Biochemical and Structural Study of the Escherichia coli Transcription Elongation Complex with Lambda-N and HK022 Nun

Lima, Joel

Lambda phages have made important contributions to current understanding of phage gene expression, viral DNA assembly, and lysogeny. The N protein of lambda phage is an anti-termination protein which functions by binding to lambda's N-Utilization (Nut) sites. The Nun protein of the HK022 phage inhibits super-infection of lambda phage to its host by arresting transcription of the lambda genome after Nut site binding. When binding to Nut sites, HK022 Nun directly competes with lambda-N to trigger early termination during transcription. Both phage proteins also bind to the ternary elongation complex of E. coli RNA polymerase. Further research into Lambda's transcription factors could lead to an understanding of the mechanisms by which lambda-N and HK022 Nun regulate the elongation step in E. coli transcription. To study these proteins, lambda-N and HK022 Nun-K106A/K107A double mutant were produced in E. coli BL21-AI cells, and purified using ion and size exclusion chromatography. A transcription assay of the purified proteins was performed and showed that HK022 Nun-K106A/K107A had reduced activity compared to HK022 Nun. Purified protein samples were prepared for cryo-electron microscopy in a collaborating lab. Cryo-electron microscopy images were run through Relion analysis for particle picking prior to manual selection of protein complexes. Following conversion to protein database (.pdb) files, 3D structures were manipulated using Pymol. This analysis produced high resolution images of HK022 Nun as well as Lambda's Nut site. Analysis on the double mutant of HK022 Nun is still in process. Ultimately this analysis could further understanding of bacterial transcription, improving development of elongation targeted treatments for bacterial infections.