Bacteriophage λ N protein inhibits transcription slippage by Escherichia coli RNA polymerase

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ABSTRACT

Transcriptional slippage is a class of error in which ribonucleic acid (RNA) polymerase incorporates nucleotides out of register, with respect to the deoxyribonucleic acid (DNA) template. This phenomenon is involved in gene regulation mechanisms and in the development of diverse diseases. The bacteriophage λ N protein reduces transcriptional slippage within actively growing cells and in vitro. N appears to stabilize the RNA/DNA hybrid, particularly at the 5′ end, preventing loss of register between transcript and template. This report provides the first evidence of a protein that directly influences transcriptional slippage, and provides a clue about the molecular mechanism of transcription termination and N-mediated antitermination.

INTRODUCTION

Errors in the transcription process can potentially lead to aberrant gene products and, ultimately, disease. One major class of error, known as transcriptional slippage, can occur during transcription elongation when the nascent ribonucleic acid (RNA) molecule shifts register, backward or forward, with respect to the template deoxyribonucleic acid (DNA) (1). RNA polymerase (RNAP) maintains an ∼8–9 base pair RNA/DNA hybrid during transcription elongation (2). RNAPs depend on this short RNA/DNA hybrid for stability and processivity of ternary elongation complexes (TECs). RNA/DNA hybrids of less than 8-bp display markedly less stability than those that are 8 bp or longer (2–4). If the RNAP shifts backward and resumes transcription, an extra nucleotide will be inserted; conversely, if the complex shifts forward a coded base will be omitted from the transcript. During elongation, transcriptional slippage is typically restricted to DNA sequences containing a single repeated nucleotide, called homopolymeric tracts, generally 8 or more in a stretch (1,5–8). When transcriptional slippage occurs within the coding sequence of a gene, the resulting transcripts can encode disrupted reading frames, changing the sense of all downstream codons (9). This type of error has been implicated in the development of a wide variety of diseases, including colon cancer, non-familial Alzheimer’s and Down’s syndrome [reviewed in (1) and (10)].

Instances of transcriptional slippage have been observed in many organisms, and certain genetic elements, such as transposons, exploit this phenomenon for use as a regulatory mechanism (5,10,11). Transcription initiation is particularly prone to slippage at short homopolymeric tracts, due in part to the limited length of the RNA/DNA hybrid compared to hybrid length during elongation (12). For example, in Escherichia coli, expression of the pyrBI operon is regulated during initiation by an iterative transcription mechanism that is related to slippage during elongation. In Thermus thermophilus, transcriptional slippage during elongation of dnaX is responsible for producing either the γ- or τ-subunits of the DNA replication machinery, both being produced from the same DNA sequence (13).

Attempts to assess the fidelity of RNAP in vivo have been hampered by the low frequency of transcription errors, the transient nature of the transcript and a comparatively high frequency of translation error (1,14). Early systems used to study transcriptional slippage utilized plasmidborne constructs containing a lacZ reporter interrupted by homopolymeric tracts of DNA (7). Long stretches of A or T nucleotides (>8 nt) are particularly prone to slippage, possibly due to the weak bonding of the RNA/DNA hybrid during transcription (5,7). A shifted RNA/DNA hybrid in a long repeating tract would be indistinguishable by polymerase from the unshifted RNA/DNA hybrid. Shifts in register may go undetected and uncorrected by RNAP and its associated nucleolytic proofreading functions, e.g. GreA and GreB, because correct pairing of the RNA/DNA hybrid is only structurally monitored up to ∼9 bp (15). Mutant β subunits (encoded by rpoB) of E. coli RNAP exist that increase or decrease the frequency of transcriptional slippage (8). One of the β mutants, P564L, demonstrated increased slippage and showed a defect in the formation of
transcription antitermination complexes with the N protein of bacteriophage λ (16–18).

The N protein is an extensively studied transcription regulatory factor that prevents both ρ-dependent and intrinsic transcription termination. N also increases the rate of transcription and reduces pausing of RNAP (19,20). The N-antitermination system has served as a model for understanding many regulatory mechanisms that modulate transcription elongation such as the Tat and TAR system of human immunodeficiency virus (HIV) and the antitermination system of bacterial ribosomal RNA operons (21–23). The N protein binds to a specific RNA hairpin structure within a regulatory RNA element known as NUT (for N utilization) (19,21). Four host proteins (NusA, NusB, NusE and NusG) interact with NUT, N and RNAP to generate a termination resistant transcription complex; however, experiments have demonstrated that N alone can modify RNAP when over-expressed in vivo or added in excess of elongation complexes in vitro (24–27).

Given the relationship between slippage and antitermination phenotypes observed in strains carrying the β P564L mutant protein, and the observation that repetitive U-rich sequences are pervasive in intrinsic transcription terminators, we tested if the N-antitermination complex exerts an effect on transcriptional slippage. Here we describe a λ-based genetic assay designed to assess transcriptional slippage by N-modified RNAP. We also use TECs assembled based genetic assay designed to assess transcriptional slippage when over-expressed in vivo or added in excess of elongation complexes in vitro (24–27).

By definition, transcriptional slippage is monitored by a specific DNA hybrid. Once a stretch of U-rich DNA is transcribed, the RNA hairpin structure is generated, and transcription continues in the direction of the strand with the lower melting temperature (28). The N protein binds to a specific RNA hairpin structure within a regulatory RNA element known as NUT (for N utilization) (19,21). Four host proteins (NusA, NusB, NusE and NusG) interact with NUT, N and RNAP to generate a termination resistant transcription complex; however, experiments have demonstrated that N alone can modify RNAP when over-expressed in vivo or added in excess of elongation complexes in vitro (24–27).

Given the relationship between slippage and antitermination phenotypes observed in strains carrying the β P564L mutant protein, and the observation that repetitive U-rich sequences are pervasive in intrinsic transcription terminators, we tested if the N-antitermination complex exerts an effect on transcriptional slippage. Here we describe a λ-based genetic assay designed to assess transcriptional slippage by N-modified RNAP. We also use TECs assembled based genetic assay designed to assess transcriptional slippage when over-expressed in vivo or added in excess of elongation complexes in vitro (24–27).

**MATERIALS AND METHODS**

**Bacterial strains and plasmids**

The N–lacZ slippage reporter strains were constructed using recombineering techniques in the parent strain ZH1041 [W3110 Δ(argF-lac)U169], with the following genetic structure around the λ prophage: gal490*/IS2 /plac8 Δatt int-lacZ-int red kil N nutL pL CI857 Δ[cro-bio] (28). Cultures were grown to OD600 ~0.4, induced at 42°C for 15 min, and prepared for electrocorporation. These cultures were then electroporated with either overlapping oligonucleotides (Supplementary Table S1) or a single oligonucleotide with 35-bp homology to the N gene, ending at the 33rd codon, a homopolymeric tract, and 35-bp of homology to the lacZ gene, starting at the 16th codon. Recombinants were selected for the loss of the prophage region containing kil and all intervening sequence between N and lacZ, by selecting on L-plates at 42°C, as described in (28). Recombinants were sequenced to confirm the desired genotype. N was expressed from the pZH124 plasmid. This plasmid is a pGB2 derivative (pSCL101 replicon), with the coding sequence of N under pLac control (28). In control strains, pGB2 alone was used as a vector-only control (29).

**β-galactosidase assay**

Cultures were grown from three independent colonies per condition overnight in L broth at 30°C, supplemented with 30-μg/ml spectinomycin when plasmids were present. Cultures were diluted 1:100 in fresh L broth and grown 1.5 h at 30°C, without antibiotic in all cases, to an OD600 of ∼0.1. Cultures were then shifted to 42°C shaking water bath and grown for an additional hour, reaching a final OD600 of ∼0.5–0.6. Cultures were transferred to an ice-water bath and chilled for 10 min. One hundred microliters of each culture was assayed for β-galactosidase (β-gal) activity as described by Miller (30).

**Procesive slippage assay**

RNAP and TEC assembly was performed as described by Kireeva and Kashlev (31). Briefly, a 9-nt synthetic RNA (5’-AUC GAG AGG-3’) was annealed to template DNA (5’-TTG GGT TCT CTA TTC GCC TCG TTT TTT CCC TCT CGA TGG CTG TAA GTA TCC TAT ACC-3’) and incubated with histidine-tagged (His-tagged) RNAP, bound to Ni-NTA agarose beads (Qiagen). The non-template strand (5’-GGT ATA GGA TAC TTA CAG -3’) was added at 10 μM, and complexes were allowed to transcribe the A tract for 10, 20, 40, 90, and 180 s, after which they were stopped by the addition of 2× loading buffer containing 7-M urea. Samples were analyzed by denaturing PAGE, visualized by phororimaging (Molecular Dynamics).

**RESULTS**

**Development of a bacteriophage λ-based transcriptional slippage assay**

We employed a genetic reporter to assess transcriptional slippage in a λ-based assay. In this system, transcriptional slippage is monitored by β-gal enzyme activity produced from a defective prophage. The construct consists of a translation fusion of lacZ to the N gene, the first gene transcribed in the λ pL operon (Figure 1). The strong pL promoter can be conditionally controlled by temperature in bacteria expressing the mutant AC1857 repressor protein (28). A homopolymeric tract was placed at the translation fusion junction between N and lacZ. To test for transcriptional slippage within the homopolymeric tracts, we fused the lacZ coding sequence one nucleotide out-of-frame with respect to the start codon of the N protein. Transcriptional slippage events can restore the reading frame of the lacZ gene. Forward slippage of the transcript, coupled with resumed transcription, would lead to the elimination of a coded nucleotide, restoring the reading frame in +1 out-of-frame constructs, whereas backward slippage of the transcript would lead to addition of a nucleotide, restoring the reading frame in −1 out-of-frame constructs.
that is incorporated into the RNA, for example A9 tracts.

We refer to the homopolymeric tracts by the nucleotide
in a row, in both the mopolymeric tracts containing 7, 8, 9, 10 and 11 adenines
and A10 constructs, we adopted it as the standard control con-
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A10 constructs, we adopted it as the standard control con-
struct. In contrast to the out-of-frame constructs, in-
frame constructs show decreasing levels of
slippage causing a shift from the correct reading frame to
an incorrect reading frame.

We tested for RNAP termination or arrest within the homopolymeric tract by designing a reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) assay that compared the abundance of transcripts containing se-
quence upstream of the A tract with the abundance of se-
quence immediately downstream of the A tract. We found no difference in upstream versus downstream RNA levels, further establishing that the homopolymeric tract itself does not cause termination or arrest (Supplementary Figure S1).

In addition to A tracts, we constructed strains that con-
tain U10, C10, or G10 tracts. The out-of-frame U10 tracts
yielded activities that were qualitatively similar to A10
tracts, whereas C10 and G10 are comparable to the non-
slippery A5GA4 tract (Table 1). These observations are con-
sistent with measurements made in a similar system by oth-
ers (7). From these data, we conclude that differences in
amino acid composition at this site in the fusion result in
negligible changes to β-gal enzyme activity, and the C and
G tracts are not slippery.

**N inhibits transcriptional slippage on homopolymeric A and U tracts**

It has been suggested that slippage of the transcript within
the elongation complex may contribute to transcription ter-
nination (9,32). To test if the N protein has an effect on transcriptional slippage, as well as preventing termination,
we expressed N from a medium-copy-number plasmid in
reporter strains that contain A7, A8, A9, A10, A11, U9 or
U10 homopolymeric tracts. In the in-frame A5GA4 control
strain, we found ~2-fold higher levels of functional β-gal
when N protein was expressed from plasmids compared to
vector-only controls, 2531 ± 87 Miller units versus 1357
(±204) Miller units, respectively. This result reaffirms the
presence of a weak terminator (see t10; Figure 1) in the N-
gene leader between NUT and the N gene, as described previ-
ously (33). These are taken to be the total levels of transcription
possible with or without antitermination, respectively. We
assayed the fusion constructs containing homopoly-
meric tracts in −1 and +1 reading frames and normalized all
β-gal measurements to the corresponding A5GA4 controls
with or without N. There was a consistent decrease in β-gal
activity when N was expressed (Table 2), despite the over-
all increase in transcription observed in A5GA4 controls
with N expressed. While both A10 and U10 tracts exhibit
extensive transcriptional slippage, N prevented slippage to
a greater extent on U tracts, which are known to make the
least stable RNA/DNA hybrids (34). The most profound effect of N was observed in the +1 out-of-frame U10 con-
structs, causing a 7.3-fold decrease in nucleotide omissions
(Table 2). N consistently inhibited nucleotide deletions to a
greater extent than insertions.

The N-leader sequence contains the NUT RNA element that is bound by N protein. Antitermination complexes are
formed when the N–NUT complex binds RNAP, along with
additional transcription elongation Nus factors (19). To test
the dependence of the anti-slippage phenomenon on the
N–NUT nucleoprotein complex, we made a mutation that
disables the NUT site (nutL7) (28). In the A5GA4 control
strain, expressing N with nutL7 yielded β-gal activities that
were similar to those containing the vector-only control and
the wild-type NUT sequence (2286 ± 75) Miller units versus
1902 ± 180] Miller units, P > 0.05, two-tailed t-test). This
result demonstrates that nutL7 effectively abolished N’s an-
titermination activity. The β-gal activity is the same with
and without N-expressing plasmids in −1 and +1 A10 out-
of-frame constructs (Table 2), therefore the inhibition of
slippage is also abolished when N cannot bind to NUT.

**N alone is capable of inhibiting transcriptional slippage in vitro**

To further confirm that N protein is capable of inhibiting transcriptional slippage, we assembled purified TECs con-
sisting of RNAP, a synthetic RNA primer, template DNA
and non-template DNA oligonucleotides containing an A5
tract and flanking sequence that is unrelated to the in vivo λ
constructs (Figure 2A). The TECs did not contain the NUT
RNA element or Nus elongation factors that are present
in the in vivo system. The RNA was annealed one base be-
fore the homopolymeric tract, and annealed transcript was
labeled at the 3′ end by the addition of α-32P GTP, which

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**Figure 1.** Diagram depicting N leader sequence and N::lacZ fusion. The 223 nt long leader sequence consists of an N protein binding site (nut) and a weak transcription terminator (tL0). The N::lacZ fusion consists of the Shine–Dalgarno (SD) sequence and the first 33 codons from the 3′ leader region of the lacZ gene, where Xn represents variable number of X nucleotides. The lacZ coding sequence is unframed. A10 constructs are used as the standard control construct. In the A5GA4 control strain, we found 2-fold higher levels of functional β-gal activity when N protein was expressed from plasmids compared to vector-only controls, 2531 ± 87 Miller units versus 1357 (±204) Miller units, respectively. This result reaffirms the presence of a weak terminator in the N−NUT fusion construct. In the −1 and +1 frames, all β-gal measurements were normalized to the corresponding A5GA4 controls with or without N. There was a consistent decrease in β-gal activity when N was expressed (Table 2), despite the overall increase in transcription observed in A5GA4 controls with N expressed. While both A10 and U10 tracts exhibit extensive transcriptional slippage, N prevented slippage to a greater extent on U tracts, which are known to make the least stable RNA/DNA hybrids (34). The most profound effect of N was observed in the +1 out-of-frame U10 constructs, causing a 7.3-fold decrease in nucleotide omissions (Table 2). N consistently inhibited nucleotide deletions to a greater extent than insertions.

The N-leader sequence contains the NUT RNA element that is bound by N protein. Antitermination complexes are formed when the N–NUT complex binds RNAP, along with additional transcription elongation Nus factors (19). To test the dependence of the anti-slippage phenomenon on the N–NUT nucleoprotein complex, we made a mutation that disables the NUT site (nutL7) (28). In the A5GA4 control strain, expressing N with nutL7 yielded β-gal activities that were similar to those containing the vector-only control and the wild-type NUT sequence (2286 ± 75) Miller units versus 1902 ± 180] Miller units, P > 0.05, two-tailed t-test). This result demonstrates that nutL7 effectively abolished N’s antitermination activity. The β-gal activity is the same with and without N-expressing plasmids in −1 and +1 A10 out-of-frame constructs (Table 2), therefore the inhibition of slippage is also abolished when N cannot bind to NUT.

**N alone is capable of inhibiting transcriptional slippage in vitro**

To further confirm that N protein is capable of inhibiting transcriptional slippage, we assembled purified TECs consisting of RNAP, a synthetic RNA primer, template DNA and non-template DNA oligonucleotides containing an A5 tract and flanking sequence that is unrelated to the in vivo λ constructs (Figure 2A). The TECs did not contain the NUT RNA element or Nus elongation factors that are present in the in vivo system. The RNA was annealed one base before the homopolymeric tract, and annealed transcript was labeled at the 3′ end by the addition of α-32P GTP, which
Table 1. The effect of homopolymeric tract on β-gal expression

<table>
<thead>
<tr>
<th></th>
<th>Out-of-frame (+1)</th>
<th>Out-of-frame (−1)</th>
<th>In-frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>A7</td>
<td>7 (1)</td>
<td>17 (1)</td>
<td>2077 (167)</td>
</tr>
<tr>
<td>A8</td>
<td>22 (1)</td>
<td>180 (16)</td>
<td>1616 (134)</td>
</tr>
<tr>
<td>A9</td>
<td>180 (15)</td>
<td>390 (86)</td>
<td>1260 (143)</td>
</tr>
<tr>
<td>A10</td>
<td>308 (19)</td>
<td>624 (37)</td>
<td>1113 (69)</td>
</tr>
<tr>
<td>A11</td>
<td>303 (28)</td>
<td>539 (46)</td>
<td>822 (55)</td>
</tr>
<tr>
<td>U9</td>
<td>145 (10)</td>
<td>307 (28)</td>
<td>1652 (95)</td>
</tr>
<tr>
<td>U10</td>
<td>431 (33)</td>
<td>323 (17)</td>
<td>791 (35)</td>
</tr>
<tr>
<td>C10</td>
<td>9 (1)</td>
<td>15 (9)</td>
<td>1246 (76)</td>
</tr>
<tr>
<td>G10</td>
<td>7 (4)</td>
<td>10 (3)</td>
<td>1647 (152)</td>
</tr>
<tr>
<td>A5GA4</td>
<td>8 (2)</td>
<td>7 (1)</td>
<td>1746 (101)</td>
</tr>
<tr>
<td>A5UA4</td>
<td>3 (1)</td>
<td>4 (1)</td>
<td>1698 (100)</td>
</tr>
</tbody>
</table>

Average β-gal activities are presented in Miller units (bold), with standard error of the mean (SEM) in parentheses, n = ≥3.

Table 2. Fold effect of N on β-gal activity

<table>
<thead>
<tr>
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<th>Fold change in percent slippage</th>
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<tbody>
<tr>
<td></td>
<td>Out-of-frame (+1)</td>
</tr>
<tr>
<td>N−/N+</td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>1.0</td>
</tr>
<tr>
<td>A8</td>
<td>3.5</td>
</tr>
<tr>
<td>A9</td>
<td>3.1</td>
</tr>
<tr>
<td>A10</td>
<td>3.6</td>
</tr>
<tr>
<td>A11</td>
<td>3.6</td>
</tr>
<tr>
<td>U9</td>
<td>6.5</td>
</tr>
<tr>
<td>U10</td>
<td>7.3</td>
</tr>
<tr>
<td>A10, nutL7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The percent of transcriptional slippage in the out-of-frame constructs was calculated by the formula: [(Miller units An N−/Miller units A5GA4 N−) ÷ (Miller units An N+/Miller units A5GA4 N+)]. A value of 1.0 indicates no change.

also extended the annealed RNA to the beginning of the homopolymeric tract. To commence the slippage assay, ATP alone was added to the TECs, thus only ATP could be incorporated into the RNA transcript. Under this condition, RNAP transcribes to the end of the A tract and continues adding adenines at the end of the A tract by a slippage mechanism (8). The reaction was stopped at selected time points, and the products were separated on a denaturing gel for visualization. This assay measures the length of RNAs over the reaction time course and, therefore, the insertion rate of additional As into the transcript. We observed distributions of transcripts longer than the expected 19 nt, and the length of transcripts steadily increased over the reaction time (Figure 2B). We quantified the bands that were >19 nt in length and assessed their position within the distribution of transcripts at each time point (see arrows in Figure 2B and C). The number of additional nucleotides present in the most abundant transcript was divided by reaction time to provide the rate of slippage. The catalytic rate of slippage was calculated as 0.25 nt/s where no N was present. To test the effect of N on slippage, 100-fold molar excess of N protein was pre-incubated with the complexes, prior to the addition of ATP. High concentrations of N are necessary for antitermination to function in vitro in the absence of NUT and Nus factors (24), so similarly high concentrations were used in these experiments. Consistent with in vivo effects, the slippage rate dropped 1.6-fold, compared to reactions lacking N (P < 0.05, two-tailed t-test).

Similar reactions were carried out on templates containing U11 tracts, and incubating with uridine triphosphate (UTP) instead of ATP. N also reduced slippage on the U11 templates (Supplementary Figure S2). To confirm that N acts as a positive elongation factor under comparable conditions, the elongation rate was compared between N+ and N− reactions in the presence of limited amounts of all four nucleotides. N+ reactions stimulated the rate of nucleotide incorporation ≥2-fold and decreased pausing (Supplementary Figure S3.), consistent with the effects measured by others (24). We also tested if the NusA elongation factor influences transcriptional slippage. We detected no change in slippage with the addition of NusA (Supplementary Figure S4).

Sequence at the 5′ end of the slippery sequence influences slippage

On A8 tracts, both forward and backward slippage will result in mismatches at the 5′ end of the hybrid, if the RNA/DNA hybrid is restricted to nine nucleotides. The interaction interface between N and RNAP has not been definitively solved; however, there is reason to believe that N interacts near the RNA exit channel (35). For these reasons, we wanted to determine first if slippage, particularly on the
The *in vitro* transcriptional slippage assay. (A) Diagram depicting the *in vitro* transcription assay to assess the rate of slippage. His-tagged (HHHHHH) RNAP was immobilized on Ni-NTA functionalized agarose beads. A 9-nt-long RNA was annealed one base upstream of the A9 tract. This RNA/DNA hybrid was added to RNAP that was immobilized on Ni-NTA agarose beads, and the non-template DNA strand was added to complete the assembly of the TEC. α-32P-labeled GTP (G* ) was added, elongating the transcript by one nucleotide and radiolabeling the transcript. The complex was washed several times to remove any un-incorporated GTP. To commence the slippage assay, TECs were incubated with ATP or UTP (depending on the homopolymeric tract). The active site is presented as a small circle at the 3′ end of the transcript. (B) A representative denaturing gel from complexes containing A9 homopolymeric tracts. Time points are given in seconds, and N+ and N− conditions are shown. Peak bands corresponding to transcripts >19-nt long are labeled with arrows; note that in the N+ condition peak bands contain fewer nucleotides for all time points. See Supplementary Figure S2 for results of *in vitro* slippage assay on U11 tracts. See Supplementary Figure S3 for controls that demonstrate that N acts as a positive elongation factor on these *in vitro* templates. (C) Histograms of samples from *in vitro* slippage assays enable determination of slippage rates. Transcript abundance, as measured by 32P counts, is presented relative to transcript length. A representative time point (40 s) is shown. Samples containing N are shown in light gray and samples lacking N are shown in black. Arrows indicate the location of the most abundant band.

shorter A9 tract, is sensitive to nucleotide composition immediately 5′ of the homopolymeric tract, and second, if such an effect is influenced by N. We constructed additional reporter strains, using the *in vivo* system described above, in which the nucleotide encoded directly upstream of the homopolymeric A8 tract was engineered to contain U, C, or G.

In control experiments without N, we found that β-gal activity in the N−*lacZ* fusion reporters was highest in the 5′-U constructs, followed by 5′-C then 5′-G (Table 3). This pattern was consistent in both −1 and +1 out-of-frame constructs. These differences may be attributed to relative stability of hybrid base pairs, where greater stability, rG/dC > rC/dG > rU/dA (34), prevents slippage to a larger extent. When N was provided, β-gal activity was reduced in all cases. In −1 out-of-frame constructs, the greatest effect of N was observed on A tracts with a 5′-U; slippage was reduced >3-fold and ~2-fold in 5′-C and 5′-G reporters, respectively. In +1 out-of-frame constructs, N appears to have a greater effect on 5′-C than on 5′-U. Along with the observation that N inhibits deletions to a greater extent than insertions (Table 2), these results provide evidence that deletions may occur through a different mechanism than insertions and that the deletion process is more sensitive to N’s influence.

**DISCUSSION**

We have described the development of a new transcriptional slippage assay, specifically designed to determine if the λ antitermination factor N affects the frequency of transcriptional slippage by RNAP. We find that a homopolymeric A tract begins to elicit a large change in β-gal activity at A8, reminiscent of the length of the 8–9-bp RNA/DNA hybrid during transcription elongation (3,4). β-gal functions as a tetramer (36) and multiple functional β-gal subunits can be produced from in-frame transcripts resulting from transcriptional slippage (7). Ribosomal frameshifting could also result in active β-gal subunits; however, the requisite sequences and downstream RNA secondary structures re-
required for ribosomal frameshifting are not predicted in this sequence (13). Moreover, because it generates only one subunit per event, ribosomal frameshifting would be less likely to produce the high local concentration of functional β-gal subunits necessary to make active β-gal tetramers (7,8,13).

We found that modification of RNAP by the N–NUT antitermination complex reduces transcriptional slippage in vivo. The greater effect of N on homopolymeric U tracts compared to A tracts supports the hypothesis that stabilization of the hybrid reduces slippage, as rU/dT pairs are more unstable than rA/dT pairs (34). N prevents insertions to a greater extent on A tracts with 5′-U than on 5′-C or 5′-G, suggesting that hybrids that are already reasonably stable are not further stabilized by N to an appreciable extent. Slippage of the transcript on short homopolymeric tracts is also expected to generate mismatches between bases at the upstream end of the RNA/DNA hybrid, which have differential stability (37) and might also contribute to the overall stability of the RNA/DNA hybrid after a slippage event. Stability of mismatches in slippage intermediates does not appear to be an overriding factor in generating insertions or deletions in the transcript. It might be expected that slippage events that result in a relatively stable upstream mismatch in the RNA/DNA hybrid, such as rG/dT (37) and rA/dC (38), would promote more insertions or deletions due to a higher RNA/DNA hybrid stability, compared to less stable mismatches. However, we observed the opposite; there was less slippage in the two contexts that would generate rG/dT or rA/dC mismatches, compared to similar out-of-frame constructs (Table 3).

In vitro, where N was present at a high concentration, N alone was capable of reducing the frequency of insertions at homopolymeric A and U tracts, despite an increase in elongation rate that might have been expected to increase the catalytic rate of slippage. The in vitro experiments were conducted on DNA templates that are unrelated to the λ genetic reporter system, and did not encode the NUT element. In vivo, N was expressed from a medium-copy-number plasmid and would not be produced at a high enough concentration to function without NUT or Nus factors (39). Indeed, we see no evidence of antitermination or slippage inhibition when NUT was disabled in vivo (see nutL7; Table 2). In vitro assays measure the rate of insertion, analogous to the frequency measurements made in the −1 out-of-frame genetic constructs. The 1.6-fold effect is similar in magnitude to the 1.9-fold effect observed on −1 out-of-frame constructs in vivo (Table 2).

N may prevent disruption of the hybrid at the 5′ end, making dissociation of RNA from the DNA template less likely, a step that appears to be important in both termination and slippage (9,32). Loss of stability in the 5′ end of the RNA/DNA hybrid plays a major role in shortening the length of the hybrid, ultimately leading to termination of transcription at intrinsic terminators (4). A shift in the RNA/DNA hybrid at the boundary of a homopolymeric tract will align non-pairing nucleotides with one another at the upstream end of the hybrid, reducing the length of correctly paired nucleotides within the hybrid. Slippage of the transcript might, therefore, also participate in the shortening of the RNA/DNA hybrid and contribute to the termination of transcription. Our findings support the hypothesis that slippage and termination of the elongation complexes are connected phenomena (9,40). It will be interesting to learn if other antitermination systems, such as the Tat/TAR in HIV (22), similarly affect transcriptional slippage.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Conflict of interest statement. None declared.

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