Protein-Protein Interactions in DNA Precursor Biosynthesis

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Because the rate of DNA chain extension during replication is quite rapid, it is unlikely that deoxyribonucleotides can migrate to replication forks by simple diffusion, and still maintain substrate saturation for replicative DNA polymerases. Using T4 bacteriophage and its host Escherichia coli as a model system, our laboratory has characterized a multienzyme complex for deoxyribonucleoside triphosphate biosynthesis and has developed evidence that this dNTP synthetase complex is juxtaposed with the replication machinery, thereby allowing direct transfer of dNTPs to polymerases, and maintaining local dNTP concentrations at optimal levels in the face of enormous pool turnover. The talk will describe several approaches we are using to identify the direct protein-protein contacts within this replication hyperstructure, including kinetic analysis, immobilized protein affinity chromatography, immunological approaches, and optical biosensor analysis.

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