論文の内容の要旨

応用生命工学専攻
平成１８年度博士課程進学
氏名　　邓 鄧 岚
指導教員　太田　明徳

論文題目

Study on molecular remodeling and function of phosphatidylethanolamine in *Saccharomyces cerevisiae*

(酵母*Saccharomyces cerevisiae*におけるホスファチジルエタノールアミンの分子リモデリングと機能に関する研究)

Biological membranes are composed of a wide variety of phospholipids with respect to their hydrophilic head moieties and hydrophobic acyl chains. The generation and maintenance of the diversity in phospholipid molecular species are accomplished by the process of synthesis, degradation, and remodeling of each phospholipid. The fatty acyl chains in phospholipids are considered to be remodeled in cells to maintain membrane homeostasis. Despite its potential importance for the structure and function of the biological membranes, the molecular mechanisms underlying the remodeling of acyl chains in phospholipids are poorly understood

Phosphatidylethanolamine (PE) is one of the abundant phospholipids, comprising 20 to 30% of total phospholipids in most eukaryotic cells. Due to its small polar head group, PE tends to form non-bilayer hexagonal-II structure under physiological condition. This property has been considered to affect dynamics of biological membrane, for instance, membrane fission and fusion. In *Saccharomyces cerevisiae*, PE is biosynthesized through two pathways. In *de novo* pathway, phosphatidylserine (PS) is decarboxylated to PE by Psd1p or Psd2p. In CDP-ethanolamine branch of the Kennedy pathway, PE is synthesized from exogenous ethanolamine or ethanolamine formed endogenously through a lipid turnover process. In *S. cerevisiae*, PE is essential for growth and deletion of both *PSD1* and *PSD2* is lethal without a PE supply through the Kennedy pathway

Kakihara in our laboratory constructed a yeast mutant, TKY12Ga, in which both *PSD1* and *PSD2* were deleted and the promoter of *ECT1* encoding a key enzyme in PE on the Kennedy pathway, was replaced with the galactose-inducible and glucose-repressible *GAL1* promoter. In a medium containing galactose as a carbon source, the expression of *ECT1* was induced and the mutant grew using PE synthesized via the Kennedy pathway in the presence of ethanolamine. In contrast, the expression of *ECT1* was repressed and cell growth was arrested in a glucose-containing medium, and PE content was reduced to 1%. However, when didecanoyl PE (diC10PE) was added to the medium containing glucose, TKY12Ga grew despite the inability to synthesize PE. Since a 10-carbon-fatty acyl residue appears to be too short to maintain the structure and function of the biological membrane,

diC10PE was assumed to be remodeled to the ones containing acyl chains of normal length to support the growth of the mutant.

This study is aimed at elucidating the mechanism of PE remodeling and the physiological role of PE in *S. cerevisiae*.

Incorporation and remodeling of PE with short fatty acyl chains in yeast

It was reported that Lem3p/Ros3p and two P-type ATPase, Dnf1p and Dnf2p were involved in the inward-directed trans-bilayer transport of fluorescent-labeled PE and lyso-PE at the plasma membrane in yeast. The involvement of these three proteins in the uptake of PE with short acyl chains was examined. Genes encoding these proteins were deleted in TKY12Ga and the growth of the null mutants in SD medium containing diC10PE was examined. Deletion of *DNF2* caused slight growth impairment in the PE-containing medium, while deletion of *DNF1* did not. However, deletion of both *DNF1* and *DNF2* conferred severer growth defect, suggesting their redundant functions in the uptake of diC10PE. In contrast, deletion of *LEM3* caused most significant growth impairment. These results indicate that the growth of TKY12Ga in SD medium containing diC10PE requires uptake of PE with short acyl chains or its metabolites from the medium and that Lem3p, Dnf1p and Dnf2p are involved in this process.

The metabolism of diC10PE, in which ethanolamine moiety was labeled with deuterium, was analyzed using ESI-MS/MS. Deuterium-labeled diC10PE was synthesized by the base exchange transphosphatidylation by phospholipase D from the didecanoyl PC and ethanol-1,1,2,2-d_4-amine. TKY12Ga was pulse-labeled with deuterium-labeled diC10PE for 15 min, and chased with non-labeled diC10PE for 15, 30, 60 and 180 min. Then, the molecular species profile of PE was determined by the neutral loss scan of *m/z* 145 to detect PEs containing ethanol-1,1,2,2,-d_4-amine phosphate headgroup in positive ion mode. After 15 min incubation with deuterium-labeled diC10PE, a portion of the deuterium-labeled diC10PE had been remodeled, as one fatty acyl chain was replaced with a longer monounsaturated C16:1 and C18:1 acyl chains. These remodeling intermediates decreased quickly during chased incubation. In addition, PEs in which two fatty acyl chains were replaced with C16:1 or C18:1 acyl chain (16:1-16:1-PE, 16:1-18:1-PE and 18:1-18:1-PE) were also detected after 15 min incubation with deuterium-labeled diC10PE. These results suggest that PE with short acyl chains was remodeled to PEs containing normal acyl chains. Analysis of structures of the remodeling intermediates in detail using product ion scan by MS/MS showed that the majority of the remodeling intermediates contained C16:1 or C18:1 acyl chain at *sn*-2 position. These results suggest that diC10PE was first remodeled at *sn*-2 position and then at *sn*-1 position. The water-soluble metabolites of diC10PE, which were partitioned in the aqueous phase were also extracted, and their molecular profiles were analyzed. After a 30-min incubation with deuterium-labeled diC10PE, the peak corresponding to monoC10PE was observed, suggesting that lyso-10:0-PE was also an intermediate of remodeling of diC10PE.

Quantification of diC10PE and decanoic acid in the whole culture and the culture supernatant using LC-MS showed that amount of decanoic acid in both of the whole culture and the culture supernatant was increased during the incubation of TKY12Ga with diC10PE, accompanied by the

decrease of diC10PE. The amount of released decanoic acid in the culture supernatant was approximately twofold of the consumed diC10PE. These results suggest that most of C10 fatty acid cleaved from diC10PE were excreted into culture medium and it could be concluded that yeast remodeled diC10PE by removing C10 fatty acid and reacylating with normal length acyl chain, but not by elongating the acyl chains.

In vitro remodeling reaction experiment was performed using cell lysate of TKY12Ga with deuterium-labeled diC10PE and palmitoleoyl-CoA. After 15 min incubation, a small portion of deuterium-labeled 10:0-16:1-PE was detected, and its amount was increased with the prolonged incubation. In addition, deuterium-labeled 10:0-18:1-PE, 16:1-16:1-PE and 16:1-18:1-PE were detected after 60 min incubation. These results suggest the PE remodeling reaction can be reconstituted *in vitro*.

Analysis of genes involved in remodeling of diC10PE

Presence of monoC10PE, 10:0-16:1-PE, and 10:0-18:1-PE as remodeling intermediates of the diC10PE suggests that the remodeling can be dissected into at least two reactions, the cleavage of acyl chains by phospholipase activities and reacylation by acyltransferase activities. Yeast genome contains five genes encoding phospholipase B, *PLB1, PLB2, PLB3, SPO1,* and *NTE1,* and a gene, *YOR022c*, homologous to a gene encoding bovine phospholipase A1. A mutant was constructed by deletion of these six genes in TKY12Ga, and its growth in SD medium containing diC10PE and remodeling of deuterium-labeled diC10PE *in vivo* and *in vitro* were examined. No defect of the sextuple mutant in growth and remodeling was observed. In addition, monoC10PE was detected in the cell extract when this mutant was incubated with diC10PE. These data suggested that other enzyme(s) is/are involved in the removal of acyl chains in diC10PE.

Ale1p was reported to have an acyltransferase activity against the *sn*-2 position in various lysophospholipids. Null mutation in *ALE1* is synthetically lethal with the deletion of *SLC1*, which encodes a lyso-PA acyltransferase. Null mutants of *ALE1* or *SLC1* under TKY12Ga background were constructed. Their growth in SD medium containing diC10PE and their remodeling of deuterium-labeled diC10PE *in vivo* and *in vitro* were examined. Both mutants were partially impaired in the growth in SD medium containing diC10PE, and also impaired in remodeling of deuterium-labeled diC10PE *in vivo* and *in vitro*, suggesting that Ale1p and Slc1p were involved in the remodeling of diC10PE. To identity genes involved in the remodeling at *sn*-1 position, same experiments were performed using null mutants of *GAT1* and *GAT2*, which encode glycerol-3-phosphate (Gly-3-P) acyltransferase that were reported to transfer fatty acid from acyl-CoA to the *sn*-1 position of Gly-3-P. However, no growth and remodeling defect was observed in these two mutants.

Analysis of mutant defective in utilization of exogenous PE

To find enzymes involved in PE remodeling reaction, analysis of mutants defective in utilization of exogenous PE will be a useful way. Kakihara mutagenized TKY12Ga with mutagen ethyl methanesulfonate (EMS) and isolated 33 mutants that have defects in growth in

SD medium containing diC10PE. M25 is one of these mutants. Analysis of M25 showed that introduction of a plasmid containing the *DCP1* gene to M25 complemented its growth defect in SD medium containing diC10PE. *DCP1* gene encodes an mRNA decapping enzyme on the major pathway of mRNA decay in *S. cerevisiae*. Nucleotide sequence analysis showed a point mutation within the highly conserved region of *DCP1* gene in M25, suggesting the possibility that Dcp1p is involved in the mRNA degradation process of the transcript(s) that is/are responsible for the utilization of exogenous PE in yeast.

Requirement of *de novo* synthesis of PE and PC for yeast sporulation

During the sporulation process of *S. cerevisiae*, meiotic progression is accompanied by *de novo* formation of the prospore membrane inside the cell. However, it remains to be determined whether *de novo* synthesis of certain species of lipids is necessary for spore formation. Requirements of PE and another major phospholipid, phosphotidylcholine (PC), in the sporulation of yeast were examined. When TKY12G, diploid of TKY12Ga, was incubated in spore medium (SM) with exogenous ethanolamine, sporulation efficiency was approximately 21%. However, no sporulation was observed in the absence of exogenous ethanolamine, suggesting that *de novo* PE synthesis was essential to yeast sporulation. On the other hand, when KEY503, a strain that synthesizes PC only *via* the CDP-choline branch of the Kennedy pathway and is therefore auxotrophic for choline because of the deletion of both *PEM1* and *PEM2* genes, was incubated in SM in the presence of choline, spore formation was efficient. In contrast, spore formation efficiency of KEY503 was decreased in the absence of choline, but the effect was less drastic than that of repression of PE synthesis. These results suggest that *de novo* synthesis of PC was also required for efficient spore formation by yeast.

Conclusion

In this study, the incorporation and metabolism of diC10PE in yeast was analyzed. Results suggest that diC10PE was remodeled to PEs containing normal acyl chains and Ale1p and Slc1p were involved in this reaction. This system, in which the remodeling process of stable isotope-labeled phospholipids containing short acyl chains in yeast is monitored by ESI-MS/MS, will be applicable to the analysis of each reaction in the acyl chain remodeling of various phospholipid and provide a valuable tool to study the transport and metabolism of PE in yeast.

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