For 25 mutant alleles of ret1, encoding the second largest subunit of yeast RNA polymerase III, we have studied the polymerase III nuclease activity, measuring both the total yield and dinucleotide product composition. Mutations affecting amino acids 309–325 gave slightly elevated nuclease activity. In region 367–376, two mutations gave 12–15-fold increased nuclease activity. Our results do not support the catalytic role in nuclease activity proposed for the conserved DDRD motif in this region (Shirai, T., and Go, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9056–9060). Mutations centered on a basic region from amino acids 480 to 490, which aligns with *Escherichia coli* ϕ− subunit sequences between Rif 

*‡* To whom correspondence should be addressed: Dept. of Genetics, University of Washington, Seattle, WA 98195-7360.

In earlier studies of *in vivo* termination by yeast RNA polymerase III, we found that genetic alteration of the second largest subunit can change this enzyme’s ability to continue transcription downstream of a U$_5$ tract. Mutant polymerases with increased read-through of an intronic U$_5$ sequence in the SUP4 YUV allele can efficiently produce biologically active suppressor tRNA$^{\text{Tyr}}$ (9, 10). These mutant polymerases also read through the U$_5$ tract in SUP4Δ94 that is placed at the location of the normal U$_5$GU$_6$ terminator, producing abnormally long pre-tRNAs that are not matured to functional tRNA$^{\text{Tyr}}$. By *in vivo* mutant screening with these same two SUP4 alleles, a large number of second-largest subunit mutations having the opposite phenotype were also obtained. These have been designated increased termination mutations (10).

RNA polymerase III preparations isolated from several yeast strains bearing increased or decreased termination mutations have an altered response to several U-rich pause sites within the SUP4 template (11). Because of our previous observation (12) that the amounts of mono- and dinucleotide exonuclease products released concurrently with the transcription reaction are directly proportional to the content of oligo(U) tracts in the transcript, we have characterized the set of ret1 mutations in the second largest pol III subunit for the specific activity and substrate preference of the 3′-exonuclease of each mutant pol III. We made analytical measurements of the dinucleotide products released during transcription by the mutant yeast enzymes. As templates, we employed the SUP4+ yeast tRNA$^{\text{Tyr}}$ gene, which contains three internal pause sites and a U$_5$GU$_6$ terminator, as well as a synthetically constructed chimeric tRNA$^{\text{Tyr}}$/RNA$^{\text{Less}}$ tRNA gene. The latter template contained a U$_6$ terminator between the A and B block internal control regions as well as the normal U$_5$ terminator at the end of the tRNA$^{\text{Less}}$ gene. For each mutant RNA polymerase III transcribing these two DNA templates, we measured both the total amount of dinucleotide released per transcript completed and the ratio between released pUpU and released pGpU.

Of the 25 ret1 alleles studied, 11 had near normal rates of dinucleotide release, one had significantly slower cleavage, and the remainder had rates of RNA cleavage ranging from 2 to 60 times that of the wild type. The largest effects were produced by mutations in two conserved motifs: DDRDYVGNKR, between amino acids 367 and 376, and GEMERDCYIA, between amino acid 1063 and 1072 (10). Qualitative effects upon the release of pGpU relative to pUpU were observed for mutations in the region from amino acids 476 to 485 (analogous to Rif cluster II of *E. coli* rpoB (13)).

**MATERIALS AND METHODS**

Reagents and Enzymes—[$\gamma$-$^{32}$P]ATP, [$\alpha$-$^{32}$P]UTP, fast protein liquid chromatography-purified ribo- and deoxy-NTPs, and the Sculptor* in *vitro* mutagenesis system were purchased from Amersham Pharmacia Biotech. Ribo-ApU and UpA were obtained from Sigma, and other ribo-oligonucleotides were from Oligos Etc. Inc. M-280 streptavidin-Dynabeads were purchased from Dynal, Inc. DNase and RNase-free bovine serum albumin was purchased from Roche Molecular Biochemi-
Reactions were stopped by the addition of EDTA to 20 mM and 1:3 (v/v) SUP4 mg/ml heparin to prevent reinitiation. The total volume of each post-reaction, samples were separated on 20% gels as described (12). To avoid possible distortions of the front bands and to verify that the original template was used, the intensities of cleavage products were compared with that of wild-type RNA polymerase. For example, mutants K310T, V309E, E325K, M312T, and T311K produced considerably more of the pyrimidine dinucleotide (pPypPy) product than did the wild type (Fig. 1, B). This increase was even more obvious in the case of mutants D370E and R376A (Fig. 1, D). The values in the second and sixth columns are normalized to a value of 1.0 for the pyrimidine dinucleotide (pPypPy) product. The other parameters were measured during the transcription reaction.

To provide a quantitative measurement of 3′-exonucleolytic action concurrent with transcription, we carried out single-round transcription with yeast RNA polymerase III from each of the ret1 mutant alleles. For this purpose, the DNA template was immobilized on magnetic beads through streptavidin–biotin interactions, were used in all transcription experiments. The volume of washing buffer (20 mM HEPES-KOH (pH 7.9), 100 mM KCl, 3 mM dithiothreitol, 0.25 mg/ml bovine serum albumin, and 10% glycerol). Since the specific activities of some of the mutants were considerably lower than that of the wild-type enzyme, transcription reactions for those mutants were proportionally scaled up to obtain an intensity of the full-length product signal approximately equal to that of the wild type. After incubation at 25 °C for 30 min, ternary complexes were magnetically concentrated and washed three times with an excess volume of washing buffer (20 mM HEPES-KOH (pH 7.9), 100 mM KCl, 3 mM dithiothreitol, 0.25 mg/ml bovine serum albumin, and 10% glycerol). Transcription was then restarted by the addition of 0.2 mM CTP, 0.2 mM ATP, 0.2 mM GTP, 15 µM [α-32P]UTP, and 0.5 mg/ml heparin to prevent reinitiation. The total volume of each post-initiation reaction was 6 µl. After transcription, the RNA and oligonucleotides made were separated on the same sample lanes. Dried gels were then subjected to PhosphorImager analysis.

Cleavage Concurrent with Transcription of the Chimeric Gene—The formation and purification of 17-mer ternary complexes on a chimeric template were performed under the conditions described above. Transcription was restarted by the addition of 0.2 mM CTP, 0.2 mM ATP, 0.4 mM GTP, 2 µM [α-32P]UTP, and 0.5 mg/ml heparin to prevent reinitiation. The total reaction volume was 6 µl. After incubation for 30 min at 25 °C, the reactions were stopped and treated as described above.

Elongation Profiles—Wild-type 17-mer ternary complexes were formed in a 15-µl reaction mixture containing 5 µl of P-11 extract, 3 µm linear double-stranded SUP4 template attached to the magnetic beads, 0.5 mM ATP, 0.5 mM CTP, and 0.3 µM [α-32P]UTP in transcription buffer (20 mM HEPES-KOH (pH 7.9), 100 mM KCl, 3 mM dithiothreitol, and 6% glycerol). For mutant polymerases with lower specific activities, transcription reactions were proportionally scaled up. After incubation at 25 °C for 30 min, ternary complexes were washed free of unbound proteins and nucleic acids as described previously (12). Transcription was restarted in the presence of ATP, CTP, and UTP, and then the resulting 17-mer complexes were purified by extensive washing of Dynabeads with the attached ternary complexes. Elongation was restarted in the presence of all four NTPs with [α-32P]UTP as the radioactive label. After elongation was completed, reactions were stopped and denatured in the presence of formamide. The samples were then loaded onto 20% polyacrylamide gels to detect both the RNA and oligonucleotides made during the transcription reaction.

Cleavage by mutant polymerases during elongation on the SUP4 tRNA5′ gene is shown in Fig. 1. Under the conditions used, the intensities of cleavage products were ~100 times higher than those of full-length RNA. For autoradiographic display of both long and short products separated on the same gel, the bottom part of gel was exposed against the film for only 2 h in comparison with 12 h for the upper part that contains completed RNA molecules.

Oligonucleotide Standards—Labeled markers were obtained as described (16) by phosphorylation of appropriate 5′-nonphosphorylated ribo-oligonucleotides with T4 nucleotidyltransferase.

RESULTS

Cleavage during Elongation of the SUP4 tRNA5′ Gene—To provide a quantitative measurement of 3′-exonuclease action concurrent with transcription, we carried out single-round transcription with yeast RNA polymerase III from each of the ret1 mutant alleles. For this purpose, the DNA template was immobilized on magnetic beads through streptavidin–biotin interactions, were used in all transcription experiments. The volume of washing buffer (20 mM HEPES-KOH (pH 7.9), 100 mM KCl, 3 mM dithiothreitol, and 6% glycerol). For mutant polymerases with lower specific activities, transcription reactions were proportionally scaled up. After incubation at 25 °C for 30 min, ternary complexes were washed free of unbound proteins and nucleic acids as described previously (12). Transcription was restarted in the presence of ATP, CTP, and UTP, and then the resulting 17-mer complexes were purified by extensive washing of Dynabeads with the attached ternary complexes. Elongation was restarted in the presence of all four NTPs with [α-32P]UTP as the radioactive label. After elongation was completed, reactions were stopped and denatured in the presence of formamide. The samples were then loaded onto 20% polyacrylamide gels to detect both the RNA and oligonucleotides made during the transcription reaction.

Cleavage by mutant polymerases during elongation on the SUP4 tRNA5′ gene is shown in Fig. 1. Under the conditions used, the intensities of cleavage products were ~100 times higher than those of full-length RNA. For autoradiographic display of both long and short products separated on the same gel, the bottom part of gel was exposed against the film for only 2 h in comparison with 12 h for the upper part that contains completed RNA molecules.

As shown Fig. 1, many of the mutants investigated have marked differences in their hydrolytic properties compared with that of wild-type RNA polymerase. For example, mutants K310T, V309E, E325K, M312T, and T311K produced considerably more of the pyrimidine dinucleotide (pPypPy) product than did the wild type (Fig. 1, A and B). This increase was even more obvious in the case of mutants D370E and R376A (Fig. 1D) and mutants I1071S and A1075V (Fig. 1E).

For quantitation, the gels were analyzed by a PhosphorImager, permitting a comparison of the amounts of full-length transcript and short cleavage products produced per round of transcription. For every mutant, at least three replicate experiments were done. The results are summarized in Table I. The three parameters we chose typify different aspects of the cleavage reaction. The first of these (Table I, second column), frequency of nucleolytic events per round of transcription, is defined as the ratio between the amounts of pPyPy product and full-length RNA for each mutant. The other parameters were the ratio between pGPu or pPAPu and the major cleavage product pPypPy. The values in the second and sixth columns were normalized to those for wild-type pol III, set at 100%, whereas the values for dinucleotide ratios were normalized to a wild-type value of 1.0.

For pol III enzymes with ret1 mutations between amino acids 300 and 325 performed cleavage several times more frequently than does the wild-type enzyme (Table I). For these, the rela-
tive amounts of different dinucleotide products remained about the same as for the wild-type polymerase. The only exception is mutant M312I, which showed a 2-fold increase in the relative production of pApU product.

Mutations in the conserved regions 370–376 and 1061–1075 caused drastic qualitative and/or quantitative changes in the cleavage reaction. Mutant polymerases from these regions performed cleavage 12–60 times more frequently than did the wild-type enzyme. Amino acid substitutions at the invariant residue Asp-370 led to a reduction in the relative yield of G-containing dinucleotide. Substitution of alanine for aspartate also caused a decrease in the relative production of pApU. Mutant I1071S cleaved 60 times more frequently than did the wild type and produced twice as high an amount of pApU, with no effect on pGpU production. In contrast, mutant A1075V demonstrated a considerably lower relative yield of pGpU product, but did not alter pApU production. Mutant R1061S demonstrated behavior that was similar to that of mutants in region 455–512. While cutting with the same frequency as the wild-type enzyme, it showed a 2-fold increase in the relative production of pApU.

Mutations in region 455–512 exhibited a very specific effect on hydrolytic activity. Mutant polymerases V483D, G485N, and K512N and the double mutant T455I/E478K produced less G-containing products than did the wild-type enzyme. In addition, mutant G485N and double mutant T455I/E478K showed a reduced production of pApU product. The frequency of cleavage events per round of transcription was unchanged for all

FIG. 1. Cleavage concurrent with post-initiation transcription of the SUP4 tRNA<sup>Tyr</sup> gene by mutant RNA polymerases. 17-mer ternary complexes were formed in the presence of 0.5 mM ATP, 0.5 mM CTP, and 0.5 mM UTP. After magnetic purification of ternary complexes, transcription was restarted by the addition of 0.2 mM CTP, 0.2 mM ATP, 0.2 mM GTP, 15 μM [α-<sup>32</sup>P]UTP, and 0.5 mg/ml heparin to prevent reinitiation. Reactions were allowed to proceed at 25 °C for 3 h. Samples were separated on 20% gels. Film was exposed to the top part of the gel for 12 h and to the bottom part of the gel for 1 h. wt, wild type.
these mutants. It is interesting that altered termination mutations in the adjoining region 512–517 showed no changes whatsoever in hydrolytic properties.

Cleavage during Elongation on the Chimeric tRNA{sup}Tyr{/sup}/tRNA{sup}Leu{/sup}MINd DNA Template—To further simplify the analysis of short cleavage products of varying composition, we analyzed 3′-exonuclease products and transcripts made on the chimeric tRNA{sup}Tyr{/sup}/tRNA{sup}Leu{/sup}MINd pol III template (12) that lacks natural arrest sites. In transcribing this template, wild-type ternary complexes elongate synchronously up to the terminator without pausing. Since the artificially constructed terminator (T{sub}7{/sub}) of the chimeric template is not as efficient as the double terminator (T{sub}7,G{Tsub}16{sub}7{sub}) of the SUP4 tRNA{sup}Tyr{/sup} gene, to prevent substantial “leakage” through the terminator, we performed elongation at a reduced UTP concentration (2 mM). Products formed during a single round of elongation on this chimeric template are shown in Fig. 2. Mutants in the region between amino acids 309 and 325 exhibited the expected severalfold increase in cleavage frequency per round of transcription. Interestingly, mutants from region 455–517, which produced less of the G-containing cleavage products during transcription of the SUP4 tRNA{sup}Tyr{/sup} gene, showed considerably higher amounts of pGpU product formed on the chimeric template than did the wild-type RNA polymerase. The sizes of the full-length products made by these mutants were also quite different from those made by the wild type. Mutants G485N, K512N, and V483D and double mutant T455I/E478K displayed pausing at sites through which wild-type polymerase and all other mutants elongated smoothly. To determine the positions on the template at which these mutants paused, we performed electrophoretic separation of the products on a long 10% polyacrylamide gel (Fig. 3A). The results show that these mutant polymerases are arrested at or near positions where the RNA transcript has a G residue at its 3′ terminus. To check if there are any other positions on chimeric template through which these “G mutants” elongate differently from the wild-type enzyme, we carried out elongation kinetics with time intervals as short as 1 s. The results (Fig. 3B) demonstrate that this pausing at G-associated positions happens throughout the gene, not only near the terminator. The pausing occurs preferentially at G residues that precede a pyrimidine.

DISCUSSION

The termination of transcription by RNA polymerase III (8, 17, and 18) and the 3′-exonuclease activity of this enzyme are both processes that respond strongly to the presence of uridylates at the 3′ terminus of nascent transcripts. Because of the shared dependence of these two processes upon U residues, we have examined a collection of altered termination mutants in yeast pol III for possible qualitative or quantitative changes in their pol III exonuclease activity. Many of these mutant enzymes have either an enhanced or reduced tendency to pause at short clusters of T residues in the non-template strand (11). Different mutant alleles vary in the time they require to complete transcription of a tDNA template; the number and length of pauses during transcription are by far the most important determinants of the time required for transcription of a tRNA gene (11).

Mutations Quantitatively Affecting Nuclease Activity—There is a distinctive range of mutant nuclease phenotypes within each of the four gene regions from which we have chosen alleles for study (Fig. 4). In the Arg-Lys-rich region 309–325, all the mutant polymerases except one have elevated nuclease activity; generally, the mutant cleavage frequency is higher than wild type by a factor of 2–3. The relative composition of cleavage products remains the same as for wild-type pol III. Continuing studies on mutants in this region show that they have large effects upon the kinetics of transcript release independently of any change in pol III exonuclease activity.

Within the conserved domain 363–367DDRDYVGNKR376 (Fig. 4), changes in invariant residues Asp-370 and Arg-376 in two cases act to strongly influence the cleavage process, yet these conserved residues are not essential for cleavage itself. Mutagenesis at these positions produced a >10-fold increase in the
number of cleavage events as well as a considerable decrease in the proportion of pGpU and pApU cleavage products. The presence of enhanced nuclease activity following such drastic changes in amino acid sequence (Arg or Asp for Ala) argues against participation of this region in the nuclease active site (1), yet it may be located very near to it.

Similar but even stronger effects were shown by two mutants from the conserved C-terminal region 1061–1081 (Fig. 4). The drastic changes exhibited by mutants I1071S and A1075V both in the frequency of cleavage events and in the production of pGpU and pApU can be explained by specific interactions of these residues with sequences at or near the RNA 3′ terminus.

**Fig. 2.** Cleavage concurrent with post-initiation transcription of the chimeric RNA pol III gene by mutant RNA polymerases. 17-mer ternary complexes were formed in the presence of 0.5 mM ATP, 0.5 mM CTP, and 0.5 mM UTP. After magnetic purification of ternary complexes, transcription was restarted by the addition of 0.2 mM CTP, 0.2 mM ATP, 0.2 mM GTP, 2 μM [α-32P]UTP, and 0.5 mg/ml heparin to prevent reinitiation. Reactions were allowed to proceed at 25 °C for 30 min. Samples were separated on 20% gels. Film was exposed to the top part of the gel for 12 h and to the bottom part of the gel for 2 h.

**Fig. 3.** Arrest sites during transcription of the chimeric RNA pol III gene by mutants with G-dependent pausing. A, arrest sites displayed by G mutants under the standard conditions. 17-mer ternary complexes were formed in the presence of 0.5 mM ATP, 0.5 mM CTP, and 0.5 mM UTP. After magnetic purification of ternary complexes, transcription was restarted by the addition of 0.2 mM CTP, 0.2 mM ATP, 0.2 mM GTP, 2 μM [α-32P]UTP, and 0.5 mg/ml heparin to prevent reinitiation. Reactions were allowed to proceed at 25 °C for 30 min. After EtOH precipitation, samples were separated on 10% gels. B, elongation profiles of G mutants. 17-mer ternary complexes were formed in the presence of 0.5 mM ATP, 0.5 mM CTP, and 0.3 mM [α-32P]UTP. After magnetic purification of ternary complexes, transcription was restarted by the addition of 0.1 mM CTP, 0.1 mM ATP, 0.2 mM GTP, and 0.2 mM UTP in the presence of heparin (0.5 mg/ml) to prevent reinitiation. Reactions were allowed to proceed at 25 °C for the times indicated. After EtOH precipitation, samples were separated on 10% gels.
In addition, previous studies (11, 19) on elongation by RNA polymerase III containing the mutant subunit R1061K/E1081D showed drastically slowed transcription due to pausing of very long duration at the normal (U-rich) pause sites. All of these effects seem entirely consistent with a role of this region, corresponding both in sequence and in placement to region I of the E. coli RpoB protein, in processive elongation.

Mutations Qualitatively Affecting Nuclease Activity—We examined 11 different mutant pol III preparations with substitution mutations between amino acids 455 and 517 (Fig. 4), a region that corresponds in position and sequence to Rifr clusters I and II in the E. coli rpoB gene (13). Approximately half of these mutant polymerases, including all with changes in the region from amino acids 476 to 485, gave altered ratios of pGpU and/or pApU to pUpU hydrolytic release. This effect was particularly strong for mutants T455I/E478K and G485N.

Most of the mutant RNA polymerases with alterations in region 476–485 showed RNA sequence specificity in their dinucleotide release preference. For the chimeric tRNA^{Tyr}/tRNA^{Leu} transcript, there was heightened pGpU production by these mutants, whereas with the SUP4 transcript, there was diminished release of pGpU for many of the mutant pol III enzymes with amino acid changes between residues 476 and 512. Between these two DNA templates, there are substantial differences in sequence context for all but one of the GU dinucleotides (Table II). For SUP4, the relative release of pApU was substantially reduced in mutant polymerases T455I/E478K and G485N, but not in V483D or K512N.

Both the differential responses of mutant polymerases with alterations in region 476–512 to the SUP4 and tRNA^{Tyr}/tRNA^{Leu} templates and their sequence preferences for exonucleolytic dinucleotide production indicate the existence of base- and amino acid-specific interactions between the conserved protein domain D and sequences at the 3’-end of nascent RNA. Earlier elongation studies with the V483D and K512N mutant RNA polymerases and the T455I/E478K double mutant showed that these enzymes had a tendency to pause at novel sites (11, 19). When tested on the chimeric tRNA^{Tyr}/tRNA^{Leu} tDNA tem-
plate, these three mutant RNA polymerases and the G485N polymerase all underwent a prolonged arrest at positions corresponding to an incorporated G residue (Fig. 3A). The further observation (Fig. 3B) that transient arrest of these mutant polymerases happens mainly at positions where G is followed by a pyrimidine may be explained by an inability of the RNA-binding region in these polymerases to accommodate consecutive RNA bases that differ in size. Finally, all four of the mutant polymerases studied in Fig. 3 (A and B) exhibit a reduced frequency of termination as compared with the wild type. This may well be an independent consequence of the RNA-binding region alteration that directly affects the termination process (19).

Null Mutations for pol III Exonuclease—A third possible phenotype, one we have not observed for any of the ret1 mutations, would be the total lack of nuclease activity. Recent experiments carried out by Chédin et al. (22) suggest that such a mutation in ret1 would likely be lethal. These authors characterized an altered form of yeast RNA polymerase III, devoid of subunit C11, that does not pause for extended periods at the U-rich pause sites, that exhibits no pol III exonuclease activity in a standard retraction assay (12, 20), and that is partially impaired in transcription termination. From their results, they conclude that the C11 subunit allows pol III to switch between an elongation mode and an RNA cleavage mode. Disruption of the gene for pol III subunit C11 is lethal for yeast (22), suggesting that pol III exonuclease is an essential function.

pol III Subunit Function in the 3′-Exonuclease Reaction—Because none of the mutant pol III enzymes we studied are devoid of nuclease activity and many have enhanced activity, it seems unlikely that any of the ret1 domains we studied encode the nuclease active site. However, the region encompassing amino acids 455–512 modulates the specificity of nuclease cutting, and the wild-type sequences in regions 367–376 and 1063–1072 appear to down-regulate the catalytic efficiency of pol III nuclease. Subunit C11, on the other hand, appears to activate an intrinsic nuclease activity that is embodied in the pol III core subunits. A number of very interesting questions regarding the relative roles in pol III nuclease of domains within ret1 and subunit C11 can now be approached by genetic means. We hope to find out, for example, whether the hyper-nuclease phenotypes of any of the mutants at positions 370, 376, 1071, and 1075 are phenotypically epistatic to the absence of subunit C11. Similarly, we might ask whether these mutations suppress the lethality of a C11 gene disruption or the slow growth of a Schizosaccharomyces pombe C11 replacement.

Relationships among Altered Termination, Pausing, and Nuclease Degradation Phenotypes—In relation to the concept of kinetic coupling between transcription elongation and transcription termination (2, 21), many ret1 mutations fall into one of two clear categories. There are a number of mutations, most corresponding in intragenic location to E. coli Rif clusters I and II, that elongate slowly because of pausing at U-rich sites and that have increased termination and increased overall pol III exonuclease activity. Among these are the mutations E478K, V511E/L516S, N513Y, and R1061K/E1081D (11, 19). These behave as expected according to the kinetic coupling model. They spend a longer time elongating across oligo(U) clusters, repeatedly transcribing and degrading RNA in those regions, thus increasing their cross-section for termination.

Quite different behavior was observed for a group of four ret1 mutations centered on residues 483 and 485. These include T455I/E478K, V483D, G485N, and K512N (Table I). The RNA polymerase III from these mutant strains combines a reduced termination phenotype with slow elongation, pauses at G residues, and has a differential lowering of cleavage at G in one context (SUP4) and an increase of cleavage at G in the chimeric template. We attribute the effects of G upon pausing and cleavage in these mutants to the bulkiness of this purine base. The 10-amino acid region encompassing these mutations is conserved within each class of RNA polymerase and contains either 3 or 4 basic amino acid residues in all eukaryotic RNA polymerases and in E. coli RNA polymerase (Fig. 5). We propose that this region engages the nascent RNA-DNA template hybrid very close to its 3′ (RNA) terminus. These contacts play an important role both in transcription elongation and in the reverse exonucleolytic process. Mutational changes such as V483D and G485N may decrease the size or flexibility of this RNA-binding domain of the protein, resulting in G-dependent pausing and exonuclease changes. At the same time, the changed geometry of this region may cause the enzyme to grasp the RNA-DNA hybrid more firmly, decreasing its tendency to terminate at U3 sequences.

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