107-LIPS

Incorporating Unnatural Amino Acid *p*Bpa Into NXF1 to Probe Protein Interactions

The export of mRNA from the nucleus to the cytoplasm is a vital process for all eukaryotic gene expression. NXF1:NXT1 are required for bulk mRNA export. Other proteins contribute to mRNA export; however, their interaction with NXF1:NXT1 is not well understood. We aim to investigate these protein interactions by incorporating the unnatural amino acid *p*-benzoyl-L-phenylalanine (*p*Bpa), a photo-reactive crosslinker, into NXF1. NXF1 is made up of four domains, the RRM, LRR, NTF2L, and UBA. The RRM domain is involved in RNA binding and is a common site for protein interactions. Thus, we generated two NXF1 mutants at the RRM domain by replacing one amino acid with a stop codon, Glu155-TAG, and Ile190-TAG. We expressed a tRNA/tRNA synthetase pair in *E. coli* with our protein that recognizes the stop codon and inserts *p*Bpa at the site of the mutation. *p*Bpa becomes reactive under ultraviolet (UV) light where it will create a covalent bond with a nearby C-H bond. We tested the crosslinking of our two *p*Bpa-incorporated NXF1 mutants to a newly identified mRNA export protein. We did not see crosslinking of this protein to either site of the RRM domain of NXF1. Despite not seeing crosslinking, we determined that *p*Bpa was successfully incorporated into the NXF1 protein due to several self-crosslinking bands seen when treated with UV. We now have two pBpa-NXF1 probes to test binding of other proteins at the RRM domain.