

## In crystallo study of the reaction mechanism in a family B DNA polymerase

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Reaction intermediates during DNA synthesis have been studied in detail using time-resolved X-ray crystallography for translesion and repair DNA polymerases. Contrary to the originally proposed two-metal-ion mechanism, a third metal ion was identified between the finger domain and the  $\alpha$ - and  $\beta$ -phosphates of the incoming nucleotide. This third metal ion was suggested to either participate in catalysis or stabilize product formation. To investigate this further in a replicative polymerase, we conducted time-resolved X-ray crystallography with DNA Polymerase epsilon, which synthesizes DNA at a much faster rate—10x to 100x higher than family Y and X polymerases. Surprisingly, no metal ion was observed between the finger domain and the  $\alpha$ - and  $\beta$ -phosphates of the incoming nucleotide in any of the solved structures with Pol epsilon. Instead, our biochemical and structural data support the original two-metal mechanism. In addition, we discovered that the 3'-OH group releases a proton, which is channeled via structural waters to a basic residue in the Palm domain. After forming a new bond with the incoming nucleotide, an acidic residue in the finger domain protonates the released pyrophosphate, stabilizing the product. In summary, it seems that metal A's role is to lower the pKa of the 3'-OH group, followed by specific residues in Pol epsilon donating or receiving a proton to catalyze this acid-base reaction.

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