot be published online. The papers are scheduled to be published within 1 or 5 years.

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Acknowledgements

I gratefully acknowledge to Prof. Hiroyuki Horiuchi for his worthy comments and suggestions. I would express my deepest gratitude and appreciation to my supervisor Assist. Prof. Ryoichi Fukuda for his strict but kindly teach, leads me to enter the world of life science.

I sincerely thank Prof. Akinori Ohta in the Department of Biological Chemistry, College of Bioscience and Biotechnology, Chubu University for his worthy suggestions.

I wish to thank Dr. Masao Tokunaga for anti-Kar2 antibody, Dr. Akihiko Nakano for anti-CPY antibody, Dr. Christopher Beh for CBY strains, Dr. Yoichi Noda for pRS314 plasmid, Dr. Hiroyuki Tachikawa for pTL511 plasmid and Dr. Machiko Kawabata for plasmid pT-HIS3.

I would also thank Dr. Takuya Katayama and Ms. Aya Mizuike who have taught me a lot from the beginning of my study in Japan till now. I must thank Ms. Keiko Takagi, Mr. Wei Sheng, Mr. Ryo Iwama, Mr. Ryo Ninomiya and all the other members in the Laboratory of Cellular Genetics, for their friendships, helpfulness and encouragement.

Last but no least, I want to express a special thank to all my family members.