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Funded by: NSF-REU Program in Biological Sciences & Biochemistry

Binding properties of X29 protein and RNA

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The Peculis Lab previously demonstrated that X29 protein binds U8 snoRNA with high affinity and specificity in Xenopus Laevis [1]. U8 snoRNA is a C/D box RNP required for pre-rRNA maturation of 5.8s and 28s rRNA in the nucleolus. X29 is a Nudix hydrolase that decaps the U8 RNA at the m^7G and $m^{227}G$ caps [2]. Together these two components may interact in vivo to regulate the rate of ribosome biogenesis and thus, the rates of cell growth and cell division. My work has focused on characterizing the interaction between these two molecules and I have been working on two interrelated projects. The first project involves generating mutations to alter one amino acid in the protein sequence. Based on crystal structure data, the amino acid tryptophan, at position F49, lies in the putative RNA binding site on the X29 protein. The mutation will substitute a tryptophan for the 'wild type' phenylalanine residue at this position. After the mutagenesis, the protein is expressed in bacteria in BL21 cells and the mutated protein is purified. We predict the protein would contain a fluorescence property such that when analyzed by flourimitry we will be able to determine RNA binding and possibly address stoichiometry and dimer formation. The second project involves cross-linking wild type X29 protein to U8 RNA and mapping the cross-link on the RNA. Cross-linking reactions followed by reverse transcription using 5' end-labeled oligo DNA primers will identify 'stops' which are UV- and protein-dependent. We will be able to map the precise nucleotide on the RNA and interpret this in the framework of the proposed secondary structure for U8 snoRNA. The results of these experiments will greatly aid the research of the X29 protein and its binding capabilities to RNA.

References

1. Tomasevic, N. And B. Peculis, Identification of a U8 snoRNA-specific binding protein. J Biol Chem, 1999. 274: p. 35914-20. 2. Ghosh, T., et al., Xenopus U8 snoRNA binding protein is a conserved nuclear decapping enzyme. Mol Cell, 2004. 13: p. 817- 828.