Replicative DNA polymerases, as exemplified by the B family polymerases from bacteriophages T4 and RB69, not only replicate DNA but also have the ability to proofread misincorporated nucleotides. Because the two activities reside in separate protein domains, polymerases must employ a mechanism that allows for efficient switching of the primer strand between the two active sites to achieve fast and accurate replication. Prior mutational and structural studies suggested that a β hairpin structure located in the exonuclease domain of family B polymerases might play an important role in active site switching in the event of a nucleotide misincorporation. We show that deleting the β hairpin loop in RB69 gp43 affects neither polymerase nor exonuclease activities. Single binding event studies with mismatched primer termini, however, show that the β hairpin plays a role in maintaining the stability of the polymerase/DNA interactions during the binding of the primer DNA in the exonuclease active site but not on the return of the corrected primer to the polymerase active site. In addition, the deletion variant showed a more stable incorporation of a nucleotide opposite an abasic site. Moreover, in the 2.4 Å crystal structure of the β hairpin deletion variant incorporating an A opposite a templating furan, all four molecules in the crystal asymmetric unit have DNA in the polymerase active site, despite the presence of DNA distortions because of the misincorporation, confirming that the primer strand is not stably bound within the exonuclease active site in the absence of the β hairpin loop.

Survival of a species necessitates a high degree of accuracy during the replication of its genome. Replicative DNA polymerases therefore possess high levels of discrimination against incorrect nucleotides. When mistakes are made, a rare but potentially problematic situation, many polymerases are equipped with a built-in exonuclease activity, which is capable of removing the mismatched base at the primer terminus. This proofreading activity increases the accuracy of the polymerase up to a hundredfold over that of the polymerase activity alone. One of the most potent exonuclease activities is found in the replicative DNA polymerases from bacteriophages T4 and RB69, which are members of the B family of DNA polymerases. These enzymes can achieve an error frequency of ~1 mistake for every 10⁶ correct incorporations (1, 2).

Because the exonuclease domain of these polymerases is located some 30–40 Å away from the polymerase active site, an intriguing question is how the mismatched primer migrates from the polymerase to the exonuclease active site. In DNA polymerases of the A family, the exonuclease domain is located on one side of the palm domain, whereas in the B family polymerases, the proofreading domain is found on the opposite side. Different mechanisms are therefore likely at work in the two families during the primer switching event that takes place during proofreading. Both families of polymerases, however, have been shown to switch active sites processively. For the B family replicative DNA polymerase from bacteriophage T4, switching from the exonuclease to polymerase active sites has been shown to be processive (3), and for the A family replicative DNA polymerase from bacteriophage T7, the switch between active sites has been shown to be processive in both directions (4). These polymerases can readily switch DNA between the exonuclease and polymerase active sites without dissociating from the duplex DNA. The A family repair polymerase (pol)⁴ from Escherichia coli can also switch active sites intramolecularly, but the predominant pathway appears to be an intermolecular pathway of dissociation from the mismatched primer terminus with rebinding of the DNA within the exonuclease active site (5). Such an arrangement makes intuitive sense because replicative polymerases have to copy much longer stretches of DNA than repair polymerases; reassembling the replication machinery every time a mispaired nucleotide must be excised is likely to be detrimental to the replication of the genome of an organism. A structural basis for the intramolecular pathway has been difficult to elucidate, as it requires a system that allows for visualization of both pre- and post-nu-

---

⁴ The abbreviations used are: pol, polymerase; β−, β hairpin loop deletion; g43, gene 43; gp43, gene product 43; PDB, Protein Data Bank; r.m.s., root mean square.
Role of the β Hairpin in RB69 gp43

A
![Diagram A]

B
![Diagram B]

FIGURE 1. Switching of the β hairpin loop in response to nucleotide incorporation opposite a lesion. A, the closed ternary complex of RB69 gp43 trapped with an incoming dTTP opposite a templating A (PDB code 1IG9 (17)). The 5′-end of the template DNA (gray) stacks against Trp-574 (gold), and the β hairpin is in an up position. B, an open binary complex of RB69 gp43 after successful incorporation of an A opposite a furan (PDB code 1RV2, chain C (15)). In this structure the 5′-end of the template is sandwiched between Phe-359 (purple) and the β hairpin, which is in a down position.

cleotide insertion events, as well as editing events. gp43, the DNA polymerase from bacteriophage RB69 (a homolog of T4, as described below), provides a unique opportunity to observe these different conformational states.

Several mutations in T4 have been shown to affect the ability of the polymerase to switch the primer strand between active sites (reviewed in Ref. 6) rather than influence the polymerization or exonuclease reactions. Of these, some are antimutators, such as the A737V substitution within the thumb domain of the enzyme (7). This mutation is proposed to cause the thumb domain to remain in a more “open” conformation, which would cause the primer to remain within the exonuclease active site for a longer period of time. This results in a polymerase that has more opportunity to degrade primers and is thus more likely to edit mistakes, albeit at the cost of editing correctly base-paired nucleotides. Other switching mutants display a mutator phenotype. Switching from the polymerase to the exonuclease active site is greatly reduced, which decreases the efficiency of the editing reaction. A common mutation pulled out of genetic selection experiments is the mutation encoding the G255S amino acid substitution in T4 (G258S in RB69) (8, 9). This single amino acid substitution leads to a higher phage mutation rate in replicating cells (8), and biochemical assays show that the exonuclease activity of the polymerase on single-stranded DNA is unaffected by the point mutation, but the rate of degradation of double-stranded DNA is reduced (9, 10). These results suggested a role for this amino acid in the formation of exonuclease complexes.

Structural studies of a T4 DNA polymerase fragment showed that the glycine residue at position 255 resides at the tip of a β hairpin loop within the exonuclease domain of the protein (11), but so far there is no structure of full-length T4 DNA polymerase in complex with DNA. The homologous polymerase from bacteriophage RB69, gp43, which shares 61% sequence identity with the T4 polymerase (12), has been crystallized in an apoform (13) and with both normal and damaged DNA (14–17). In the ternary complex of the polymerase with a chain terminated primer/template and an incoming dTTP, the fingers domain is closed, and the β hairpin segment is found in an “up” confor-

mation and does not associate with the DNA (Fig. 1A). The 5′ single-stranded template DNA in this structure stacks against Trp-574 at the junction of the palm and fingers domain (17). Similarly, in a binary complex of the polymerase bound to DNA containing a templating furan (a chemically stable abasic site analog (18)) and a chain terminated primer, the fingers domain is open, and the β hairpin is also in an up conformation (16) with no interaction with the single-stranded template DNA. Using an extendable primer, a binary polymerase-DNA complex was captured in which an A was incorporated opposite furan (15). In this structure, four discrete complexes were observed in the asymmetric unit of the crystal.

Collectively, the structural studies suggest that the β hairpin loop holds the template strand in place, while the primer strand unwinds from the template and moves toward the exonuclease active site. In an attempt to understand the role of this β hairpin segment in forming exonuclease complexes, we created a β hairpin loop deletion (β−) variant (I253G, Δ254−260) of RB69 gp43. Biochemical assays indicate that neither the polymerase nor exonuclease activities of the enzyme are affected by the deletion variant. Instead, it is the ability of the polymerase to remain associated with the DNA during formation of exonuclease complexes that is reduced in this variant, which in turn leads to a higher tolerance for the generation of errors. A structural analysis of this polymerase variant is consistent with the biochemical data, as it reveals a marked preference for keeping the primer DNA in the polymerase active site after a misincorporation event.

EXPERIMENTAL PROCEDURES

Enzymes and Oligonucleotides—Plasmids expressing full-length and exonuclease-deficient (D222A/D327A) RB69 g43 with a C-terminal His₅ tag were obtained from Dr. James Karam (Tulane University). The C-terminal His₅-tagged RB69 g43 β− (I253G, Δ254−260) construct was designed as follows. The cDNA for wild type RB69 pol from plasmid pRB.43 (19) was amplified by PCR and inserted into the appropriately restricted pET21 vector (Invitrogen), and DH5α cells were transformed with this plasmid. After isolation of the pET21 vector containing the RB69 g43 insert, the I253G replacement and the Δ254−260 deletion in the g43 insert were carried out using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA). The mutagenic oligonucleotide forward and reverse primers were 48 residues long and had 24 residues of the wild type
Role of the β Hairpin in RB69 gp43

RB69 gp43 flanking the site of the deletion, and residue 253 was changed from Ile to Gly. After transforming DH5α cells with the PCR product, plasmid p281 was purified and sequenced. The altered gp43 sequence was found to be correct and had no adventitious substitutions in the gp43 insert. BL21-DE3 cells were then transformed with this plasmid and the RB69 β-variant expressed after induction of the cells with isopropyl 1-thio-β-d-galactopyranoside. The exonuclease-deficient variant (D222A/D327A) of the β- polymerase was made using standard site-directed mutation techniques, and its sequence was checked in its entirety. All enzymes were purified according to a previously published protocol (20).

All oligonucleotides were purchased from The Midland Certified Reagent Co. (Midland, TX) and were purified on 16% (w/v) polyacrylamide gels containing 8 M urea and de-salted on a Sep-Pak cartridge. Duplexes were formed by mixing primer and a 20% excess of template in a buffer containing 10 mM Tris-HCl (pH 7.5) and 50 mM NaCl, heating to 90 °C for 5 min, and slowly cooling to room temperature. Primer strands for biochemical assays were labeled at the 5′-end with tetrachlorofluorescein fluorophore attached to the 5′-end with tetrachlorofluorescein for visualizing product bands. The template sequence was 5′-TTGGCGXXTTATGACGCCGGCG-3′, where the X was either a thymine or a furan. The furan residue is the chemically stable abasic site analog used in previous biochemical studies (20, 21) and structural (15, 16) studies. The primer strand sequences were 5′-GCCGCTGTCTACAAG-3′ for the properly paired primer/template and 5′-GCCGCTGTCTACAC-3′, 5′-GCCGCTGTCTACCC-3′, and 5′-GCCGCTGTCTCCCC-3′ for the single, double, and triple mismatches, respectively. As a control experiment, the same primers were annealed to the shorter 18-mer template used for crystallization experiments. The results from the kinetics experiments described below were indistinguishable between the longer 21-mer template and the shorter 18-mer template used for crystallization experiments (data not shown).

Polymerase and Exonuclease Assays—All time points from 6 ms to 8 min were taken using an RQF-3 rapid quencher apparatus (KinTek Corp., Austin, TX). For all reactions, syringe A contained 2000 nM enzyme and 500 nM DNA (either duplex DNA or single-stranded primer alone) in a buffer of 25 mM Tris acetate (pH 7.5), 50 mM NaCl, 1 mM 2-mercaptoethanol, and 0.5 mM EDTA. Syringe B contained 20 mM magnesium acetate and 250 μM dNTP in an equivalent buffer. For incorporation reactions opposite furan, 2 mM dATP was used as the incoming dNTP, and for exonuclease assays the dNTPs were omitted. Both halves of the reaction were pushed into the reaction chamber with the same buffer and were quenched by the addition of 500 mM EDTA. Ten microliters of each quenched reaction were mixed with 10 μl of formamide, and product bands were separated from the unreacted primers on 16% polyacrylamide gels with 8 M urea. Gels were scanned on a Bio-Rad molecular imager FX at the Alexa 532 setting to excite the tetra- chlorofluorescein fluorophore attached to the 5′-end of the primer DNA. Relative intensities of DNA bands were quantified using Quantity-One software (Bio-Rad), and all data were plotted using Prism 4 (GraphPad Software, San Diego, CA). The relative intensities of each band were calculated by dividing the intensity of the band by the total intensities of all bands at a given time point. For nucleotide incorporation assays, the percent incorporation was plotted against time and fit to a single exponential equation \( p = A(1 - e^{-kt}) \) to determine the observed rate constant. The results from the kinetics experiments described below served as control reactions

Heparin Trap—For single binding events, heparin (porcine intestinal mucosa, M, 3000; Sigma) was added to the Mg2+/dNTP half of the reaction at 2 mg/ml. Control reactions were run in which heparin was preincubated with the DNA and dNTPs, and the reaction was started by addition of enzyme. No products were observed in these reactions demonstrating the efficacy of the heparin trap (data not shown). For multiple binding events, heparin was omitted.

Crystallization—The C-terminal His6-tagged exonuclease-deficient (D222A/D327A), β-variant (I253G, D222A/D327A), and variant (D222A/D327A) was annealed to a complementary 14-mer oligonucleotide (5′-GCCGCTGTCTACAAG-3′). The polymerase (100 μM) was mixed with the annealed primer/template (100 μM) and 2 mM of dATP. Hanger drops were set up by mixing 0.5 μl of reaction mix with 0.5 μl of reservoir solution (9% (w/v) polyethylene glycol 2000 monomethyl ether, 100 mM sodium acetate, 125 mM magnesium sulphate, 100 mM HEPES (pH 7.1), 2 mM β-mercaptoethanol, and 14% (v/v) glycerol) and were equilibrated against 1 ml of reservoir solution at 20 °C. After about 10 days, prismatic monoclinic (P21) crystals grew to maximum dimensions of 120 × 80 × 80 μm3. Crystals were cryoprotected by increasing the concentration of glycerol and polyethylene glycol 2000 monomethyl ether to 17% (v/v) and 10% (w/v), respectively. The crystals were incubated in the cryoprotecting solution for 5 min prior to flash cooling in liquid nitrogen.

Data Collection—X-ray diffraction data to 2.4-Å Bragg spacings were measured at λ = 0.98 Å and at a temperature of 100 K at beamline ID23-D of the Advanced Photon Source, using a MAR m300 CCD detector. The data were processed and scaled using HKL 2000 (22). The unit cell parameters (a = 133.07 Å, b = 123.06 Å, c = 164.56 Å, and β = 96.78°) are very similar to those reported for our previously published structure (PDB code 1Rv2 (15)), and accordingly the crystal asymmetric unit contains four polymerase-DNA complexes. The data collection statistics are summarized in Table 1.

Structure Determination and Refinement—The structure was determined by using as a model our previously published RB69 gp43 structure devoid of all non-protein residues (PDB code 1Rv2 (15)). Structure refinement consisted of cycles of model building using COOT (23), followed by positional refinement, simulated annealing with torsion angle molecular dynamics, and individual B factor refinement all performed with CNS (24). The only amino acid residue in the disallowed...
event. Based on the structural evidence, we hypothesized that the polymerase switches active sites as the result of a misincorporation primer and template strands of duplex DNA when the polymerase complexes to be hindered by the deletion of the exonuclease active site. If this hypothesis was correct, we would expect the formation of an extended primer strand moved toward the exonuclease active site. Previous biochemical and structural work has suggested a role for the hairpin loop in separating the duplex DNA to the polymerase active site and extend the newly formed mismatched primer termi-

region of the Ramachandran plot is Thr-622, a residue that is always found in a strained conformation because of its proximity to two active site aspartates Asp-621 and Asp-623 (15, 17). Molecules A and B are complete (residues 1–903); in molecule C residues 900–903 are missing, and in molecule D residues Leu-538, Gln-539, and 899–903 are disordered. No density was always found in a strained conformation because of its proxim-

The results therefore support our hypothesis that the β hairpin loop is playing a direct role in maintaining the association between control experiments with an exonuclease-deficient mutant, we were unable to detect any extended mismatches at the dNTP concentration used (within a limit of detection of about 1 fmol of fluorescently labeled primer) demonstrating that excision of the mismatch must occur before the primer can be measurably extended (data not shown). Therefore, for both sets of measurements, only fully excised mismatches can be considered as extendable substrates. For a single mismatch any excision product of length n = 1 or shorter will result in extendable duplex DNA; for a double mismatch anything n = 2 or shorter is extendable, and for a triple mismatch products n = 3 or shorter are extendable, although in these experiments excision products shorter than n = 3 were not observed.

For the first measurement, the total amount of mismatched primer successfully excised and extended during a single binding event, dividing the total intensities of all the extended bands (n + x) plus the completely corrected primers (n – x, as described above) by the sum total of all bands (extended (n + x), excised (n – x), and original primer (n)), shows that there is a 2–3-fold decrease in the amount of excised and extended DNA between the wild type and β- variant polymerases for all three mismatch lengths (Fig. 2A; Table 2, 1st column). For the second measurement, the amount of extended primer relative to the amount of correctly excised primer, dividing the intensities of the extended bands (n + x) by the sum of the intensities of the extended and fully excised bands (n – x, as described above) shows no statistical difference between the two enzymes (Fig. 2A; Table 2, 2nd column). For both enzymes about 75% of all properly excised mismatches were extended. Because these experiments were performed in the presence of a heparin trap, the decrease in the total amount of mismatched primer that is processed by the polymerases is attributed to an increase in the dissociation of the β- variant from the DNA. The fact that the amount of extended primer as a proportion of the fully excised mismatch is the same for both enzymes suggests that they dissociate from the DNA at equal rates during the switch from the exonuclease back to the polymerase domain. Thus a phenotype for the β- variant is only observed when the polymerase must separate the duplex DNA and place the primer within the exonuclease active site. But once the primer is properly placed in the exonuclease active site and the mismatched primer terminus is removed, both polymerases are able to extend the primer DNA to the polymerase active site and extend the newly formed primer/template with equal efficiencies. Under conditions where the protein was free to dissociate and re-bind the DNA, i.e. in the absence of heparin, the excision of all three lengths of pre-formed mismatches followed by primer extension was indistinguishable between the wild type and β- variant (Fig. 2B). By the end of the time course, all of the originally mismatched primers had been corrected and fully extended. Under conditions of multiple association and disassociation events, the β hairpin loop deletion phenotype is not observed, but with single hit conditions, as described above, there is a clear distinction between the two polymerases in terms of how much mismatched primer can be corrected. The multiple turnover experiments therefore support our hypothesis that the β hairpin loop is playing a direct role in maintaining the association between

### TABLE 1
Data collection and refinement statistics

|                                | Furans-dAMP, β− variant |
|--------------------------------|--------------------------|
| **Data collection**            |                          |
| Space group                    | P2₁                      |
| **Cell dimensions**            |                          |
| a, b, c (Å)                    | 133.07, 123.06, 164.56   |
| β (°)                          | 96.78                    |
| Resolution (Å)                 | 50 to 2.37 (2.45 to 2.37) |
| R<sub>merge</sub> (%)          | 8.2 (41.0)               |
| I stalls (%)                   | 14.7 (2.72)              |
| Completeness (%)               | 96.8 (80.6)              |
| Redundancy (%)                 | 5.2 (3.5)                |
| **Refinement**                 |                          |
| Resolution (Å)                 | 50 to 2.37               |
| No. of reflections             | 1,063,368 (210,758)      |
| R<sub>work</sub>/R<sub>free</sub> (%) | 22.51/26.80               |
| No. of atoms                   | 32,472                   |
| Protein                        | 28,951                   |
| DNA                            | 2652                     |
| Water                          | 869                      |
| **Average B-factors (Å<sup>2</sup>)** |                        |
| Protein (A, B, C, and D)       | 55.3, 72.2, 51.5, 111.1  |
| DNA (A, B, C, and D)           | 94.6, 77.6, 59.5, 125.3  |
| Water                          | 54.2                     |
| **R.m.s. deviations**          |                          |
| Bond lengths (Å)               | 0.0065                   |
| Bond angles (°)                | 1.082                    |

<sup>a</sup> Highest resolution shell is shown in parentheses.
<sup>b</sup> The number of all reflections is shown. Unique reflections is in parentheses.

RESULTS

Formation of Exonuclease Complexes Is Affected by the β Hairpin Deletion—Previous biochemical and structural work has suggested a role for the β hairpin loop in separating the primer and template strands of duplex DNA when the polymerase switches active sites as the result of a misincorporation event. Based on the structural evidence, we hypothesized that the β hairpin loop was holding the single-stranded template DNA in the polymerase active site, while the incorrectly extended primer strand moved toward the exonuclease active site. If this hypothesis was correct, we would expect the formation of exonuclease complexes to be hindered by the deletion of the β hairpin loop. To test this hypothesis, we synthesized primers ending with one, two, or three mismatches at the 3′-end. In order for extension of the primer to occur, the mismatches must first be removed by the exonuclease activity of the enzyme, followed by switching the primer back into the polymerase active site and subsequent addition of nucleotides to the growing primer. A heparin trap was used to ensure that a single binding event occurs (3, 4), and the resulting partitioning assay can be used to measure the following: 1) the total amount of DNA processed (excision followed by extension), and 2) the amount of extension relative to the amount of excision. In contrast, we were unable to detect any extended mismatches at the dNTP concentration used (within a limit of detection of about 1 fmol of fluorescently labeled primer) demonstrating that excision of the mismatch must occur before the primer can be measurably extended (data not shown). Therefore, for both sets of measurements, only fully excised mismatches can be considered as extendable substrates. For a single mismatch any excision product of length n = 1 or shorter will result in extendable duplex DNA; for a double mismatch anything n = 2 or shorter is extendable, and for a triple mismatch products n = 3 or shorter are extendable, although in these experiments excision products shorter than n = 3 were not observed.

For the first measurement, the total amount of mismatched primer successfully excised and extended during a single binding event, dividing the total intensities of all the extended bands (n + x) plus the completely corrected primers (n – x, as described above) by the sum total of all bands (extended (n + x), excised (n – x), and original primer (n)), shows that there is a 2–3-fold decrease in the amount of excised and extended DNA between the wild type and β- variant polymerases for all three mismatch lengths (Fig. 2A; Table 2, 1st column). For the second measurement, the amount of extended primer relative to the amount of correctly excised primer, dividing the intensities of the extended bands (n + x) by the sum of the intensities of the extended and fully excised bands (n – x, as described above) shows no statistical difference between the two enzymes (Fig. 2A; Table 2, 2nd column). For both enzymes about 75% of all properly excised mismatches were extended. Because these experiments were performed in the presence of a heparin trap, the decrease in the total amount of mismatched primer that is processed by the polymerases is attributed to an increase in the dissociation of the β- variant from the DNA. The fact that the amount of extended primer as a proportion of the fully excised mismatch is the same for both enzymes suggests that they dissociate from the DNA at equal rates during the switch from the exonuclease back to the polymerase domain. Thus a phenotype for the β- variant is only observed when the polymerase must separate the duplex DNA and place the primer within the exonuclease active site. But once the primer is properly placed in the exonuclease active site and the mismatched primer terminus is removed, both polymerases are able to extend the primer DNA to the polymerase active site and extend the newly formed primer/template with equal efficiencies. Under conditions where the protein was free to dissociate and re-bind the DNA, i.e. in the absence of heparin, the excision of all three lengths of pre-formed mismatches followed by primer extension was indistinguishable between the wild type and β- variant (Fig. 2B). By the end of the time course, all of the originally mismatched primers had been corrected and fully extended. Under conditions of multiple association and disassociation events, the β hairpin loop deletion phenotype is not observed, but with single hit conditions, as described above, there is a clear distinction between the two polymerases in terms of how much mismatched primer can be corrected. The multiple turnover experiments therefore support our hypothesis that the β hairpin loop is playing a direct role in maintaining the association between
the polymerase and the DNA as the primer DNA unwinds and moves to the exonuclease active site.

The β Hairpin Loop Does Not Play a Role in Polymerase Activity—The polymerase activity of the enzymes, both with and without the β hairpin loop, was studied independently by changing two of the catalytic aspartates in the exonuclease active site (Asp-222 and Asp-327) to alanines. When the exonuclease-deficient enzymes were mixed with undamaged, correctly paired primer/template DNA and dNTPs, the rates of extension, as well as the total amount of extension, were equivalent between the two enzymes under single hit conditions (Fig. 3; Table 3). The observed rate constants for extension past the primer terminus were 166 s⁻¹ in the presence of the β hairpin loop and 155 s⁻¹ in the absence of the β hairpin loop. In the presence of the heparin trap, the intensities of the various extension lengths do not vary significantly between the two enzymes over several experiments. The $k_{obs}$ values are likely equivalent to $k_{pol}$ because the concentration of dNTP was about 6-fold greater than the $K_D$ value for dAMP incorporation opposite template T. These results indicated that the β hairpin loop plays no significant role in the rate of primer extension or the processivity of the enzyme as it extends a primer.

The β Hairpin Loop Does Not Play a Significant Role in the Exonuclease Reaction on Single-stranded DNA—Single-stranded DNA binds predominantly in the exonuclease active site and can therefore be used to study the exonuclease reaction separately from the polymerase reaction. Under single hit conditions, the rate constants were similar for both wild type polymerase and the β− variant (Table 3), and for the most part, neither enzyme was processive past one excision event on single-stranded DNA (Fig. 4A). The total amount of primer degraded by 1 base, however, was greater for

![Image](https://example.com/image.png)

**TABLE 2**

Proportion of extended versus unextended primers

For single (1C), double (2C), and triple (3C) mismatches, two values are given. The first is the sum of the intensities of the extended primer bands ($n + x$) and the intensities of the excised primer bands resulting in an extendable primer ($n - 1$ or shorter for a single mismatch, $n - 2$ or shorter for a double mismatch, and $n - 3$ for a triple mismatch) divided by the total intensities for all primer bands, including the original primer of length $n$. The second value is the intensities of all extended primers divided by the sum of the intensities of the various extension bands. These reactions were carried out under single hit conditions with both wild type (WT) and the β hairpin deletion (β−) variant of RB69 gp43.

|        | Extended/(extended + excised) | (Extended + excised)/total |
|--------|-------------------------------|---------------------------|
|        | ($n + x$)/($n + (n - 1) + (n - 2) + (n - 3)$) | ($(n + x) + (n - 1) + (n - 2) + (n - 3))/total$ |
| 1C     | WT 82 ± 0.5%                  | 83 ± 4%                   |
|        | β− variant 43 ± 4%            | 47 ± 2%                   |
| 2C     | WT 48 ± 2%                    | 51 ± 3%                   |
|        | β− variant 21 ± 11%           | 25 ± 14%                  |
| 3C     | WT 25 ± 2%                    | 27 ± 5%                   |
|        | β− variant 9 ± 5%             | 10 ± 1%                   |

![Figure 2. The β hairpin loop plays a role in the switch from the polymerase active site to the exonuclease active site.](https://example.com/figure.png)
the wild type polymerase. About 75% of the single-stranded DNA was cleaved by 1 base by wild type polymerase compared with 52% for the \( /H9252 \)/H91002 variant. This suggested that the dissociation constant for the \( /H9252 \)/H91002 variant had increased, and the polymerase was less stably bound to the single-stranded DNA. In multiple binding event reactions, the degradation of primer was similar for both enzymes with the wild type polymerase showing a \( k_{obs} \) of 31 \( s^{-1} \) and the \( /H9252 \)/H91002 variant 44 \( s^{-1} \) for excision of the 3'-terminal nucleotide residue (Fig. 4B; Table 3). These values are in keeping with the \( k_{exo} \) value of 24 \( s^{-1} \) determined previously for RB69 gp43 degrading duplex DNA containing four consecutive mismatches (26).

**TABLE 3**

| Rate constants were measured using four variants of the RB69 gp43 as follows: wild type polymerase (WT), \( /H9252 \) (I253G, \( /H9004 \)/D254–260), \( /H9252 \)/H91002 (D222A/D327A), and \( /H9252 \)/H91002. The polymerization and exonuclease rate constants (\( k_{obs} \)) were calculated as described under “Experimental Procedures.” pol represents extension past a properly paired primer/template under single hit conditions; F represents single incorporation of A opposite furan under multiple hit conditions, and \( /H9252 \)/H91002 represents rates of degradation of single-stranded primer under multiple hit conditions. Standard deviations were calculated from three independent experiments. |
|---|
| | \( k_{obs} \) (\( s^{-1} \)) |
| pol | WT exo | \( 166 \pm 8 \) |
| | \( /H9252 \)/H91002 | \( 155 \pm 10 \) |
| F | WT exo | \( 0.16 \pm 0.01 \) |
| | \( /H9252 \)/H91002 | \( 0.15 \pm 0.01 \) |
| exo | WT | \( 31 \pm 3 \) |
| | \( /H9252 \)/H91002 | \( 44 \pm 5 \) |

**FIGURE 3.** The \( /H9252 \) hairpin deletion does not affect polymerase activity under single hit conditions. For each reaction, 2000 nM polymerase was preincubated with 500 nM properly paired, undamaged primer/template DNA at 25 °C. Reactions were started by addition of 250 \( \mu M \) dNTP, 20 mM magnesium acetate, and 2 mg/ml heparin. Final concentrations were 1000 nM enzyme, 250 nM DNA, 125 \( \mu M \) dNTP, 10 mM magnesium acetate, and 1 mg/ml heparin. Reactions were quenched with 500 mM EDTA at the times indicated. The starting primer band is labeled \( n \). The graph is a plot of the intensities of the bands extended past the primer divided by the intensities of all bands against time and fit to a single exponential equation. WT, wild type; exo-, exonuclease-deficient.

**FIGURE 4.** The \( /H9252 \) hairpin deletion does not affect exonuclease activity on single-stranded DNA. A, single turnover reactions. For each reaction, 2000 nM polymerase was preincubated with 500 nM single-stranded primer DNA at 25 °C. Reactions were started by addition of 20 mM magnesium acetate and 2 mg/ml heparin. Final concentrations were 1000 nM enzyme, 250 nM DNA, 10 mM magnesium acetate, and 1 mg/ml heparin. Reactions were quenched with 500 mM EDTA at the times indicated. B, multiple turnover reactions. All reactions were performed as in A except that heparin was omitted. The starting primer band is labeled \( n \), and excision products are labeled as \( /H9252 \)/H91002, \( /H9252 \)/H91002, \( /H9252 \)/H91002, and \( /H9252 \)/H91002. The graph is a plot of the intensities of the remaining full-length primer divided by the intensities of all bands against time and fit to a single exponential equation. WT, wild type.
cleaved about 72% of the primer termini (Table 4). The rate constants for cleavage of the terminal primer nucleotide are, again, similar between the two enzymes (Table 4). For the triple mismatch, both enzymes were able to cleave nearly all of the primer termini in a single binding event (Fig. 5C), but the β− variant showed a slightly reduced rate constant for this reaction (Table 4).

For each of the mismatched duplexes, the amount of extendable product resulting from mismatch excision is an indicator of how well the mismatches are corrected by the two polymerases. As described for the partitioning assay (Fig. 2; Table 2), for a single mismatch, any degradation product equal to or shorter than \( n - 1 \) should result in a properly paired duplex that would be a suitable substrate for extension. For the double mismatch, \( n - 2 \) and \( n - 3 \) lengths are extendable, and for the triple mismatch, only the \( n - 3 \) length product is extendable. Standard deviations were calculated from three independent experiments.

Table 4
Observed first-order rate constants and cleavage products for exonuclease reactions on mismatched DNA duplexes

| Mismatch Length | WT          | β− variant | 1C | 2C | 3C |
|-----------------|-------------|------------|----|----|----|
|                 | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 | Experiment 5 |
|                 | 1C          | 2C         | 3C |
| k_{obs} (s⁻¹)   | % cleaved   | % (n - 1) + (n - 2) + (n - 3) formed |
| WT              | 32 ± 2      | 78 ± 1     | 76 ± 3 |
| β− variant      | 28 ± 4      | 37 ± 1     | 37 ± 4 |
|                 | 24 ± 1      | 95 ± 1     | 95 ± 4 |
|                 | 28 ± 1      | 72 ± 1     | 72 ± 4 |
|                 | 21 ± 1      | 97 ± 2     | 97 ± 4 |
|                 | 15 ± 5      | 92 ± 1     | 92 ± 4 |

The β− variant incorporates nucleotides opposite abasic sites more stably than the wild type protein. The previous data suggested that the β− variant would be more likely than wild type to incorporate nucleotides opposite a noninstructive lesion, in accordance with the mutator phenotype observed for the β hairpin mutant G255S in T4 (8). Incorporation reactions were therefore run in which an incoming nucleotide was added opposite a templating furan. Abasic sites are noninstructive lesions that are strong blocks to replication (27) and are likely to be mutagenic when bypassed (28, 29). These lesions are among the most prevalent within the genome, and this, combined with
Role of the β Hairpin in RB69 gp43

FIGURE 6. The β hairpin deletion allows for stable incorporation opposite a furan. A, multiple turnover reactions for incorporation of a dAMP opposite furan by wild type (WT) and β− variant polymerases. For each reaction, 2000 nM polymerase was preincubated at 25 °C with 500 nM primer/template DNA containing a furan residue in the templating position. Reactions were started by addition of 2 μM dATP and 20 mM magnesium acetate. Final concentrations were 1000 nM enzyme, 250 nM DNA, 1 mM dATP, and 10 mM magnesium acetate. Reactions were quenched with 500 mM EDTA at the times indicated. B, β hairpin deletion does not affect incorporation rates opposite furan. These reactions were run as in A except that exonuclease (exo−)−deficient polymerases were used. The graph is a plot of the intensities of the extended bands divided by the intensities of all bands against time and fit to a single exponential equation. For all reactions the starting primer band is labeled n, and the extension opposite furan is labeled +1 and indicated by the horizontal line.

ded by about 20 s whereas the and without the rate because exonuclease-deficient polymerases both with and without the β hairpin loop exhibited nearly identical values, 0.16 and 0.15 s−1, respectively, for incorporation of an A opposite furan (Fig. 6B; Table 3). Thus the stable incorporation of an A opposite furan in the presence of an active exonuclease site is most likely because of the reduced ability of the protein to switch a mistakenly extended primer into the exonuclease active site and a subsequent reduction in the idling turnover rate at the site of the lesion.

The Crystal Structure of the β− Variant Revealed That All Four Complexes Have DNA in the Polymerase Active Site—Previous structural work had shown that the β hairpin loop had moved in response to the incorporation of an A opposite a furan and was contacting the single-stranded template DNA upstream from the lesion (15) (Fig. 1). Because this arrangement was not seen in the ternary complex with normal DNA (17) nor in the binary complex with a furan in the templating position but no nucleotide incorporated opposite (16), we presumed that the β hairpin loop was, in effect, holding onto the template DNA, while the primer unwound to enter the exonuclease site. Therefore, crystallization studies were carried out to compare the position of the primer and template strands of DNA when the polymerase makes a mistake, and the β hairpin loop is either intact
Role of the β Hairpin in RB69 gp43

A β+, molecule B (exo)

B β+, molecule C (pol)

C β−, molecule B (pol)

D β−, molecule C (pol)

FIGURE 7. In the crystal structure of the β hairpin deletion variant, all four molecules have DNA in the polymerase active site. A and B, two of the four complexes found in the asymmetric unit of the previously solved furan-dAMP binary complex (PDB code 1RV2 (15)). One complex has DNA in the exonuclease active center (molecule B) (A); the other has DNA in the polymerase active site (molecule C) (B). C and D, the equivalent complexes from the structure of the β− variant inserting an A opposite furan. In this structure none of the four complexes within the asymmetric unit has DNA in the exonuclease (exo) domain. The polymerase domains are colored red for the palm, green for the thumb, blue for the fingers, cyan for the exonuclease (with the β hairpin in black), and orange for the N-terminal domain. The primer strand is shown in magenta and the template in dark blue. The tip of the β hairpin loop was disordered in A.

TABLE 5
Comparison of r.m.s. deviations (Å) between equivalent domains of the full-length and β− variant polymerases

|                | β+, molecule B (exo) vs. β−, molecule B (pol) | β+, molecule C (pol) vs. β−, molecule C (pol) |
|----------------|----------------------------------------------|-----------------------------------------------|
| N terminus     | 0.81                                         | 0.44                                          |
| Exonuclease    | 3.74                                         | 0.62                                          |
| Palm           | 0.33                                         | 0.25                                          |
| Fingers        | 1.72                                         | 0.71                                          |
| Thumb          | 6.43                                         | 0.33                                          |

olog in the crystal obtained with the β−, exo− variant (this study) occupies the same position in the crystal asymmetric unit, and in both cases, DNA is found in the polymerase active site (Fig. 7, B and D). Although the two molecules share much similarity, with r.m.s. deviations less than 0.7 Å (Table 5) for each domain (N-terminal, exonuclease, palm, fingers, and thumb), some differences are seen in the organization of the 5′-end of the template. The guanine at position 2 in the tem-

plate strand stacks with Phe-359 in both structures, but cytosine 1 in the β− complex lost its interaction with the β hairpin loop, more specifically with Ile-253 and Glu-219. Cytosine 1 stacks with Trp-574 instead. A comparable stacking arrangement was described in the ternary complex with normal DNA, where the β hairpin does not contact the 5′-end of the template strand (Fig. 1A) (17). The same observation could be made when molecules A from both structures were compared (low r.m.s deviations and similar stacking of the template base at the 5′ terminus). This was in contrast to what we observed when we compared molecules B from the full-length and β− complexes (Fig. 7, A and C). The differences between these complexes were significant. In the full-length enzyme the DNA is in the exonuclease active site, whereas in the β− variant the DNA stayed in the polymerase active site, and the r.m.s. deviations between the exonuclease domains and thumb domains are 3.7 and 6.4 Å, respectively (Table 5). The movements of the exonuclease and thumb domains appear to be required to allow the primer DNA to switch between active sites (14, 15, 17); thus large differences between the relative domain positions of the two molecules were not unexpected. In molecule B of the β− complex, we observed the same interactions between the 5′-end of the template with the polymerase as in molecules A and C.

Even though the protein-DNA complexes with and without the β hairpin loop crystallized under nearly identical conditions with similar unit cell parameters (15), deletion of the β hairpin loop caused differences in the composition of the crystal asymmetric unit. Stable positioning of the primer DNA within the exonuclease active site was prevented in the β− variant, and as a result, all four complexes had DNA in the polymerase active site. The duplex DNA formed after incorporation opposite a furan was distorted compared with standard B-form DNA, as observed in the previous structures with an A opposite furan (15). This distortion and the concomitant loss of minor groove contacts between the DNA and the polymerase were postulated to be the signal for the primer strand to switch active sites. In the β− structure, although the DNA is distorted in the vicinity of the polymerase active site, the primer strand does not switch and remains within the confines of the polymerase active site.
Role of the β Hairpin in RB69 gp43

DISCUSSION

The accurate replication of the genome involves the interplay between error avoidance and error correction mechanisms. If the wrong dNTP is incorporated across the templating base in the active site, the geometry of the nascent base pair will be distorted such that in-line attack by the 3′-OH of the primer onto the α-phosphate of the incoming dNTP cannot readily occur. If the incorrect base is incorporated, many replicative DNA polymerases have a built-in exonuclease activity that degrades DNA in a 3′→5′ direction to remove the misincorporated base. This activity is especially potent in the replicative DNA polymerases from bacteriophages T4 and RB69. The exonuclease activity increases fidelity of these polymerases by almost 2 orders of magnitude over the fidelity obtained by the polymerase active site alone and is itself almost 1000 times more rapid at degrading DNA than the exonuclease domain of the E. coli Klenow fragment, a repair DNA polymerase (26).

The partitioning assay described in Fig. 2 provided significant insight into the role of the β hairpin loop of the RB69 DNA gp43 exonuclease domain during proofreading. Because excision of the terminal mismatches must occur before extension, and because we used a heparin trap to sequester unbound polymerases, the difference observed between the wild type and β− variant polymerases demonstrates that in the absence of the β hairpin loop the polymerase is more likely to dissociate from the DNA when it attempts to unwind the primer/template duplex. Once the mismatched primer is corrected, however, the total amount of primer extended relative to the amount of primer properly corrected is indistinguishable between the two enzymes. Both enzymes extend about 75% of the mismatches that are corrected by the exonuclease activity of the enzymes (Table 2). These results clearly show that the β hairpin loop plays a role in keeping the polymerase associated with the DNA as the mismatched primer unwinds from the template and is bound within the exonuclease active site. But once excision occurs, the absence of the β hairpin loop has no effect on the amount of corrected primer that is returned to the polymerase active site for extension.

The role of the β hairpin loop in maintaining the association between the polymerase and DNA is also supported by the experiments shown in Fig. 5. As the length of the mismatch increases, the total amount of fully corrected primer decreases for both polymerases, and this reduction is greater for the β− variant. The amount of the terminal mismatched nucleotide cleaved, however, becomes indistinguishable between the two polymerases as the mismatch length increases. With a single mismatch, the β− variant cleaves significantly less of the primer terminus (Fig. 5A; Table 4). With three consecutive mismatches, however, the β− variant cleaves as much of the primer terminus as wild type (Fig. 5C; Table 4). The increase in total primer cleaved corresponds to the amount of DNA already unwound when the experiment begins. With a single mismatch, the polymerase is likely actively engaged in assisting in the unwinding of the primer, and during a single binding event, more of the wild type polymerases are able to separate the mismatched primer and bind it within the exonuclease active site than the β− variant, and thus more of the primer can be cleaved. With the triply mismatched duplex, however, the primer is likely to be in an already unwound state and bound in the exonuclease domain, thus relieving the polymerase of the requirement to actively separate the primer from the template such that the β hairpin deletion phenotype is not observed.

Single binding event experiments using a heparin trap also showed a reduced association between the β− variant and single-stranded DNA. With this substrate (Fig. 4A), both wild type and the β− variant polymerases are only able to efficiently cleave a single base during a single encounter with the DNA (less than 5% of the DNA was cleaved by more than one nucleotide), demonstrating that the single-stranded DNA does not readily translocate within the exonuclease active site. The total amount of single-stranded primer that is cleaved by the β− variant polymerases is about 25% less than for the wild type polymerase, so although we propose from the structural studies that the β hairpin loop is associating with the single-stranded template, it clearly has some influence on the ability of the polymerase to bind single-stranded primers within the exonuclease active site. It is plausible that the β hairpin loop helps maintain the primer DNA within the exonuclease active site during the editing reaction.

Whether the β hairpin loop plays a direct role in binding the template DNA, the primer strand, or both during formation of exonuclease complexes may be a matter of interpretation. In our previous structural work with an abasic site in the template, we observed an interaction between the β hairpin loop and the single-stranded template DNA as the primer DNA moved away from the polymerase active site (15), and another group (14) observed the β hairpin loop acting as a wedge to separate the primer and template strands. The experiments performed in this work, however, clearly show that the role of the β hairpin loop is to maintain the association between the polymerase and DNA when a mismatched primer must be bound within the exonuclease active site. All kinetic rates measured for wild type and β− variant polymerases are similar for the two enzymes, and it is only the total amount of mismatches that are properly processed that differs between the two enzymes. In addition, the partitioning assay in Fig. 2 and Table 2 suggests that the β hairpin loop could assist the polymerase in separating the primer strand from the template but would play no observable role in the return reaction. Unwinding 4 bp of the duplex DNA, as observed in the crystal structure of an editing complex (15), is energetically unfavorable, and assistance from the β hairpin loop may be required to achieve the switching efficiency required for accurate and processive DNA synthesis in response to an error. Presumably the free energy cost of unwinding is offset by the favorable free energy stabilization provided by the β hairpin loop. Reassociation of the duplex DNA after error correction is likely to be an energetically favorable reaction that does not require assistance from the β hairpin loop to efficiently return the primer 3′-end to the polymerase active site for continued extension.

The effect we observe upon deleting the β hairpin loop is not absolute; this variant is still able to process and extend mismatches in the presence of a heparin trap. We do, however, observe a 2–3-fold reduction in the ability of the polymerase to
remain associated with the DNA as it binds DNA in the exonuclease active site in response to an error at the primer terminus. This difference is likely to be important, as the hairpin loop appears to be ubiquitous in the B family DNA polymerases. All B family DNA polymerases whose structures have been solved to date show a similarly placed hairpin loop in the same orientation with respect to the polymerase and exonuclease active sites as in RB69 gp43 (Fig. 8A). These include the replicative DNA polymerases from bacteriophages T4 (11) (Fig. 8B) and φ29 (30) (Fig. 8C), herpes simplex virus (31) (Fig. 8D), the archaea Thermococcus sp. 9°N-7 (32), Pyrococcus kodakaraensis KOD1 (33), Thermococcus gorgonarius (34), Sulfolobus solfataricus (35) (Fig. 8E), and Desulfurocococcus tok (36) (Fig. 8F), as well as pol II from E. coli (PDB code 1Q8I) (Fig. 8G). Although all of the B family DNA polymerases appear to possess a similar hairpin loop located in the same position within their exonuclease domain, not all may play the same role as in RB69 gp43. The β hairpin in bacteriophage φ29, for example, is part of an extended β sheet that forms the floor of the exonuclease active site (Fig. 8C), whereas the β hairpin loop in organisms such as RB69 is separate from the active site β sheet and is free to move up and down (Fig. 1, A and B). The β hairpin loop in φ29 also interacts with a loop from the palm domain and is proposed to form part of the tunnel that wraps around the single-stranded template. Thus the φ29 β hairpin appears to be involved in strand separation of duplex DNA ahead of polymerization (30), whereas the β hairpin loop in RB69 gp43 is involved in DNA strand separation subsequent to a misincorporation event. In φ29 DNA polymerase, it is the thumb domain that appears to coordinate primer extension and proofreading (37).

The β hairpin loop from S. solfataricus is significantly shorter than that of RB69 (Fig. 8E), which would affect any putative role in separating the primer and template DNA. Although shorter loop regions are a hallmark of proteins of thermophilic origin,
all the other thermophiles whose structures are known have a β hairpin loop that is very similar in length to that of RB69 (compare the β hairpin loops in Figs. 8, F and A).

Although duplex DNA is similarly oriented with respect to the fingers, palm, and thumb domains in both A and B family DNA polymerases, their exonuclease domains are on opposite sides of the palm domain, implying that the two families are unlikely to use the same mechanism to separate the primer DNA from the template strand. Alignment of the exonuclease domains of RB69 (B family) and T7 (A family) DNA polymerases shows highly conserved active site architecture but, in place of a β hairpin loop, T7 has a helix, which has no association with the duplex DNA (Fig. 8H). This is not unexpected because the exonuclease domain of T7 DNA polymerase lies on the opposite side of the palm domain as compared with the B family DNA polymerases (38).

Structural studies have shown large movements of protein domains, especially of the exonuclease and thumb domains, that could allow for intramolecular switching of the primer DNA in both directions between active sites (14, 17, 37). Upon incorporation of a dAMP opposite a templating furan (15), two unique polymerase-DNA complexes with distorted DNA were observed along with movements of the exonuclease and thumb domains. In both of these post-insertion structures, the duplex DNA was still bound within the polymerase active site, but the primer DNA had pulled away from the catalytic residues. A third protein-DNA complex was also observed that had 4 bases of the primer separated from the template DNA and switched into the exonuclease active site. Taken together, the crystal structures of RB69 DNA polymerase before and after incorporation of an A opposite a furan suggest a plausible pathway along which the primer DNA could move from the polymerase to the exonuclease active site and back again without the need to dissociate from the polymerase. In addition to large domain rearrangements, a conformational change of the β hairpin loop of the exonuclease domain was also observed where the loop was contacting the single-stranded template DNA (15) (Fig. 1B). This interaction between the β hairpin loop and the template DNA was absent in the ternary complex of the polymerase incorporating a correct nucleotide (17) (Fig. 1A) and in the open binary complex with a furan in the templating position with no incoming nucleotide (16).

In our current structure of the β variant in complex with DNA containing a furan-dAMP mismatch, all four molecules in the crystal asymmetric unit show DNA residing in the polymerase active site. Despite nearly identical experimental conditions and crystal packing parameters, no editing complexes are observed as they had been when the full-length β hairpin loop was present (15). The duplex DNA, however, is still distorted from that seen in the ternary complex, which suggests that in the absence of the β hairpin loop the polymerase is unable to efficiently unwind the mismatched primer. Instead the duplex DNA remains primarily in the polymerase active site but in a distorted conformation that is not amenable to efficient extension. Based on the structural studies to date and this work, we propose that the role of the β hairpin loop may be to maintain the association between the polymerase and template DNA during formation of exonuclease complexes in response to error formation.

The use of the β hairpin loop to hold onto the single-stranded template during active site switching makes sense from a kinetic standpoint. Dissociation of the polymerase from the DNA will lead to a pause in replication and the potential disruption of the other elements associated with the complete replisome. The difference we observe in dissociation from the DNA between wild type and the β variant was about 2-fold, an advantage that may be sufficient to prevent excessive pausing when misincorporation events occur.

Acknowledgments—We thank Dr. Christopher Francklyn for use of the Kintek instrument; Stephen Corcoran, Dr. Ward Smith, and Dr. Ruslan Sanishvili for data collection assistance at the GM/CA CAT beamline at APS; Dr. Mark A. Roule for help with structure refinement; and Dr. Linda Reha-Krantz for critically reading the manuscript. GM/CA CAT has been funded in whole or in part by NCI Grant Y1-CO-1020 and NIGMS Grant Y1-GM-1104 from the National Institutes of Health. Use of the Advanced Photon Source was supported by the United States Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-ENG-38.

REFERENCES

1. Kunkel, T. A., Loeb, L. A., and Goodman, M. F. (1984) J. Biol. Chem. 259, 1539–1545
2. Drake, J. W. (1969) Nature 221, 1132
3. Reddy, M. K., Weitzel, S. E., and von Hippel, P. H. (1992) J. Biol. Chem. 267, 14157–14166
4. Donlin, M. J., Patel, S. S., and Johnson, K. A. (1991) Biochemistry 30, 538–546
5. Joyce, C. M. (1989) J. Biol. Chem. 264, 10858–10866
6. Reha-Krantz, L. J. (1998) Genetics 148, 1551–1557
7. Spaccapioli, P., and Nossal, N. G. (1994) J. Biol. Chem. 269, 438–446
8. Reha-Krantz, L. J. (1988) J. Mol. Biol. 202, 711–724
9. Stocki, S. A., Nonay, R. L., and Reha-Krantz, L. J. (1995) J. Mol. Biol. 254, 15–28
10. Marquez, L. A., and Reha-Krantz, L. J. (1996) J. Biol. Chem. 271, 28903–28911
11. Wang, J., Yu, P., Lin, T. C., Konigsberg, W. H., and Steitz, T. A. (1996) Biochemistry 35, 8110–8119
12. Wang, C. C., Yeh, L. S., and Karam, J. D. (1995) J. Biol. Chem. 270, 26558–26564
13. Wang, J., Sattar, A. K., Wang, C. C., Karam, J. D., Konigsberg, W. H., and Steitz, T. A. (1997) Cell 89, 1087–1099
14. Shamoo, Y., and Steitz, T. A. (1999) Cell 99, 155–166
15. Hogg, M., Wallace, S. S., and Doublet, S. (2004) EMBO J. 23, 1483–1493
16. Freisinger, E., Grollman, A. P., Miller, H., and Kiser, C. (2004) EMBO J. 23, 1494–1505
17. Franklin, M. C., Wang, J., and Steitz, T. A. (2001) Cell 105, 657–667
18. Takeshita, M., Chang, C. N., Johnson, F., Will, S., and Grollman, A. P. (1987) J. Biol. Chem. 262, 10171–10179
19. Bebenek, A., Dressman, H. K., Carver, G. T., Ng, S., Petrov, V., Yang, G., Konigsberg, W. H., Karam, J. D., and Drake, J. W. (2001) J. Biol. Chem. 276, 10387–10397
20. Hogg, M., Cooper, W., Reha-Krantz, L., and Wallace, S. S. (2006) Nucleic Acids Res. 34, 2528–2535
21. Berdis, A. J. (2001) Biochemistry 40, 7180–7191
22. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
23. Emmsley, P., and Cowtan, K. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 2126–2132
24. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta

Role of the β Hairpin in RB69 gp43
Role of the β Hairpin in RB69 gp43

Crystallogr. Sect. D. Biol. Crystallogr. 54, 905–921
25. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, Delano Scientific, San Carlos, CA
26. Wang, C. X., Zakharova, E., Li, J., Joyce, C. M., Wang, J., and Konigsberg, W. (2004) Biochemistry 43, 3853–3861
27. Schaaper, R. M., Kunkel, T. A., and Loeb, L. A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 487–491
28. Sagher, D., and Strauss, B. (1983) Biochemistry 22, 4518–4526
29. Boiteux, S., and Laval, J. (1982) Biochemistry 21, 6746–6751
30. Kamtekar, S., Berman, A. J., Wang, J., Lazaro, J. M., de Vega, M., Blanco, L., Salas, M., and Steitz, T. A. (2004) Mol. Cell 16, 609–618
31. Liu, S., Knafles, J. D., Chang, J. S., Waszak, G. A., Baldwin, E. T., Deibel, M. R., Jr., Thomsen, D. R., Homa, F. L., Wells, P. A., Tory, M. C., Poorman, R. A., Gao, H., Qiu, X., and Seddon, A. P. (2006) J. Biol. Chem. 281, 18193–18200
32. Rodriguez, A. C., Park, H. W., Mao, C., and Beese, L. S. (2000) J. Mol. Biol. 299, 447–462
33. Hashimoto, H., Nishioka, M., Fujiwara, S., Takagi, M., Imanaka, T., Inoue, T., and Kai, Y. (2001) J. Mol. Biol. 306, 469–477
34. Hopfner, K. P., Eichinger, A., Engh, R. A., Laue, F., Ankenbauer, W., Huber, R., and Angerer, B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3600–3605
35. Savino, C., Federici, L., Johnson, K. A., Vallone, B., Nastopoulos, V., Rossi, M., Pisani, F. M., and Tsernoglou, D. (2004) Structure (Lond.) 12, 2001–2008
36. Zhao, Y., Jeruzalmi, D., Moarefi, I., Lighton, L., Lasken, R., and Kuriyan, J. (1999) Structure (Lond.) 7, 1189–1199
37. Perez-Arnaiz, P., Lazaro, J. M., Salas, M., and de Vega, M. (2006) Nucleic Acids Res. 34, 3107–3115
38. Doublie, S., Tabor, S., Long, A. M., Richardson, C. C., and Ellenberger, T. (1998) Nature 391, 251–258
39. Humphrey, W., Dalke, A., and Schulten, K. (1996) J. Mol. Graphics 14, 27–38