Veerendra Koppolu, J Bacteriol Parasitol 2017, 8:6(Suppl)
DOI: 10.4172/2155-9597-C1-041

Role of N-terminal domain of Rns from Enterotoxigenic *Escherichia coli* in transcription activation

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Enterotoxigenic *Escherichia coli* (ETEC) is a diarrhea causing pathogen which causes ~500,000 deaths/ year worldwide. Rns is a transcriptional activator of the AraC family and is required for virulence gene activation in ETEC. Rns has 2 domains: an amino terminal domain (NTD) and a DNA binding domain (DBD). Current evidence indicates that Rns functions as a monomer and may not require an effector to activate transcription. The main goal of this study is to identify the role of Rns-NTD in transcription activation. In previous work, two individual mutations I14T and N16D, near the amino terminus of Rns and far from known DNA-binding determinants, abolished Rns activation at its own promoter by hindering the binding of Rns to DNA. We have found that deleting the Rns-NTD (residues 1-156) leads to loss of Rns activity in vivo. Sequence analysis of Rns closest homologs showed that a region of the NTD (residues 12- 30) shares high identity (74%), much higher than the 26% overall NTD identity. Further, this region aligns with a region in the ToxT structure that contacts the DBD. We hypothesize that residues in this region contact Rns DBD, causing structural rearrangements in the DBD that facilitate DNA binding. One test of our hypothesis is site-directed random mutagenesis to identify mutants in this region that are defective in transcription activation. To date, we have mutagenized residues 12 to16 and identified eight variants (at positions I12, I14, N15 and N16) that decreased Rns activity by > 2-fold, indicating that these residues are important for Rns activity. On the other hand, none of the mutants of K13 had decreased Rns activity. We will perform Western blots to eliminate unstable variants from consideration. We have purified Rns DBD to near homogeneity by adding a solubility tag, GB1, at the C-terminus of the DBD. The purified Rns DBD will be used for antibody generation and structural studies. Our electrophoretic mobility shift analysis (EMSA) with purified Rns DBD-GB1 showed that Rns DBD can bind to DNA *in vitro* at sufficiently high protein concentrations.

Biography

Veerendra Koppolu is a scientist at MedImmune LLC (AstraZeneca). He has 10 years of experience in biological sciences research. Research interests include Assay development (Cell/Protein/Immune), Protein engineering, purification and characterization, Drug target mechanism studies, Cell culture process development.

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