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Isolation and Characterization of Transcription Fidelity Mutants

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Abstract

Accurate transcription is an essential step in maintaining genetic information. Error-prone transcription has been proposed to contribute to cancer, aging, adaptive mutagenesis, and mutagenic evolution of retroviruses and retrotransposons. The mechanisms controlling transcription fidelity and the biological consequences of transcription errors are poorly understood. Because of the transient nature of mRNAs and the lack of reliable experimental systems, the identification and characterization of defects that increase transcription errors has been particularly challenging. In this review we describe novel genetic screens for the isolation of fidelity mutants in both *S. cerevisiae* and *E. coli* RNA polymerases. We obtained and characterized two distinct classes of mutants altering NTP misincorporation and transcription slippage both *in vivo* and *in vitro*. Our study not only validates the genetic schemes for the isolation of RNA polymerase mutants that alter fidelity, but also sheds light on the mechanism of transcription accuracy.

For over 50 years it has been apparent that, while genetic information in most organisms is stored in DNA, access to that information involves the synthesis of RNA. Jacob and Monod's postulation of a messenger RNA, combined with the demonstrated roles of transfer RNAs and ribosomal RNAs in translation provided key early steps in the recognition of the roles of RNAs as essential in biological information flow [1] (see also Watson's Nobel lecture). More recent demonstrations of regulatory roles of small RNAs and the importance of RNA structural motifs in the fates of mRNAs serve to further emphasize the expectation that accurate synthesis of RNAs is necessary for the preservation of biological information. The search for mutations that reduce the fidelity of transcription began soon after the discovery of mRNA. However, relatively little progress has been made determining the features of RNA polymerases that control the fidelity of transcription. The unstable nature of mRNAs has made it difficult to develop genetic screens for mutants that alter transcription fidelity. Errors made in one message that result in a desired phenotype are rare and transient. However, recent analysis of the human transcriptome reveals that transcription error rates could be substantially higher than previously recognized [2]. An additional challenge for isolating transcription fidelity mutants is to identify phenotypes that are likely to result from transcription errors rather than from errors of protein translation as error rates in that process

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are probably higher than for transcription. In most selections or screens, the desired phenotype is dependent on the continued production of messages with the same mistake. If the mistake has to happen at a particular base in the mRNA leading to a desired phenotype, that will be very rare in wild type cells; perhaps only 1 in 100,000 transcripts would have the desired mistake. For an error-prone RNA polymerase to make mistakes at a unique position of a reporter gene so as to restore function to even 1% of the level of the "corrected" reporter gene would require a 1,000-fold increase in the error rate. Such an elevated error rate would potentially result in unwanted mistakes in most other transcripts as well. Hence, genetic approaches to identifying error-prone RNA polymerases require very sensitive reporters and/or genes with sequence motifs that promote high error rates to facilitate detection of transcription mistakes. Here we describe a collaboration among the Court, Jin, Kashlev, and Strathern laboratories to develop genetic screens for transcription fidelity mutants in RNA polymerase and biochemical assays that demonstrate their error-prone RNA synthesis. These approaches address two different kinds of transcription errors, those that reflect loss of register (slippage) of the RNA polymerase, and errors that reflect the incorporation of incorrect bases.

Transcriptional Slippage.

We developed assays for fidelity of transcription that derive from observations made by Wagner et al 1990 [3] They showed that a *lacZ* gene with an out-of-frame insertion of runs of 11 A's (11A) or 11T gave levels of LacZ activity consistent with high levels of slippage (25–30% of the in-frame construct). They showed directly, by sizing the RNA, that *E. coli* RNA polymerase (RNAP) undergoes high levels of slippage on DNA template containing homopolymeric runs of adenosine (A) or thymidine (T), In contrast, they saw no evidence of slippage with a similar construct in yeast.

Because homopolymeric runs of A or T are sequences at which there is an elevated risk for transcriptional errors, we built sensitized reporters for the identification of error-prone RNA polymerases based on the inclusion of homopolymeric runs. Dr. Strathern's laboratory made *lacZ, HIS3* and *TRP1*-based reporters in *S. cerevisiae* that incorporated runs of A or T (Fig. 1A). Due to concerns about the reported lower fidelity of RNA polymerase in the initial phases of transcription [4], we designed the reporters so that spacer sequences containing the slippery sites (homopolymeric runs) were well downstream of the promoter and inserted between the open reading frame (ORF) for the maltose binding protein (*malE*) and the reporter ORFs. For example, we compared the expression of a *malE-lacZ* fusion gene that has a spacer of 10 adenosines, named 10A(0) (0 in parentheses designates that the spacer sequence is in-frame relative to the *lacZ* ORF), with a reporter that has an extra base in the spacer, named 11A(+1) (+1 in parentheses indicates a one-base addition leading to out-of-frame relative to the *lacZ* ORF as defined by Wagner et al [3]). With the 11A(+1) reporter, a slippage error deleting one adenosine is required to become in-frame with the *lacZ* ORF. The 11A(+1) reporter produces about 1% of the LacZ activity seen for the in-frame reporter. We used these reporters to screen for Pol II mutants that make yeast sensitive to 6-azauracil. This drug is commonly used for isolation of RNA polymerase mutants defective in transcription elongation, This approach identified *rpb1-N488D* as an allele with a seven-fold elevated expression of LacZ activity from the 11A(+1) reporter compared to wild type RNA

polymerase. A genetic screen for increased growth on media lacking histidine with an 11A(+1) *malE-HIS3* reporter identified the *rpb1-M487V* mutation that also increases expression of the 11A(+1) *malE-lacZ* reporter four-fold. The genetic screen with a 10A(+1) *malE-TRP1* reporter identified several *rpb2* mutations near the DNA side of the RNA/DNA hybrid that elevate slippage during transcription (Fig. 2). Dr. Kashlev's laboratory demonstrated that RNA polymerase from strains carrying the *rpb1-N488D*, *rpb1-M487V*, or *rpb2-R1122S* alleles show elevated transcriptional slippage frequency on templates with homopolymeric runs.

Dr. Jin's laboratory developed similar reporters for transcriptional slippage in *E. coli* based on chromosomal fusions of a portion of *lacI* with the *lacZ* gene. Fine tuning this screen required balancing promoter strength and the length of the homopolymeric run. One of these optimized reporters uses a weak version of the *tac* promoter and a 9A(−1) spacer between the *lacI* and *lacZ* ORFs (Fig. 1B). In wild type strains, this reporter results in a white color (Lac^-) phenotype on MacLac indicator plates. A screen of a library of mutagenized *rpoB* variants for colonies that had a red color (Lac^+) phenotype identified *rpoB* mutants that cluster in the elongation complex along the RNA strand of the 9-bp RNA-DNA hybrid region (Fig. 2). Some of these *E. coli* RNAP variants are near the region of *rpoB* that can mutate to resistance to rifampicin (Rif^R). A genetic screen for transcription fidelity defects identified some rif^R *rpoB* mutants as candidates for reduced fidelity [5,6]. A survey of extensive Rif^R *rpoB* alleles [7,8] identified only a subset of these *rpoB* mutations including Q513P, D516N, S522Y, P564L and I572N, which either increase or decrease slippage. These results indicate that these amino acids near the rifampicin-binding site of RNAP are important for controlling slippage (Fig. 2). Quantitation of the *lacZ* expression found changes of up to three-fold are due to slippage by some RNAP mutants relative to wild type. Biochemical analyses of the *E. coli* RNAP mutants by Dr. Kashlev's laboratory demonstrated that mutants with increased slippage phenotype exhibit higher frequency of insertion, as expected for a selection based on a reporter that is missing a base, 9A(−1). Mutants with reduced slippage phenotype lower the frequency of insertion (data not shown). Biochemical characterization not only validates the genetic schemes clustering of the amino acid residues involved in control of slippage at the interface with for the isolation of RNAP mutants that altered transcription slippage, but also sheds light on the mechanism underlying the process. The high the RNA-DNA hybrid strongly support the idea that the loss of transcription register involves transient separation of the RNA and DNA strand in the elongation complex.

The scheme in Fig. 2 shows mapping of the amino acid residues involved in control of slippage in the X-ray structure of the ternary elongation complex (TEC) by the yeast Pol II and bacterial RNAP. It is noteworthy that the positions of the *rpoB* mutations in *E. coli*, which cause slippage are different from those identified in the corresponding yeast polymerase subunit, *RPB2*. It remains to be determined whether this difference relative to the location of the RNA-DNA hybrid in the corresponding TEC reflects a difference between *S. cerevisiae* and *E. coli* in the slippage mechanism, or the difference in isolating/screening for slippage events that result in shorter versus longer RNAs.

In vitro analysis of transcriptional slippage.

Kashlev's laboratory designed an *in vitro* assay to capture the transient slippage events within the A-tract and to address the slippage directionality. They employed the promoter-independent assembly of a TEC by *Ec*RNAP and yeast Pol II [9] at 1-bp distance upstream of the A-tract (Fig. 3A). The TEC was chased with unlabeled ATP and [$\alpha-^{32}$P] CTP. This strategy resulted in the unimpeded transcription of the entire 11A-tract followed by the incorporation of the labeled CMP and halting of TEC due to the lack of the next NTP substrate (GTP). The transcribed sequence contained two cleavage sites for Ribonuclease A (RNase A) at each end of the 11A-tract lacking pyrimidines (RNase A cleaves at the 3' end of C and U residues). The upstream cleavage site was located in the RNA primer used for TEC assembly, and the downstream site was generated by the incorporation of the labeled CMP beyond the end of the 11A-tract. Incorporation of the label enabled identification of the slippage products in the gel after cleavage of the purified RNA with easy RNase A (Fig. 3A, the bottom panel). This assay revealed that Pol II isolated from the yeast *rpb1-N488D* slippage mutant showed increased production of transcripts differing in length from the encoded transcript. It is noteworthy that on an 11A-tract the wild type Pol II shows a bias toward making longer transcripts and wild type *E. coli* RNAP shows a bias toward producing shorter transcripts (Fig. 3B). This assay also demonstrates that the *rpb1-N488D* mutation increases slippage in both directions (Fig. 3A, insertions or deletions) while several other *rpb1* mutations alter the bias toward one direction (data not shown). The similar results were obtained for the slippage mutants of *E. coli* RNAP (data not shown).

Making transcription errors cause permanent consequences.

As mentioned above, one of the major challenges in developing screens for transcription fidelity mutants is the transient nature of each error. Screens based on suppression of nonsense alleles have been problematic and controversial [6,10,11,12]. Because phenotypic changes based on rare and short-lived transcription errors are difficult to detect, we previously developed a retrotransposition fidelity assay in which errors of transcription become fixed as permanent changes of genotype and phenotype [13]. We are developing other assays based on a related principle: transcription errors that lead to a permanent genetic change but without a requirement for reverse transcriptase. These novel assays are based on suppression of frameshift and missense alleles of the Cre recombinase coupled with a selectable Cre-dependent reporter gene.

In one of the schemes, suppression of *cre* frameshift alleles is used to identify transcriptional slippage mutants (Fig. 4). Screening for this class of mutants has the advantage that the out-of-frame transcripts do not produce inactive Cre recombinase to inhibit the assay. We made Cre slippage reporters based on fusion to the maltose binding protein similar to those described above. While an in-frame fusion of *malE* and *cre* is active, the out-of-frame *malE-cre* fusion with an 8A(+1) tract shows greatly reduced activity. To monitor the Cre activity, we created another reporter based on the *ADE6* gene with a functional artificial intron that contains a single recognition site (lox) for Cre recombinase. The intron is efficiently removed from the *ADE6-AIlox* transcript so that the cells are Ade$^+$. A second version of that gene, *ade6-AIlox::neo^R*, was also made with the *neo^R* gene flanked by two lox sites inserted

into the artificial intron causing the cells to be Ade^{-}. Cre recombinase efficiently excises the *neo^{R}* gene generating the functional *ADE6-AIlox* allele. In yeast that carry an *ade2* mutation, the Cre-mediated switch from *ade6-AIlox::neo^{R}* to *ADE6-AIlox* results in a colony color change from white to red. Colonies that start with the *ade6-AIlox::neo^{R}* allele and an *rpb1* mutation that causes increased slippage show an increase in the number of red sectors.

Our efforts to identify Pol II variants with increased misincorporation are focused on the use of missense mutations causing a substitution at the active site tyrosine of Cre recombinase [14]. These mutant Cre recombinases are catalytically inactive. For this assay we created a *his3*-based reporter that requires a Cre-mediated inversion to generate a functional *HIS3* allele. Mutations in the active site tyrosine reduce the production of His^{+} cells to the level seen in the absence of Cre. Cre was chosen for the important reason that it is a tetramer and its activity is dependent on four active sites. Thus occasional translation errors cannot be expected to generate a tetramer four functional subunits. In contrast, a rare transcription error that restored the tyrosine codon could allow the production of multiple active subunits. It was necessary to develop conditions where the level of transcription is on the order of one transcript per cell so that the proteins derived from rare functional transcripts are not in competition with inactive proteins. We used the promoter of the *HO* gene to drive the *cre* gene because *HO* is not expressed in the first cell cycle of yeast daughter cells, and is restricted to a short portion of the cell cycle in mother cells. We have used this screen to identify *rpb1* mutations that elevate the frequency of Cre-mediated switches to His^{+}. Several map to the trigger loop region of Rpb1 and include the previously identified fidelity mutant *rpb1-E1103G* [13].

In vitro analysis of transcription fidelity.

Kashlev's laboratory developed several assays to monitor the ratio of incorporation of the correctly templated base versus misincorporation events [13]. We previously demonstrated that the *rpb1-E1103G* mutation and deletion of the nonessential Rpb9 subunit result in Pol II enzymes with an elevated misincorporation rate (Fig. 5 and [13,15]). The *rpb1-E1103G* allele came from a collection of mutations obtained by random mutagenesis of *RPB1* and selected for sensitivity to the nucleotide-depleting drug 6-azauracil (6AU). The *rpb1-E1103G* mutation was distinguished from other 6AU-sensitive *rpb1* mutations by causing synthetic lethality with the deletion of the *DST1* gene coding for the putative transcription error-correction factor TFIIS [16]. The synthetic phenotype with deletion of *DST1* gene suggested that this mutation might decrease transcription fidelity because of the demonstrated role of TFIIS in error correction *in vitro* [17]. We isolated and characterized the mutant Pol II *in vitro*. For the quantitative tests of fidelity, we followed the experimental approaches previously established for pre-steady-state analyses of DNA polymerases. Briefly, fidelity of NTP selection by Pol II was determined by the relative rates of correct and incorrect substrate incorporation at a given DNA position. The greater the difference between the two rates indicates the higher fidelity. The substrate incorporation rate at a given substrate concentration was determined by the *apparent* maximum incorporation rate k_{cat} and an *apparent* specificity constant, k_{cat}/K_m. The k_{cat}/K_m determined for a correct NTP substrate and for the incorrect substrates at a given DNA position allows quantification of

the fidelity of the polymerase, $(k_{cat}/K_m)^{correct}$ / $(k_{cat}/K_m)^{incorrect}$. By measuring single NTP incorporation rates for a range of concentrations of the matched and mismatched substrates, we established that *rpb1-E1103G* mutation reduced Pol II ability to discriminate against transition- and transversion-type mismatches two- to ten-fold [13].

Based on the available crystal structures, Rpb1 Glu1103 residue is located in the flexible trigger loop domain of the Pol II active site (Fig. 5). This element folds on the NTP substrate bound to Pol II, prevents its dissociation from the active site and participates in phosphodiester bond formation [13]. We proposed that interaction of Glu1103 with another Rpb1 Thr1095 residue stabilizes the opened conformation of the trigger loop thus allowing the incorrect NTP to dissociate from the active site before the enzyme proceeds to bond formation, which creates a substrate selection checkpoint (Fig. 5). Importantly, an Rpb1 T1095A substitution also decreased fidelity of Pol II *in vitro*, as did E1103G substitution [18]. Glu1103/Thr1095 interaction appears to be one of several contacts supporting the open conformation of the trigger loop. Indeed, analyses of catalytic properties of Pol II lacking a non-essential subunit Rpb9 revealed that it has a fidelity defect, which was similar, though somewhat milder than those observed for the *rpb1-E1103G* mutant. Accordingly, Pol IIΔ9 was characterized by decreased fidelity *in vivo* [11] and *in vitro* [15]. Both phenotypes are consistent with the previously published model that interaction of Rpb9 with the trigger loop may stabilize its open state [15] (Fig. 5). These observations supported the proposed role of the trigger loop in the interactions with the incoming NTP substrate based on the crystal structure analyses of the elongation complexes of Pol II [19]. Our recent analysis showed that Glu1103 residue might have another role in the fidelity control distinct from the trigger loop mobilization (data not shown). The Rpb9 subunit forms a part of the DNA-binding clamp in Pol II. The better DNA clamping may additionally improve fidelity by restricting thermal motion of the transcribed template. In summary, our *in vitro* results validated the regulatory interactions involved in control of Pol II fidelity identified by our genetic screens.

Consequences of transcription errors.

One might expect mutations that reduce the fidelity of transcription to have widespread consequences. Having identified several mutants that satisfy the genetic and biochemical criteria for elevated transcription error rates, the Court, Jin and Strathern laboratories are focused on determining what additional phenotypes are caused by these mutations. For example, *rpoB-P564L*, which shows elevated slippage in our assays, was identified by other groups as a potential transcription fidelity mutant (*rpoB-ACK*) [5,6]. That allele has also been shown to reduce the stability of F' maintenance, block the growth of bacteriophage T4, and restrict the growth of bacteriophage lambda at 32°C [20]. Because the *rpoB-P564L* mutant supports the growth of λ*nin5* phage, a derivative of lambda, which bypasses the need for N-mediated antitermination, at the restrictive temperature, it suggests the RpoB mutant is defective in N-mediated antitermination [21]. In addition, *rpoB-P564L* has been identified as a mutation that gives a growth advantage to cells in aging colonies [22] and prevents replication arrest in the absence of DksA, a transcription factor important for resolving conflicts between DNA replication and transcription machinery [23]. In the yeast system, the *rpb1-E1103G* allele causes a dependence on TFIIS, the product of the *DST1* gene. Similarly, mutations that block TFIIS binding or activity, such as *rpb1-E1230K*, cannot be combined

with the *rpb1-E1103G*. It remains to be determined whether these cells die as a result of error catastrophe in which the cells become overwhelmed with defective RNAs and proteins, or simply that transcription is blocked by the stalled polymerases due to an abundance of errors without the ability to use TFIIS for restarting transcription. A mutation in *rpoC* of *E. coli* at the position D1143 that corresponds to yeast *rpb1-E1103* also misincorporates nucleotides during transcription and has an enhanced defect in combination with *greA* and *greB* mutations. Strains carrying this *rpoC* mutation show increased frequency of reversion of an IS2 insertion mutation in the leader of the *gal* operon. Cells carrying *rpoC-D1143*G and deletions of *greA* and *greB* have even greater effects on genetic instability. This observation raises the possibility that reduced fidelity of transcription could lead to increased levels of mutagenesis and genetic instability. Together, the pleiotropic effects of these transcription fidelity mutants illustrate the importance of maintaining accuracy in RNA synthesis.

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Abbreviations:

RNAP	*E. coli* RNA polymerase
Pol II	yeast RNA polymerase

REFERENCES

[1]. Jacob F, Monod J, Genetic regulatory mechanisms in the synthesis of proteins, J Mol Biol 3 (1961) 318–356. [PubMed: 13718526]

[2]. Li M, Wang IX, Li Y, Bruzel A, Richards AL, Toung JM, Cheung VG, Widespread RNA and DNA sequence differences in the human transcriptome, Science 333 (2011) 53–58. [PubMed: 21596952]

[3]. Wagner LA, Weiss RB, Driscoll R, Dunn DS, Gesteland RF, Transcriptional slippage occurs during elongation at runs of adenine or thymine in Escherichia coli, Nucleic Acids Res 18 (1990) 3529–3535. [PubMed: 2194164]

[4]. Pal M, Luse DS, Strong natural pausing by RNA polymerase II within 10 bases of transcription start may result in repeated slippage and reextension of the nascent RNA, Mol Cell Biol 22 (2002) 30–40. [PubMed: 11739720]

[5]. Libby RT, Gallant JA, The role of RNA polymerase in transcriptional fidelity, Mol Microbiol 5 (1991) 999–1004. [PubMed: 1659650]

[6]. Blank A, Gallant JA, Burgess RR, Loeb LA, An RNA polymerase mutant with reduced accuracy of chain elongation, Biochemistry 25 (1986) 5920–5928. [PubMed: 3098280]

[7]. Jin DJ, Gross CA, Mapping and sequencing of mutations in the Escherichia coli rpoB gene that lead to rifampicin resistance, J Mol Biol 202 (1988) 45–58. [PubMed: 3050121]

[8]. Garibyan L, Huang T, Kim M, Wolff E, Nguyen A, Nguyen T, Diep A, Hu K, Iverson A, Yang H, Miller JH, Use of the rpoB gene to determine the specificity of base substitution mutations on the Escherichia coli chromosome, DNA Repair (Amst) 2 (2003) 593–608. [PubMed: 12713816]

[9]. Komissarova N, Kireeva ML, Becker J, Sidorenkov I, Kashlev M, Engineering of elongation complexes of bacterial and yeast RNA polymerases, Methods Enzymol 371 (2003) 233–251. [PubMed: 14712704]

[10]. Shaw RJ, Bonawitz ND, Reines D, Use of an in vivo reporter assay to test for transcriptional and translational fidelity in yeast, J Biol Chem 277 (2002) 24420–24426. [PubMed: 12006589]

[11]. Nesser NK, Peterson DO, Hawley DK, RNA polymerase II subunit Rpb9 is important for transcriptional fidelity in vivo, Proc Natl Acad Sci U S A 103 (2006) 3268–3273. [PubMed: 16492753]

[12]. Jeon C, Agarwal K, Fidelity of RNA polymerase II transcription controlled by elongation factor TFIIS, Proc Natl Acad Sci U S A 93 (1996) 13677–13682. [PubMed: 8942993]

[13]. Kireeva ML, Nedialkov YA, Cremona GH, Purtov YA, Lubkowska L, Malagon F, Burton ZF, Strathern JN, Kashlev M, Transient reversal of RNA polymerase II active site closing controls fidelity of transcription elongation, Mol Cell 30 (2008) 557–566. [PubMed: 18538654]

[14]. Guo F, Gopaul DN, van Duyne GD, Structure of Cre recombinase complexed with DNA in a site-specific recombination synapse, Nature 389 (1997) 40–46. [PubMed: 9288963]

[15]. Walmacq C, Kireeva ML, Irvin J, Nedialkov Y, Lubkowska L, Malagon F, Strathern JN, Kashlev M, Rpb9 subunit controls transcription fidelity by delaying NTP sequestration in RNA polymerase II, J Biol Chem 284 (2009) 19601–19612. [PubMed: 19439405]

[16]. Malagon F, Tong AH, Shafer BK, Strathern JN, Genetic interactions of DST1 in Saccharomyces cerevisiae suggest a role of TFIIS in the initiation-elongation transition, Genetics 166 (2004) 1215–1227. [PubMed: 15082542]

[17]. Thomas MJ, Platas AA, Hawley DK, Transcriptional fidelity and proofreading by RNA polymerase II, Cell 93 (1998) 627–637. [PubMed: 9604937]

[18]. Walmacq C, Cheung A, Kireeva M, Lubkowska L, Ye C, Gotte D, Strathern JN, Carell T, Cramer P, Kashlev M, Mechanism of Translesion Transcription by RNA Polymerase II and Its Role in Cellular Resistance to DNA Damage, Molecular Cell in press (2012).

[19]. Wang D, Bushnell DA, Westover KD, Kaplan CD, Kornberg RD, Structural basis of transcription: role of the trigger loop in substrate specificity and catalysis, Cell 127 (2006) 941–954. [PubMed: 17129781]

[20]. Jin DJ, Gross CA, Characterization of the pleiotropic phenotypes of rifampin-resistant rpoB mutants of Escherichia coli, J Bacteriol 171 (1989) 5229–5231. [PubMed: 2670912]

[21]. Jin DJ, Cashel M, Friedman DI, Nakamura Y, Walter WA, Gross CA, Effects of rifampicin resistant rpoB mutations on antitermination and interaction with nusA in Escherichia coli, J Mol Biol 204 (1988) 247–261. [PubMed: 2464690]

[22]. Wrande M, Roth JR, Hughes D, Accumulation of mutants in “aging” bacterial colonies is due to growth under selection, not stress-induced mutagenesis, Proc Natl Acad Sci U S A 105 (2008) 11863–11868. [PubMed: 18701713]

[23]. Tehranchi AK, Blankschien MD, Zhang Y, Halliday JA, Srivatsan A, Peng J, Herman C, Wang JD, The transcription factor DksA prevents conflicts between DNA replication and transcription machinery, Cell 141 (2010) 595–605. [PubMed: 20478253]

[24]. Vassylyev DG, Vassylyeva MN, Zhang J, Palangat M, Artsimovitch I, Landick R, Structural basis for substrate loading in bacterial RNA polymerase, Nature 448 (2007) 163–168. [PubMed: 17581591]

[25]. Kettenberger H, Armache KJ, Cramer P, Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIS, Mol Cell 16 (2004) 955–965. [PubMed: 15610738]

Highlights

- Accurate RNA synthesis is necessary for preservation of biological information
- Studying transcription fidelity is a challenge due to the transient nature of mRNAs
- Genetic schemes for isolation of fidelity RNA polymerase mutants are developed
- Biochemical assays for detection of transcription errors are designed
- The consequences of transcription errors by the fidelity mutants are discussed

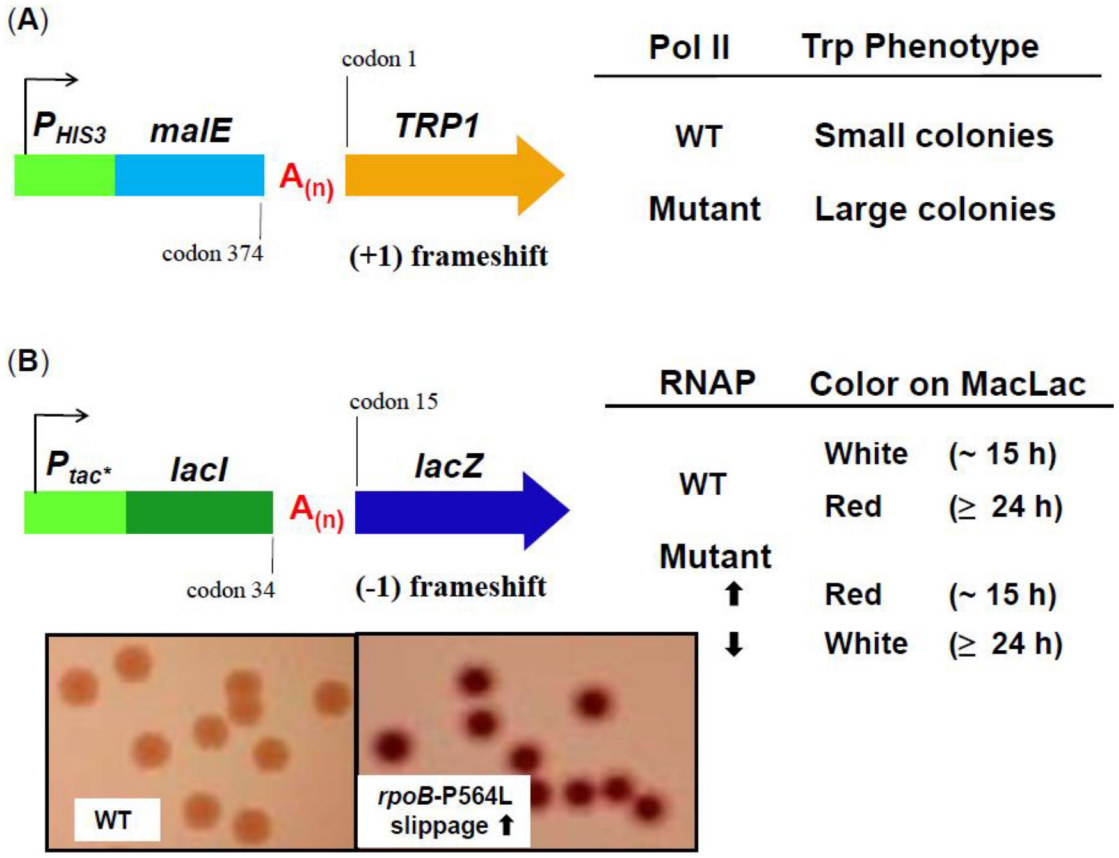

Fig. 1.

Schematic illustration of a set of reporters used for the isolation/screening of RNA polymerase slippage mutants. (**A**) The yeast *malE-trp1* reporter driven by the *HIS3* promoter and with a spacer containing runs of A [$A_{(n)}$] is shown. In a (+1) out-of-frame construct, wild type (WT) formed only small-sized colonies on Synthetic Complete plates lacking tryptophan; however, Pol II slippage mutants appeared as larger-sized colonies. (**B**) The *E. coli* chromosomal *lacI-lacZ* reporter driven by a mutant P*tac* promoter (P*tac**) and with a slippery spacer $A_{(n)}$ is shown. In a 9A(−1) contruct, WT formed white color colonies on MacLac indicator plates after 15 h incubation and red color after more than 24 h. Slippage-enhancing (↑) RNAP mutants were red color after shorter periods of incubation while slippage-reducing (↓) RNAP mutants maintained white color even after a prolonged incubation.

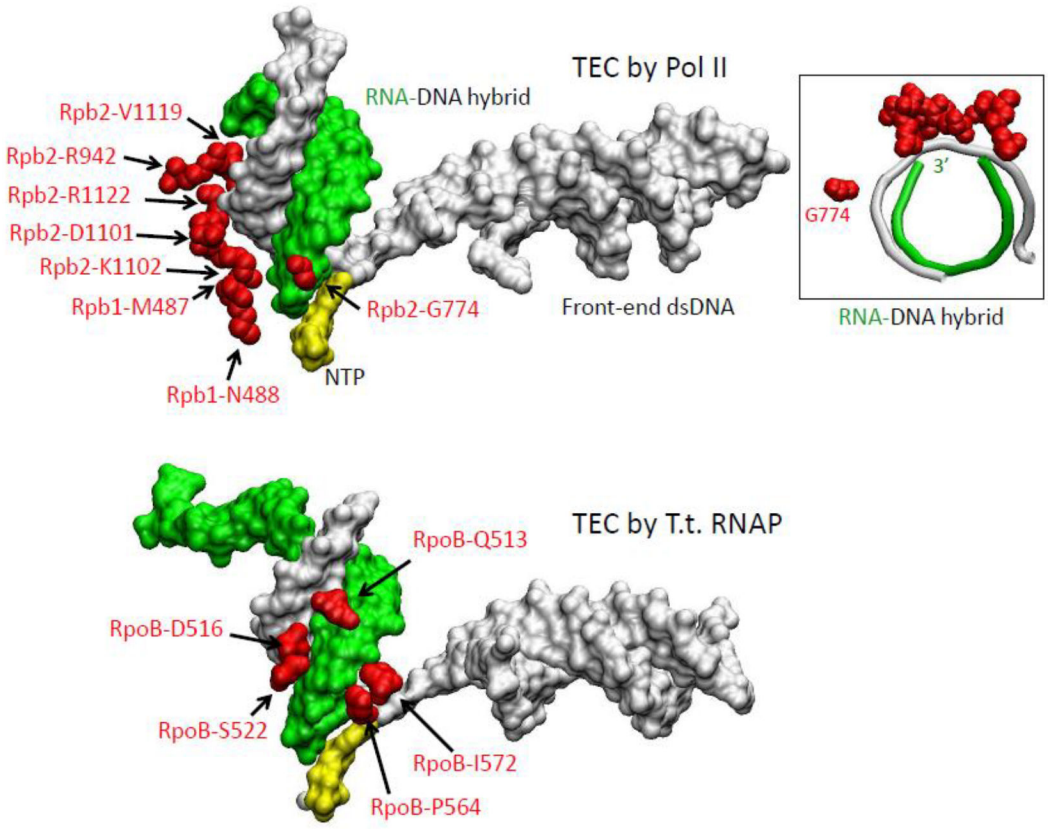

Fig. 2.

Mapping of the amino acid residues involved in control of slippage in the structure of TEC by Pol II and bacterial RNAP. *On the top:* Structure of the RNA and DNA stands in Pol II TEC (from PDB: 2E2H, [19]). Three amino acid residues involved in control of slippage are highlighted in red (in Rpb1 and Rpb2 subunits). Template DNA/front-end DNA duplex (*gray*), RNA (green) and the incoming NTP in the active center (yellow) are shown. The rest of the structure is omitted for simplicity. The inset shows a view along the long axis of the RNA-DNA hybrid in TEC with the 3' end of the RNA marked. *On the bottom:* The structure of TEC by *T. thermpohilis* RNAP (from PDB: 2O5J, [24]). The corresponding residues in the β subunit of *E. coli* RNAP carrying slippage mutations are shown in red. The other elements of TEC are color-coded the same as in the Pol II structure.

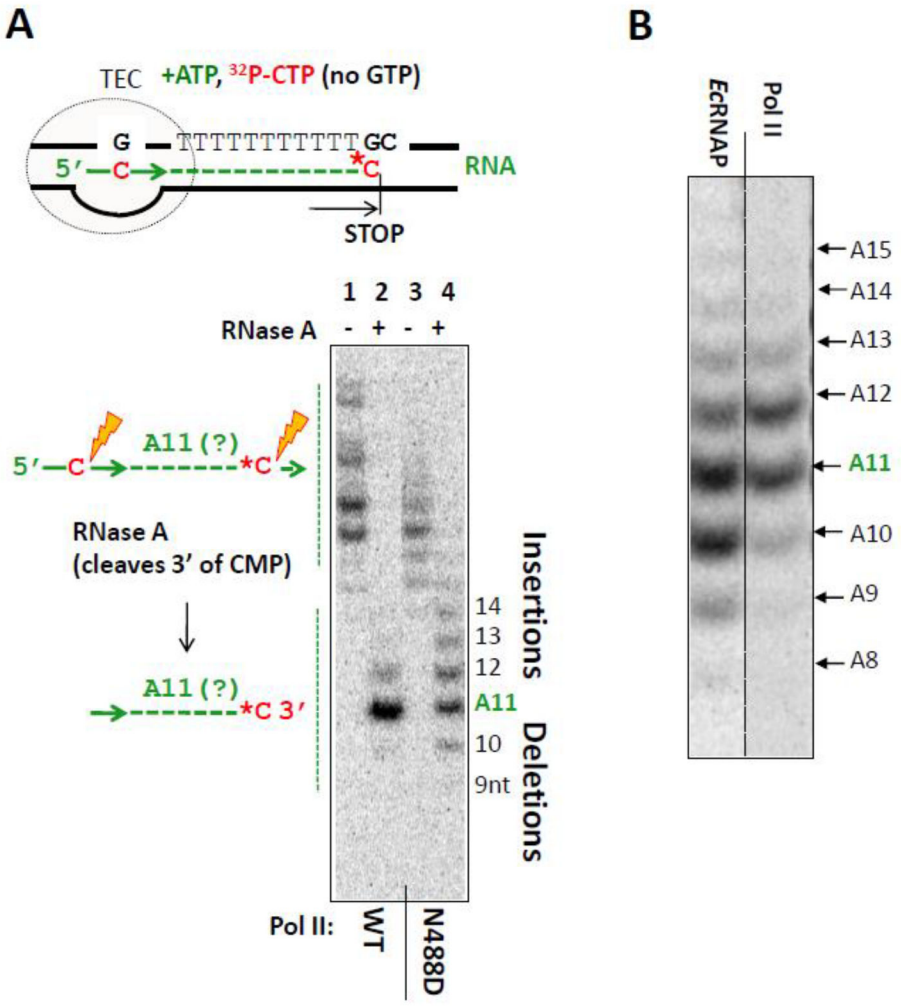

Fig. 3.

Test for slippage *in vitro*. (**A)** The cartoon shows the assembled TEC by yeast Pol II before incubation with 10μM ATP and 100μM labeled CTP. The horizontal arrow denotes the DNA sequence for halting of TEC due to the lack of GTP. The two cleavage CMP sites for RNase A (red) in the nascent RNA are shown. The different pattern of the digested RNA products was expected to accumulate depending on the slippage directionality. The bottom panel displays the actual data for wild type Pol II and *the* Rpb1-N488D mutant exhibiting the increase of the deletions and insertions in the 11A-tract. The corresponding length of the A-tract in the RNA is shown on the right side of the gel. Note, that digestion with RNase A allowed an unambiguous identification of the slippage products (lanes 2 and 4), which was impossible in the non-digested samples (lanes 1 and 3) because Pol II partially transcribed across the stop site due to the cross-contamination of the commercial stocks of ATP and CTP with residual amount of GTP. (**B)** Yeast Pol II and *Ec*RNAP have the opposite slippage directionality in the 11A-tract under the identical transcription conditions.

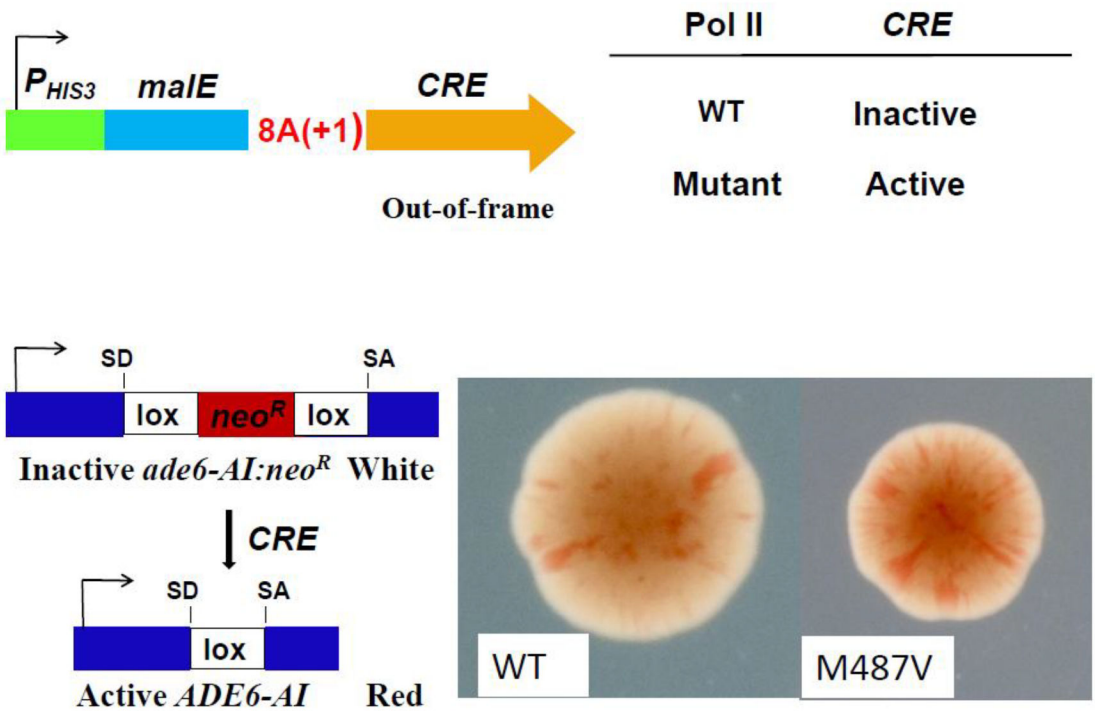

Fig. 4.

Schematic illustration of the Cre-mediated capture of transcription errors. The 8A(+1) *malE-cre* reporter is out-of-frame, generating nonfunctional Cre recombinase. Transcriptional slippage can restore the reading-frame of Cre. The active Cre in turn removes neo^R from the second reporter *ade6-AIlox::*neo^R, generating active *ADE6-AI*. In an *ade2* background, an *ade6-AIlox::*neo^R colony is white and *ADE6-AI* are red colonies. Red sectors, representing transcription slippage errors that produce active Cre, are more frequent in transcription slippage mutants. SD (splicing donor site) and SA (splicing acceptor site).

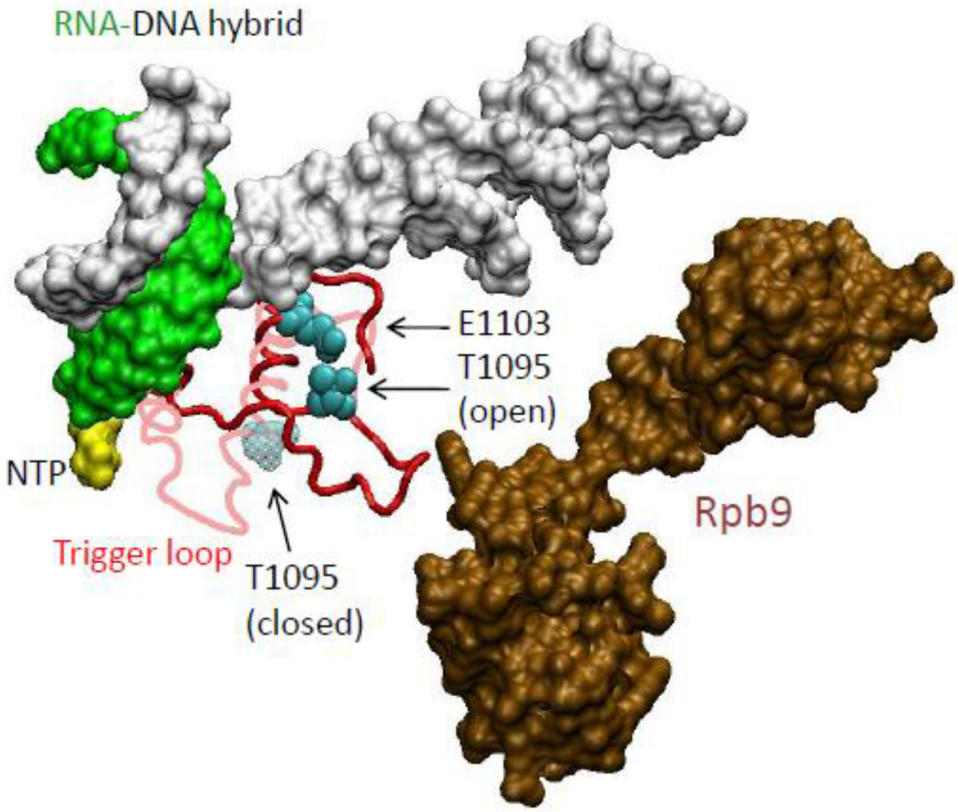

Fig. 5.

The *rpb1-E1103G* mutation highlights the role of the trigger loop in Pol II fidelity. Glu1103 residue of the trigger loop and Rpb9 subunit play a role in stabilization of the opened state of the Pol II active site, and attenuate sequestration of substrate NTP leading to the improved fidelity. The closed (shaded pink) and opened (red) states of the trigger loop are superimposed from the crystal structures of the elongation complex by the yeast Pol II [PDB: 2E2H and 1Y1V, respectively [19,25]]. Glu1103 and Thr1095 interact in the opened conformation of the trigger loop, but this interaction is disrupted by the loop closure on the NTP (yellow). Rpb9 subunit of Pol II (gold) forms a putative interaction with the opened trigger loop. All other elements are colored the same as in Fig. 2.