2022

Exploring the Functionality of Putative Bop3 Post-Translational Modifications

Liliya Tkachuk
liliya.tkachuk@pop.belmont.edu

Rebecca Adams PhD
Belmont University, rebecca.adams@belmont.edu

Exploring the Functionality of Putative Bop3 Post-Translational Modifications

Liliya Tkachuk and Rebecca Adams, PhD

All eukaryotic cells require that transcribed mRNAs undergo export form the nucleus to the cytoplasm where they can be translated into proteins. This process requires a host of proteins which are conserved between the unicellular budding yeast, *S. cerevisiae*, and humans. During this process, Mex67 and other associated proteins facilitate the mRNA to travel across the nuclear pore complex (NPC), doorways embedded in the nuclear envelope. Upon the exit of mRNA, Mex67 is released and recycled back into the nucleus to provide the export of more mRNA. This release occurs through the action of Dbp5, whose activity is regulated through additional proteins, Gle1, Nup42, and Ipk1. In the absence of *NUP42* and *IPK1* (*nup42*Δ*ipk1*Δ mutants), mRNA export cannot occur at high temperatures, leading to a growth defect. Previous studies have shown that overexpression of *BOP3*, a nonessential gene, can rescue this growth defect. However, Bop3 is not widely studied, and its function is not known. Bioinformatic analysis has uncovered several high confidence sites of post translational modification (PTM): two sites of phosphorylation and two sites of SUMOylation. PTM is a process wherein proteins can have additional chemical groups or small proteins covalently attached following translation, and this process regulates protein function. We hypothesize that the putative sites of PTM in Bop3 may have a role in regulating mRNA export. To test our hypothesis, these sites were altered using PCR mutagenesis to inhibit or mimic the PTM function in a *BOP3* plasmid. The resulting collection of wild-type and mutant plasmids were then transformed into two mutant yeast strains to assess the resulting functionality of the altered bop3 protein. We observed growth of Bop3 mutants overexpressed in *nup42*Δ*ipk1*Δ mutants. From this, the S231A mutant was

found not to rescue the temperature sensitive growth defect. We also identified and analyzed Bop3 orthologues for evolutionary conservation, and S231 was found to be highly conserved. This suggests that maintaining the phosphorylation of this residue is important for Bop3 function. Further research may aim to discover what kinases phosphorylate this site and uncovering how Bop3 functions during the mRNA export process.

Keywords: bop3; budding yeast; mRNA export; nuclear pore complex; post translational modification; phosphorylation; SUMOylation; bioinformatics; temperature sensitivity