A Second Proliferating Cell Nuclear Antigen Loader Complex, Ctf18-Replication Factor C, Stimulates DNA Polymerase η Activity

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Replication factor C (RFC) loads the clamp protein PCNA onto DNA structures. Ctf18-RFC, which consists of the chromosome cohesion factors Ctf18, Dcc1, and Ctf8 and four small RFC subunits, functions as a second proliferating cell nuclear antigen (PCNA) loader. To identify potential targets of Ctf18-RFC, human cell extracts were assayed for DNA polymerase activity specifically stimulated by Ctf18-RFC in conjunction with PCNA. After several chromatography steps, an activity stimulated by Ctf18-RFC but not by RFC was identified. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis revealed the presence of two DNA polymerases, η and λ, in the most purified fraction, but experiments with purified recombinant proteins demonstrated that only polymerase (pol) η was responsible for activity. Ctf18-RFC alone stimulated pol η, and the addition of PCNA cooperatively increased stimulation. Furthermore, Ctf18-RFC interacted physically with pol η, as indicated by co-precipitation in human cells. We propose that this novel loader-DNA polymerase interaction allows DNA replication forks to overcome interference by various template structures, including damaged DNA and DNA-protein complexes that maintain chromosome cohesion.

Clamp and clamp loader proteins recruit target proteins to DNA structures, such as replication forks and repair intermediates. A representative eukaryotic clamp, PCNA,2 is loaded onto replicating DNA ends by a loader complex, RFC, and functions as a processivity factor for the replicative DNA polymerases δ and ε (1). RFC is a heteropentameric protein complex composed of a large subunit (RFC1) and four small subunits (RFC2 to -5), all of which belong to the AAA + ATPase family (2, 3). Structural and biochemical studies have demonstrated that an ATP-dependent conformational change in RFC promotes loading of PCNA onto template DNA (4–8). Three other clamp loader complexes that are involved in checkpoint responses (Rad17-RFC), sister chromatid cohesion (Ctf18/Chl12-RFC), and maintenance of genome stability (Elg1-RFC) have been identified in eukaryotes (9, 10). Ctf18/Chl12, hereafter referred to as Ctf18, is required for precise chromosome transmission in yeast and is highly conserved in eukaryotes (11, 12). Unlike other loader complexes, Ctf18-RFC also associates with the chromosome cohesion factors Dcc1 and Ctf8 to form a heteroheptameric complex, although functions for the additional subunits have not been identified (13)3; we refer to the heptameric and pentameric complexes as Ctf18-RFC and Ctf18-RFC(5s), respectively. Ctf18-RFC loads functional PCNA onto the 3’ end of a primer-template DNA duplex, and like RFC, it stimulates pol δ activity in vitro. Nonetheless, the functions of these two PCNA loaders are clearly distinguishable. For example, PCNA loaded by Ctf18-RFC can fully support DNA synthesis by pol δ on a primed M13mp18 single-stranded DNA (M13) DNA template, but Ctf18-RFC cannot substitute for RFC in promoting SV40 DNA replication in vitro with crude human protein fractions (14). These results suggest that Ctf18-RFC functions in the chromosome cohesion pathway by activating specific target proteins, such as DNA polymerases. Indeed, DNA pol σ, ε, and α have been implicated in the chromosome cohesion (15–17), although biochemical evidence for these activities has not been reported.

Several eukaryotic DNA polymerases have been identified recently. Most belong to the novel DNA polymerase X and Y families, based on conserved motifs distinguishable from those seen in the B family, which include the major replicative DNA polymerases (18). Some of these carry out translesion DNA synthesis (TLS), which incorporates nucleotides (nt) at damaged sites with low fidelity. The primary role of these polymerases is to function as a backup system for more conventional DNA repair to avoid unnecessary cell death in the event of severe DNA damage. However, some may have more diverse functions. For example, targeted mutation of a TLS polymerase,
pol ζ, which is an exceptional member of the B family, results in embryonic lethality in mice, suggesting that it functions during development (19). Thus, our knowledge of the functions of these novel DNA polymerases remains limited. PCNA interacts with and stimulates the DNA synthesis activities of many of these polymerases (20), but the mechanism of their recruitment by PCNA to particular DNA sites and the determinants of selectivity are not known.

In this report, we fractionated human cell lysates to isolate DNA synthesis activities, which allowed us to identify pol η as a potential target for the second PCNA loader, Ctf18-RFC. Furthermore, we show that Ctf18-RFC directly interacts with pol η and stimulates its activity, indicating a novel role for the loader complex. These results also demonstrate that pol η, identified as a TLS polymerase, has more diverse functions in regulating replication fork progression.

**EXPERIMENTAL PROCEDURES**

Fractionation of Ctf18-RFC-stimulated DNA Polymerase Activity from Human Cell Lysates—Two representative experiments (1 and 2) were carried out under essentially the same conditions. Human 293 cell cytoplasmic extracts (S100) were prepared from 24- or 16-liter suspension cultures for experiments 1 and 2, respectively, as described (21). Three fractions eluting at 0.24, 0.35, and 0.68 M NaCl in buffer H (25 mM HEPES-NaOH (pH 7.5), 1 mM EDTA, 0.01% Nonidet P-40, 10% glycerol, 0.1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin) from a phosphocellulose column (P-11; Whatman, Middlesex, UK) were prepared from lysates (22) and dialyzed against buffer H containing 25 mM NaCl and 20% sucrose, and their DNA synthesis activity was measured. The 0.35 M NaCl fraction was further fractionated by stepwise elution with 0.025, 0.1, 0.2, 0.3, and 0.5 M NaCl on a Q-Sepharose column (Amerham Biosciences, Buckinghamshire, UK) in buffer H. The eluates were dialyzed against buffer H containing 25 mM NaCl and 20% sucrose, and DNA synthesis activity was measured. The 0.1 M NaCl fraction was then applied to a linear NaCl gradient (0.025–0.6 M) in buffer H on a mini-S column (Amersham Biotechnology, Inc. (Santa Cruz, CA), and the monoclonal anti-FLAG M2 antibody (F-3165) was from Sigma. Monoclonal antibodies reacting with pol α (STK1) (29) and pol ε (CRL-2284) were prepared from mouse hybridoma cell lines purchased from the ATCC (Manassas, VA). The rabbit anti-Ctf18 serum has been described (14). An anti-human pol η monoclonal antibody, 5H10, prepared with a His-tagged human pol η fragment of amino acid residues 1–511 was used for immunoblotting.

DNA Synthesis Activity—DNA synthesis with 30 ng of M13 DNA (Takara, Shiga, Japan) annealed with a 20-mer sequencing primer (GTTGTAAAACGACG) was assayed in a reaction mixture (5 μl) containing 30 mM HEPES-NaOH (pH 7.5), 7 mM MgCl₂, 30 mM NaCl, 0.5 mM dithiothreitol, 1 mM ATP, and 0.1 mM each dNTP with [α-32P]dUTP, 20 ng of PCNA, 100 ng of RPA, and the indicated amounts of protein at 37 °C for 1 h. One-third of the reaction was spotted on DE81 paper (Whatman) to measure incorporated radioactivity (30). DNA synthesis products were taken from the same reactions using a 32P-labeled 20-mer primer and the indicated amounts of pol η at various NaCl concentrations. After the reaction, the products were denatured with formamide and loaded onto 13.5% polyacrylamide gels containing 7 M urea in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA). After electrophoresis, radioactivity was measured with a Fuji Image analyzer LAS5000 (Fuji-film, Tokyo, Japan). To measure the processivity of DNA synthesis, a 32P-labeled singly primed M13 DNA substrate was preincubated with pol η (0.1 μg/ml) without dNTP at 90 mM NaCl for 10 min at 37 °C. After preincubation, activated calf thymus DNA (Amersham Biosciences) at final concentrations of 0.125, 0.25, 0.5, and 1 mg/ml was added to the mixture as a DNA trap, along with a 0.25 mM concentration of each dNTP. After a 10-min incubation at 37 °C, the reactions were stopped,

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*Stimulation of DNA Polymerase η by Ctf18-RFC*

Pol η, with a C- or N-terminal His tag, was expressed from a baculovirus as previously described (28) or was prepared from pBacPAKHispol (Clontech) by inserting the pol η cDNA immediately after a His tag cassette. Both His-tagged pol η proteins were prepared from insect cells as previously described (28). The N- and C-terminal His-tagged pol η were used for the pull-down analysis in Fig. 5A and DNA synthesis analyses in Figs. 2C, 3, and 4. The plasmid pETHispolA for His-tagged pol η (His-pol η) expression was constructed by insertion of the pol η cDNA into pET20b (Novagen (Merck)), which resulted in an N-terminal fusion of the His tag. His-pol η was prepared from E. coli Rosetta (DE3) (Novagen) carrying pETHispolA using DEAE-Sepharose, Ni²⁺-nitrilotriacetic acid-Sepharose high performance, and fast desalting columns (Amersham Biosciences).

Concentrations and purities of purified proteins were estimated from intensities of protein bands in an SDS-polyacrylamide gel using bovine serum albumin as a standard, and proteins of greater than 95% purity were used in this work.

**Antibodies**—Antibodies to the catalytic subunits of human pol δ (C-20, sc-8797; polyclonal) and to RFCp37 (RFC4; sc-20996; polyclonal) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and the monoclonal anti-FLAG M2 antibody (F-3165) was from Sigma. Monoclonal antibodies reacting with pol α (STK1) (29) and pol ε (CRL-2284) were prepared from mouse hybridoma cell lines purchased from the ATCC (Manassas, VA). The rabbit anti-Ctf18 serum has been described (14). An anti-human pol η monoclonal antibody, 5H10, prepared with a His-tagged human pol η fragment of amino acid residues 1–511 was used for immunoblotting.

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and the products were electrophoresed in a 19% polyacrylamide gel. DNA synthesis with poly(dA)/oligo(dT) (20:1; 6 μM nt) was assayed in a reaction mixture (5 μl) containing 30 mM HEPES-NaOH (pH 7.5), 7 mM MgCl₂, 90 mM NaCl, 0.5 mM dithiothreitol, 0.05 mM [α-³²P]dTTP, 100 ng of RPA, and 45 ng of loader complexes, and the incorporated radioactivity was measured after incubation at 37 °C for 1 h. Experiments were performed with or without 20 ng of PCNA or 1 mM ATP.

Interaction of Clamp Loaders and Pol η—About 100 ng of FLAG-tagged RFC or Ctf18-RFC produced by baculovirus systems was prebound with 3 μl of anti-FLAG beads (anti-FLAG M2-agarose affinity beads; Sigma) at 0 °C for 1 h. After four washes with 50 mM 2-agarose affinity beads; Sigma) at 0 °C for 1 h. After four washes with 50 mM NaOH (pH 7.5), 7 mM MgCl₂, 90 mM NaCl, 0.5 mM dithiothreitol, 0.05 mM [α-³²P]dTTP, 100 ng of RPA, and 45 ng of loader complexes, and the incorporated radioactivity was measured after incubation at 37 °C for 1 h. Experiments were performed with or without 20 ng of PCNA or 1 mM ATP.

Expression of FLAG-Ctf18 and Green Fluorescent Protein (GFP)-tagged Pol η in Human 293 Cells and Co-immunoprecipitation—Ctf18 or pol η with N-terminal FLAG or GFP tags was constructed by inserting these cDNAs into pcDNA3 or pEGFP-C1 (Invitrogen). The resulting plasmids, pcDNA-FLAG-Ctf18 and pEGFP-pol, were transfected into 293 cells (10⁵ cells/ml) with FuGENE 6 (Roche Applied Science). After incubation at 37 °C for 72 h, the cells were washed three times with phosphate-buffered saline three times. The cells were then suspended in 250 μl of modified CSK buffer (10 mM Pipes (pH 7.0), 300 mM sucrose, 0.1% Triton X-100, 1 mM MgCl₂, 1 mM EDTA, 0.1 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin) and centrifuged for 5 min at 700 g at 4 °C. The supernatant was obtained as the lysate. GFP-tagged pol η in 25 μl of lysate was bound with 1 μl of agarose beads conjugated to anti-GFP antibodies (MBL, Aichi, Japan) at 0 °C for 1 h. After four washes with modified CSK buffer, bound proteins were eluted with 10 μl of SDS loading buffer, separated in a 12.5% SDS-polyacrylamide gel, detected by immunoblotting with anti-FLAG, anti-p37, or anti-pol η antibodies, and quantified using Fuji Image Gauge software (Fujiﬁlm).

RESULTS

Identification of a DNA Polymerase Activity Stimulated by Ctf18-RFC and PCNA—To identify target DNA polymerases for Ctf18-RFC and PCNA, we analyzed crude fractions of human 293 cell extracts for DNA synthesis activity that was specifically stimulated by these proteins. We isolated a DNA polymerase-enriched fraction (the 0.35 M fraction) eluting between 0.24 and 0.35 M NaCl from a phosphocellulose column (22). DNA synthesis activity was measured in the presence of singly primed M13 DNA, RPA, PCNA, and RFC or Ctf18-RFC (Fig. 1A) under assay conditions in which an equivalent stimulation of purified pol δ-dependent DNA synthesis could be observed with RFC or Ctf18-RFC, as previously described (Fig. 1B, c) (14). Stimulation was not observed with Ctf18-RFC, whereas the addition of RFC increased activity about 6-fold compared with that from reactions lacking loader complexes (Fig. 1A; 0.35 M) (14). We further fractionated the 0.35 M fraction on a Q-Sepharose column by stepwise elution with 0.025, 0.1, 0.2, 0.3, and 0.5 M NaCl fractions (lanes 2–6) containing 0.05, 0.2, 0.8, 1.6, and 0.9 mg/ml proteins, respectively, obtained from experiment 1. Bottom, immunoblotting with an anti-pol η antibody using 2-μl aliquots of fractions as in the top, except that the proteins (1.6, 0.05, 0.1, 0.9, 1.5, and 0.8 mg/ml) were obtained from experiment 2. An arrow indicates the migration position of 0.5 ng of recombinant His-pol η (lane 7).

FIGURE 1. Detection of Ctf18-RFC-stimulated DNA polymerase activity in human cell lysates. A, DNA synthesis activity with 2 μl each of the 0.24 M, 0.35 M, and 0.68 M NaCl fractions of 293 cell lysates from a phosphocellulose column (5, 1.2, and 0.9 ng of protein/ml, respectively) and their buffer control (Bu). The fractions were incubated with RFC or Ctf18-RFC or neither, in the presence of PCNA and singly primed M13 DNA as a template. Means ± S.D. were obtained from two independent experiments. Buffer controls did not show any detectable incorporation even with RFC or Ctf18-RFC, indicating no contaminating polymerase activity in these protein samples. B, titrations of Q-Sepharose 0.025 m (a) and 0.1 M NaCl (b) fractions and purified pol δ (c). Results with RFC, Ctf18-RFC, or neither in the presence of PCNA and singly primed M13 DNA are shown. The abscissas are volumes of added fractions or amounts of pol δ. C, top, immunoblotting for pol α, δ, and ε with 2-μl aliquots of the phosphocellulose 0.35 M NaCl fraction (lane 1) and the Q-Sepharose 0.025, 0.1, 0.2, 0.3, and 0.5 M NaCl fractions (lanes 2–6) containing 0.05, 0.2, 0.8, 1.6, and 0.9 mg/ml proteins, respectively, obtained from experiment 1. Bottom, immunoblotting with an anti-pol η antibody using 2-μl aliquots of fractions as in the top, except that the proteins (1.6, 0.05, 0.1, 0.9, 1.5, and 0.8 mg/ml) were obtained from experiment 2. An arrow indicates the migration position of 0.5 ng of recombinant His-pol η (lane 7).

1B, c) (14). Stimulation was not observed with Ctf18-RFC, whereas the addition of RFC increased activity about 6-fold compared with that from reactions lacking loader complexes (Fig. 1A; 0.35 M) (14). We further fractionated the 0.35 M fraction on a Q-Sepharose column by stepwise elution with 0.025, 0.1, 0.2, 0.3, and 0.5 M NaCl and measured DNA polymerase activities (Fig. 1B and supplemental Fig. S1A) as well as the levels of the catalytic subunits of pol α, δ, and ε by immunoblotting (Fig. 1C, top). The 0.025 M NaCl fraction did not contain detectable DNA polymerase activity or pol α, δ, or ε subunits (Fig. 1, B (a) and C (lane 2)). Instead, DNA polymerase activities and various levels of pol α, δ, and ε were seen in the 0.2, 0.3, and 0.5 M NaCl fractions (Fig. 1C, lanes 4–6, and supplemental Fig. S1A). However, polymerase activities in these fractions were not specifically stimulated by Ctf18-RFC. In contrast, although the 0.1 M fraction did not contain detectable pol α, δ, or ε (Fig. 1C, lane 3), the low DNA polymerase activity in this fraction appeared to be specifically stimulated by Ctf18-RFC (Fig. 1B, b). The Q-Sepharose 0.1 M NaCl fraction was further fractionated on a mini-S column with a linear NaCl gradient from 0.025 to 0.6 M. DNA polymerase activity specifically stimulated by
PCNA (Fig. 2) was specifically and strongly stimulated by Ctf18-RFC and the presence of PCNA. The fractions (0.2–0.5 M NaCl) contained pol η activity to be distinguished. Therefore, we identified all proteins in the pooled peak fractions by LC/MS/MS analysis. The glycerol gradient was divided into 38 fractions (2–4 M NaCl fraction pool). Every other fraction (0.5–0.8 M NaCl) was characterized by RFC, Ctf18-RFC, or neither on singly primed M13 in the presence of PCNA. The 0.35 M NaCl fraction pool (Fig. 2 graphs) and 0.45 M NaCl fraction pool (Fig. 2 graphs) were used to distinguish the DNA polymerase activity. The fractions (2–4 M NaCl) were analyzed by 10% SDS-polyacrylamide gel electrophoresis in a 10% SDS-polyacrylamide gel, and stained with Coomassie Brilliant Blue. As shown in Fig. 2C, the pol η activity with singly primed M13 DNA was efficiently stimulated by Ctf18-RFC but only slightly by RFC, similar to what was seen for the most purified polymerase fraction (Fig. 2C, a). In contrast, pol λ exhibited much lower DNA synthesis activity and was not obviously stimulated by either Ctf18-RFC or RFC (supplemental Fig. S2B). Thus, the protein we purified exhibited DNA polymerase activity equivalent to that of pol η.

Co-purification of Pol η with the DNA Polymerase Activity Stimulated by Ctf18-RFC and PCNA—We asked whether pol η co-purified with polymerase activity during purification. We detected a band of ~75 kDa in the 0.35 M fraction of the phosphocellulose column by immunoblotting with an anti-pol η antibody (Fig. 1C, bottom). Protein bands around 50 kDa co-purified with pol η in subsequent steps. Since the abundance of these bands relative to that of the 75-kDa protein differed from preparation to preparation, they probably represent degraded forms of pol η, which have been reported to appear as a 54-kDa protein in purified human pol η preparations (32). Q-Sepharose fractions from 0.1 to 0.5 M NaCl contained varying amounts of pol η, indicating its heterogeneous migratory behavior in this type of chromatography, probably due to the presence of various complexes or modifications (33–36). Although the 0.2–0.5 M NaCl fractions contained pol η, co-purifying pol δ (Fig. 1C, lanes 4–6), which exhibited prominent DNA synthesis activity in the presence of RFC, seemed to mask other DNA polymerase activities. Therefore, we could not detect Ctf18-RFC-stimulated activity in these fractions with our initial approaches, even in the presence of pol η, but we did observe Ctf18-RFC-stimulated DNA polymerase activity from the 0.2 M fraction following subsequent fractionation (data not shown). The sum of the intensities of the 75 and 50 kDa bands in the mini-S and glycerol gradient sedimentation fractions was compatible with the observed Ctf18-RFC-stimulated DNA polymerase activity (Fig. 2, A and B, bottom). In the mini-S chromatography step, minor populations of pol η could be detected at lower NaCl concentrations, in fractions in which weak Ctf18-RFC-stimulated DNA polymerase activity was present, indicating the coincidence of pol η with this activity.

We used immunoblotting band intensities of recombinant pol η in Fig. 2B as a standard to estimate the concentration of Ctf18-RFC formed minor peaks at the 0.25 and 0.35 M fractions and a major peak at the 0.45 M NaCl fraction (Fig. 2A, graph). We further processed the 0.45 M fraction by glycerol gradient sedimentation and observed a single DNA polymerase activity peak in a fraction around 60 kDa (Fig. 2B, graph). Titration of this peak fraction demonstrated that DNA polymerase activity was specifically and strongly stimulated by Ctf18-RFC and PCNA (Fig. 2C and supplemental Fig. S1B).

The Polymerase Stimulated by Ctf18-RFC and PCNA Is Pol η—Staining of proteins in the glycerol gradient fractions revealed that they contained too many protein bands to allow a single band co-eluting with the activity to be distinguished. Therefore, we identified all proteins in the pooled peak fractions by LC/MS/MS analysis. The glycerol gradient was divided into 38 gel slices, and the proteins in each slice were identified following in-gel trypsin digestion (Fig. 2D). The raw data were used to query the MASCOT protein data base, which identified various human proteins with high scores (data not shown). Among these, pol η and pol λ were prominent as DNA polymerases with highly significant scores. The graphs in Fig. 2D indicate the numbers of peptides identified as pol η and λ in each gel slice, which represents the approximate abundance of each protein. Peaks corresponding to pol η and λ appeared around 75 and 65 kDa, which roughly correspond to their predicted peptide lengths, respectively. To determine which polymerase was responsible for activity, we prepared recombinant human pol η and λ. Because the DNA synthesis activity of recombinant pol η with poly(dA)/oligo(dt) was 20 times higher than that of recombinant pol λ (supplemental Fig. S2A), we evaluated the activities of both polymerases in the presence of Ctf18-RFC and PCNA by using 20-fold more pol λ than pol η. As shown in Fig. 2C (b), pol η activity with singly primed M13 DNA was efficiently stimulated by Ctf18-RFC but only slightly by RFC, similar to what was seen for the most purified polymerase fraction (Fig. 2C, a). In contrast, pol λ exhibited much lower DNA synthesis activity and was not obviously stimulated by either Ctf18-RFC or RFC (supplemental Fig. S2B). Thus, the protein we purified exhibited DNA polymerase activity equivalent to that of pol η.
pol η in glycerol gradient fraction 12 as 0.5 ng/μl, based on the sum of the two bands. Titration of fraction 12 and recombinant pol η showed that the Ctf18-RFC-stimulated activity became roughly equivalent when 0.25 ng of pol η in this fraction and 0.05 ng of recombinant pol η were assayed (Fig. 2C). The 5-fold lower specific activity of the fraction might be due to partial inactivation during preparation or to the presence of an inhibitory activity.

The Ctf18 complex exists in two forms, Ctf18-RFC and Ctf18-RFC(5s), depending on the presence or absence of Dcc1 and Ctf8. We therefore asked whether the specific stimulation of pol η might be due to the presence of these additional subunits. However, the two Ctf18-RFC complexes showed no significant differences in their stimulatory activity (Fig. 2C). Thus, Ctf18-RFC(5s) is sufficient, and the two additional subunits are not necessary, for the stimulation of pol η activity.

Synthesis of DNA by Ctf18-RFC-stimulated Pol η—To study the details of Ctf18-RFC-stimulated pol η DNA synthesis, we incubated M13 DNA annealed with a 32P-labeled primer with the native pol η-containing fraction and recombinant pol η in the presence of PCNA and loader proteins and electrophoresed the products in a polyacrylamide gel as shown in Fig. 3A. At 30 mM NaCl, we observed products generated by 50 ng/ml pol η that were longer (about 100 nt; lane 4) in the presence of Ctf18-RFC than in the presence of RFC or in the absence of both (around 8 nt; lanes 2 and 3), similar to what we observed in the previous incorporation experiment with 30 mM NaCl (Fig. 2C, a). Under the same conditions, but with 10 ng/ml recombinant pol η (lane 10), we observed almost the same products specific to Ctf18-RFC. At 0.1 and 1 μg/ml (lanes 18 and 27), recombinant pol η alone synthesized longer products (around 30 and 180 nt, respectively), and these products were elongated by up to 180 and over 200 nt, respectively, in the presence of Ctf18-RFC (lanes 20 and 29). In contrast, the addition of RFC only slightly elongated the DNA at either concentration of recombinant pol η (Fig. 3A, lanes 19 and 28). Alkaline agarose gel analysis of the same products of lanes 27–29 clearly demonstrated that DNA synthesis was stimulated more by Ctf18-RFC than by RFC, with products reaching about 1500 nt (Fig. 3B). Thus, Ctf18-RFC stimulates pol η over a wide range of pol η concentrations.

We studied the DNA elongation modes of pol η in the presence of 90 and 150 mM NaCl, which are close to physiological salt concentrations. At 90 mM NaCl, DNA synthesis with 10 ng/ml recombinant pol η alone was limited to 1–2 nt, which increased to 8 and 100 nt with 0.1 and 1 μg/ml recombinant pol η, respectively (lanes 11, 21, and 30). These products were elongated slightly when RFC was included (lanes 12, 22, and 31), whereas the presence of Ctf18-RFC led to extensive synthesis, with an increase in length of about 10-fold with both 10 ng/ml and 0.1 μg/ml recombinant pol η (lanes 13 and 23). The same stimulation was observed with the native pol η fraction (lanes 5–7). Thus, Ctf18-RFC specifically stimulates pol η in the presence of 30–90 mM NaCl. However, at 150 mM NaCl, pol η activity was significantly blocked, and synthesis of only 2–3 nt was observed with 0.1 μg/ml recombinant pol η and of 10–25 nt with 1 μg/ml recombinant pol η (lanes 24 and 33). Under these conditions, the extent of stimulation by Ctf18-RFC was severalfold lower than at lower salt conditions (lanes 26 and 35). RFC also exhibited a severalfold stimulation of synthesis at 150 mM NaCl at higher pol η concentrations, almost equivalent to that seen with Ctf18-RFC (lanes 25 and 34). These results are consistent with those of another group, which demonstrated that the addition of RFC and PCNA stimulates DNA synthesis by recombinant yeast and human pol η in the presence of 150 mM NaCl (37, 38). These observations are comparable with the stimulation of pol η by RFC and PCNA at comparable concentrations of pol η (1 μg/ml; lane 34) and under the same salt condition.

PCNA and ATP Are Required for the Stimulation of Pol η by Ctf18-RFC—We tested the requirements for PCNA at 90 mM NaCl (Fig. 4A). In the presence of PCNA, products were elongated from about 8 nt up to 200 nt as the concentration of Ctf18-RFC increased (lanes 7–9). Furthermore, the addition of Ctf18-RFC in the absence of PCNA also increased product lengths to 30 nt (lanes 2–4), clearly indicating that stimulation is dependent on Ctf18-RFC. In contrast, RFC had a biphasic effect on pol η DNA synthesis in the presence of PCNA that depended on the concentration of RFC. At a lower RFC concentration (6 μg/ml; lane 10), products between 10 and 30 nt...
These results are consistent with the product analysis shown in Fig. 4. Furthermore, PCNA is involved in the regulation of pol η activity differentially but probably through direct interactions in both cases. Furthermore, PCNA is involved in the regulation of pol η activity by Ctf18-RFC or RFC. To address whether the loading of PCNA is required for stimulating DNA synthesis, we omitted PCNA or ATP from the reaction (Fig. 4B). We employed poly(dA)/oligo(dT) as a template in the presence of RPA to avoid the addition of dATP, which can substitute for ATP. With this template, we also observed efficient and specific stimulation of pol η by Ctf18-RFC in the presence of both PCNA and ATP (Fig. 4B, a). However, in the absence of either PCNA or ATP or both, stimulation decreased to only about 1.5-fold (Fig. 4B, b–d).

These results are consistent with the product analysis shown in Fig. 4A, which demonstrates that Ctf18-RFC alone stimulates pol η DNA synthesis and that PCNA loaded by Ctf18-RFC cooperatively enhances stimulation.

Next, we examined whether this stimulation is due to an increase of processivity or to an increase in the frequency of initiation of DNA synthesis by pol η. We added excess activated calf thymus DNA as a trap for pol η dissociating from the template DNA after formation of template-pol η complexes (Fig. 4C) (37, 39). Without the trap DNA, pol η DNA synthesis was stimulated 10-fold with or 2-fold without PCNA, resulting in elongation products of around 80 and 20 nt, respectively (Fig. 4C, lanes 2, 7, 9, and 11). However, synthesis was efficiently reduced by the addition of increasing amounts of the trap DNA to only a single nt (lanes 3–6, 8, 10, and 12). These results clearly indicate that stimulation of pol η by Ctf18-RFC is caused by an increase in its initiation frequency and not of its processivity.

Interaction of Ctf18-RFC with Pol η—Potential direct interactions of Ctf18-RFC and RFC with pol η were examined by a pull-down assay (Fig. 5A). Recombinant pol η was mixed with anti-FLAG antibody beads pre-bound with FLAG-RFC or Ctf18-RFC, and pol η bound to the beads was detected by immunoblotting. Both RFC and Ctf18-RFC bound with pol η in the presence of 0.15 M NaCl (lanes 7–9 and 11–13), and the binding efficiencies of RFC and Ctf18-RFC to pol η were almost the same.

We further evaluated the interaction of Ctf18-RFC and pol η in vivo by immunoprecipitation using a GFP-pol η fusion (GFP-pol η). GFP-pol η and FLAG-tagged Ctf18 were transiently co-expressed in 293 cells, followed by fixation with 0.1% formaldehyde and extraction and incubation with agaro beads conjugated to anti-GFP antibodies, and Ctf18 remaining on the beads was detected. FLAG-Ctf18 specifically interacted with GFP-pol η but not with GFP in vivo (Fig. 5B, lanes 2 and 4). These results strongly suggest that pol η and Ctf18 functionally interact in vivo.

DISCUSSION

Detection of Pol η Obtained from Human Cell Extracts as a DNA Polymerase Stimulated by Ctf18-RFC—To identify potential targets of the Ctf18-RFC loader complex, we examined lysates of human cells for Ctf18-RFC-stimulated DNA polymerase activity. We purified fractions in which activity was found and identified pol η and λ in the most purified fraction by LC/MS/MS analysis.

Of these, only pol η is responsible for activity, since recombinant pol η exhibits the same Ctf18-RFC-dependent stimulation as the native polymerase that we purified. However, our results do not
Stimulation of DNA Polymerase η by Ctf18-RFC

(A) 

- 

RFC 

Ctf18-RFC loader 

Input 

Bound 

pol η 

p140 

Ctf18 

p37 

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 Lane 

(B) 

Expression 

[ IN B IN B ] 

Ctf18 

GFP-pol η 

GFP 

Input 

Bound 

1 2 3 4 5 Lane 

FIGURE 5. A, interaction of pol η with Ctf18-RFC or RFC. Anti-FLAG beads prebound with FLAG-RFC (lanes 6–9) or FLAG-Ctf18-RFC (lanes 10–13) or unbound (lanes 2–5) were mixed with purified His-pol η, and the bound proteins were detected by immunoblotting with anti-pol η, anti-FLAG, or anti-RFC p37 antibodies. Lanes 1, 14, and 15, 6 ng of His-pol η and 30 ng of purified RFC and Ctf18-RFC, respectively. B, co-immunoprecipitation of GFP-pol η and FLAG-Ctf18 expressed in 293 cells. Anti-GFP beads were mixed with cell lysates co-expressing GFP and FLAG-Ctf18 (lanes 1 and 2) or GFP-pol η and FLAG-Ctf18 (lanes 3 and 4), and proteins bound to the beads were detected by immunoblotting with anti-FLAG (Ctf18), anti-pol η (GFP-pol η), or anti-GFP (GFP) antibodies. IN and B, the bands from 10% of the input lysates and in bound fractions, respectively.

rule out the possibility that other DNA polymerases can also be stimulated by Ctf18-RFC or that other factors are involved in the activity of native pol η fractions.

At earlier fractionation steps, RFC stimulated polymerase activity more strongly than did Ctf18-RFC, although purified pol δ was stimulated at similar levels by both RFC and Ctf18-RFC. Consistent with our previous observation (14), the addition of a crude fraction blocked the stimulation of pol δ by Ctf18-RFC but not by RFC. Based on this result, we predict that specific factor(s) in these fractions direct RFC to load PCNA for pol δ DNA synthesis.

Stimulation of Pol η by Ctf18-RFC—Our results demonstrate that between 30 and 90 mM NaCl, a range close to physiological ionic concentrations, pol η is efficiently stimulated by Ctf18-RFC, but to a more limited extent by RFC, in the presence of PCNA (Fig. 3). Previous reports indicated that PCNA interacts with pol η and stimulates DNA synthesis in the presence of RFC (37–39). We obtained a result essentially consistent with these reports when we used 1 μg/ml recombinant pol η at 150 mM NaCl; under these conditions, both RFC and Ctf18-RFC stimulated pol η severalfold (Fig. 3A, lanes 33–35). Thus, Ctf18-RFC and PCNA stimulate pol η over a wide range of salt and pol η concentrations, and the combination of RFC and PCNA stimulates pol η only at high salt and pol η concentrations.

Ctf18-RFC and RFC appear to stimulate pol η through different mechanisms (Fig. 4). RFC had a biphasic effect on DNA synthesis in the presence of PCNA, in that pol η activity was stimulated at a low RFC concentration but inhibited at a high RFC concentration (Fig. 4A, lanes 7, 10, and 11). This may be because RFC can stimulate pol η activity indirectly by loading PCNA, but it inhibits activity by direct interaction, as shown in Fig. 5A. Indeed, we observed a monophasic inhibition of pol η by RFC in the absence of PCNA (Fig. 4A, lanes 2, 5, and 6). Ctf18-RFC exhibited three stimulation modes (Fig. 6): first, an efficient stimulation that depends on PCNA loading, as observed by the requirement for PCNA and ATP (b, cooperative stimulation); second, an about 2-fold stimulation without PCNA and ATP through a direct interaction of Ctf18-RFC with pol η (a, unilateral stimulation by Ctf18-RFC); and third, a severalfold stimulation under high salt and high pol η concentrations (c, unilateral stimulation by PCNA). The third mode is probably the same as that seen with RFC (f) and may represent a direct interaction of pol η with DNA-loaded PCNA, which is sufficient to stimulate pol η severalfold, because loader complexes dissociate from PCNA under high salt conditions. The first and second modes are Ctf18-RFC-specific and are significant for pol η function. In this way, the nuclear concentrations and localizations of RFC and Ctf18-RFC contribute to the recruitment of functional DNA polymerases at appropriate DNA synthesis sites. For example, Ctf18-RFC (or, alternatively, the Ctf18-RFC and PCNA complex) first binds to an initiation site, which might be a Ctf18-RFC-specific DNA structure, and then attracts pol η to this site. In contrast, RFC might inhibit the initiation of pol η-dependent DNA synthesis at “normal” sites, and instead it recruits the major replicative polymerases.

It has been reported that pol η exhibits distributive DNA synthesis and that its processivity is less than 4 nt with or without PCNA and RFC (37, 38, 40). In our experiments, the DNA
products synthesized by pol η in the presence of Ctf18-RFC appeared to be longer than those observed in its absence. However, DNA synthesis in the presence of excess trap DNA clearly demonstrated that the processivity of pol η was 1–2 nt with or without Ctf18-RFC and PCNA, indicating that the stimulation of pol η by Ctf18-RFC is due to a more frequent initiation of pol η-dependent synthesis.

*Interaction of Pol η and PCNA Loader Complexes*—We demonstrated that pol η directly interacts with Ctf18-RFC or RFC and is regulated by these factors even in the absence of PCNA. Although we have not mapped the target subunits of the loader complexes involved in this interaction, it is likely that the largest subunits, RFC1 and Ctf18, mediate the individual effects on pol η. The clamp loader complex RFC was recently reported to have novel functions through its interactions with other factors. For example, RFC interacts with DNA ligase I and negatively regulates its activity (41). A histone deposition protein, Asf1, binds directly to RFC, is recruited to DNA, and may play a role in replication-coupled chromatin assembly or replication fork progression (42). RFC1 is also suggested to regulate transcription, since it has been found to interact with several transcription factors (43–46). Thus, RFC probably has multiple functions in cell proliferation, which affect DNA replication, repair, transcription, and chromatin remodeling, and Ctf18-RFC also probably has roles other than in PCNA loading.

A Possible Functional Link between Pol η and Chromosome Cohesion—Deficiencies in the human pol η gene cause the xeroderma pigmentosum variant syndrome, and a major biochemical function of pol η is to synthesize past pyrimidine dimer lesions by inserting the correct base opposite the damage site (20, 28, 32, 48–50). Our data suggest that pol η might also have a role in the chromosome cohesion pathway. However, the only genetic evidence supporting this has been analysis of the eso1 gene in the fission yeast *Schizosaccharomyces pombe*. This gene encodes a novel acetyltransferase that acetylates cohesin proteins (51) and is highly homologous to *Sacccharomyces cerevisiae* Ctf7 (also called Ecol), which is essential for the establishment of sister chromatid cohesion during S phase (52–55). Interestingly, the N-terminal two-thirds of Eso1 is highly homologous to pol η, and the C-terminal one-third is highly homologous to Ctf7 (54), suggesting a functional link between pol η and chromosome cohesion. However, the pol η-homologous region in Eso1 does not appear to be essential for chromosome cohesion, since it can be deleted without loss of function. Even so, pol η could still be involved in the chromosome cohesion pathway. For example, if pol η has a redundant function or plays a modulating role in this pathway, no obvious defects would be observed. We observed nuclear coexpression of pol η and Ctf18 in human cells. Our preliminary immunofluorescence microscopy data also demonstrate partial nuclear co-localization of pol η with Ctf18 (data not shown), suggesting that they have a functional link in vivo. In relation to this notion, preliminary data indicate that Rad30 may be required for damage-induced cohesion after γ-irradiation in G2 phase. Thus, our observed interaction between Ctf18-RFC and pol η may be relevant to this pathway.

Possible Functional Coordination of Pol η and Ctf18-RFC in Multiple Processes That Maintain Chromosome Integrity—Our results also suggest that Ctf18-RFC is required for the DNA damage response pathway or other novel reactions involving pol η. Previous work demonstrated that stalling of pol δ and/or ε leads to checkpoint signaling through the redundant functions of Rad17-RFC and Ctf18-RFC (56). A genome-wide DNA integrity network study also demonstrated the importance of Ctf18-RFC in the DNA damage response pathway (57). Thus, Ctf18-RFC may monitor the status of replication forks by interacting with pol η and simultaneously enhancing its TLS activity. Relevant to this idea, the mismatch repair protein MSH2, which interacts with pol η, recruits it to U:G mismatch sites and stimulates its DNA synthesis activity (58). Thus, pol η may interact with various proteins that monitor aberrant DNA structures.

Pol η is also implicated in the 3′ strand extension of D loop structures during mitotic homologous recombination in vivo (59) and in vitro (60) (reviewed in Ref. 61). Interference studies of cohesin in various species demonstrate that cohesin functions not only in cohesion but also in DNA repair and recombination (62–65). For example, the cohesin complex can be recruited to regions surrounding double strand breaks and can function in their repair (66–68). Thus, pol η may be functionally significant in the cohesion process leading to DNA recombination.

In this study, we have demonstrated a novel functional link between pol η and Ctf18-RFC that is suggestive of a protein network that coordinates DNA repair, recombination, and chromosome cohesion reactions with replication fork progression. Ctf18-RFC and RFC probably recognize intermediate DNA structures created by stalled replication forks at sites of damage or of cohesion and recruit not only PCNA but also pol η and interacting proteins. Subsequently, their assembly would switch DNA synthesis to alternative modes to allow replication through these structures. Our observations suggest that, in addition to PCNA, PCNA loader complexes are a functional core that maintains replication forks at several interfering structures. Further studies on the molecular mechanism of the DNA polymerase switch mediated by PCNA and PCNA loader complexes are necessary to understand the complexity of the homeostatic mechanisms that regulate replication fork activity at various chromosomal structures.

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**REFERENCES**

1. Waga, S., and Stillman, B. (1998) *Annu. Rev. Biochem.* 67, 721–751
2. Neuwald, A. F., Aravind, L., Spouge, J. L., and Koonin, E. V. (1999) *Genome Res.* 9, 27–43
3. Iyer, L. M., Leipe, D. D., Koonin, E. V., and Aravind, L. (2004) *J. Struct. Biol.* 146, 11–31
4. Shiomi, Y., Usukura, J., Masamura, Y., Takeyasu, K., Nakayama, Y., Obuse,
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C, Yoshikawa, H., and Tsurimoto, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14127–14132
5. Ellison, V., and Stillman, B. (2001) Cell 106, 655–660
6. O’Donnell, M., Jeruzalmi, D., and Kuriyan, J. (2001) Curr. Biol. 11, R935–R946
7. Bowman, G. D., O’Donnell, M., and Kuriyan, J. (2004) Nature 429, 724–730
8. Miyata, T., Oyama, T., Mayanagi, K., Ishino, S., Ishino, Y., and Morikawa, K. (2004) Nat. Struct. Mol. Biol. 11, 632–636
9. Kim, J., and MacNell, S. A. (2003) Curr. Biol. 13, R873–R875
10. Majka, J., and Burgers, P. M. (2004) Prog. Nucleic Acids Res. Mol. Biol. 78, 227–260
11. Kouprina, N., Tsoiuladze, A., Koryabin, M., Hieter, P., Spencer, F., and LariJonov, V. (1993) Yeast 9, 11–19
12. Kouprina, N., Kroll, E., Kirkillov, A., Bannikov, V., Zakhariey, V., and LariJonov, V. (1994) Genetics 138, 1067–1079
13. Bermudez, V. P., Maniwa, Y., Tappin, I., Ozato, K., Yokomori, K., and Hanaoka, F. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10237–10242
14. Shiomi, Y., Shinozaki, A., Sugimoto, K., Usukura, J., Obuse, C., and Tsurimoto, T. (2004) Genes Cells 9, 279–290
15. Wang, Z., Castano, I. B., De Las Penas, A., Adams, C., and Christians, M. F. (2000) Science 289, 774–779
16. Edwards, S., Li, C. M., Levy, D. L., Brown, J., Snow, P. M., and Campbell, J. L. (2002) Mol. Cell. Biol. 22, 7233–7248
17. Zhou, Y., and Wang, T. S. (2004) Annu. Rev. Biochem. 73, 133–163
18. Bemark, M., Khamlichi, A. A., Davies, S. L., and Neuberger, M. S. (2000) Curr. Biol. 10, 1213–1216
19. Prakash, S., Johnson, R. E., and Prakash, L. (2005) Annu. Rev. Biochem. 74, 317–353
20. Stillman, B., and Gluzman, Y. (1985) Mol. Cell. Biol. 5, 2051–2060
21. Tsurimoto, T., and Stillman, B. (1989) Mol. Cell. Biol. 9, 609–619
22. Ohta, S., Shiomi, Y., Sugimoto, K., Obuse, C., and Tsurimoto, T. (2002) J. Biol. Chem. 277, 40362–40367
23. Matsumoto, M., Hatakeyama, S., Oyama, K., Oda, Y., Nishimura, T., and Takeda, S. (2001) Genes Cells 6, 1023–1027
24. Matsumoto, M., Hatakeyama, S., Oyama, K., Iwai, S., and Hanaoka, F. (2000) J. Biol. Chem. 275, 14127–14132
25. Fukuda, K., Morioka, H., Imaiou, S., Ikeda, S., Ohtsuka, K., and Hanaoka, F. (1999) J. Biol. Chem. 274, 28098–28105
26. Sonoda, E., Matsusaka, T., Morrison, C., Vagnarelli, P., Hoshi, O., Ushiki, K., and Kisker, C., (2001) Nature 407–415
27. Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., and Nasmyth, K. (1999) Genetics 1557–1568
28. Pan, X., Ye, P., Kondo, T., Nakada, D., Matsumoto, K., and Sugimoto, K. (2001) Mol. Cell. Biol. 21, 4371–4377
29. Nol, T., Kando, T., Nakada, D., Matsumoto, K., and Sugimoto, K. (2001) Mol. Cell. Biol. 21, 5388–5394
30. Wang, X., Yu, D., Wang, X., Bader, J. S., and Boeke, J. D. (2005) Cell 124, 1069–1081
31. Wang, Z., Castano, I. B., De Las Penas, A., Adams, C., and Christians, M. F. (2000) Science 289, 774–779
32. Edwards, S., Li, C. M., Levy, D. L., Brown, J., Snow, P. M., and Campbell, J. L. (2002) Mol. Cell. Biol. 22, 7233–7248
33. Zhou, Y., and Wang, T. S. (2004) Mol. Cell. Biol. 24, 9568–9579
34. Hubscher, U., Maga, G., and Spadari, S. (2002) Annu. Rev. Biochem. 71, 133–163
35. Lehmann, A. R. (2005) FEBS Lett. 579, 873–876
36. Murakumo, Y., Kanjo, N., Akagi, I., Matsumot, C., Hanaoka, F., and Ohmori, H. (2004) Genes Cells 9, 523–531
37. Tanaka, S., Hu, S. Z., Wang, T. S., and Korn, D. (1982) J. Biol. Chem. 257, 8386–8390
38. Tsurimoto, T., and Stillman, B. (1999) Proc. Natl. Acad. Sci. U. S. A. 97, 1023–1027
39. Shikata, K., Ohta, S., Yamada, K., Obuse, C., Yoshikawa, H., and Tsurimoto, T. (2001) J. Biochem. (Tokyo) 129, 699–708
40. Masutani, C., Araki, K., Sonoda, E., Yamada, A., Kusumoto, R., Iwai, S., and Hanaoka, F. (1999) EMBO J. 18, 3491–3501
41. Guo, C., Fischhaber, P. L., Luk-Paszyc, M., Iwai, S., Zhou, J., Kamiya, K., Kischer, C., and Friedberg, E. C. (2003) EMBO J. 22, 6621–6630
42. Ohashi, E., Murakumo, Y., Kanjo, N., Akagi, I., Masutani, C., Hanaoka, F., and Ohmori, H. (2004) Genes Cells 9, 523–531
43. Lehmann, A. R. (2005) FEBS Lett. 579, 873–876
44. Yuasa, M., Masutani, C., Hirano, A., Cohn, M. A., Yamaizumi, M., Nakatani, Y., and Hanaoka, F. (2006) Genes Cells 11, 731–744
45. Harakcska, L., Johnson, R. E., Unk, L., Phillips, B., Hurwitz, J., Prakash, L., and Prakash, S. (2001) Mol. Cell. Biol. 21, 7199–7206