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Title: Functions of the Ubiquitin-like protein Hub1

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Abstract:

Hub1 is an evolutionarily conserved ubiquitin-like protein whose deletion is lethal in S. pombe but not in Saccharomyces cerevisiae. It has a role in pre-mRNA splicing. It is hypothesized that it binds to pre-mRNA and regulates splicing. We wanted to confirm it further by TRIBE (targets of RNA-binding proteins identified by editing) - a method used to find RNA binding targets of a protein (if any). We fused Hub1 with ADARcd (catalytic domain of Adenosine deaminases acting on RNA) followed by transformation of the engineered plasmid in the genomically deleted Hub1 strain. It was used to check whether Hub1 binds to three different target pre-mRNAs or not in S. pombe. But the strategy did not work in our model organism. Besides splicing, Hub1 seems to have a role in decarboxylation of cinnamic acid derivatives (CAD). It binds to the ferulic acid decarboxylase Fdc1 and negatively regulates its activity. We did experiments with different strains of S. cerevisiae and figured out where the Hub1 deletion effect on decarboxylation is the most. Then we overexpressed Fdc1, Pad1 along with deletion of Hub1 and did spot assays under different concentrations of 4-Methoxycinnamic acid and trans-Cinnamic acid to compare the growth of engineered strain with that of control. On spot assay plates, growth rescue was observed by Hub1 deletion. We did growth curve experiments also. In both types of experiments, Hub1 deletion seems to have a positive effect on decarboxylation.

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