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Kinetics vs. equilibrium binding: Determining the mechanism of transcriptional activation of Polymerase II

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The mechanism of transcriptional activation of Polymerase II (Pol II) can be studied in a unique way by using a model transcriptional activator, TFIIIA-VP16, whose DNA-binding domain is derived from the zinc finger protein TFIIIA. In previous research, the equilibrium binding constants of wild-type TFIIIA-VP16 and two mutants, L148F and L148H, were determined, along with the half lives of the DNA-protein binding complexes containing these proteins. It was found that the equilibrium constants of the mutants were changed only slightly relative to that of wild-type TFIIIA-VP16 (Kd = 0.4nM). However, the half-lives of the mutants, L148F and L148H, showed a 20 fold and 6 fold increase over the wild type, respectively. Activation of Pol II by L148F and L148H showed a 20 fold and 3 fold increase over the wild type.

The question to be answered is why Pol II activation increased so greatly when the equilibrium binding affinity, the factor that should determine site occupancy, differed so little. There are three research components to answering this question: 1) Determine the in vivo concentration of wild type and mutant TFIIA-VP16 and compare this to the equilibrium binding constant. If the in vivo concentration is much higher than the equilibrium binding constant, then the activation binding site should be fully occupied regardless of whether the TFIIIA-VP16 is mutated or not. This would imply that the activation is controlled by a kinetic parameter, not an equilibrium binding parameter. 2) Determine the in vivo kinetics of dissociation of wild type and mutant TFIIIA/ DNA complexes and compare to the in vitro dissociation rates. 3) Determine the in vivo occupancy of TFIIA-VP16 binding sites by the activator protein. The first two components are the subject of this study.

Western blotting techniques using pure TFIIIA as a standard were done to determine the number of TFIIIA-VP16 molecules per cell as well as the concentration. Results show that the average in vivo concentration of TFIIIA-VP16 and mutants is 4.8 μM, roughly 10,000 times higher than the equilibrium binding constant (0.4nM). This suggests that the activation binding site absolutely should be full regardless of whether the TFIIIA-VP16 is mutated or not, which means activation isn't based solely on an equilibrium binding parameter. To determine the in vivo kinetics of dissociation, TFIIIA-VP16 is being placed under the control of a copper inducible promoter so that production of the protein can be turned on or off. Reporter gene activity will be assayed following induction or repression of either wild type or mutant TFIIIA-VP16. These assays are currently in progress.