DNA replication in Mycobacterium tuberculosis: structural and biochemical characterization of the replicative DNA polymerase DnaE1 and its unique PHP-exonuclease

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High fidelity DNA synthesis is essential for all organisms and depends on a proofreading 3'-5' exonuclease that is associated with the replicative DNA polymerase. Contrary to humans and the model organism *Escherichia coli*, the proofreading activity in the major pathogenic bacteria *Mycobacterium tuberculosis* (Mtb) is performed by an intrinsic domain of the replicative DNA polymerase DnaE1, the PHP domain. The mechanism of action of the PHP-exonuclease is unknown. Resistance to antibiotic in Mtb is caused by point mutations that occur during DNA replication. Hence, understanding the mechanisms that regulate replication fidelity in Mtb is crucial in the search for new therapies to treat one of the major global health problems. We have solved the crystal structure of the Mtb DnaE1 polymerase and biochemically characterized its PHP-exonuclease. We have identified a tri-nuclear Zn center in the PHP domain coordinated by nine conserved residues. Interestingly, the PHP active site in DnaE1 shows remarkable similarities to the active site of *E. coli* endonuclease IV (Endo IV), an enzyme that also cleaves DNA. All these observations allow us to propose a mechanism for DNA hydrolysis by the PHP-exonuclease based on the mechanism of action of Endo IV. Finally, the unique PHP-exonuclease active site of Mtb appears an attractive target for specific inhibition as is not found in eukaryotes, making the likelihood of cross reactivity to human exonucleases small. Therefore, this work provides new insights in understanding replication fidelity in Mtb and will be a valuable tool in the development of novel treatments that target DNA replication in *Mycobacterium tuberculosis*.