Functional dissection of RNA polymerase active sites by deep mutational scanning

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How does each TL residue work to promote transcription?

The conserved trigger loop promotes fast and accurate transcription by its flexible nature

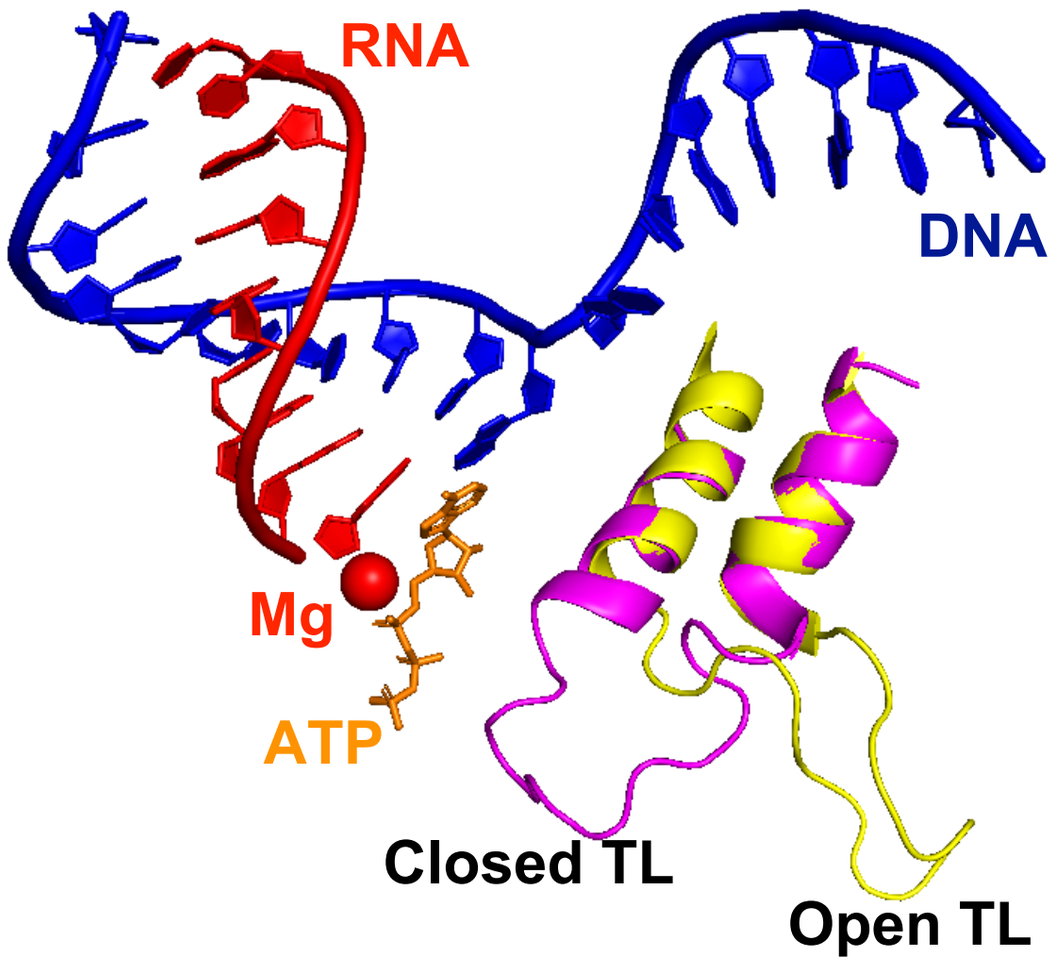

Figure 1. Structures of open (PDB:5C4X) and closed TLs (unpublished structure from Calero lab) are shown in the context of RNA synthesis scaffold.

Deep mutational scanning and phenotypic analysis by high throughput sequencing

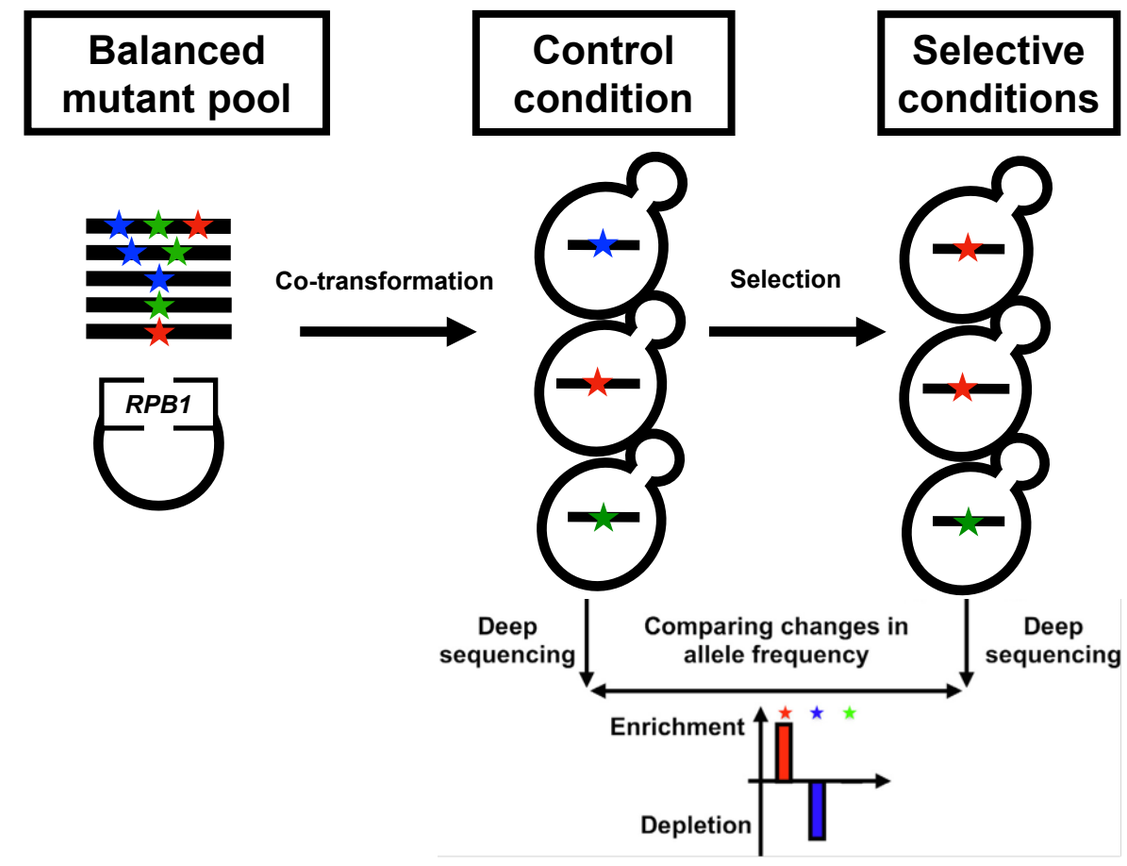

Figure 2. To phenotype 12000 mutants in parallel, a balanced variant pool is amplified and co-transformed into yeast. Fitness selection assays are performed on the yeast *RPB1* variant pools. The variant distribution shifts due to differing variant growth rates under particular conditions are captured by deep sequencing and represent the corresponding phenotypes.

Collaborate Pol II TL residues are indicated by phenotype landscapes

The Pol II TL single mutant growth fitness landscape

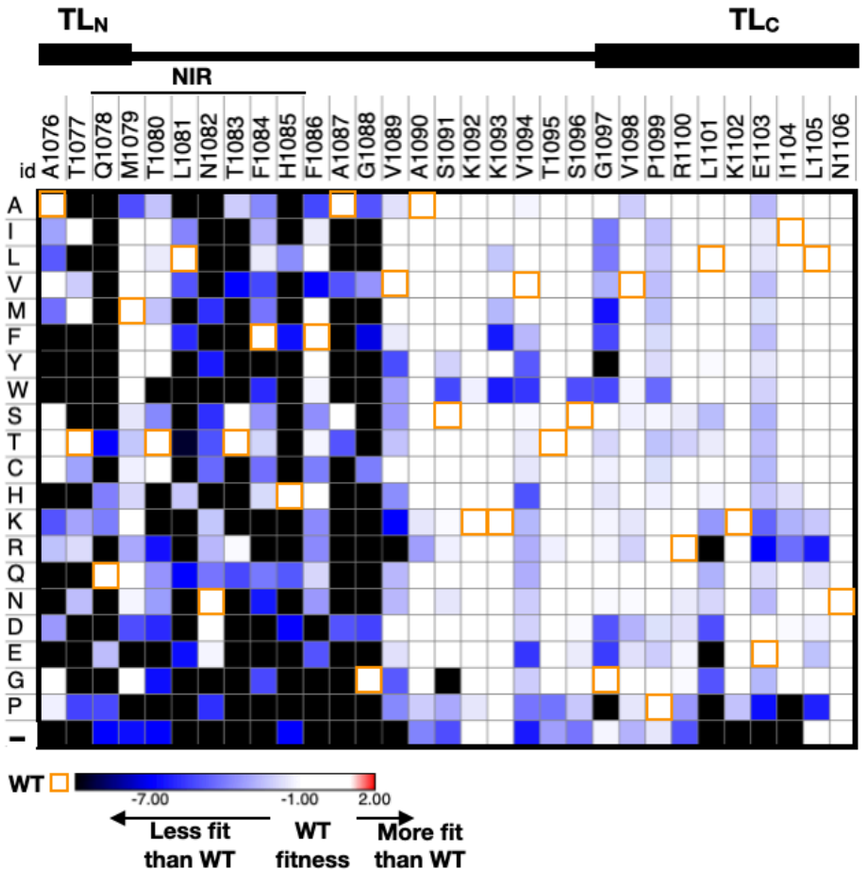

Figure 3. Upper panel: The TL comprises alpha-helical regions(TL_N and TL_C), a loop tip and some regions that transition between coil and alpha helix. **Lower panel:** The Pol II TL fitness (growth) landscape reveals higher mutational sensitivity in TL_N terminal including the nucleotide interacting region (NIR) than TL_C terminal.

The Pol II TL mutant genetic interaction landscape

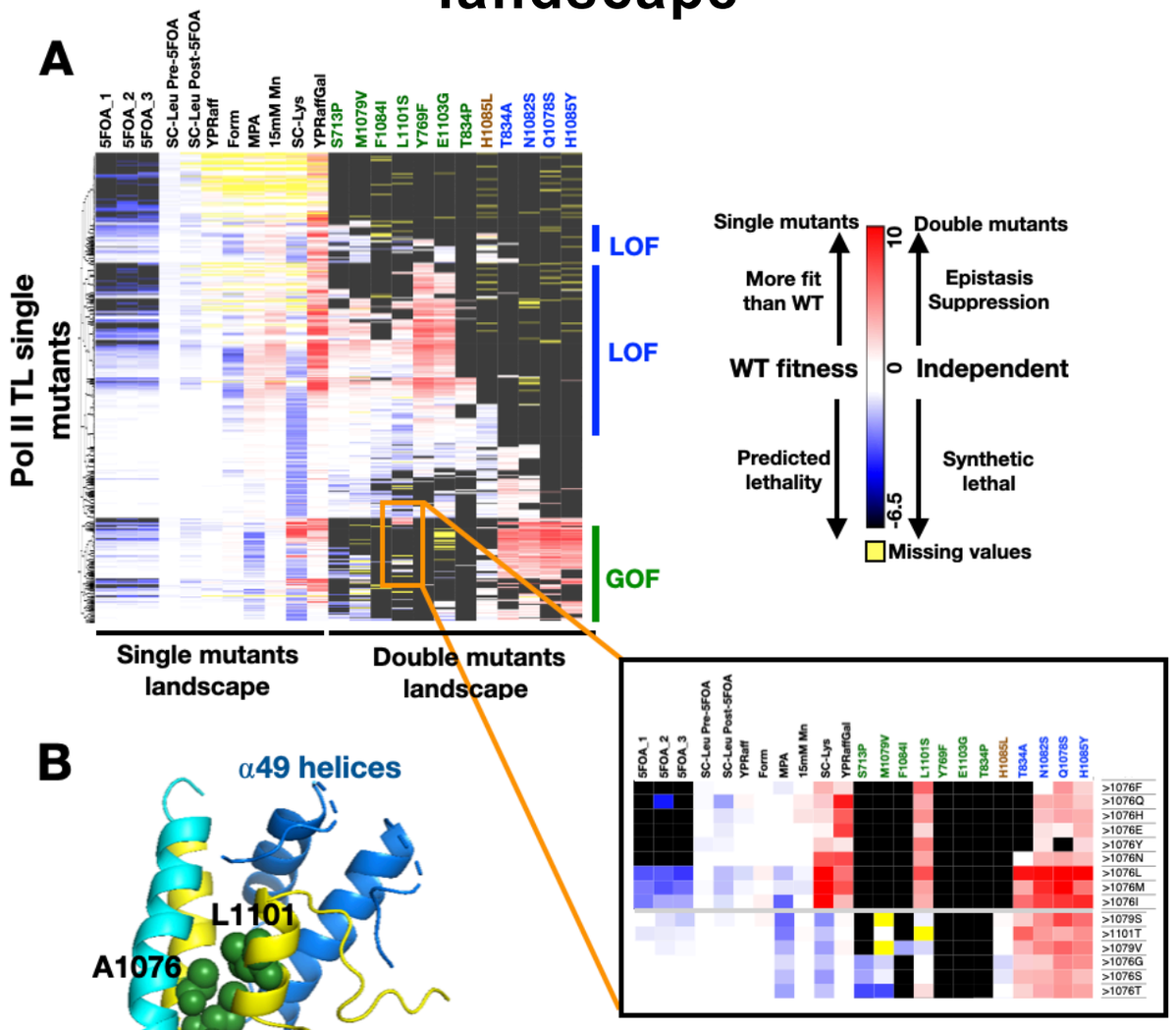

Figure 4. A. Genetic interaction analysis discloses residues packing TL helices collaboratively to stabilize the open, catalytic-disfavoring state of TL. **B.** The Structure of the collaborate residues.

Collaborate Pol II TL residues are indicated by phenotype landscapes

Enzyme-specific epistasis within Pol II limits the compatibility of TL mutations

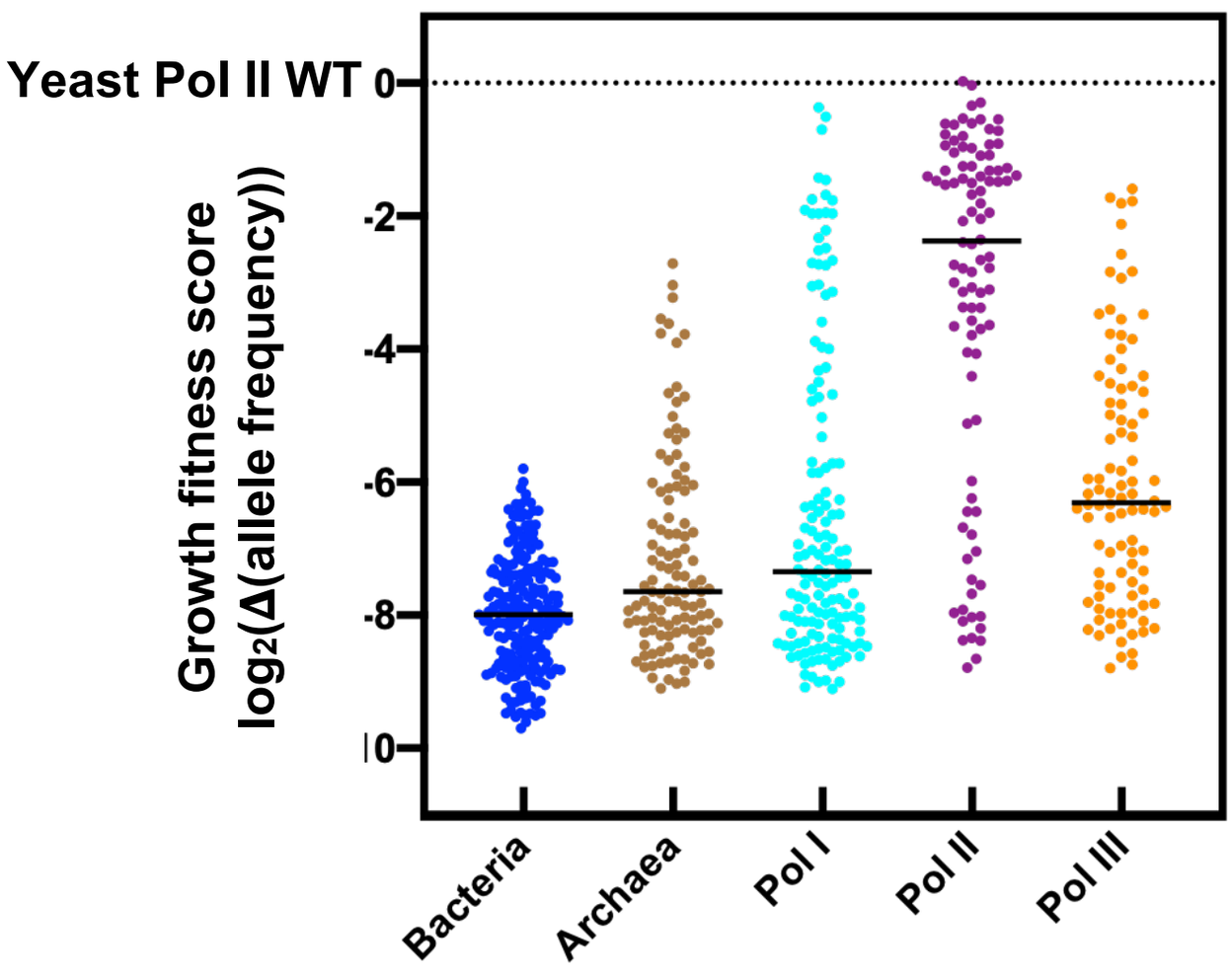

Figure 5. The TLs of Bacteria, Archaea, and Eukaryotic species have incremental compatibility in yeast Pol II background. The incompatible TL alleles indicate particular genetic interaction between TL residues and yeast Pol II enzymatic background.

Ongoing experiments

- Comparison of yeast Pol I, Pol II, and Pol III mutants phenotypic landscapes.
- Exploration of co-evolved Pol II TL residues.
- Investigation of genetic interaction between the Pol II TL and BH.

Collaborators

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References

- **Qiu, C. and C.D. Kaplan,** Functional assays for transcription mechanisms in high-throughput. Methods, 2019. 159-160: p. 115-123.