Hiroshima University

The roles of membrane contact sites in ceramide trafficking and intracellular homeostasis

セラミドの輸送と細胞内ホメオスタシスにおける
膜接触部位の役割に関する研究

Dissertation

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(summary)

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Summary

Sphingolipids are a diverse class of membrane lipids that cover a large range of functions depending on their structural maturation and subcellular localization. Ceramide, a structurally simple sphingolipid precursor, is known as a pro-apoptotic signaling molecule that induces the programmed cell death upon accumulation in the endoplasmic reticulum (ER). In contrast, mature and structurally complex sphingolipids are essential for expanding the plasma membrane during cell growth. Producing enough ceramides in the ER to supply sphingolipids to the plasma membrane without exceeding ceramides homeostatic range is a difficult task for the cell and has been implicated in the prevention of uncontrolled cell proliferation. Henceforth, mechanisms that mitigate ceramide accumulation have attracted research interest due to their role in promoting cancer and resistance to apoptosis-inducing chemotherapeutic agents. The budding yeast *Saccharomyces cerevisiae* has been extensively used to study ceramides as it is an easily genetically manipulable model organism that shares significant homologies with human cells in the regulation of sphingolipid homeostasis and apoptosis.

The conversion of ceramide into complex sphingolipids by the Golgi-resident inositolphosphorylceramide (IPC) synthase Aur1 is described as the primary method to keep ER ceramide levels in check. To reach the Golgi, ceramides are either packaged together with GPI-anchored proteins into COPII vesicles of the secretory pathway or are transported to the Golgi via non-vesicular transport involving ER-Golgi membrane contact sites (MCSs). While less important under ideal growth conditions, non-vesicular ceramide transport and ER-Golgi MCS formation is induced upon stress conditions that are associated with elevated ceramide levels. Henceforth, non-vesicular ceramide transport is to date the best-known mechanism to actively respond to ceramide toxicity. In mammalian cells, ceramide transport at ER-Golgi MCSs is well characterized, exemplified by the identification of the transfer protein CERT and the detailed description of its transport mechanism. One feature of ceramide transport in mammals is that distinct ceramide subspecies are sorted into different pathways, with long-chain ceramides occupying vesicular transport and short-chain ceramides being dependent on CERT-mediated non-vesicular transport. Whether ceramide subclasses are similarly divided into distinct pathways in yeast is unknown and will be addressed in the first chapter of this thesis. In yeast, a direct counterpart to CERT is also yet to be identified, but several stress inducible ER-Golgi tethers were shown to promote non-vesicular ceramide transport. Tethering proteins (‘tethers’) bind to either of two opposing membranes, thereby establishing physical connections and forming a MCS. The identified tethers related to non-vesicular ceramide transport in yeast include the

tricalbin proteins (Tcb1, Tcb2 and Tcb3) and Nvj2, which normally localize to ER-PM and ER-vacuole MCSs, respectively, but are recruited upon ER stress to ER-Golgi MCSs.

Besides ceramide conversion into IPC, ceramide conversion into acyl-ceramide by neutral lipid synthases Dga1 and Lro1 was shown as an alternative way to reduce toxic ceramide accumulation. The acylation of ceramide enables its uptake in lipid droplets (LDs), which has been proposed as a potent mechanism to reduce ceramide toxicity in mammals and instigated resistance to chemotherapy-induced apoptosis. Interestingly, both deletion of *NVJ2* and tricalbins provoke responses involving LD. Deletion of *NVJ2* together with *DGA1* and *LRO1* results in a growth defect, whereas deletion of *TCB3* induced accumulation of lipid droplets and acyl-ceramide. Both results were attributed to a compensatory role of LDs in sequestration of access ceramides caused by reduced non-vesicular ceramide transport. However, given the minor role of non-vesicular transport in cells with functional vesicular transport, slight ceramide accumulation caused by loss of partially redundant tethers alone may not sufficiently explain these effects. Instead, these results may indicate a direct link between non-vesicular ceramide transport and LD function. I hypothesize that tethering proteins are involved in acyl-ceramide export from LDs, and attribute acyl-ceramide accumulation in the tricalbin deletion strain to jammed acyl-ceramide traffic out of LDs. Combined with the role of Tcb3 and Nvj2 in non-vesicular ceramide transport to the Golgi, I propose that tethers may aid acyl-ceramide export from LDs to the Golgi. The validation of this model was addressed in chapter II of my thesis.

Besides controlling transport and homeostasis of ill-fated ceramides, MCSs have been implicated in the regulation of various other cell functions, that mostly promote the short-term, direct, and unidirectional communication between organelles. This function is in stark contrast to the main mode of cellular regulation, the adjustment of gene expression. Most pathways targeting gene expression are initiated by signaling cascades that relatively slowly propagate signals radially throughout the entire cell adjusting cell metabolism in the medium to long-term. However, since MCSs are bottlenecks in signaling and metabolic pathways, it is conceivable that MCSs further regulate long-term metabolic adjustment through gene expression. To address this hypothesis, I investigated the role of MCSs in gene regulation in chapter III of my thesis.

In the present thesis, I set out to identify novel functions of MCSs in ceramide trafficking and intracellular homeostasis and present the results of the following three projects:

Chapter I: Ceramide sorting into non-vesicular transport is independent of acyl chain length in budding yeast.

Chapter I addresses whether short and long-chain ceramides in yeast are sorted into vesicular and non-vesicular transport, respectively, in the same way as in mammals. I employed a yeast *GhLag1* strain in which the endogenous ceramide synthase is replaced by the cotton derived *GhLag1* gene, resulting in the production of short chain C18 rather

than C26 ceramides. I show that block of vesicular transport through ATP-depletion or the use of temperature-sensitive *sec* mutants caused a reduction in IPC synthesis to similar extent in WT and *GhLag1* backgrounds. Since the remaining IPC synthesis is a readout for non-vesicular ceramide transport, my results indicate that non-vesicular ceramide transport is neither blocked nor facilitated when only short chain ceramides are present. Therefore, I propose that the sorting of ceramide into non-vesicular transport is independent of acyl chain length in budding yeast.

Chapter II: Lipid droplets participate in ER-to-Golgi non-vesicular ceramide transport in yeast

In chapter II, I investigated whether LDs in yeast are directly involved in non-vesicular ceramide transport. I discovered tethering proteins that localize to a novel three-way MCS including the ER, LD and Golgi. Moreover, I showed that block of vesicular transport in LD synthesis defective strains caused growth defects and reduced IPC synthesis, indicating that LDs are involved in non-vesicular ceramide transport to the Golgi and alleviate ceramide accumulation. Based on these results I propose a model in which non-vesicular ceramide transport requires the intermediate conversion of ceramide into acyl-ceramides and their uptake into lipid droplets.

Chapter III: The tricalbin family of membrane contact site tethers is involved in the transcriptional responses of *S. cerevisiae* to glucose

In chapter III, I performed RNA-sequencing on *Saccharomyces cerevisiae* cells lacking tricalbin proteins to investigate the role of MCSs for gene expression. My results indicate that in the *tcb1*Δ*2*Δ*3*Δ strain, pathways responsive to a high-glucose environment, including glycolysis, fermentation, amino acid synthesis, and low-affinity glucose uptake, are upregulated. Conversely, pathways crucial during glucose depletion, such as the tricarboxylic acid (TCA) cycle, respiration, and high-affinity glucose uptake, are downregulated. In addition, I demonstrate that the altered gene expression of *tcb1*Δ*2*Δ*3*Δ in glucose metabolism correlates with increased growth, glucose consumption, CO_2 production, and ethanol generation. In conclusion, these findings reveal that tricalbin protein deletion induces a shift in gene expression patterns mimicking cellular responses to a high-glucose environment. This suggests that MCSs play a role in signaling pathways that modulate gene transcription in response to glucose.

Conclusions and outlook

This study demonstrates that MCSs are not only versatile tools for stress adaptation but also play a crucial role in regulating the glucose signaling pathways that promote growth. Additionally, the newly uncovered link between lipid droplet function and non-vesicular ceramide transport addresses the longstanding question of how cells prevent the accumulation of toxic ceramides during periods of high ceramide synthesis. Temporary acyl-ceramide storage may reduce the risk of ceramide toxicity while facilitating the non-

vesicular transport of acyl-ceramides from the reserved LD-pool. Overall, this research establishes a foundation for two novel areas in MCS research: the role of contact sites with lipid droplets in sphingolipid transport and homeostasis, and the involvement of MCSs in gene regulation.