A novel arginine metabolic pathway regulated by the yeast *N*-acetyltransferase Mpr1

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Report: The yeast *N*-acetyltransferase Mpr1 enhances arginine production *via* *N*-acetyl-glutamic acid synthesis by inducing the transcription of ornithine acetyltransferase Arg7.

Research aims

Recent studies have focused on the physiological functions of free amino acids in addition to the function of complete proteins. Previously, we demonstrated that the yeast *N*-acetyltransferase Mpr1 confers oxidative stress tolerance to yeast *via* arginine biosynthesis[1] and determined its crystal structure. We also succeeded in enhancing Mpr1-dependent arginine production by thermostabilization using a structure-based rational design[2]. However, the mechanism underlying Mpr1-dependent arginine synthesis remains unclear. This study aimed to clarify the molecular mechanism of the Mpr1-dependent arginine biosynthesis pathway.

Methods

The yeast *Saccharomyces cerevisiae* L5685 strain, derived from Σ1278b strain and harboring the *MPR1* gene in its genome, was used as a wild-type strain in this study. The gene disruption was performed via homologous recombination using an antibiotic resistance gene. Arg5 and Arg6 localize and function in the mitochondria and are encoded in the *ARG5,6* single gene. Therefore, the Arg6-deficient strain was constructed by deletion of *ARG5,6* gene followed by the introduction of the plasmid expressing the *ARG5,6* gene encoding Arg6 with an amino acid substation Asp251Glu in its catalytic center. *MPR1* overexpression was performed via the multi-copy type plasmid harboring the *MPR1* gene under the control of a GPD promoter. The yeast cell was cultured in a complete synthetic medium at 30 °C by omitting designated amino acids and/or nucleic acid bases, as required. RT-PCR was performed to analyze the transcription level of *ARG7*. The recombinant purified Mpr1 from *E. coli* was used for its enzymatic reaction *in vitro*. Novel substrates of

Mpr1 were screened via colorimetric assay using Ellman's reagent to detect its reaction product CoA. The reaction product *N*-acetyl-proline was identified via LC-MS analysis.

Results

The *S. cerevisiae* *Δarg2* strain showed remarkable growth defect under Arg-depleted conditions (Arg auxotrophy). Our previous study showed that Mpr1 is involved in one of the steps between Arg2 and Arg8 in the arginine biosynthesis pathway (Figure). The growth of *Δarg2* and *Δarg5,6* strains was evaluated in order to examine which specific step involves Mpr1. *MPR1* overexpression compensated for the Arg auxotrophy of *Δarg2* but not of the *Δarg5,6* strain. Subsequent analysis showed that *MPR1* overexpression did not compensate for Arg auxotrophy of the strain expressing the unfunctional Arg6 mutant which has the amino acid substitution at its catalytic center. In contrast, the Arg auxotrophy of the *Δarg2* strain was reversed by supplementation of *N*-acetyl-glutamate (NAG) to the medium. Interestingly, NAG is the reaction product of the Arg-catalyzed enzymatic reaction. These results suggested that Mpr1 accelerates Arg biosynthesis via NAG production.

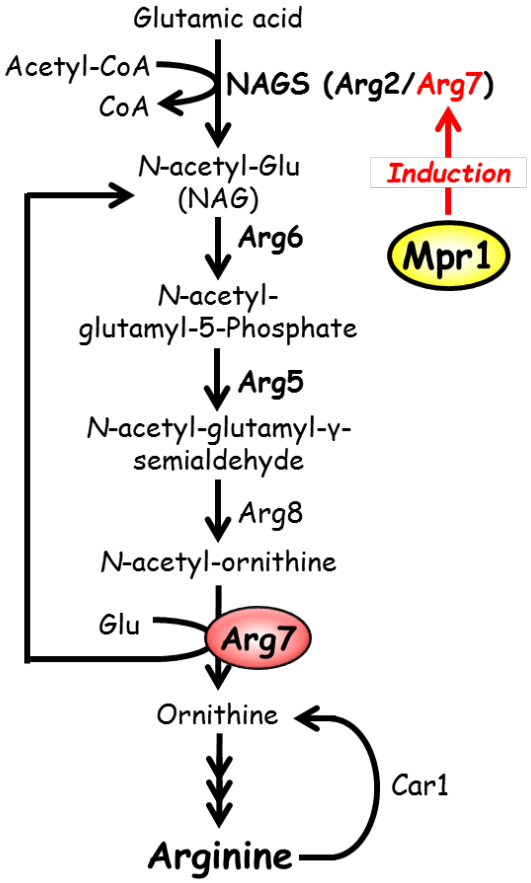

Figure Arginine biosynthesis in yeast
In yeast Saccharomyces cerevisiae, Arg is synthesized from a starter compound glutamic acid, through its acetylation by *N*-acetyl-glutamate synthase (NAGS) Arg2, phosphorylation by Arg6, amino group transfer by Arg8, and deacetylation by ornithine acetyltransferase (OAT) Arg7, followed by further several reactions. Arg is degraded to ornithine by Car1.

Ornithine acetyltransferase (OAT) Arg7, which catalyzes the deacetylation reaction of *N*-acetyl-ornithine, had been suggested to exert NAGS activity like Arg2[3]. The overexpression of *ARG7* compensated for Arg auxotrophy of the *Δarg2* strain, indicating that Arg7 possesses NAGS activity. RT-PCR analysis indicated that *MPR1* overexpression induced the transcription of *ARG7*. On the basis of the results of this study, we conclude that Mpr1 increases NAG production via Arg7's NAGS activity, which induces the transcription of *ARG7*, leading to the enhancement of Arg biosynthesis.

Conclusion

In this study, we used a growth test to evaluate Arg auxotrophy using arginine biosynthesis gene disruptants, mutants, and RT-PCR analysis. We found that Mpr1 increases NAG production through an increase in NAGS activity in Arg7, which occurs via transcriptional induction of *ARG7*, resulting in the enhancement of Arg biosynthesis. Our previous study suggested that Mpr1 exists in both the cytosol and mitochondria; no reports have suggested that Mpr1 translocates to the nucleus. Screening for novel substrates of Mpr1 performed in this study found that proline is converted to *N*-acetyl-proline by Mpr1 under basic conditions. As of now, there are no studies on the biological significance of *N*-acetyl-proline. Thus, its physiological functions are unclear. However, it is possible that *N*-acetyl-proline is involved in Mpr1-dependent *ARG7* induction. Indeed, since some *N*-acetyltransferases acetylate both small molecules and proteins as their substrates[4], Mpr1 might induce *ARG7* similarly, by acetylating certain proteins.

In future studies, Arg7 protein levels will be evaluated in order to test the conclusion made here. Localization analysis of Mpr1 and/or chromatin immunoprecipitation analysis is required to examine whether Mpr1 is directly involved in *ARG7* induction. Physiological analyses of *N*-acetyl-proline and evaluation of *N*-acetylation of the proteins involved in *ARG7* induction by Mpr1 will be performed to clarify the molecular mechanism of Mpr1-dependent *ARG7* transcriptional upregulation.

References

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