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論文題目

Study on transcriptional regulation of fatty acid metabolism in *Yarrowia lipolytica*

（酵母 *Yarrowia lipolytica* における脂肪酸代謝の転写制御機構に関する研究）

The yeast *Yarrowia lipolytica* efficiently utilizes various hydrophobic substrates such as *n*-alkanes and fatty acids as a sole carbon source. Because of these properties and qualification as a GRAS (Generally Regarded As Safe) organism by FDA, it has been a target of intensive study for industrial application and used as a model organism to study the metabolism of hydrophobic substrates and its regulation.

In *Y. lipolytica*, *n*-alkanes are metabolized to fatty acids through sequential terminal oxidation initiated by cytochromes P450 (P450ALKs). Fatty acids synthesized endogenously or incorporated from culture media are activated to fatty-acyl CoA and metabolized by β-oxidation in peroxisome. Transcription of *ALK1*, a primary P450ALK, is induced by *n*-alkanes. It is positively regulated by the hetero-complex composed of two basic helix-loop-helix (bHLH) transcription factors, Yas1p and Yas2p, via Alkane Responsive Element 1 (ARE1) and negatively by Yas3p. Likewise, transcription of a number of genes involved in the metabolism of fatty acids is activated in the presence of fatty acids. However, the molecular mechanism of the transcriptional activation in response to fatty acids has been concealed in *Y. lipolytica*. The aim of this study is to elucidate the mechanism of transcriptional regulation by fatty acids in *Y. lipolytica*.

1. Search for genes involved in the fatty acid utilization in *Y. lipolytica*

In Fungi, the fatty acid utilization is transcriptionally regulated by transcription factors of two distinct families. One is Oaf1p-Pip2p system, most extensively characterized in the yeast *Saccharomyces cerevisiae*, in which the Zn_2Cys_6 transcription factors, Oaf1p and Pip2p, together with the C_2H_2 zinc finger protein Adr1p, regulate the transcription of genes in response to fatty acids. An alternative is FarA system, originally reported in the filamentous fungus *Aspergillus nidulans*, in which FarA, belonging to another Zn_2Cys_6 transcription factor family, activates the transcription of genes involved in fatty acid utilization.

Orthologs of *OAF1* of *S. cerevisiae* were searched in *Y. lipolytica* genome database. Translation products of *YALI0F13321g*, *YALI0D10681g*, *and YALI0D17988g* exhibited similarity with Oaf1p only in their zinc cluster domains at 38, 45 and 38% identities, respectively. Deletion mutants of these genes, Δ*yali0f13321g*, Δ*yali0d10681g*, Δ*yali0d17988g*, Δ*yali0f13321g*Δ*yali0d10681g*, and Δ*yali0d17988g*Δ*yali0d10681g*, were constructed and their growth on oleic acid was analyzed. However, no growth defect was observed, in marked contrast to *S. cerevisiae*, in which deletion of *OAF1* or *PIP2* causes a severe growth defect on oleic acid. These results indicated that fatty acid utilization of *Y. lipolytica* is regulated by a mechanism distinct from that of *S. cerevisiae*.

Next, a deletion mutant of *YALI0D126281g*, an ortholog *farA* of *A. nidulans* was constructed and its growth was analyzed. Δ*yali0d126281*g strain grew normally on glycerol, glucose, *n*-tetradecane, and *n*-hexadecane. Δ*yali0d12628g* also grew on *n*-decane and *n*-dodecane with slight growth impairments. In contrast, it exhibited severe growth defects on lauric acid and myristic acid. The growth of Δ*yali0d12628g* on oleic acid was partially impaired. The growth defects of the Δ*yali0d12628*g strain on fatty acids were complemented by introduction of *YALI0D12628g* with its native promoter on a low copy plasmid. These results suggest that *YALI0D12628g* plays an important role in fatty acid utilization in *Y. lipolytica*. Thus, this gene was designated as *POR1* (*Primary Oleate Regulator 1*).

2. Characterization of Por1p

The RLM-RACE analysis suggested that an open reading frame of *POR1* is 2751 bp in size and encodes a 916-amino acid protein. Two introns of 80 bp and 90 bp were found in *POR1*. Por1p has three structural features: a fungal Zn_2Cys_6 binuclear cluster domain (amino

acids 32 to 71), a fungal specific transcription factor domain (amino acid 327 to 422), and a glutamine-rich (Q-rich) domain (amino acid 661 to 742). The first and second domains are conserved in orthologs of FarA. The Q-rich domains were found in Ctf1p of *Candida albicans* and an ortholog in *Neurospora crassa*, but not in FarA and FarB of *A. nidulans*.

As shown previously, *PAT1*, *POT1, POX2* and *PEX5,* which are involved in β-oxidation or peroxisome proliferation, were induced by oleic acid. However, the expression of these genes was distinctly diminished in the Δ*por1* strain grown on oleic acid. *POR1* was expressed on all carbon sources in the wild-type strain. In agreement with the results of northern blot analysis, the reporter β-galactosidase activities under the control of *PAT1* promoter were increased by the growth on oleic acid and *n*-decane in the wild-type cells. In contrast, reporter activities in the Δ*por1* strain were much less induced on oleic acid and partially on *n*-decane. These results suggest that Por1p functions in the transcriptional activation in response to fatty acids.

Next, the localization of Por1p fused with GFP at its C-terminus, expressed from a low copy plasmid under the control of its promoter, was observed in living cells. The fluorescent signal was enriched in the nuclear periphery in cells grown on glucose. Interestingly, Por1p-EGFP was also observed as one or a few spots at the nuclear periphery in live cells and this punctuate signals were more prominent in cells grown on oleic acid. When cells were fixed, fluorescent signal was observed irrespective of carbon sources. These results suggested that Por1p localizes to the periphery of inner nuclear membrane.

The deletion analysis of *PAT1* promoter using *lacZ* as a reporter gene was performed to identify a DNA element responsible for the transcriptional regulation by Por1p. The results suggested that the sequence CGAGCCGA at -173 to -166 in the *PAT1* promoter is important for the transcriptional activation by Por1p in response to fatty acid.

3. Other mechanisms involved in the regulation of fatty utilization in *Y. lipolytica*

The deletion mutant of *POR1* exhibited weak growth on oleic acid. In addition, slight transcriptional activation of *POT1*, *PAT1*, *POX1*, and *PEX5* by oleic acid was observed in the Δ*por1* cells. These results indicated the presence of another mechanism(s) for the transcriptional activation by oleic acid in *Y. lipolytica.*

Transcription of genes involved in fatty acid utilization is repressed by glucose, inconsistent to *ALK1*, transcription of which is not repressed by glucose. The reporter assay using *PAT1* promoter in the cells cultured on glucose, oleic acid, and glucose with oleic acid

suggested that derepression from glucose significantly contribute the expression of *PAT1* in the presence of oleic acid.

The ortholog of *ADR1* of *S. cerevisiae* was searched in *Y. lipolytica* genome database and *YALI0D18678g* deduced to encode a protein belonging to the member of C_2H_2 Zinc finger protein was identified. This gene was designated as *CFU1*, (Control of Fatty acid Utilization 1). The reporter activity under the control of *PAT1* promoter was decreased in Δ*cfu1* and Δ*por1*Δ*cfu1* on oleic acid and *n*-decane. These results suggested that the Cfu1p is involved in transcriptional regulation of *PAT1* together with Por1p.

4. Conclusion

In this study, two genes, *POR1* and *CFUI*, in involvement of the transcriptional regulation of fatty acid utilization in *Y. lipolytica* were identified and characterized. Although the mechanisms of fatty acid sensing and glucose derepression still remain unclear, this study will contribute to the understanding of entire regulatory mechanism of fatty acid utilization in *Y. lipolytica*.

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