Cryo-EM Structure of Human DNA Polymerase d Inhibited by Aphidicolin: Implications for Cancer and Structure-Based Drug Design

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Cancer remains a leading cause of death worldwide, accounting for approximately 10 million deaths annually, and is strongly associated with genome instability and replication stress. Aphidicolin is a diterpenoid small-molecule inhibitor that targets B-family DNA polymerases and is widely used to induce replication stress, a key driver of genome instability in cancer; however, the structural basis by which it inhibits the human lagging-strand replicase, DNA polymerase d (Pol d), has remained unknown. In this study, the complete four-subunit human Pol d holoenzyme was reconstituted in complex with PCNA, primer–template DNA, and aphidicolin, assembled under controlled biochemical conditions, and purified using size-exclusion chromatography to isolate the fully assembled fraction. Single-particle cryo-electron microscopy was then used to determine the structure at 3.1 Å resolution following standard image processing and refinement procedures. The structure reveals aphidicolin bound within the incoming nucleotide-binding pocket of the polymerase active site, where it distorts the templating base and sterically blocks nucleotide incorporation, while the enzyme adopts an open and inactive conformation that prevents the conformational changes required for catalysis. 3D Variability Analysis (3DVA) was performed to explore conformational heterogeneity and to probe the dynamics of the interaction between Pol d and aphidicolin. These findings provide the first structural view of aphidicolin inhibiting human Pol d within its complete holoenzyme context, establish a mechanistic framework for understanding replication stress, and support structure-based drug design and comparative analysis across replicative DNA polymerases.

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