Multicopy suppressor screen

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Method Article

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Abstract

Introduction

To identify mutations that restore the activity of a defective ammonium transporter, natural suppressors or supprssors generated by mutagenesis can be identified.

Procedure

1) Select suppressor mutants from the 31019b yeast strain\(1) carrying the high copy number plasmid pDR-AtAMT1;1Thr460Ala by growing at 28ºC for 8 days on solid YNB medium supplemented with 3% glucose and 2 mM ammonium chloride. 2) Pick suppressors and amplify in liquid YNB medium supplemented with 3% glucose and 2 mM ammonium. 3) Isolate plasmid from yeast by CTAB extraction and use for _E. coli_ transformation. 4) Amplify plasmids in _E. coli_, isolate and sequence.

Anticipated Results

When an inactive transporter **Trans** is subjected to a multi-copy suppressor screen, point mutations \(single base changes, primarily) may occur spontaneously in the sequence of **Trans**. Such a spontaneous mutation occurs first in a single plasmid molecule, yielding a single **Trans-Mut** sequence. If **Trans-Mut** does not contribute to transport and growth, the cell will die and the sequence will be lost. If **Trans-Mut** does improve transport, it will initially function in \(**Trans**)$_2$\(**Trans-Mut**)$_1$ complexes, as **Trans** is present in vast excess. If activity is sufficient to lead to growth, **Trans-Mut** will replicate sufficiently to be observed by plasmid DNA sequencing. Reversion to the wild-type sequence in a single plasmid molecule would yield \(**Trans**)$_2$\(**wt**)$_1$ and \(**Trans**)$_1$\(**wt**)$_2$ complexes at the outset \(**wt**$_3$ would be a sufficiently small portion of the population that it would not contribute to growth). The absence of wild-type revertants from the suppressor screens suggests that these complexes are inactive.

References

\(1) Marini, A. M., Soussi-Boudekou, S., Vissers, S. & André, B. A family of ammonium transporters in Saccharomyces cerevisiae. _Mol. Cell. Biol._ **17**, 4282-4293 \(1997).