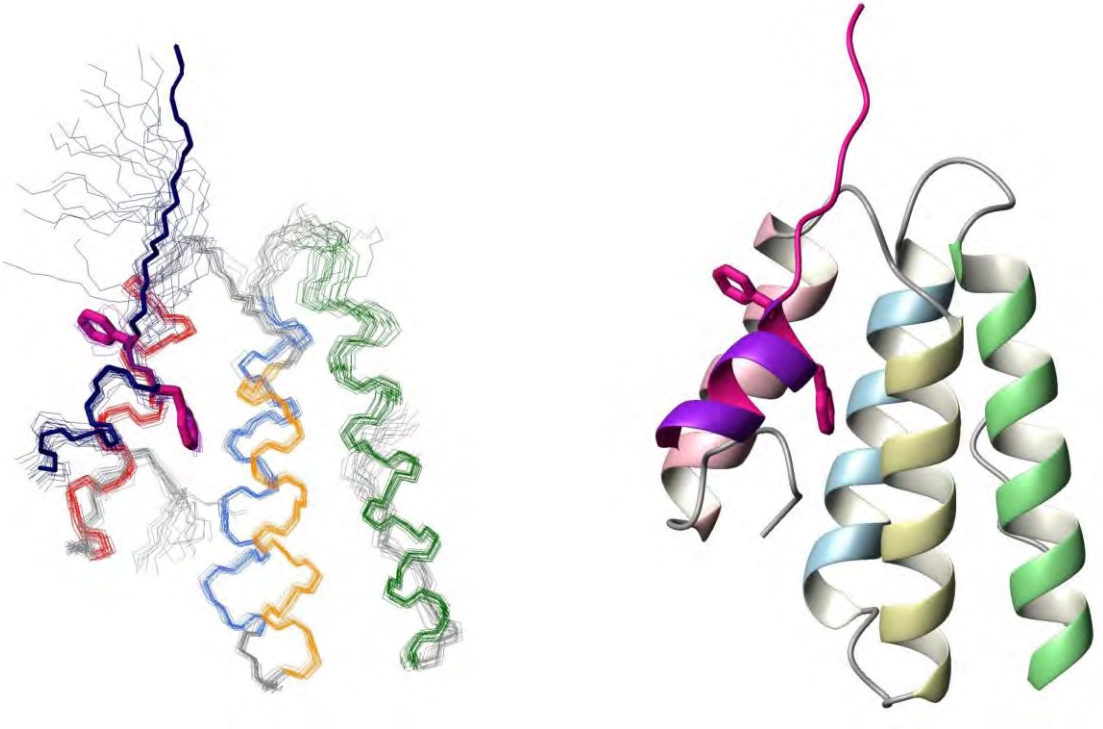
Structure and Interactions of Translesion Synthesis DNA Polymerases



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What are TLS DNA Polymerases?

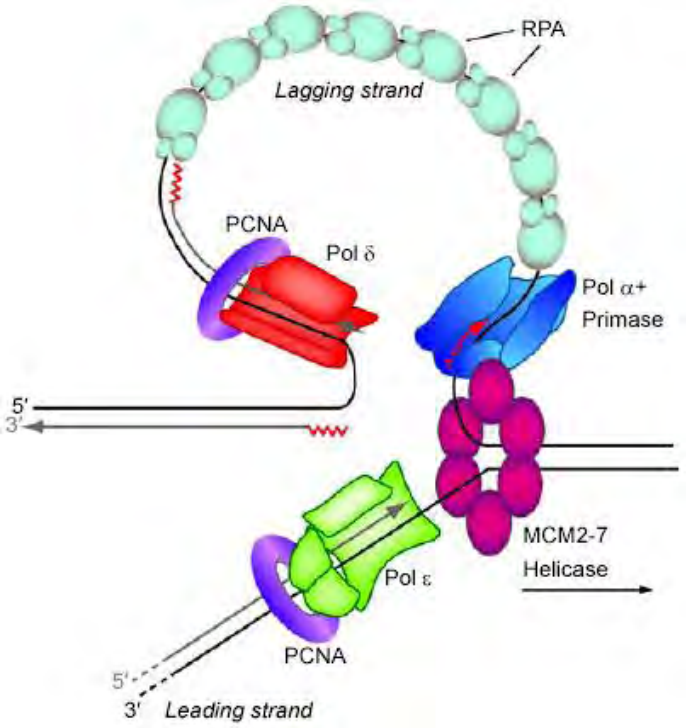
- They are a key component of DNA replication pathways. Translesion synthesis is a DNA damage tolerance process.
- However, certain types of DNA damage (lesions, dimers) persist and can halt DNA replication. Replicative DNA polymerases, cannot bypass most types of DNA damage due to restricted active sites.
- Thus, organisms have evolved DNA damage tolerance pathways, which employ certain TLS enzymes that temporarily leave DNA damage unrepaired.
- TLS polymerases have relaxed active sites that can replicate through various types of DNA damage. However, they replicate undamaged DNA at an extremely low fidelity, incorporating errors roughly every 10 to 1000 base pairs.

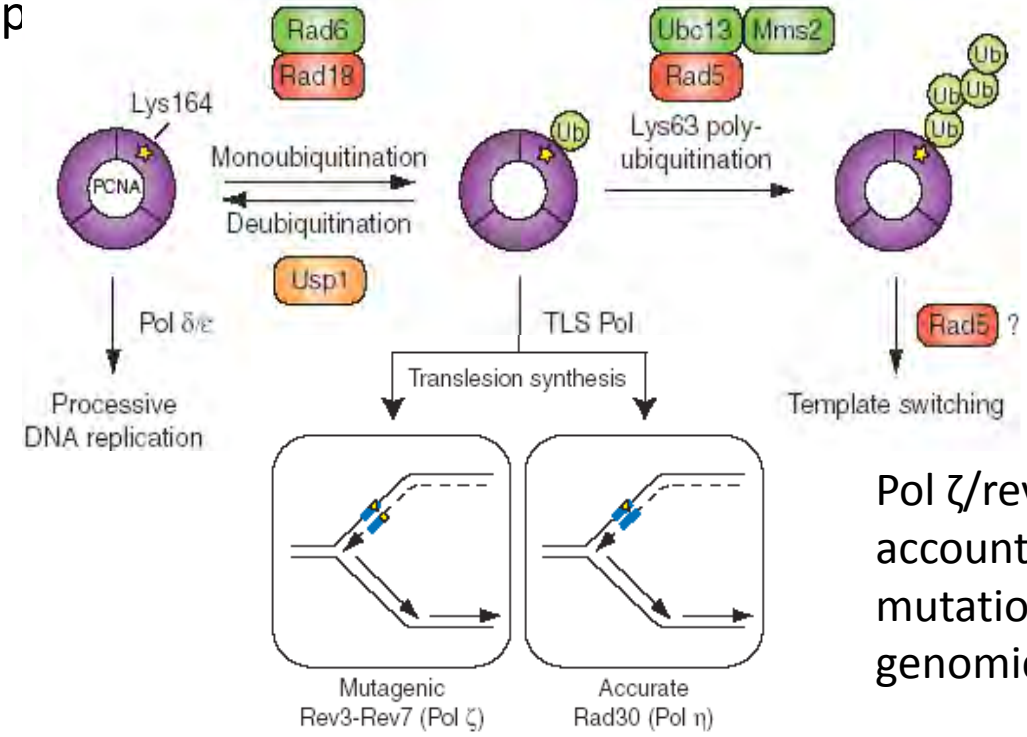
DNA Polymerases

Bulk DNA Replication **Replicative B-family:** **Pol δ, Pol ε**	**Translesional Synthesis** **TLS Y-family:** **Rev1, pol η, pol ι, pol κ** **TLS B-family:** **Pol ζ**
•High fidelity, low error rate, about one in every 10^6 to 10^8 base pairs. •Contains 3' to 5' proofreading activity •Restricted binding site, cannot bypass DNA lesions. •Therefore proceeds more slowly/ ineffective in highly damaged DNA	•Low fidelity, high error rate, one in every 10 to 1000 base pairs. •No proofreading activity •Relaxed binding site, can bypass DNA lesions •Effective at replicating highly damaged DNA, however is mutagenic and can incorporate new mutations in the DNA through mismatch base pairs.

Basic Mechanism of TLS - Eukaryotes

- The switch to TLS polymerases is facilitated by interaction with PCNA, a ring shaped protein involved in replication, that otherwise serves as an activator and binding platform.
- Upon mono-ubiquitination of PCNA at lysine 164 at a stalled replication fork, replicative polymerases are exchanged for TLS p

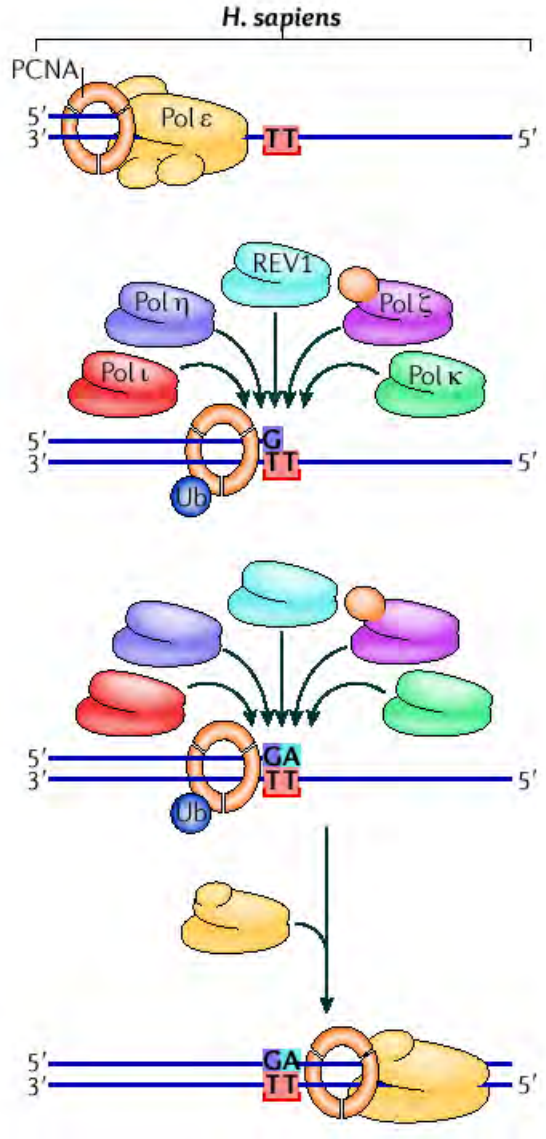




Pol ζ/rev1 TLS branch accounts for 90% of mutations introduced in genomic DNA.

DNA damage tolerance pathways and PCNA ubiquitination

Basic Mechanism of TLS -Eukaryotes

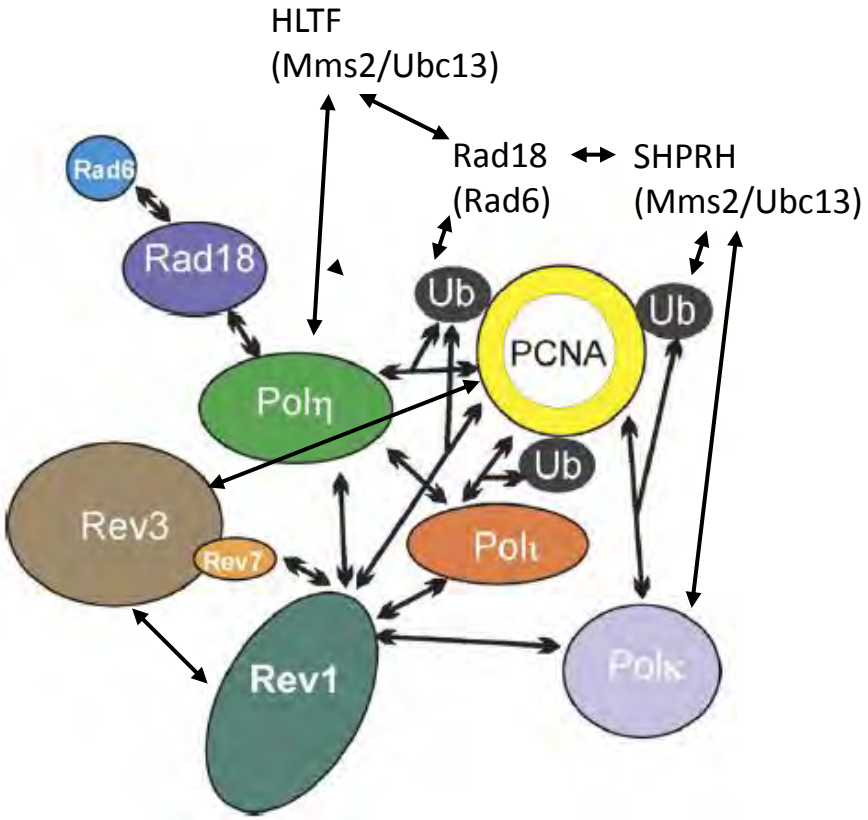


- Upon PCNA mono-ubiquitination, one or multiple TLS polymerases are recruited to the site of DNA damage, where they replace replicative polymerases, **Pol δ** and **Pol ε**.
- **Pol ζ** extends
- **Pol η**, **Pol ι**, **Pol κ** are inserters
- **Rev1** structural, interacts with all other proteins

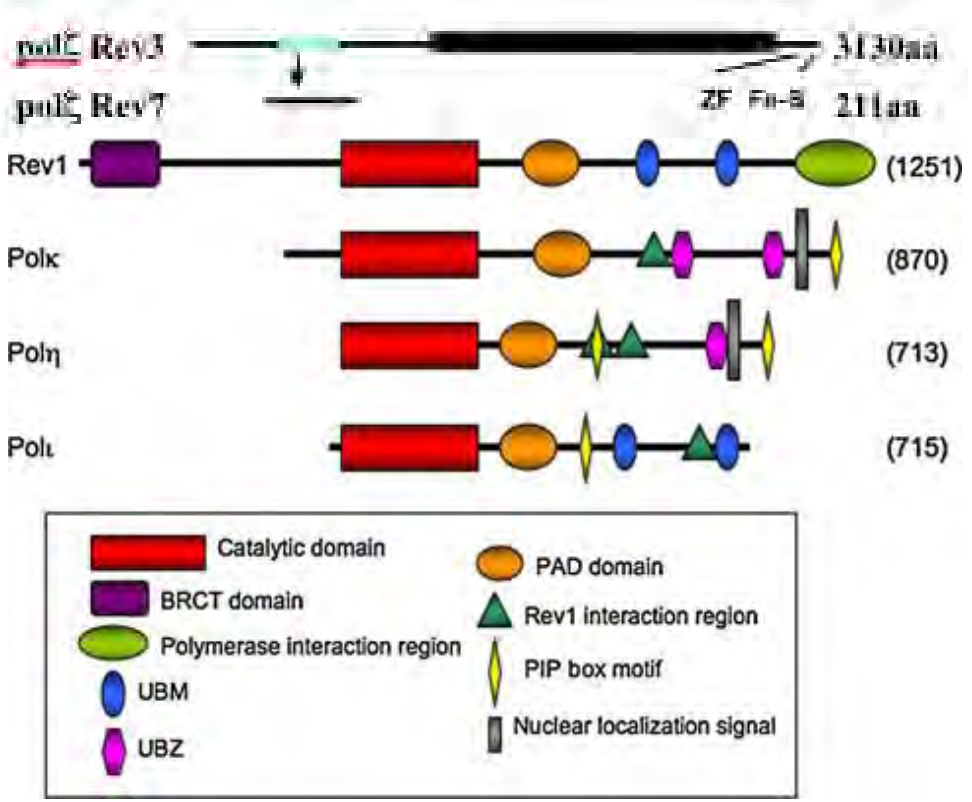
Research Question

- Why is any of this important?
  - The translesional synthesis pathway is responsible for up to 90% of mutations introduced into the genome.
  - DNA damage leads to TLS replication which leads to more mutations, etc., resulting mutagenesis and of tumor cells.
- What are the specific protein-protein interactions responsible for the recruitment of TLS enzymes, specifically error-prone Rev 1 and Pol ζ?
- Application - Interfere or inhibit the recruitment of TLS enzymes in highly mutated genomes in order to sensitize cancer cells to chemotherapy.

The Project



Interactions between proteins involved in TLS



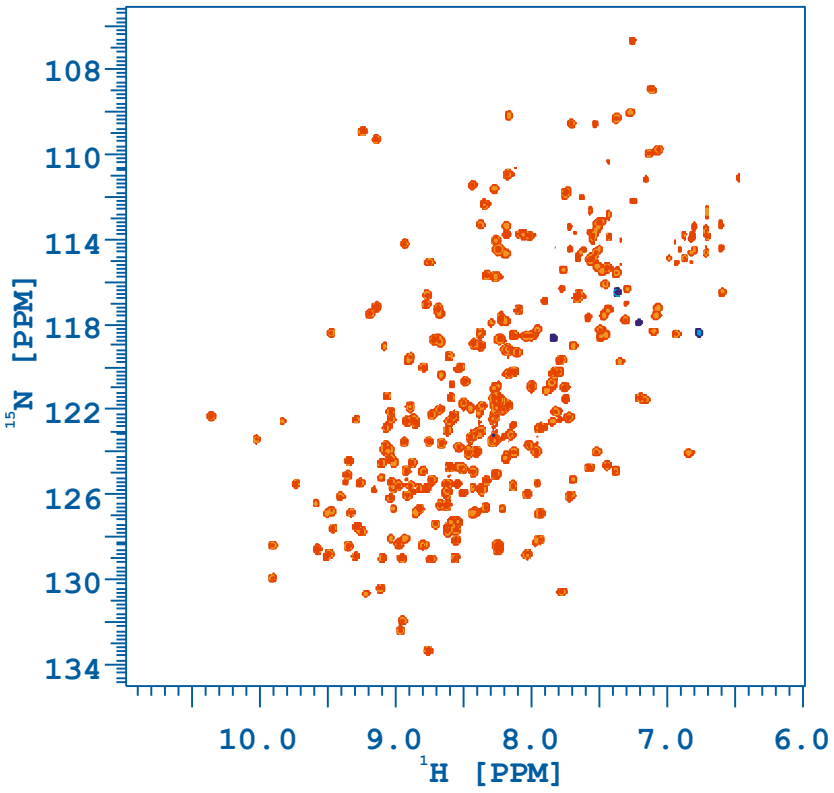
Domain architecture of TLS polymerases

- TLS is coordinated through an intricate network of protein-protein interactions. Determining a detailed, structural characterization aimed at understanding these mechanisms is our goal.
- TLS polymerases contain several binding domains and motifs that facilitate their interaction with each other and with PCNA. We can find the specific amino acid sequence of these domains using protein NMR spectroscopy.

Protein NMR

- What is protein NMR?
  - "Fingerprint" of a protein
  - Each peak represents an amino acid
  - Can involve different techniques and methods depending on size of the protein sample and resonance assignments.
  - Use of N15 and C13 labeled protein to map chemical shifts, labeled amine groups resonate at a different frequency
  - Requires highly purified protein grown on isotopic media.

NH TROSY - scPCNA (100 kDa)

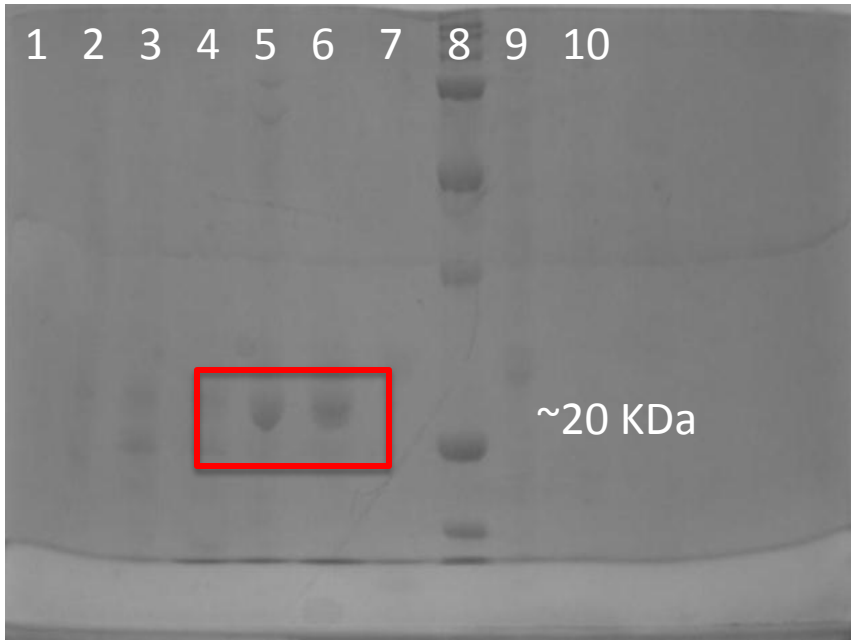


Work and Data

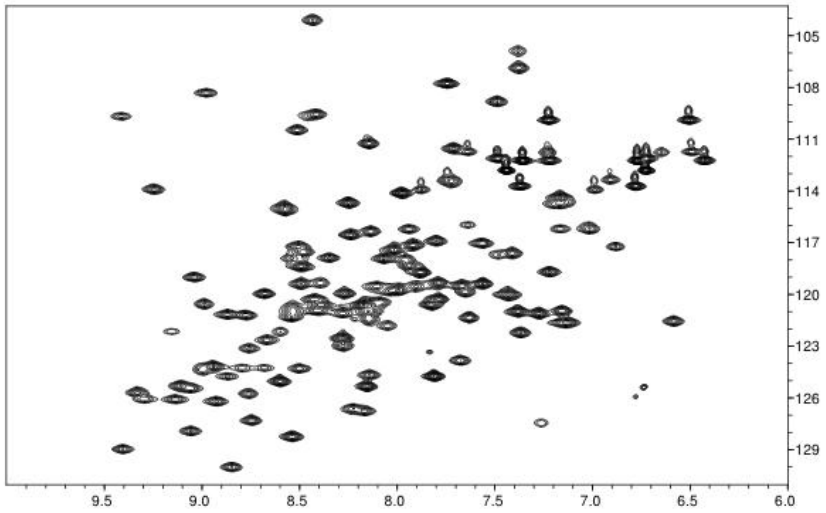
- Structural biology labs are very slow!
- Worked specifically on hPCNA, Polζ hUBZ, and Rev1 hPAD protein domains.
- Grew both labeled and unlabeled samples of proteins via transformation of *e. coli* w/ protein plasmid, spun down
- Isolated protein via Ni^{2+} column, TEV and DDT enzyme cleavage and FPLS gel filtration
- Collected fractions and ran samples on gel electrophoresis
- Concentrated for NMR spectroscopy or titrations

Rev1 hPAD domain

hPAD FPLC Gel Fractions



hPAD HSQC Spectrum



^{15}N

^{1}H

Speculations

- Titrations
  - Hypothesize that Polζ UBZ binds ubiquitin to Rev1 hPAD domain
  - Upon addition of substrate, signals shift
  - Can map binding interface by watching peaks move

Citations/Special Thanks

- Image Sources
  - Chang, Cimprich (2009) *Nat. Chem. Bio* 2, 82-90
  - McCulloch & Kunkel (2008) Cell Research 18, 148-161
  - Sale, Lehmann, Woodgate (2012) *Nat. Rev. MCB* 13, 141-152
  - Lehmann et al (2007) *DNA Repair* 6, 891-899
  - Guo et al (2009) *Cell. Mol. Life. Sci.* 66, 2363-238
- Thank you to Dmitry, Irena, Yulia, Sasha, Luciana and Brandon for the wonderful experience of working with you in the lab.
- Have a great rest of the summer!