Calf 5′ to 3′ Exo/Endonuclease Must Slide from a 5′ End of the Substrate to Perform Structure-specific Cleavage*

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Calf 5′ to 3′ exo/endonuclease, the counterpart of the human FEN-1 and yeast RTH-1 nucleases, performs structure-specific cleavage of both RNA and DNA and is implicated in Okazaki fragment processing and DNA repair. The substrate for endonuclease activity is a primer annealed to a template but with a 5′ unannealed tail. The results presented here demonstrate that the nuclease must enter the 5′ end of the unannealed tail and then slide to the region of hybridization where the cleavage occurs. The presence of bound protein or a primer at any point on the single-stranded tail prevents cleavage. However, biotinylation of a nucleotide at the 5′ end or internal to the tail does not prevent cleavage. The sliding process is bidirectional. If the nuclease slides onto the tail, later binding of a primer to the tail traps the nuclease between the primer binding site and the cleavage site, preventing the nuclease from departing from the 5′ end. A model for 5′ entry, sliding, and cleavage is presented. The possible role of this unusual mechanism in Okazaki fragment processing, DNA repair, and protection of the replication fork from inappropriate endonucleolytic cleavage is presented.

A 5′ to 3′ exonuclease purified from calf has been shown to cooperate functionally with calf DNA polymerase α to perform nick translation (Siegal et al., 1992). In this reaction, exonucleolytic activity on a downstream primer required synthesis from an upstream primer. The exonuclease was later found to be much more active on a nicked compared with a gapped double-stranded DNA substrate (Murante et al., 1994). This suggests that stimulation of nucleolytic activity by DNA synthesis resulted from continuous generation of the favored substrate.

The calf 5′ to 3′ exonuclease has been implicated in processing of Okazaki fragments (Turchi et al., 1994). A DNA template, primed with an oligoribonucleotide that had been extended at the 3′ end with DNA, was used as a model Okazaki fragment. Calf RNase H1 removed the initiator RNA with a 5′ to 3′ exonuclease then could remove the remaining ribonucleotide, creating a 5′ DNA. Extension of an upstream primer by polymerization in the presence of DNA ligase I allowed the two primers to be joined, as expected on the lagging replication fork strand in vivo.

In addition to the above activities, the 5′ to 3′ exonuclease could also cleave endonucleolytically (Murante et al., 1994). The substrate for this reaction was a DNA primer-template, having a downstream primer with a noncomplementary, unannealed region at the 5′ end. Additionally, an upstream primer was annealed directly adjacent to the first annealed nucleotide of the downstream primer. Endonucleolytic cleavage removed the unannealed region as an intact segment. Cleavage occurred either at the point of annealing to make a nicked substrate or one nucleotide downstream to make a 1-nucleotide gap. The 5′ to 3′ exonuclease domains of Escherichia coli DNA polymerase I and Thermus aquaticus (Taq) DNA polymerase (Yamichev et al., 1993) are functionally homologous to the calf nuclease. The homologous nuclease has also been purified from murine cells and designated cca nuclease (Goulian et al., 1990) and flap endonuclease (FEN-1) (Harrington and Lieber, 1994). The corresponding Hel a cell nuclease has been purified (Ishimi et al., 1988; Waga et al., 1994; Robins et al., 1994; Murray et al., 1994), and called maturation factor I (Waga et al., 1994) and DNase IV (Robins et al., 1994). The homolog of this mammalian protein has been identified in Saccharomyces cerevisiae and named the RTH-1 nuclease (Sommers et al., 1995; Johnson et al., 1995). Knockout mutants are temperature-sensitive for DNA replication and repair of methylmethane sulfonate lesions (Sommers et al., 1995). RTH-1 nuclease was also found to be necessary for the stability of DNA repeats in yeast, suggesting that mutation of the human counterpart could impair the mismatch repair pathway that protects from colorectal cancer (Johnson et al., 1995).

In this report, we explore the substrate structure requirements for endonuclease activity of the calf nuclease. We show that the nuclease must slide over the 5′ end of the unannealed tail of the primer and then along the single-stranded tail to the point of cleavage. The sliding action is bidirectional. The presence of a primer or protein on the tail prevents entry of the nuclease or traps a nuclease that is already on the tail. Cleavage is observed on tails with covalent adducts on nucleotides either at the 5′ end or within the tail. A model is proposed that has implications for the biological role of this unusual nuclease.

**Experimental Procedures**

Materials—Unlabeled deoxyribonucleotides and cloned E. coli single-stranded binding protein were purchased from Pharmacia Biotech Inc. Radiolabeled nucleotides (3000 mCi/mmol) were purchased from DuPont NEN. Biotinylated oligonucleotides were synthesized by the University of Rochester Core Nucleic Acid Laboratory. Other synthetic DNA oligonucleotides were obtained from Genosys (The Woodlands, TX). Sequenase® (version 2.0) and T4 polynucleotide kinase were from U.S. Biochemical Corp. Proteinase K was obtained from Boehringer Mannheim. All other reagents were from Sigma unless otherwise noted.

The calf 5′ to 3′ exonuclease was purified through hydroxyapatite chromatography as described previously (Murante et al., 1994). Final specific activity was 180,000 units/mg, with one unit defined as the amount of exonuclease required to release 1 pmol of [32P]TMP from

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The abbreviations used are: SSB, single-stranded binding protein.

TABLE I
Oligonucleotide sequences

| Templates | Primers | Oligonucleotide sequences |
|-----------|---------|--------------------------|
| T1 (44-mer) | | GCACTGGCCGTCGTTTTACGGTGACTGGGAAA ACCTCGGG |
| T2 (49-mer) | | GCCCAAGTCAGCTGTGTTAAGACGGTGTGACTG |
| Upstream Primers | U1 (24-mer) | GCCAGGGTTTTCGAGTGTCAGCA |
| | U2 (25-mer) | GCCAGGGTTTTCGAGTGTCAGACCA |
| | U3 (25-mer) | GCCAGGGTTTTCGAGTGTCAGACCA |
| | U4 (26-mer) | GCCAGGGTTTTCGAGTGTCAGACCA |
| | U5 (18-mer) | TTCCACATCACGGACCTT |
| Downstream Primers | D1 (18-mer) | GTAAAACGACGGCCAGTG |
| | D2 (30-mer) | TCAGTGCTGCAAGTAAAACGACGGCCAGTG |
| | D3 (43-mer) | CGTACGGAGCTAGCTTTCCAGTAAGAAAGCGACCAGCCAGTG |
| | D4 (96-mer) | CGACTTAGAGTACCCGGGTACCGAGCTGAAAT |
| | D5 (33-mer) | TCGCCCGTTTCGAGCTGTAGTTAAATCTGCCG |
| Blocking Primers | B1 (18-mer) | CAGCTCTAGTCGCTAGC |
| | B2 (20-mer) | CGGGGAGCTCTAGATCG |
| | B3 (21-mer) | GGGGAAATCGCGGTCGTAAGC |
| | B4 (23-mer) | ACGGGCATGTAATTAAGCAG |

5′-[32P]dUTP d16dA2000, in 15 min at 37°C.

DNA Substrates—According to the manufacturer’s protocol, T4 polynucleotide kinase was used to 5′-phosphorylate the specified oligonucleotides with either γ-[32P]-radiolabeled ATP or unlabeled ATP. Oligomer sequences are listed in Table I, and primer-template substrates were constructed as described in the figure legends. Annealed downstream primers were sometimes radiolabeled by the addition of a single 5′-terminus using [α-32P]dCTP and 10 units of Sequenase.

Enzyme Assays—The standard nuclease reaction was described previously (Murante et al., 1994) and contained 60 μl BisTris, pH 7.0, 5% glycerol, 100 mg/ml bovine serum albumin, 5 mM β-mercaptoethanol, and 5 fmol of substrate in a volume of 25 μl. Reactions were initiated by the addition of 2.25 units of nuclease and incubation at 37°C. For exonuclease assay, proteinase K (14.3 μl, respectively). Each of the four different substrates in Fig. 3 were prepared as described by Ishimi et al. (1993). The authors proposed this effect with a substrate having a tail that was 25 nucleotides in length with the blocking primer annealed to the 5′-most 17 nucleotides of the tail, leaving a single-stranded gap of 8 nucleotides between the 3′ end of the blocking primer and the expected site of cleavage. Thus, the blocking primer did not need to be juxtaposed to the cleavage site. Similar results were obtained when examining the endonucleolytic activities of the nuclease domains of E. coli DNA polymerase I and Taq polymerase by Lyamichev et al. (1993). The authors proposed that these bacterial enzymes must track or slide along the single-stranded tail to the point of cleavage.

RESULTS
Activity of the 5′ to 3′ Exo I Endonuclease on Primers Either Fully Annealed or with Tails—Rates of exonuclease versus endonuclease cleavage were compared on substrates with either a fully annealed downstream primer or with a 5′ tail (Fig. 1). Measurement of these activities was also performed on substrates in which the 5′ terminus of the downstream primer was either hydroxyl-terminated or phosphorylated. Overall, endonuclease cleavage of tailed primers was as much as 4-fold more efficient than exonuclease cleavage of fully annealed primers. Cleavage of tailed primers was slightly more rapid in the presence of a hydroxyl group at the 5′ end versus phosphate (8.6 and 6.8% of initial substrate cleaved per min, respectively). In contrast, the 5′ moiety present on the fully annealed downstream primer had a substantial effect on its efficiency as a substrate. The phosphorylated substrate was digested at an initial rate of 2.2% cleaved per min. However, essentially no degradation (0.17% cleaved per min) occurred when the 5′ end was hydroxyl-terminated. Rates became nonlinear over the duration of the time course, indicating a loss of enzyme activity.

Primers Bound to the 5′ Tail Inhibit Cleavage by 5′ to 3′ Exo I Endonuclease—Fig. 2A shows that the presence of a primer annealed to the 5′ tail blocks cleavage. Harrington and Lieber (1994), working with the human enzyme FEN-1, reported this effect with a substrate having a tail that was completely double-stranded. The tail of our substrate was 25 nucleotides in length with the blocking primer annealed to the 5′-most 17 nucleotides of the tail, leaving a single-stranded gap of 8 nucleotides between the 3′ end of the blocking primer and the expected site of cleavage. Thus, the blocking primer does not need to be juxtaposed to the cleavage site. Similar results were obtained when examining the endonucleolytic activities of the nuclease domains of E. coli DNA polymerase I and Taq polymerase by Lyamichev et al. (1993). The authors proposed that these bacterial enzymes must track or slide along the single-stranded tail to the point of cleavage.

To determine whether the calf nuclease slides along the 5′...
tail, we employed a substrate with a 73-nucleotide tail. The longer single-stranded region allowed us to position primers at three distinct locations along the tail: primer B2 was annealed at the 5' end, primer B3 was annealed in the middle, and primer B4 was juxtaposed to the site of cleavage. Fig. 2B shows a time course experiment in which the nuclease was able to cleave off the 73-nucleotide tail in the absence of a blocking primer (lanes 1–6). When the 5' end of the tail was blocked with Primer B2, essentially no cleavage was observed (Fig. 2B, lanes 7–12). In this case, the single-stranded region of the tail between the 3' end of the blocking primer and the site of cleavage was 53 nucleotides compared with only 8 nucleotides with the first substrate tested. It would seem that the nuclease cannot enter in the middle of the tail, even if a long single-stranded region is available. Primer B2 forms a duplex up to the very 5' end of the tail. Its blocking capability implies that either the nuclease must enter at the 5' end of the single-stranded region of the tail or must recognize the 5' end of the tail before it can enter at another location.

Distinguishing a Sliding versus Looping Mechanism for Cleavage—An alternative to the sliding or tracking of the enzyme along a single-stranded tail is that the enzyme need only bind or associate with the 5' end of the tail to be activated. In the activated state, it could then bind directly to the point of cleavage and commence catalysis (see Fig. 8A). If after activation it remained bound to the 5' end of the tail, it could carry the 5' end back to the point of cleavage. The 5' end of the tail would then be looped back to the cleavage point just prior to release of the cleaved tail. If the cleavage mechanism involved an activation or looping process, primers B3 and B4 should not have the same inhibitory effect as primer B2. However, cleavage of substrates having these primers was inhibited (see Fig. 3A, lanes 5 and 7) just as efficiently as cleavage of the substrate with primer B2. Additionally, the presence of a primer with no complementarity to the tail (Fig. 3A, lane 1) did not stop cleavage. These results support a mechanism in which the nuclease enters at the 5' end and requires the entire length of the tail to be single-stranded to allow the enzyme a passage to the site of cleavage (see Fig. 8B).

Trapping the Nuclease on the Tail with Primers—The even lanes in Fig. 3A show reactions in which the substrate was preincubated with nuclease prior to the annealing of a blocking primer B1 anneals to the 5' end of D3 and leaves a single-stranded gap of 8 nucleotides between itself and the point at which the tail anneals to the template. A time course with substrate lacking a blocking primer is shown in lanes 1–6; substrate with the blocking primer is in lanes 7–12. B shows nuclease activity on a substrate with a 73-nucleotide tail (D4:U2:T2). Primer B2 hybridizes to the 5' tail at a position that generates a single-stranded gap of 53 nucleotides. Lanes 1–6 show a time course with substrate lacking a blocking primer; lanes 7–12 have substrate with the blocking primer. Substrate structures and oligonucleotide composition are shown above the appropriate lanes. Downstream primers D3 and D4 were 5' radio-labeled, and reaction conditions were as described under “Experimental Procedures.” Size markers at the left indicate product length in nucleotides.

![Graph](http://www.jbc.org/)

**Fig. 1.** Measurement of rates for exonucleolytic and endonucleolytic degradation of either 5' phosphorylated or nonphosphorylated downstream primers. Rates of exonucleolytic and endonucleolytic activity were determined using substrates pictured in the graph inset. The exonuclease substrate (I) consisted of primers U2 and D1 annealed directly adjacent to each other on template T1. See Table I for the key to oligonucleotide sequences in this and subsequent figures. The endonuclease substrate (II) had an unannealed 5' tail of 12 nucleotides that formed when primer D2 was annealed downstream of U2 on T1. Downstream primers D1 and D2 were 3' end 32P-labeled. Assays were performed as described under “Experimental Procedures” with 5 fmol of substrate and 2.25 units of calf 5' to 3' nuclease per reaction. In the graph, plots of exonucleolytic activity (circles) were derived from calculations of percent substrate degraded by a single nuclease; endonucleolytic activities (triangles) were based on the amount of substrate cleaved to products 18 or 19 nucleotides long from the appropriate substrate-D3 and leave a single-stranded gap of 8 nucleotides between itself and the point at which the tail anneals to the template. A time course with substrate lacking a blocking primer is shown in lanes 1–6; substrate with the blocking primer is in lanes 7–12. B shows nuclease activity on a substrate with a 73-nucleotide tail (D4:U2:T2). Primer B2 hybridizes to the 5' tail at a position that generates a single-stranded gap of 53 nucleotides. Lanes 1–6 show a time course with substrate lacking a blocking primer; lanes 7–12 have substrate with the blocking primer. Substrate structures and oligonucleotide composition are shown above the appropriate lanes. Downstream primers D3 and D4 were 5' radio-labeled, and reaction conditions were as described under “Experimental Procedures.” Size markers at the left indicate product length in nucleotides.
Experimental Procedures. No prebinding (primer and the addition of Mg²⁺) by trapping the enzyme on the 5’ end and primers are indicated as in gel analysis was used to detect enzyme-substrate complexes. Preincubation and primers have mobilities is shifted than that observed without any blocking primer. We interpret this result as a trapping of the nuclease on the substrate, with it caught on the single-stranded region between the blocking primer and the cleavage site. This result also indicates that movement of the nuclease on the tail is bidirectional, with nuclease molecules continuously sliding onto and off of the 5’ end of the tail.

Primer B4 is not able to trap the nuclease, possibly because it does not leave any single-stranded space for the enzyme to occupy. This interpretation implies that the binding of primer B4 forces bound nucleases to move in the direction of the 5’ end of the tail, where they fall off. Alternatively, the primer induces direct dissociation of the nuclease from the region of the tail that is complementary to primer B4. Interestingly, a 33-nucleotide region of the tail between the 5’ end and the primer remained available to bind the nuclease after primer B4 was annealed, but no shift was observed. This suggests that the nuclease has greater affinity for the cleavage site than for various positions along the tail.

Effect of Single-stranded Binding Protein on Endonuclease Activity—Single-stranded DNA is generally coated with protein in vivo (Kornberg and Baker, 1992). SSB from E. coli can bind single-stranded segments as short as 4 nucleotides (Ruyechan and Wetmur, 1976) and reportedly functions to stimulate both DNA replication and repair. We therefore examined the effect that SSB has on the endonucleolytic capacity of the calf exonuclease. In an experiment depicted in Fig. 4, endonuclease cleavage of substrates with 5’ tails either 12 or 25 nucleotides in length was tested. Cleavage of the shorter
tailed substrate was not affected by SSB in the reaction (Fig. 4, lane 2), whereas a significant inhibition resulted if the substrate had a 25-nucleotide tail. These results show that protein bound to the tail can inhibit cleavage. Possibly the nuclease can compete successfully with the SSB for binding if the tail is sufficiently short.

SSB can unwind duplexed DNA through cooperative binding of additional SSB molecules. In our experiment, it was possible that SSB displaced the oligomer with the 25-nucleotide tail from the substrate, disrupting the recognition site for cleavage. We therefore sampled each of the reactions before the addition of nuclease and performed gel shift analysis on a native gel. As seen in lanes 2 and 5 of Fig. 4B, the mobility of the different substrates was shifted by SSB. When samples containing SSB were treated with proteinase K, along with a 1000-fold excess of unlabeled primer strand, the SSB was digested away, and any displaced labeled strands were unable to reanneal. Lane 3 of Fig. 4B shows that almost all of the short tailed substrate was still present as an annealed primer-template after proteinase K treatment, but less than half of the longer tailed substrate could be recovered as primer-template in lane 6 of Fig. 4B. The displacement of the tailed strand from the second substrate would decrease the concentration of substrate in the primer-template configuration and result in a smaller amount of cleavage product. Nevertheless, inhibition of cleavage was very effective and could not be accounted for solely by this strand displacement phenomenon.

Because all of the longer tailed substrate was shifted by SSB and yet some cleavage product could still be detected (Fig. 4A, lane 4), the nuclease may either have some capacity to act on tails bound by protein or be able to displace SSB even when it is bound to the longer substrate. With the shorter tailed substrate, although a majority of the substrate was bound by SSB, we were unable to obtain a complete shift. A time course experiment measuring cleavage in both the presence and absence SSB revealed very similar rates; this also supports the conclusion that the nuclease can cleave a substrate when SSB is bound to or equilibrating with the tail.

Effects of Biotin Adducts and Streptavidin on Endonucleolytic Activity—To avoid the complication of strand displacement associated with SSB, we attempted to inhibit the nuclease by using substrates with primers having a biotinylated base at a selected position, either at the 5’ end or middle of the downstream primer. Quantitative biotinylation of the substrate is evident because the biotinylation changes the gel mobility of the substrate. Streptavidin is a 60-kDa protein that recognizes and tightly binds biotin (Green and J oynson, 1970). Substrates were incubated with streptavidin in 25-fold molar excess, resulting in quantitative gel shifts of the biotinylated substrates (data not shown). This demonstration that protein was bound to all of the biotinylated oligonucleotides. In Fig. 5, the nuclease is shown to cleave 5’ tails with biotin added at either the 5’ terminus or at the mid-point of the tail (lanes 9–12 and 17–20, respectively) or on the upstream primer (data not shown). This demonstrates that the nuclease can traverse a nucleotide in which the mass of the base has been increased by about a factor of four by the biotin and linking moiety.

When streptavidin was allowed to bind to the biotinylated substrates prior to addition of the nuclease, no cleavage was observed with substrates that were biotinylated at either of the two downstream primer sites (Fig. 5, lanes 13–16 and 21–24). Cleavage still occurred on the substrate with a biotinylated upstream primer (data not shown). These results indicate that although an adduct larger than the base can be passed, when the adduct is protein sized, the sliding mechanism is thwarted.

Analysis of the Upstream Primer-dependent Specificity of Endonucleolytic Cleavage—Previously, we observed that a primer placed immediately upstream of the annealed portion of the downstream primer resulted in an increase in either exonuclease or endonuclease activity. Here we have attempted to determine whether the 3’ OH group on the upstream primer is a structural feature that is essential for the stimulation to occur. Substrates were prepared having either a hydroxyl or hydrogen present on the 3’ carbon of the upstream primer. As illustrated in Fig. 6, the lack of a hydroxyl group does not significantly reduce cleavage after the first annealed nucleotide. What we observe instead is the complete absence of the minor product, a 12-nucleotide fragment resulting from some cleavage occurring 5’ of the first annealed nucleotide. This surprising result shows that although the 3’ OH of the up-

**Fig. 5. Effects of biotin adducts and streptavidin binding on endonucleolytic activity.** Time course experiments on substrates that were preincubated with or without streptavidin are shown. Substrate structure, nucleotide position of biotin substitutions, and complexes formed with streptavidin are depicted above the autoradiograph. Biotin (b) was covalently bound to the 1st and 7th nucleotides from the 5’ end of the downstream primer. Streptavidin, in a 25-fold molar excess, was preincubated with 5 fmol of substrate for 5 min at 37 °C in 1 x binding buffer (12% glycerol, 12 mM HEPES-NaOH, pH 7.9, 4 mM Tris-Cl, pH 7.9, 60 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol) (lanes 5–8, 13–16, and 21–24). Endonuclease reactions were then initiated by the addition of purified calf 5’ to 3’ exonuclease according to “Experimental Procedures.” Lanes 1–8, nonbiotinylated downstream primer; lanes 9–16, biotin-substituted primer at position 1; lanes 17–24, biotin-substituted primer at position 7.
stream primer is not essential to stimulate cleavage, it influences the positioning of the cut. Stimulation of activity may lie in the ability of the enzyme to sense uninterrupted base pairing associated with nick-like structures.

Because the 3' hydroxyl was not responsible for stimulation dependent on nicked structure, we next examined the importance of base pairing of the upstream primer near the cleavage site. In Fig. 7, the left panel shows the structure and sequence of the substrates tested. The right panel illustrates the cleavage products generated after digestion with the calf nuclease for 20 min at 37°C. In the absence of upstream primer (Fig. 7, right, lane 2), little activity was observed. The major and minor products obtained from the substrate with a fully annealed upstream primer correspond to the positions described earlier. When the terminal 3' nucleotide of the upstream primer formed a 1-nucleotide mismatched tail, the cut was primarily before the first annealed nucleotide (Fig. 7, right, lane 4). Unlike previous results, the 1-nucleotide gap between the annealed portions of the upstream and downstream primer formed by this 3' mismatch caused more rather than less cleavage. If the annealed regions of the upstream and downstream primers were directly adjacent but an extra mismatched nucleotide formed a 3' tail on the upstream primer, the downstream primer was cleaved after its first annealed nucleotide (Fig. 7, right, lane 5). Finally, an upstream primer with a 3' nucleotide tail and a 1-nucleotide gap before the downstream primer generated the longest cleavage product. Though this primer was only a single nucleotide longer than the primer used in lane 5 (Fig. 7, right), the cut was positioned 2 nucleotides downstream.

**DISCUSSION**

The calf 5' to 3' exo/endonuclease is a member of the RTH-1 class of eukaryotic nucleases that perform structure-specific cleavage of DNA and have important roles in DNA replication and repair. The substrate for endonuclease activity is a primer-template in which the primer has a noncomplementary, unannealed 5' end region. Here we show that in order to catalyze cleavage, the nuclease must slide over the 5' end of the unannealed tail, move down the length of the tail to the point of annealing with the template, and then carry out cleavage. The sliding process is bidirectional, such that nucleases can also slide off of the tail at the 5' end. Evidence is also presented that the nuclease binds with highest stability to the point of cleavage. The presence of biotin adducts on the tail do not affect either recognition of the 5' end or the sliding process. However, protein bound to the tail, either SSB or streptavidin, has an inhibitory effect on cleavage, presumably by blocking the movement of the nuclease to the site of cleavage. The steps taken by the nuclease to carry out this process are depicted in Fig. 8.

Biochemical and genetic evidence suggests that nucleases of this class are components of the DNA replication machinery. Studies of simian virus 40 DNA replication in vitro indicated the requirement of the human 5' to 3' exo/endonuclease in the removal of the RNA primer before joining of viral lagging strand DNA segments (Ishimi et al., 1988; Waga et al., 1994). The mouse nuclease was able to partially remove RNA from initiator RNA primers made and elongated with DNA by DNA polymerase α in vitro (Goulian et al., 1990). Using a model Okazaki fragment substrate consisting of an initiator RNA elongated by DNA and annealed to a template, we showed that two nucleases can effect complete RNA removal (Turchi et al., 1994). Mammalian RNase H1 cleaves off the initiator RNA as an intact segment, leaving only a single ribonucleotide at the RNA-DNA junction (Turchi et al., 1994; Huang et al., 1994). The 5' to 3' exo/endonuclease can then remove the last ribonucleotide. Null mutants of the counterpart nuclease in S. cerevisiae, the RTH-1 nuclease, are temperature-sensitive for DNA replication (Sommers et al., 1995). The appearance of dumbbell-shaped cells with a nucleus at the isthmus as the terminal phenotype is characteristic of mutations in DNA polymerases δ.
and ε (reviewed in Bambara and Huang (1995)). The RTH-1 mutation causes hyper-recombination, symptomatic of the presence of long-lived breaks in the chromosome (Sommers et al., 1995). These would be expected if joining of Okazaki fragments was delayed by the mutation.

The sensitivity of the RTH-1 mutants to damage by the agent methylmethane sulfonate (Sommers et al., 1995), which adds to bases, suggests that the RTH-1 class of nuclease participate in repair of base damage. The demonstration that null mutants in RTH-1 increase the instability of simple repetitive DNA indicates a role for this nuclease in the mismatch repair pathway (Johnson et al., 1995). Mutations in the mammalian mismatch repair pathway are associated with colorectal cancers (Reenan and Kolodner, 1992; Prolla et al., 1995). This suggests that the human RTH-1 counterpart applies its unique cleavage specificity for repair surveillance that prevents colorectal cancer.

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Fig. 8. Models of nuclease binding to tailed substrates. A, looping model. The enzyme binds at the cleavage site but must also interact with the 5′ terminus of the single-stranded tail for cleavage to occur. B, threading model. The enzyme must bind and enter at the 5′ terminus of the single-stranded tail (I). Movement of the enzyme on the tail is bidirectional (II). Cleavage can occur when the enzyme slides down to the point at which the tail is annealed to a template (III).
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