T7 RNA POLYMERASE MUTANTS A TOOL FOR UNIFYING THE MODELS OF ABORTING CYCLING DURING TRANSCRIPTION INITIATION

Transcription is a fundamental part of genetic regulation. The RNA polymerases that accomplish this function vary in structure, size and complexity, but must all carry out the same basic functions. The simplest of these is the single subunit family of enzymes exemplified by the RNA polymerase from bacteriophage T7 (which is closely related to the two-subunit system from mitochondria and chloroplasts). This relatively simple RNA polymerase undergoes a large conformational change in its structure as a part of its mechanism and there are crystal structures available for the complex poised at various stages of transcription.

The transcription process has been conceptualized as occurring in three distinct phases: 1) initiation, which includes the binding of enzyme and DNA at a specific sequence (promoter binding), abortive cycling and promoter release; 2) elongation, where stability of the enzyme/DNA/RNA complex is essential; and 3) termination, where the complex dissociates, releasing product. While there is a wealth of structural information that has lead to specific models for function, key elements of these models remain untested. During the transition from initiation to elongation, for example, RNA polymerases are typically unstable and release abortive products. Structures (static) have led to energetic models (dynamic) such as DNA scrunching and steric clash as the driving force for aborting cycling. Our group has disproven these models, and the mechanism remains unresolved.

My research is focused on elucidating the mechanism of abortive cycling in a mutant T7 RNA polymerase, P266L, which curiously, shows dramatically reduced abortive cycling. Previous research suggested a decrease in promoter binding affinity in the mutant and proposed this as a part of the P266L mechanism. My recent findings using a much more sensitive fluorescence anisotropy binding assay demonstrate clearly that promoter binding is not reduced in the mutant. Our current hypothesis is that the mutant has a lower (kinetic) barrier to the required conformational change. I have designed homemade accessories to measure directly the kinetics of the conformational change via FRET distance measurements. In a preliminary result, I have found that the P266L enzyme/DNA/RNA complex dissociates at a slower rate during initial transcription. I will couple these measurements to rapid kinetic analyses of product formation and release. I am also exploring a collaboration with Professor Alejandro Heuck, to achieve a more detailed understanding of distributions of species along the kinetic pathway. The results of this research should lead to a unified understanding of the process of aborting cycling, a key mechanistic step that is common to all the RNA polymerases from the prokaryotic to the eukaryotic organisms.

As an MCB student in a Chemistry lab, carrying out fluorescence and transcription assays in a complex biological system, the multidisciplinary environment that CBI offers is
essential to my professional development. I have enjoyed very much participating fully in this wonderful program.