A Critical Role of the 3’ Terminus of Nascent DNA Chains in Recognition of Stalled Replication Forks*[§]

Toshimi Mizukoshi§§, Taku Tanaka§§, Ken-ichi Arai**, Daisuke Kohda‡‡, and Hisao Masai§§

From the Department of Structural Biology, Biomolecular Engineering Research Institute, Suita, Osaka 565-8874, Japan, the Department of Cell Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo 113-8613, Japan, the Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

Arrest of replication forks by various internal and external threats evokes a myriad of cellular reactions, collectively known as DNA replication checkpoint responses. In bacteria, PriA is essential for restoration of stalled replication forks and recombinational repair of double-stranded DNA breaks and is a candidate sensor protein that may recognize arrested forks. Here, we report that PriA protein specifically recognizes 3’ termini of arrested nascent DNA chains at model stalled replication forks in vitro. Mutations in the putative “3’ terminus binding pocket” present in the N-terminal segment of PriA result in reduced binding to stalled replication fork structures and loss of its biological functions. The results suggest a mechanism by which stalled replication forks are recognized by a sensor protein for checkpoint responses.

Progression of replication forks is stalled by a variety of internal and external causes, including DNA damage and depletion of precursors for DNA synthesis. The arrested replication forks elicit checkpoint responses that enable cells to repair damage, restore replication forks, and restart DNA replication (1, 2). A number of eukaryotic gene products have been identified that participate in this process. The initial phase of this response is the recognition of the arrested forks by a sensor protein, which may be recruited to the site of the fork arrest and transduce various signals for further downstream cellular responses. However, the nature of the protein that detects the stalled replication forks and the mechanism by which they are recognized are still unclear.

In Escherichia coli, PriA, a DEXH-type DNA helicase originally discovered as a protein essential for replication of a small single-stranded phage DNA, dX174, is believed to play a key role in rescuing the stalled replication forks (1, 2–5). The priA null cells display numerous phenotypes, including low viability and sensitivity to DNA damaging agents such as UV, γ-ray, and mitomycin C (6–10). These defects may reflect a critical role played by PriA in resumption of DNA replication after arrest of ongoing replication forks (11). In E. coli, in fact, arrest of ongoing replication forks induces a recombination-dependent mode of DNA replication, known as inducible or constitutive stable DNA replication (iSDR) or cSDR, respectively, which requires PriA protein (8). It was shown that the dX174-type primosome could be assembled on a model D-loop structure in vitro (12, 13). It has been proposed that PriA may recognize recombination intermediates generated as a result of fork arrest as well as the arrested replication fork per se and facilitate reassembly of replication forks (8, 14, 15). Consistent with this, PriA recognizes and binds specifically to DNA structures such as those mimicking D-loop (intermediates of homologous recombination reactions) or arrested replication forks (16–18). However, it has not been known what structural features of D-loop or arrested DNA replication fork structures are recognized by PriA. In this report, we show that PriA recognizes the 3’-end of DNA and have identified critical residues for this recognition within the N-terminal DNA binding domain. We propose the presence of a 3’ terminus binding pocket on PriA protein, which plays a critical role in detecting stalled replication forks.

EXPERIMENTAL PROCEDURES

Purification of Protein Samples—Wild-type PriA-(1–181) and mutant PriA-(1–181) were expressed and purified as previously described (19). PriA-(1–105) was prepared in the same manner as PriA-(1–181). The N-terminal histidine tag of these proteins was removed by thrombin treatment (1:100, w/w) for Biacore and NMR analyses. Uniformly 15N-labeled PriA-(1–105) was prepared from E. coli cells carrying an expression plasmid grown in M9 minimal medium containing 1 g/liter of 15NH4Cl. Uniformly 15N,13C,H-labeled PriA-(1–105) was prepared using M9 minimal medium containing 1 g/liter of 15NH4Cl, 2.5 g/liter U-13C glucose in 99% H2O. To exchange the amide deuteriums to protons, the purified protein was denatured in 6 M guanidine hydrochloride, followed by dialysis against a buffer solution (25 mM sodium phosphate (pH 5.5), 150 mM KCl, and 10 mM dithiothreitol) for refolding. The protein was repurified by HEP-heparin-agarose column (Amersham Biosciences). The level of deuterium incorporation for non-exchangable hydrogens was determined to be about 83% by matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis. RecG protein was purified from a strain bearing pAP201, as described previously (20).

Biacore Analyses of DNA Binding—The streptavidin (SA) sensor chips with less nonspecific interaction were prepared as follows. A CM5 sensor chip (Biacore) was activated by EDC solution for 40 min. SA (50 μg/ml) in 10 mM sodium acetate (pH 5.3) was injected onto the activated CM5 surface. The remaining N-
FIG. 1. Specific recognition of the 3′ terminus of DNA by PriA protein. A, BIACore analysis of oligonucleotide binding of the N-terminal 181-residue polypeptide of PriA. Upper, 17-mer oligonucleotide (dA17) was immobilized on an SA-coated sensor chip (purchased from BIACORE) via a biotin moiety attached on either 5′- or 3′-end. The 3′-hydroxyl group was modified with a phosphate group, where indicated, to sterically sequester the 3′-terminal end of the oligonucleotide. SA and biotin are indicated by gray semicircles and red triangles, respectively. Lower, purified PriA-(1–181) was injected at a concentration of 200 nM. The interaction with the oligonucleotide on the sensor surface in the three different designs was monitored. Tailing in the sensorgram pattern during the dissociation phase is due to nonspecific interaction of PriA with the carboxyl group remaining on the surface of a commercially available SA-coupled sensor chip. In the inset, the calculated dissociation constants are shown. B, D-loop substrates, as indicated, were prepared and were used for gel shift assays with PriA (upper) or RecG (lower) protein of the amounts indicated. The concentration of potassium glutamate for DNA binding assays was 80 mM, which decreased affinity to the substrate compared with other assays where it was 40 mM. Decreased intensities of the shifted bands in lanes 12, 18, and 24 of PriA and in lanes 15, 16, 21, and 22 of RecG are due to generation of complexes that did not form specific shifted bands but migrated as smeared bands in the gel. Upon longer exposure, these complexes are visible in these lanes (data not shown). C and D, arrested fork substrates (105 and 41 nucleotides long in C and D, respectively) were prepared and were used for gel shift assays with PriA protein. The graphs in C and D show the quantification of PriA binding to 3′-free (blue) and 3′-phosphorylated (yellow) A-fork structures. The 3′-ends of DNA are shown in purple, and the phosphate group at the 3′-end is indicated by yellow circles. The radioisotope labelings at the 5′-ends of oligonucleotides are indicated by red asterisks. The positions of the free DNAs are shown by the arrows.
hydroyxysuccinimide ester groups were inactivated by treatment in 1 m ethanolamine solution for another 40 min, and SA non-specifically adsorbed on the chip was removed by three 1-min washes in 50 mm NaOH and 1 m NaCl, resulting in reduced level of undesired free carboxyl groups. The HBS-EP buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA, and 0.005% (v/v) surfactant P20) was run at 37 °C at a flow rate of 8 μl/min for immobilization. A 17-mer oligonucleotide with/without terminal phosphate modification was immobilized on an SA-coated sensor chip via a biotin moiety attached on either its 5'- or 3'-end. See Fig. 1A for details. The amount of the immobilized oligonucleotide was adjusted to about 150 resonance unit (RU) for details. The amount of the immobilized oligonucleotide was adjusted to about 150 resonance unit (RU) for details. The amount of the immobilized oligonucleotide was adjusted to about 150 resonance unit (RU). HBS-EP buffer was used at a flow rate of 30 μl/min at 20 °C for measurement of protein binding. Regeneration of the sensor surface was achieved by three 5-s washes with 10 m NaOH. The sensor responses obtained were corrected by subtraction of those recorded by injection of the buffer alone. The dissociation constants were determined by a global fitting method using the BIAevaluation software 3.1 on the basis of the BIAcore experiments using the sensor chips prepared as above which show low nonspecific interactions.

RESULTS AND DISCUSSION

We have shown via gel shift assays that the N-terminal 181 residue polypeptide of PriA can bind to a D-loop mimic DNA in vitro (19). To determine the affinity of PriA and its truncated polypeptides with various forms of DNA, we developed a BIAcore assay system where the biotin-labeled substrate DNA was immobilized on a sensor chip through streptavidin (Fig. 1A). The PriA N-terminal polypeptide exhibited binding not only to structured DNA mimicking D-loop (data not shown), but also to single-stranded oligonucleotide (17-mer) immobilized to a sensor chip through its 5'-end with K_d = 4.9 μM. In contrast, the same oligonucleotide immobilized through its 3'-end did not serve as a ligand. This suggested a possibility that the 3' terminus may play a crucial role in recognition of DNA by PriA protein. Therefore, we next generated an oligonucleotide whose 3'-end was phosphorylated and immobilized it on a sensor chip through its 5'-end (Fig. 1A). PriA interacted with this 3'-phosphorylated oligonucleotide only very weakly, supporting our hypothesis that a 3' terminus is a critical determinant for DNA binding of PriA. The above results suggest a possibility that PriA recognizes the ends of non-elongated DNA chains resulting from arrested replication forks or recombination intermediates. To directly test this hypothesis, we conducted gel shift assays with structured DNA mimicking these structures. First, we generated a D-loop-like structure bearing either a 3'-invading or a 5'-invading strand at the fork and examined the binding of PriA by gel shift assays (16, 19). PriA bound to the 3'-invading substrate with much higher affinity (Fig. 1B, upper panel, lanes 1–6 and 13–18). PriA does not form a stable complex with a single-stranded oligonucleotide with a free 3'-end in gel shift assays, and therefore, the presence or absence of a free 3'-end of the invading strand in the 5'-invading D-loop does not affect the gel shift pattern (Fig. 1B, upper panel, lanes 13–18 and 19–24). We then generated a similar D-loop substrate with a 3'-invading strand whose 3'-end was phosphorylated. PriA formed rather smeared complexes on this substrate, and the K_d was estimated to be more than one order of magnitude lower than that for the non-phosphorylated substrate (Fig. 1B, upper panel, lanes 7–12).

DNA substrates more closely mimicking arrested fork-like structures were tested next. They were 105-nucleotide-long Y-fork structures carrying either a nascent leading strand or a nascent lagging strand or both at the fork (A-fork[3'], A-fork[5'], or A-fork[3', 5'], respectively). PriA generated a specific complex in gel shift assays with A-fork[3'] or A-fork[3', 5'] with high affinity (K_d = 1.2 and 1.6 nM, respectively; Fig. 1C, lanes 1–5 and 16–20). It bound to A-fork[5'] with less affinity (with K_d of 8 nM; Fig. 1C, lanes 11–15). Notably, when the 3' terminus of the lagging strand was blocked by phosphorylation (A-fork[3'-P]), affinity was reduced to the level similar to that of A-fork[5'] (K_d = 8 nM; Fig. 1C, lanes 6–10). Inhibitory effect of 3'-phosphorylation of the leading strand was further enhanced when shorter A-fork substrates (41 nucleotides long) were used (Fig. 1D; K_d = 3 and 32 nM, respectively, for A-fork 41 [3'] and A-fork 41 [3'-P]). Furthermore, PriA bound to a 5'–extension structure with K_d = 16 nM and phosphorylation of the 3' terminus at the single-strand to double-strand junction reduced the affinity (Supplementary Fig. S1). These results indicate that the 3' terminus of DNA present at the double-strand to single-strand junction is important for high affinity binding of PriA to fork structures and support our hypothesis that PriA protein specifically recognizes the 3' terminus of the nascent leading strand at the fork, expected to be generated from arrest of replication fork progression.

RecG has been implicated in the processing of stalled replication forks in collaboration with PriA (15, 22, 23). It is also a DEXH-type DNA helicase and is highly conserved in eu- bacteria (24). RecG was shown to bind to structured DNAs including the Holliday junction and D-loop-like structures (16, 25). To examine whether the recognition of a 3' terminus in an arrested fork-like structure is unique to PriA, we have purified RecG and examined its binding to the above substrates. RecG protein bound more efficiently to the 5'-invading D-loop (regardless of the presence or absence of 3'-phosphorylation) than to the 3'-invading D-loop (Fig. 1B, lower panel, lanes 1–6 and 13–24). Furthermore, it bound to the 3'-invading D-loop with a phosphorylated 3'-end as efficiently as that with a 3'-hydroxyl group (Fig. 1B, lower panel, lanes 7–12). Thus, specific recognition of a 3' terminus appears to be unique to PriA. This is consistent with the absence of similarity in the N-terminal segments of PriA and RecG. Previous report on quantitative measurement of interaction of E. coli PriA protein with single-stranded DNA showed that this interaction is independent of DNA size from 8-mer to 24-mer (26), which is expected if PriA primarily recognizes an end of DNA.

We have further truncated the N-terminal 181 residue DNA binding segment and showed by BIAcore measurement that the N-terminal 105 residue segment (PriA-(1–105)) could bind to the 17-mer oligonucleotide in a manner dependent on the presence of a free 3' terminus, although its binding affinity was lower than PriA-(1–181) (K_d = 20 μM and data not shown), and D-loop binding could not be detected in gel shift assays. We then analyzed binding of PriA-(1–105) to an 8-mer oligonucleotide, carrying a free 3'-hydroxyl terminus or a phosphorylated 3' terminus, by NMR. The 3'-hydroxyl DNA, but not the 3'-phosphorylated DNA, induced significant changes in the 1H-15N HSQC spectrum of 15N-labeled PriA-(1–105) (Fig. 2A).
particular, we have identified Asp17, Tyr18, and Gly37 as residues whose cross-peaks disappeared during the titration due to the exchange broadening mechanism, indicative of the largest chemical shift changes. Comparison of PriA-related sequences in various bacteria indicated that these residues are among a set of well conserved residues present very near the N terminus (Fig. 2B). Particularly, FDY (16–18), GCRVRVPFG (29–37), and WAADYY (83–88) are well conserved. The conservation of the WAADYY motif was noted previously (19). The selective effects of the 3'-H11032-free DNA on the subset of the cross-peaks suggests the specific recognition of the 3'-terminal end of single-stranded DNA by PriA through a defined binding pocket. The three amino acids identified are most likely candidate residues in this putative binding pocket.

We mutagenized Phe36 and Gly37 (to alanine or leucine and alanine or asparagine, respectively) and purified the mutant N-terminal fragments (residue 1–181). Phe36 was also selected for mutagenesis, since it is a conserved aromatic residue that we predicted may interact with nucleotide bases through stacking. We could not obtain Asp17 and Tyr18 mutant polypeptides due to formation of inclusion bodies. One-dimensional 1H NMR spectra indicated that the folding of the purified mutant proteins was maintained (data not shown). All the mutant polypeptides lost binding (F36A, G37A, and G37N) or showed reduced binding (F36L) to the 17-mer DNA in the BIAcore measurements (Fig. 2C). This result suggests that these residues constitute a part of a putative "3' terminus binding pocket" of PriA protein.

We then tried to generate full-length PriA carrying mutations in the putative 3'-terminus binding pocket. We constructed four mutants and purified two of them (F36G, V46G(#2) and L12G, F16G, Y18G(#4)). We have shown that, by gel filtration analyses, the purified wild-type as well as these mutant proteins exist as monomers and exhibit similar cleavage pattern upon partial tryptic digestion (data not shown).
Fig. 3. PriA proteins with mutations in the putative 3’ terminus binding pocket bind poorly to D-loop/ arrested fork-like structures and show reduced biological activities. A, four mutant PriA proteins carrying amino acid substitutions in the putative 3’ terminus binding pocket were constructed, as shown in the top drawing. Two of them, F36G, V46G(#2) and L12G, F16G, Y18G (#4), along with the wild-type protein, were purified and were used in gel shift assays with a synthetic D-loop (upper) or arrested fork (lower) DNA as a substrate. The positions of the free DNAs are shown by the arrows. B, ATP hydrolysis activities of wild-type and mutant PriA proteins. The wild-type, F36G, V46G(#2), and L12G, F16G, Y18G(#4) mutant PriA proteins were assayed in standard reaction mixtures for ATPase assays in the presence of D-loop DNA (3’-hydroxy or 3’-phosphorylated) as indicated in the figure. C, abilities of the mutant proteins to support iSDR. iSDR was measured in priA1::kan strain carrying the wild-type or mutant PriA plasmid as indicated.

shown), indicating that the overall structures are not altered by these mutations. They bound to D-loop and A-fork with affinity 2–3-fold lower than that of the wild type (Fig. 3A).

We also examined ATPase activity of the mutant protein. PriA displays ATP hydrolysis activity in the presence of its target sequence such as n’-pas (primosome assembly site) or D-loop (27). Consistent with low affinity of PriA to D-loop with a 3’-phosphorylated 3’-invading strand, PriA hydrolyzed ATP rather inefficiently in the presence of this effector DNA compared with the regular D-loop. In contrast, F36G, V46G(#2) and L12G, F16G, Y18G(#4) showed low ATPase activity even in the presence of a normal D-loop structure (Fig. 3B). This also indicates the importance of the 3’ terminus binding pocket for productive interaction of PriA with its target sequences.

We propose that binding of PriA to arrested forks or related structures may be triggered by recognition of the 3’ terminus of the leading strand or invading strand of an arrested fork or D-loop, respectively, with its putative 3’ terminus binding pocket. This interaction, which is rather unstable in E. coli, will be further stabilized by the residues 106–181 (as indicated by the fact that PriA(1–181), but not PriA(1–105), is able to form a complex with D-loop in gel shift assays) and DNA helicase domain, which will make contact with other structures of arrested forks (19). More stable interaction with single-stranded DNA was reported for Bacillus subtilis PriA protein (28).

We expressed the mutant PriA proteins in the priA1::kan strain and examined their biological activities. The priA1::kan strain grows very slowly, exhibits low viability, and is sensitive to DNA damaging agents such as UV or γ-ray, and subsets of the cells are highly filamentous (6, 7, 9). This strain is completely deficient in iSDR and cSDR (8). The wild-type PriA protein expressed from a miniR1-based vector, pHM6050, can completely correct these deficiencies. However, G29L, G37L, G44L(#1), R31A, V32A, V34A, P35A, F36A(#3), and L12G, F16G, Y18G(#4), expressed on the same vector, did not restore growth, morphology, and SDR (Fig. 3C and Supplementary Fig. S2). F36G, V46G(#2), which bound to an arrested fork structure with affinity about half the wild-type level, could restore most of these functions but restored iSDR to the level 70% of the wild type. The 3’ terminus binding pockets of these mutant proteins may interact with an end of DNA with different affinity and stability, which may not be obvious in gel shift assays using the full-length proteins carrying other DNA binding surfaces, and this may result in differential biological activities of these mutants. Sensitivity to UV of the priA null mutant was corrected by #2 mutant, but only partially by other mutants (data not shown). Residual resistance to UV with the pocket mutants may suggest that the forks arrested by UV could be recognized by these mutants to certain extent.

We also showed that #1, #3, and #4 mutants could not rescue the extreme growth defect of priA1::kan gyrB(ts) double mutant (Ref. 29 and data not shown), in agreement with an essential role of the 3’ terminus binding pocket of PriA in recovery of replication forks arrested during the course of DNA replication. Thus, proper and efficient recognition of the 3’ terminus of an arrested replication fork or that of the invading strand within D-loop by the PriA N-terminal segment is critical for various biological responses induced by unscheduled arrest of ongoing replication forks.

Our results suggest that PriA specifically recognizes the replication forks with an arrested leading strand. When the lagging strand polymerase encounters a lesion, the leading strand synthesis may continue for a while in the absence of lagging strand synthesis. This sort of uncoupled leading strand synthesis was previously observed in plasmid DNA replication (30). It may be arrested due to constraint on the fork architecture, thus generating a non-elongated DNA end of the nascent leading strand. If double-stranded breaks are generated at the site of arrested forks, actions of recombination proteins would process them into recombination intermediates, which would also contain an end derived from the invading assimilated strand (15).

The ability of PriA to recognize the 3’ terminus of a hybridizing strand would be highly suitable for efficient and specific recognition of stalled replication forks, processed replication forks, or D-loop. Our results can explain the lack of binding of PriA to the Holliday junction or three-way junction structures
which do not possess a 3’ terminus at the junction (16, 17). They can also explain the increased helicase activity on the lagging strand arm by the presence of a leading strand fragment without a gap (15).

Monitoring the state of DNA replication is crucial for successful completion of DNA replication of the entire genome. The recognition of the stalled replication forks is the initial step for successful completion of DNA replication of the entire genome. The recognition without a gap (15).

Acknowledgments—We thank Dr. Gerard Zurawski (DNAX Research Institute) for critical reading of the manuscript and valuable suggestions. We thank Drs. Hideo Shinagawa and Yong-Woon Han (Osaka University) for construction of PriA and other technical assistance. We thank Dr. Naoko Sugata and other members of our laboratory for useful comments.

REFERENCES
1. Cox, M. M., Goodman, M. F., Kreuzer, K. N., Sherratt, D. J., Sandler, S. J., and Marians, K. J. (2000) Nature 404, 37–41
2. Boddy, M. N., and Russell, P. (2001) Curr. Biol. 11, R953–R956
3. Lee, E. H., Masai, H., Allen, G. C., Jr., and Kornberg, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4620–4624
4. Nurse, P., DiGate, R. J., Zavitz, K. H., and Marians, K. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 87, 4615–4619
5. Marians, K. J. (2000) Trends Biochem. Sci. 25, 185–189
6. Lee, E. H., and Kornberg, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3029–3032
7. Nurse, P., Zavitz, K. H., and Marians, K. J. (1991) J. Bacteriol. 173, 6686–6693
8. Masai, H., Asai, T., Kubota, Y., Arai, K., and Kogoma, T. (1994) EMBO J. 13, 5338–5345
9. Kogoma, T., Cadwell, G. W., Barnard, K. G., and Asai, T. (1996) J. Bacteriol. 178, 1258–1264
10. Sandler, S. J., Samra, H. S., and Clark, A. J. (1996) Genetics 143, 5–13
11. Sandler, S. J., and Marians, K. J. (2000) J. Bacteriol. 182, 9–13
12. Liu, J., and Marians, K. J. (1999) J. Biol. Chem. 274, 25033–25041
13. Liu, J., Xu, L., Sandler, S. J., and Marians, K. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3552–3555
14. Jones, J. M., and Nakai, H. (2000) Mol. Microbiol. 36, 519–527
15. Gregg, A. V., McGlynn, P., Jaktaji, R. P., and Lloyd, R. G. (2002) Mol. Cell 9, 241–251
16. McGlynn, P., Al-Deib, A. A., Liu, J., Marians, K. J., and Lloyd, R. G. (1997) J. Mol. Biol. 270, 212–221
17. Nurse, P., Liu, J., and Marians, K. J. (1999) J. Biol. Chem. 274, 25026–25032
18. Masai, H., Denke, J., Furui, Y., Tanaka, T., and Arai, K. (1999) Biochimie (Paris) 81, 847–857
19. Tanaka, T., Mizukoshi, T., Taniyama, C., Kohda, D., Arai, K., and Masai, H. (2002) J. Biol. Chem. 277, 38062–38071
20. Fukuzoh, A., Iwasaki, H., Ishioka, K., and Shinagawa, H. (1997) EMBO J. 16, 263–269
21. Inoue, H., Nojima, H., and Okayama, H. (1990) Gene (Amst.) 86, 23–28
22. McGlynn, P., and Lloyd, R. G. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8227–8234
23. Singleton, M. R., Scaife, S., and Wigley, D. B. (2001) Cell 107, 79–89
24. Sharples, G. J., Ingleston, S. M., and Lloyd, R. G. (1999) J. Bacteriol. 181, 5543–5550
25. Whiting, N. C., and Lloyd, R. G. (1998) J. Biol. Chem. 273, 19729–19739
26. Jezewska, M. J., Rajendran, S., and Bujalowski, W. (2000) J. Biol. Chem. 275, 27865–27873
27. Tanaka, T., Taniyama, C., Arai, K., and Masai, H. (2003) Genes Cells 8, 251–261
28. Polard, P., Marsin, S., McGovern, S., Velten, M., Wigley, D. B., Ehrlich, S. D., and Brundage, C. (2002) Nucleic Acids Res. 30, 1593–1605
29. Grompone, G., Ehrlich, S. D., and Michel, B. (2003) Mol. Microbiol. 48, 845–854
30. Staudenbauer, W. L., Scharzinger, E., and Lanka, E. (1979) Mol. Gen. Genet. 177, 115–129
A Critical Role of the 3′ Terminus of Nascent DNA Chains in Recognition of Stalled Replication Forks

Toshimi Mizukoshi, Taku Tanaka, Ken-ichi Arai, Daisuke Kohda and Hisao Masai

J. Biol. Chem. 2003, 278:42234-42239.  
doi: 10.1074/jbc.C300285200 originally published online August 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.C300285200

Alerts:
• When this article is cited  
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

Supplemental material:  
http://www.jbc.org/content/suppl/2003/08/20/C300285200.DC1

This article cites 30 references, 16 of which can be accessed free at  
http://www.jbc.org/content/278/43/42234.full.html#ref-list-1