Unexplored C-terminus of Pol ε Possibly Functions in Cancer and FILS Syndrome

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Mutations of an organism's genetic code results in dysfunctional proteins, the major structural and functional units of the cell. When proteins controlling the cell-cycle and replication are mutated, uninhibited cell proliferation may occur – ultimately leading to cancerous tumors. A single mutation is not enough to cause cancer. However, mutations that disrupt DNA replication and repair facilitate the accumulation of additional mutations that together may lead to metastasizing tumors. DNA polymerase ε (Pol ε), an enzyme responsible for elongating the leading strand during DNA replication, has commonly been found mutated in many cancer cell lines. Pol ε is made of four protein subunits – Pol2 being the most essential. The importance of Pol2 is manifest in how genetically conserved it is – even between humans and yeast. Mutations of Pol2's N-terminus, near the catalytic core, are the most commonly associated with cancer. However, this raises the question of what role the ESSENTIAL C-terminus of Pol2 plays in DNA replication. FILS syndrome, a rare genetic disorder causing skin and facial abnormalities, immunodeficiency, and short stature, is characterized by a specific mutation in the C-terminus of Pol2. To investigate whether the C-terminus of Pol2 interacts with other DNA replication proteins, several strains of Saccharomyces cerevisiae were prepared with C-terminal mutations in Pol2 by using CRISPR-Cas9. mRNA was then extracted, reverse transcribed to cDNA, and analyzed with quantitative PCR to determine whether any mutations affected DNA replication proteins. Our results show that two specific mutations cause a drastic increase in mRNA expression of DNA replication proteins suggesting that the C-terminus of Pol2 is integral to the regulation of DNA replication. These results could explain the replication defects observed in the FILS patients.