In Crystallo Observation of Three Metal Ion Promoted DNA Polymerase Misincorporation

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Error-free replication of DNA is essential for all life. Despite the proofreading capability of several polymerases, intrinsic polymerase fidelity is in general much higher than what base-pairing energies can provide. Although researchers have investigated this long-standing question with kinetics, structural determination, and computational simulations, the structural factors that dictate polymerase fidelity are not fully resolved. Previous time-resolved results showed that during correct nucleotide incorporation, three Mg2+ ions are required for aligning the primer terminus 3'-OH with the □-phosphate of the incoming nucleotide and for promoting phosphoryl transfer. To fully understand the mechanism of polymerase fidelity, we visualized the DNA misincorporation process catalyzed by DNA polymerase η at atomic resolution with X-ray time-resolved crystallography. We were able to capture reactant and product states of polymerase □ with Mg2+ or Mn2+ throughout the catalytic process. The incorrect nucleotide dG binds as a wobble base to the templating dT; binding of the A-site metal ion induces primer 3'-OH-□-phosphate alignment; and binding of the C-site metal ion promotes nucleotidyl transfer. Furthermore, we observed that C-site metal ion binding preceded the nucleotidyl transfer reaction and proved that the C-site metal ion is strictly required for misincorporation. Moreover, in crystallo titration of the A-site metal suggests that Mn2+ is better than Mg2+ at aligning the 3'-OH of the primer for nucleophilic attack. Thus, our work suggests that 3'-OH-□-phosphate alignment is key for misincorporation, explains the mechanism of Mn2+-promoted misincorporation, and highlights the essential but separated roles of the three metal ions in DNA synthesis.

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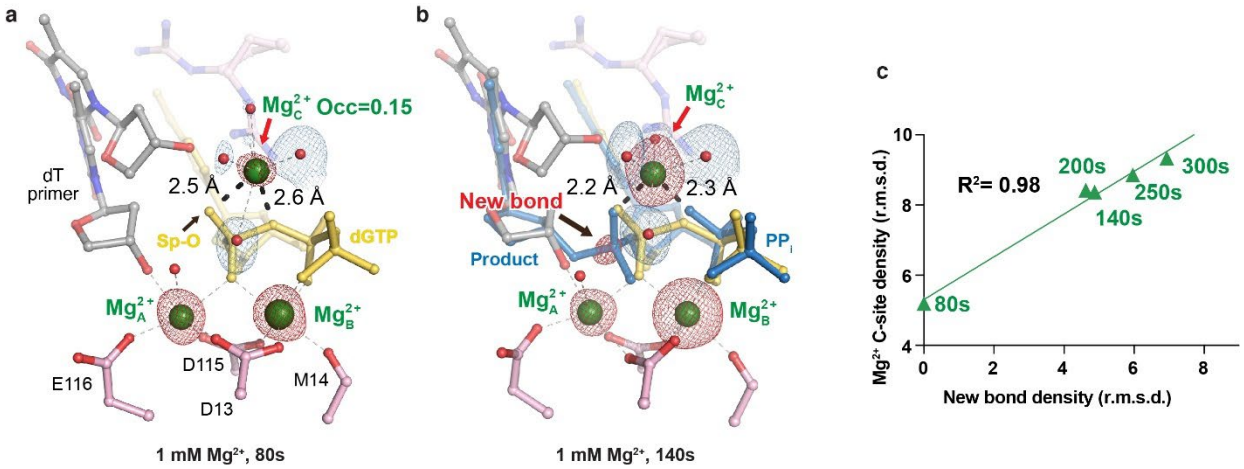

Figure 1