Investigating the Physiological Effects of Mutations in the Proposed Backtrack Site of Yeast RNA Polymerase II

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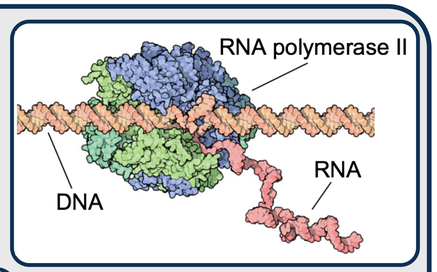
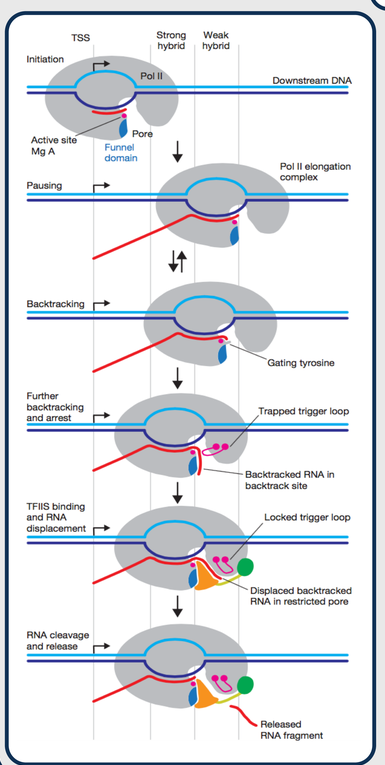
ABSTRACT

Transcription, the first step of gene expression, is a process fundamental to all known forms of life. In eukaryotic cells, the enzyme RNA polymerase II (Pol II) executes transcription by moving forward along the DNA and transferring the encoded genetic information to messenger RNA. However, Pol II also backtracks on the DNA, causing transcription to become arrested. When backtracking occurs, Pol II slides backwards on the DNA, displacing nascent RNA from the active site into a proposed "backtrack site," comprising residues in the Pol II subunits Rpb1 and Rpb2 that interact with the RNA. The resulting stable "arrested complex" must be reactivated for elongation to continue. While backtracking has been implicated in numerous processes essential for regulating gene transcription, its physiological relevance is not yet certain. Using *Saccharomyces cerevisiae* (Baker's yeast), we have engineered individual and combinations of mutations in the Rpb1 region of the backtrack site to disrupt the protein-RNA interactions that arise from backtracking. Through phenotypic and growth comparisons between wild-type and mutant strains, we examine how impairing the binding of RNA to the backtrack site affects yeast fitness and various Pol II functions *in vivo*, providing further insight into the possible functions of Pol II during backtracking and arrest.

BACKGROUND

Pol II is a 12-subunit enzyme essential for cell survival and proliferation, as it executes the first step of gene expression by synthesizing messenger RNA (the precursor for proteins) from DNA.





During transcription, Pol II moves forward along the DNA and adds nucleotides to the growing end of the RNA. However, Pol II can also backtrack on the DNA. Backtracking by one to two nucleotides typically occurs due to nucleotide misincorporation, causing transcription to pause[1]. Pol II can intrinsically cleave misincorporated nucleotides to resume elongation and ensure transcription fidelity. Backtracking beyond two nucleotides is averted by favorable RNA base-stacking interactions and by a "gating tyrosine" residue[2]. However, extensive backtracking may occur, causing nascent RNA to bypass the gating tyrosine and intrude further into the backtrack site where it ultimately binds. Such binding leads to transcription arrest. For transcription to resume, the transcription factor TFIIS must cleave backtracked RNA.

QUESTIONS

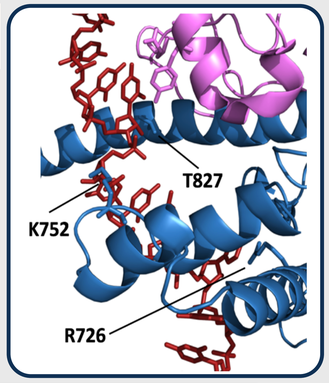
- **What is the importance of the Pol II backtrack site?**
- **Can Pol II transcribe DNA efficiently if backtracking and arrest are impaired?**
- **How do backtrack site mutations affect cell fitness and gene transcription?**

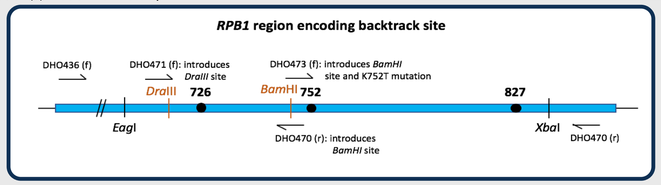
EXPERIMENTAL APPROACH

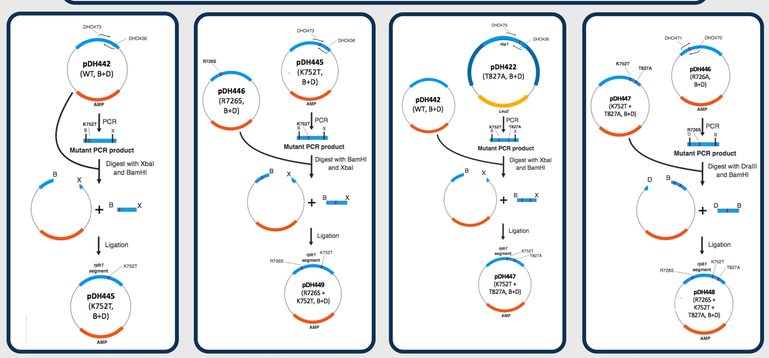
***rpb1* mutagenesis**: Residues in the Rpb1 region of the backtrack site were mutated to disrupt the protein-RNA interactions that form during backtracking and arrest (shown in crystal structure of arrested Pol II[2]).

Mutation	Method of Mutagenesis
R726S	Random, obtained in screen
K752T*	Site-directed
T827A	Site-directed
R726S + K752T*	Site-directed
K752T + T827A*	Site-directed
R726S + K752T + T827A*	Site-directed

*Mutation(s) made in this study.







K752T **R726S+K752T** **K752T+T827A** **R726S+K752T+T827A**

After mutagenesis, homologous recombination was used to introduced the mutated *rpb1* sequence into a larger plasmid containing the entire *RPB1* gene. Strains were cured of a second plasmid containing the wild-type *RPB1* gene by treatment on media containing 5-fluorotic acid (5-FOA). Interestingly, our double and triple mutants did now grow on 5-FOA (data not shown). After mutant strains were generated, they were characterized using *in vivo* assays that target cell fitness and various Pol II functions.

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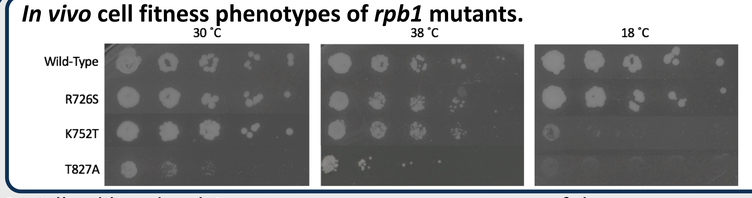
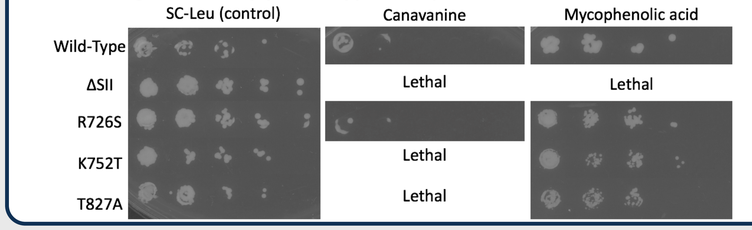
RESULTS

***In vivo* cell fitness phenotypes of *rpb1* mutants.**



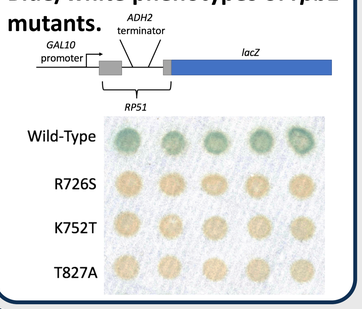
Serially diluted *rpb1* mutants were grown at stressful temperatures to observe their relative fitness. At normal and high temperatures (30˚C and 38˚C), R726S and K752T mutants grew similar to wild-type (WT), while T827A grew less well. R726S grew similar to WT at cold temperatures, whereas K752T and T827A grew much worse than WT.

***In vivo* drug sensitivity phenotypes of *rpb1* mutants.**



Serially diluted *rpb1* mutants were grown with either canavanine (which targets transcription fidelity)[3] or mycophenolic acid (MPA) (which targets elongation speed and start site recognition)[4]. R726S was the only mutant to grow on canavanine, although it grew less well than WT, suggesting that transcription fidelity is severely compromised in these mutants. Mutants did not exhibit sensitivity to MPA, suggesting that elongation speed is not affected by these mutations. ΔSII is a control strain lacking factor TFIIS.

Blue/white phenotypes of *rpb1* mutants.



rpb1 strains containing plasmids with the *lacZ* reporter gene were screened for defects in transcription termination. Blue colonies indicate defects in termination, while white colonies indicate improved termination efficiency. WT colonies appear green. All *rpb1* mutants appeared white, suggesting the Pol II variants terminate more efficiently, possibly due to slower elongation.

CONCLUSIONS & FUTURE DIRECTIONS

Conclusion:

- Rpb1 residues in the backtrack site of Pol II are essential for cell viability and play an important role in ensuring transcription fidelity
- Disrupting protein-RNA interactions that arise from backtracking affects various Pol II functions, including its ability to proofread RNA, recognize start sites, and terminate transcription

Future Directions

- Assess transcriptional error rates of Pol II mutants *in vitro*
- Assess the elongation rates of Pol II mutants *in vitro*

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