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

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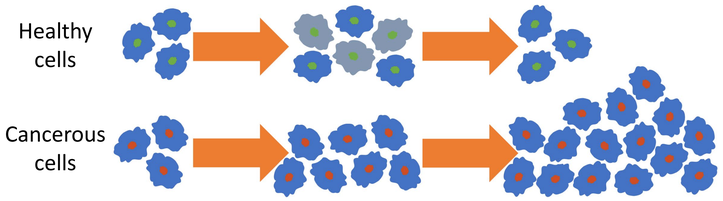
Mutations of an organism's genetic code results in dysfunctional proteins, the major structural and functional units of the cell. When proteins controlling the cell-cycle and replication are mutated, uninhibited cell proliferation may occur – ultimately leading to cancerous tumors. A single mutation 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.

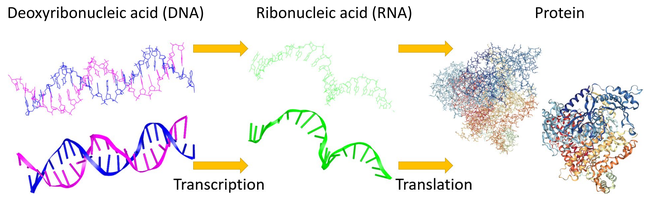
Introduction

33% of Americans will develop some form of cancer in their lifetime [1]. One thing is consistent in all cancers: mutations must take place that allow cells to proliferate while ignoring normal cell regulation.

Cellular Homeostasis



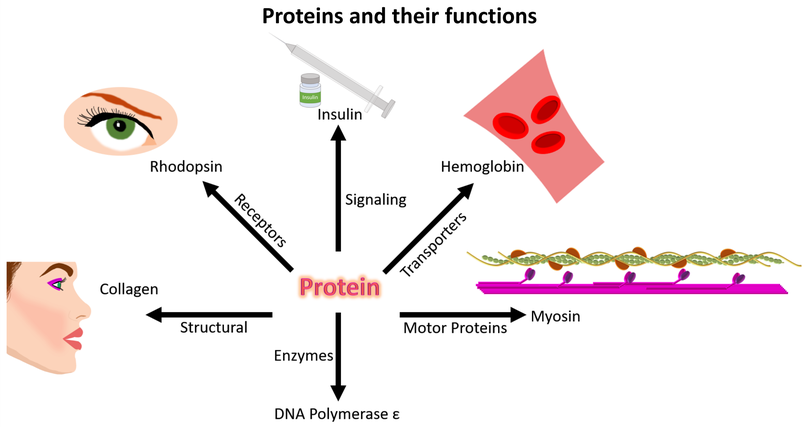
Central dogma of Molecular Biology



https://www.ncbi.nlm.nih.gov/Structure/icn3d

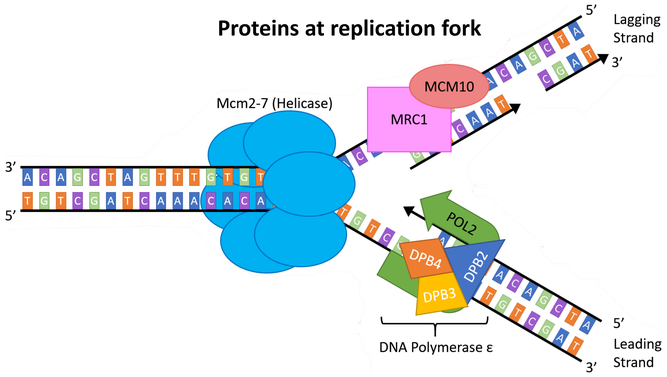
Proteins serve as the major functional subunits of the cell. When mutations affect protein structure, important processes can be compromised

Proteins and their functions



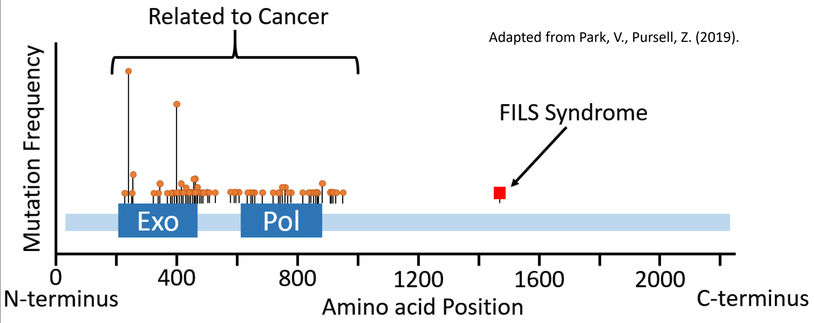
DNA polymerase ε (Polε), a four subunit (Pol2, Dpb2, Dpb3, and Dpb4) enzyme necessary for DNA replication. Mutations in the N-terminus of POL2 have been associated with cancer [2]. Polε is highly conserved from yeast to humans. Previous work has indicated that Mcm10 may associate with the C-terminus of Pol2 [3]. Furthermore, it is known that Mcm2-7 and Mrc1 play essential roles in replication. DNA polymerase ε dysfunction is linked to genome instability, cancer, and FILS Syndrome (facial dysmorphism, immunodeficiency, livedo, and short stature) [4].

Proteins at replication fork



Goal

Is the C-terminus of Pol2 Involved in Cancer and FILS Syndrome?



Materials & Methods

Yeast Strains

Table 1: Four *S. cerevisiae* strains with mutations in *POL2* were constructed by using CRISPR-Cas9 gene editing.

Strain	Pol2 Mutation	Summary
41	G1425A, E1428A	Glycine and Glutamic acid exchanged for Alanine at 1425 and 1428
42	W1280A, Q1283A	Tryptophan and Glutamine exchanged for Alanine at 1280 and 1283
46	W1272A	Tryptophan exchanged for Alanine at 1272
50	I1349A	Isoleucine exchanged for Alanine at 1349

Primer Design

Table 2: Primers were designed using NCBI-BLAST and Northwestern University's Oligo Calc.

Gene	Sequence	Length	Tm (°C)	G/C %
POL2	5′-3500 TTC CAA TCC TGT TCC AAG GGT TGA AC-3′ 3′-TTC CAA TCC TGT TCC AAG GGT TGA AC 3779-5′	26 26	59.3 56.6	46.2 46.2
MCM10	5′-701 TTC CAA TCC TGT TCC AAG GGT TGA AC-3′ 3′-TTC CAA TCC TGT TCC AAG GGT TGA AC 982-5′	27 24	58.2 55	44.0 45.8
MCM6	5′-209 TTC CAA TCC TGT TCC AAG GGT TGA AC-3′ 3′-GTC AAA AGA ACA GGG TGT TCT TCT G 505-5′	25 24	58.9 55.3	48.0 45.8
MRC1	5′-13 TTG CAT GCT TTG TCC TCG TTG AC-5′ 3′-CTT TAA CTT CGG ATT TCT CCA GCT C 327-5′	23 25	55.3 56.0	48.0 44.0
ACT1	5′-325 GCT GCT TTG GTT ATT GAT AAC GGT TC-3′ 3′-GTC AAA AGA ACA GGG TGT TCT TCT G 626-5′	26 25	56.7 56.2	42.3 44.0

Quantitative PCR

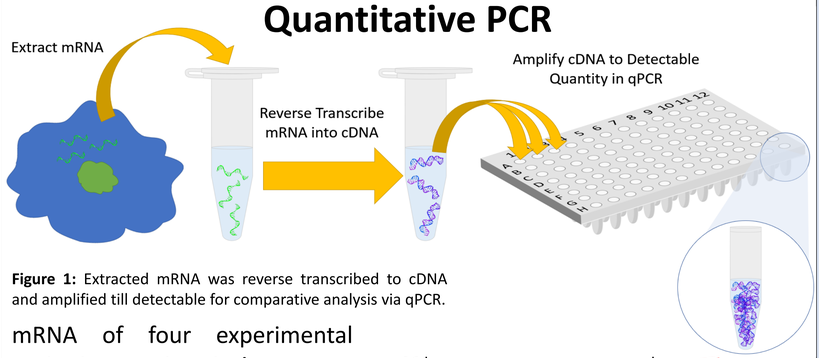


Figure 1: Extracted mRNA was reverse transcribed to cDNA and amplified till detectable for comparative analysis via qPCR.

mRNA of four experimental mutant yeast strains were isolated via Qiagen RNeasy extraction kit and then reverse transcribed into cDNA. The synthesized cDNA was used in qPCR with primers selecting for *ACT1*, a housekeeping gene serving as a control. Data was compiled with QuantStudio 3 while excel was used to calculate normalized expression rates.

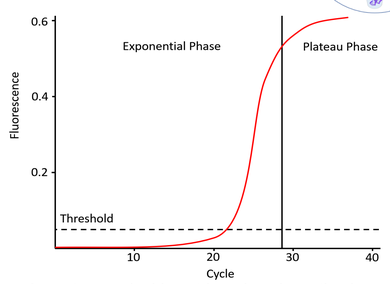
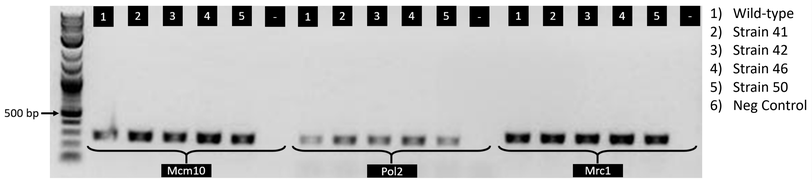


Figure 2: DNA doubles with each cycle until a detectable threshold is reached. The change in fluorescent signal indicates total quantity of DNA as it grows exponentially.

Results

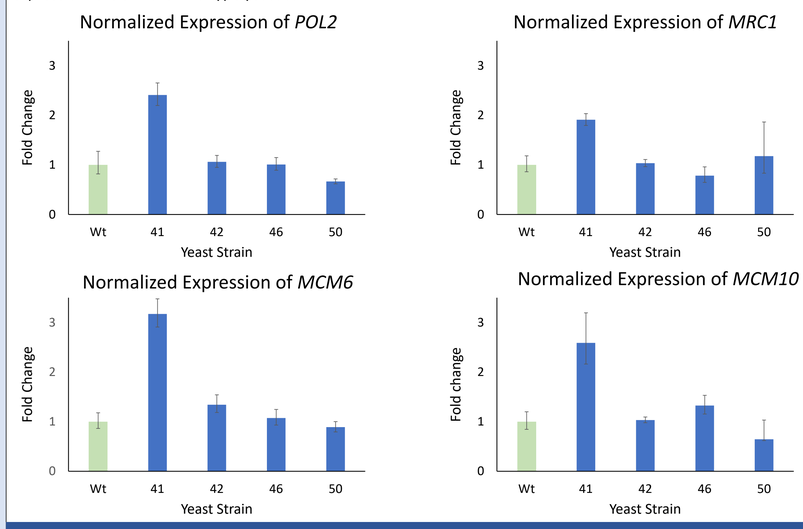
Primer Validation

Figure 3: RNA was isolated from yeast strains harboring wild-type Pol2 (DB035) and mutant Pol2 genes using RNeasy Mini Kit (Qiagen). Genes involved in DNA replication were amplified by Polymerase chain reaction using cDNA synthesized from the isolated mRNA.

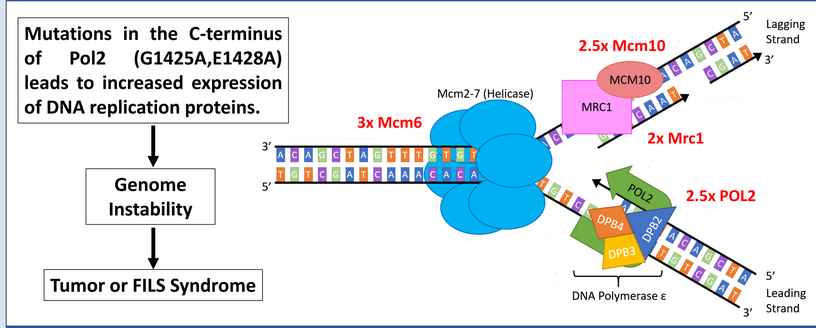


Gene Expression by Quantitative PCR

Figure 4: Evaluation of Pol2, Mrc1, Mcm10 and Mcm6 mRNA expression in wild-type and Pol2 mutated yeast strains was done using qRT-PCR. Data are represented as mean ± SE and normalized to the ACT1 transcript and to the gene expression levels in the wild type yeast strain.



Summary



Future Directions

- Design primers targeting the other Pol ε subunits
- Western Blot analysis to compare protein expression
- Flow cytometry to investigate how mutations affect cell cycle

Acknowledgements

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