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The Werner and Bloom Syndrome Proteins Catalyze Regression of a Model Replication Fork†

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ABSTRACT: The premature aging and cancer-prone diseases Werner and Bloom syndromes are caused by loss of function of WRN and BLM proteins, respectively. At the cellular level, WRN or BLM deficiency causes replication abnormalities, DNA damage hypersensitivity, and genome instability, suggesting that these proteins might participate in resolution of replication blockage. Although WRN and BLM are helicases belonging to the RecQ family, both have been recently shown to also facilitate pairing of complementary DNA strands. In this study, we demonstrate that both WRN and BLM (but not other selected helicases) can coordinate their unwinding and pairing activities to regress a model replication fork substrate. Notably, fork regression is widely believed to be the initial step in responding to replication blockage. Our findings suggest that WRN and/or BLM might regress replication forks in vivo as part of a genome maintenance pathway, consistent with the phenotypes of WRN- and BLM-deficient cells.

RecQ helicases are critical for genome stability, although their exact metabolic functions are currently unclear. Humans possess five RecQ family members, including the WRN, BLM, and RECQ4 proteins defective in Werner (WS),[1] Bloom (BS), and Rothmund-Thomson (RTS) syndromes, respectively (*1*−*3*). These syndromes are characterized by early onset and increased frequency of cancer; WS is also notable for premature appearance of other age-related phenotypes including atherosclerosis, cataracts, osteoporosis, and diabetes. Cells derived from WS, BS, and RTS patients show increased chromosomal aberrations that vary in nature between syndromes, suggesting at least partially non-overlapping roles in genome maintenance.

RecQ helicases share strong homology within defined sequence motifs characteristic of many DNA-dependent ATPase and unwinding enzymes. WRN and BLM unwind DNA with a 3′ to 5′ polarity and have similar DNA substrate specificity, preferring unusual DNA structures including forks, bubbles, D-loops, triplexes, Holliday junctions, and G-quartets (*4*−*6*). Recently, WRN, BLM, and several other RecQ helicases were shown to possess strand annealing activity (*7*−*10*). Coordination between unwinding and strand annealing by WRN or BLM has been demonstrated to mediate strand exchange (*9*) and likely underlies their ability to branch migrate Holliday junctions (*11*, *12*). The N-

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[1] Abbreviations: WS, Werner syndrome; BS, Bloom syndrome; RTS, Rothmund−Thomson syndrome; HU, hydroxyurea; PAGE, polyacrylamide gel electrophoresis; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); bp, base pairs; nt, nucleotides.

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terminus of WRN harbors a 3′ to 5′ exonuclease activity unique among human RecQ helicases (*13*), although its requirement for WRN function in vivo remains controversial.

WRN and BLM have been postulated to act in DNA metabolic pathways dealing with replication fork blockage and replication restart (*4*, *5*). In this regard, both WS and BS cells have S phase abnormalities compared to their normal counterparts (*14*−*17*). WRN-deficient cells are hypersensitive to agents that inhibit replication, including hydroxyurea (HU), topoisomerase inhibitors, and interstrand crosslinkers (*18*−*20*). BLM-deficient cells are also hypersensitive to selected genotoxins (*21*−*23*). In normal cells, WRN relocalizes upon treatment with HU and DNA damaging agents to foci containing replication factors and newly replicated DNA (*11*, *24*, *25*), suggesting that it is recruited to stalled replication forks. Following HU and certain DNA damaging treatments, BLM migrates to nuclear foci that also contain PCNA (presumably associated with replication sites) and the MRN complex (*26*−*28*). Thus, several lines of evidence suggest that WRN and/or BLM may function in response to replication blockage.

Theoretically, pathways dealing with replication blockage are initiated by fork regression, a process requiring unwinding of parental−daughter duplex arms with concomitant repairing of parental strands and pairing of nascent daughter strands to generate a Holliday junction or "chicken foot" structure (*29*−*31*). This action might then facilitate several options for repair or circumvention of the obstacle to fork progression. The finding that WRN and BLM possess both DNA unwinding and strand pairing capability suggests that these enzymes might be well-suited to regress replication forks, a hypothesis that we tested here on a model replication fork substrate. Importantly, WRN and BLM catalyzed rapid and coordinated production of parental and daughter duplexes, the latter specifically reflective of fork regression events. Our findings suggest that WRN or BLM may participate in regression of blocked replication fork regression, consistent with the DNA damage hypersensitivity, S phase abnormalities, and genomic instability of WS and BS cells, respectively.

EXPERIMENTAL PROCEDURES

Enzymes. The WRN-E84A protein possesses normal DNA-dependent ATPase and helicase activities but contains a mutation that eliminates its 3′ to 5′ exonuclease activity (*13*); conversely, the WRN-K577M protein contains a mutation that abolishes its ATPase and helicase activities but retains exonuclease activity (*32*, *33*). These proteins were overexpressed and purified as described previously (*9*). Wild type BLM and BLM-D795A protein containing a mutation that abolishes ATPase and helicase activities were overexpressed in yeast and purified as previously reported (*34*). Rep, PriA, UvrD, RecG, and RecQ from *Escherichia coli* were gifts from Kenneth Marians (Sloan-Kettering), Steve Matson (University of North Carolina), Robert Lloyd (University of Nottingham), and Jim Keck (University of Wisconsin) and were expressed and purified to homogeneity as specified in earlier reports (*35*−*38*). Protein concentrations were determined by the Bradford assay and/or SDS−PAGE using standards of known concentration.

DNA Substrate Preparation. Oligomers were purchased from Midland Certified Reagent Company (Midland, TX), and their nucleotide sequences (in 5′ to 3′ orientation) were as follows: 70lag = GCTATCGTACATGATATCCTCACACTCTGAATAGCCGAATTCTTAGGGTTAG-GGTTAACATCAAGTCACG, 27lag = GAGTGTGAGGATATCATGTACGATAGC, 30lag = TCAGAGTGTGAGGATATCATGTACGATAGC, and 70lead = CGTGACTTGATGTTAACCCTAACCCTAAGAATTCGGCTTAAGTGAGTGTGAGGATATCATGTACGATAGC, and 27lead = GCTATCGTACATGATATCCTCACACTC. The 70lag and 27lead oligomers were radiolabeled at their 5′ ends using ^{32}P-γ-ATP and T4 polynucleotide kinase (New England Biolabs). In separate annealing reactions, labeled 70lag and 27lead were combined with 27lag and 70lead oligomers, respectively, heated to 95 °C, and slow-cooled to generate lagging and leading parental−daughter partial duplexes. These partial duplexes were then incubated together for 18−24 h at 25 °C to generate replication fork substrate that was extracted from a gel slice following native 8% polyacrylamide gel electrophoresis (PAGE). Fork substrates constructed with 30lag oligomer (instead of 27lag) were used where indicated. For use as substrates in annealing reactions as well as markers for specific DNA products, other labeled structures were similarly prepared, including 70lag/70lead (parental duplex), 27lead/27lag (daughter duplex), 70lag/27lag, 70lag, and 27lead. Concentrations of DNA species were determined from the specific activities of their radiolabeled strands, noting that 70lag and 27lead were labeled with approximately equal efficiency.

Enzymatic Assays. For fork regression assays, fork substrate (100 pM) was preincubated with enzyme (WRN-E84A, WRN-K577M, BLM, BLM-D795A, UvrD, PriA, Rep, RecG, or RecQ, concentrations given in the figure legends) in reaction buffer (40 mM Tris, pH 7.0, 4 mM $MgCl_2$, 1 mM ATP, 0.1% Nonidet P40, 100 μg/mL bovine serum albumin, 5 mM dithiothreitol) for 5 min at 4 °C and then transferred to 37 °C for the specified times. ATP was replaced by ATPγS (1 mM) or omitted from selected reactions. Reactions were stopped using one-sixth volume of helicase dyes (30% glycerol, 50 mM EDTA, 0.9% SDS, 0.25% bromphenol blue, 0.25% xylene cyanol) and analyzed by native 8% PAGE. Gels were vacuum-dried, and DNA products were visualized and quantified using a Storm 860 phosphorimager and ImageQuant software (GE Healthcare). Radioactivity associated with intact fork, leading daughter strand, parental duplex, and daughter duplex products was determined for each reaction. The percentage of each product with respect to the molar amount of original fork substrate was quantitated, with (Figure 1C) or without (Figure 2B) subtraction of background amounts of DNA species (other than intact fork) present in reactions without enzyme. In annealing assays, labeled 70lag or 27lead (100 pM) in reaction buffer minus ATP was incubated for 5 min on ice without or with WRN-E84A or BLM at amounts specified in the figure legends. Immediately after addition of unlabeled 70lead or 27lead (100 pM), respectively, reactions were incubated at 37 °C for 15 min then stopped with helicase dyes including excess unlabeled 70lag or 27lead (10 nM) to prevent further annealing. DNA products were separated and visualized as described above.

RESULTS

Construction of Replication Fork Substrate. A model replication fork structure was constructed to test the hypoth-

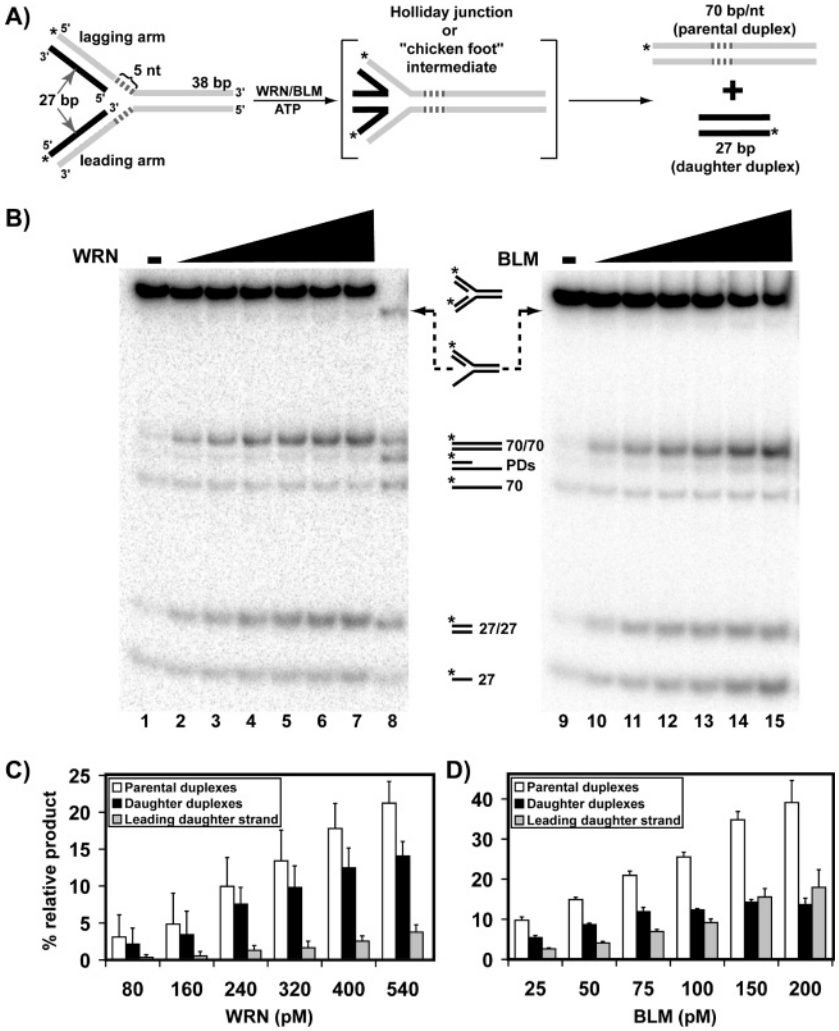

FIGURE 1: Actions of WRN-E84A and BLM on a replication fork substrate. (A) Structure of the replication fork substrate and potential regression products. The replication fork substrate (at left) with homologous leading and lagging parental−daughter duplex arms was constructed from four oligomers (70lag, 27lag, 70lead, 27lead); 70lag and 27lead were 5′ radiolabeled (indicated by asterisks). Solid lines indicate entirely complementary parental (gray) and daughter (black) strands, while dashed lines represent 5 nt noncomplementary sequences. At right, diagram of fork regression pathway by which WRN-E84A and BLM can result in formation of 70 bp (parental) and 27 bp (daughter) duplex products. (B) As detailed in Experimental Procedures, reactions containing replication fork substrate (100 pM) and WRN-E84A (left; 0, 80, 160, 240, 320, 400, 540 pM) or BLM (right; 0, 25, 50, 75, 100, 150, 200 pM) were incubated 5 min at 37 °C and DNA products were analyzed by native PAGE. Specific preformed DNA structures (depicted between panels, PDs = parental−daughter partial duplexes) were loaded as markers (lane 8). (C and D) Bar graph representation of the amounts (percentage with respect to the molar amount of original substrate) of parental duplex (white), daughter duplex (black), and leading daughter strand (gray) formed at various concentrations of WRN-E84A (C) or BLM (D), after subtraction, where applicable, of background amounts present in reactions without enzyme. Data are the mean and standard deviation of four independent experiments performed as in B.

esis that the unwinding and strand pairing activities of specific RecQ helicases might act coordinately to regress stalled replication forks. Creation of this substrate required separate formation of leading and lagging parental−daughter partial duplexes followed by low temperature annealing of these partial duplexes, yielding a forked structure (Figure 1A, left) containing a 38 bp parental region, two completely homologous 27 bp parental−daughter (lagging and leading) arms, and 5 nt of noncomplementary sequence on each arm at the fork junction to prevent spontaneous branch migration. The homology between lagging and leading arms not only mimics actual replication structures but also permits pairing between daughter strands and "re-pairing" of parental strands during putative fork regression (Figure 1A, right). To track potential production of multiple DNA species, this substrate contained two radiolabels of approximately equal specific activity on the lagging parental (70lag) and leading daughter (27lead) strands. Proper formation of this structure was confirmed using appropriate restriction enzymes followed by gel electrophoresis (data not shown).

Actions of WRN and BLM on Replication Fork Substrates. Replication fork substrates were then treated with various DNA helicases including WRN and BLM. Since wild type WRN contains a 3′ to 5′ exonuclease activity that might complicate our analysis of DNA products, an exonuclease-deficient mutant protein, WRN-E84A (subsequently referred to as WRN), was used for almost all assays described below. Since our fork substrate contains three distinct duplex regions, a helicase could potentially unwind one or more of these duplexes resulting in various products when analyzed by native PAGE. Forward unwinding of the fork would yield two parental−daughter partial duplexes (70lag/27lag and 70lead/27lead, or PDs) of identical mobility, while unwinding of either parental−daughter arm would result in a three-

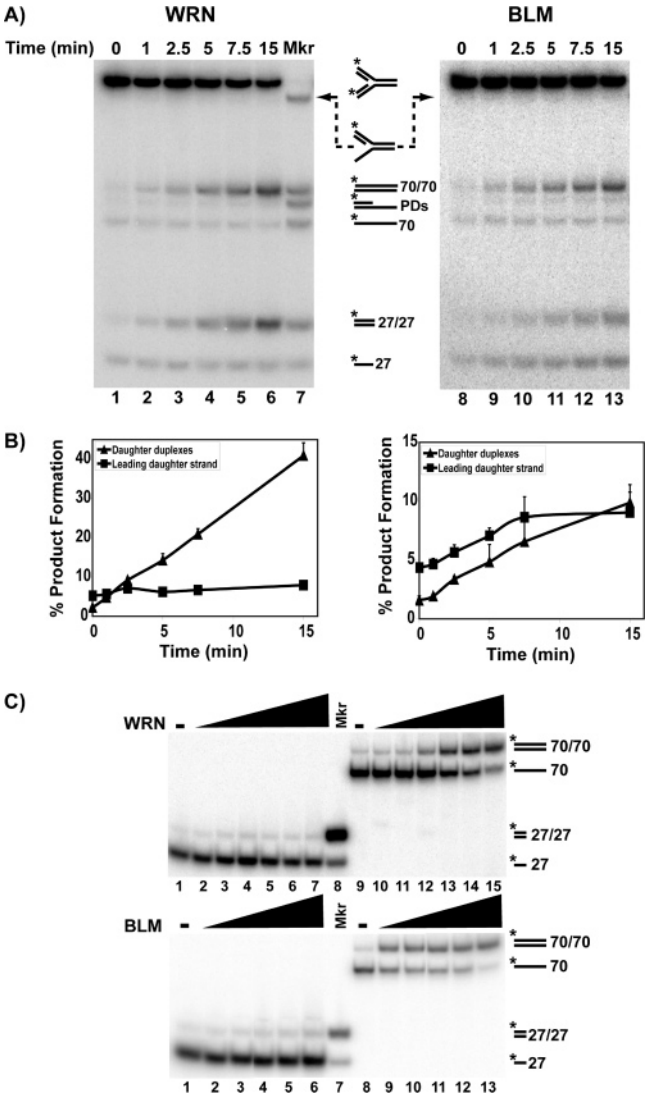

FIGURE 2: Daughter duplex formation by WRN-E84A and BLM is a highly coordinated process. (A) Reactions containing replication fork substrate (100 pM) and either WRN-E84A (240 pM, left) or BLM (50 pM, right) were incubated for the indicated times at 37 °C and analyzed as described in Experimental Procedures. Specific preformed DNA structures (depicted between panels) were loaded as markers (lane 7). (B) Graphs representing the amounts (percentage with respect to molar amounts of original fork substrate) of daughter duplex (solid triangles) and leading daughter strand (solid squares) formed by either WRN-E84A (left) or BLM (right) at varying times. Data are the mean of two experiments performed as described in A. (C) Annealing reactions containing 0.1 nM each of either 27lead and 27lag (left panel) or 70lag and 70lead (right panel) and WRN-E84A (0, 0.16, 0.32, 0.64, 3, 6, and 12 nM, top) or BLM (0, 0.36, 0.72, 1.5, 3 and 6 nM, bottom). Preformed 27lead/27lag duplex was loaded as an annealing marker (top panel, lane 8 and bottom panel, lane 7).

stranded fork structure plus a 27-mer (only visible here if the leading daughter strand was displaced). It is notable that minor amounts of particular DNA species (such as the leading daughter strand) are present in our fork substrate preparations. However, at low enzyme concentrations (beginning with sub-equimolar amounts with respect to DNA substrate), WRN and BLM produced within 5 min primarily two DNA species that comigrated precisely with parental (70lag/70lead) and daughter (27lead/27lag) duplex markers (Figure 1B). Particularly in reactions with BLM, there was some formation of leading daughter strand, but forward fork unwinding or formation of other DNA species by WRN or BLM was negligible at these enzyme concentrations. Quantitation of parental duplex, daughter duplex, and leading daughter strand produced with increasing WRN and BLM concentrations is plotted in Figure 1, panels C and D, respectively. Importantly, daughter duplex could only result from the type of DNA transaction required to initiate fork regression and chicken foot formation, i.e., unwinding of both lagging and leading parental−daughter arms combined with pairing of daughter strands. Although parental duplex is produced from the same exact event, it could also result from unwinding of both daughter strands without their pairing (for example, see results for UvrD and Rep below). Therefore, enzymatic production of daughter duplex from our fork substrate is specifically associated with regression capability. The amount of daughter duplex increases with increasing enzyme concentration, and, at the highest WRN or BLM concentration tested here, is 14.1 or 13.7%, respectively, compared to the amount of original substrate. However, as the molar excess of WRN or BLM to substrate increases, the amounts of leading daughter strand increase. At these concentrations, neither WRN nor BLM can unwind the blunt-ended daughter duplex (data not shown), consistent with reports that fully duplex DNA molecules are poor substrates for these enzymes (*33*, *39*). Thus, production of leading daughter strand probably results from some uncoupling between daughter strand unwinding and daughter duplex formation in the presence of excess enzyme. Notably, a much lower proportion of leading daughter strand is produced by WRN than BLM, indicating that WRN may be more suited to perform regression of this fork structure.

As mentioned above, daughter duplex production must result from unwinding of both lagging and leading parental−daughter arms combined with pairing of daughter strands. Since WRN and BLM possess both unwinding and annealing activities (*7*, *9*), formation of daughter duplexes could be either two unwinding steps followed independently by an annealing step or a coordinated reaction involving both activities within one functional enzymatic unit. To examine the mechanism of daughter duplex formation, two different strategies were employed. The first was to perform kinetic experiments with WRN or BLM on our fork substrate to determine whether complete unwinding (detected by increased formation of leading daughter strand) might precede daughter duplex formation. Limiting concentrations of WRN and BLM were used to reduce the likelihood of unlinked enzymatic events. With WRN, there was no significant increase in leading daughter strand product (over amounts present in the fork substrate preparation alone) at the earliest time points and only a marginal increase thereafter (Figure 2A, lanes 1−7 and B, left panel). In contrast, daughter duplex formation was evident by 1 min and increased linearly with time. The lack of a transient increase in the amount of leading daughter strand suggests that WRN-mediated unwinding and pairing of both daughter strands are intimately linked. When the same analysis was performed with BLM (Figure 2A, lanes 8−13 and B, right panel), the results were not as clear. Formation of both the daughter duplex and the leading daughter strand products was observed at the earliest time point and both increased gradually with time. This result could indicate that BLM either mediates some uncoupled unwinding along with concerted regression or unwinds and

anneals the daughter strands in sequential and possibly independent steps.

The second strategy was to test whether annealing could actually occur independently of unwinding by determining how WRN or BLM acted on the isolated daughter strands. These reactions were performed without ATP to prevent unwinding of potentially annealed product; the same molar amounts of leading and lagging strand oligomers were used as present in the context of the fork substrate, even though this significantly exceeds the concentration of daughter strands that could be displaced at any time during our fork regression assays. Significantly, WRN and BLM each readily annealed the longer (70 nt) parental strands but not the complementary 27 nt daughter strands (Figure 2C). These results indicate that annealing by WRN and BLM is dependent upon oligomer length, in agreement with other studies (*7* and our unpublished results). Most importantly for this study, the inability of WRN or BLM to anneal the isolated daughter strands suggests that daughter duplex formation during regression cannot occur independently of unwinding. These results, combined with our kinetic studies, indicate that daughter duplex production upon incubation of either WRN or BLM with our replication fork substrate must be a concerted process that directly links unwinding of the parental-daughter arms to pairing of daughter strands. WRN apparently performs this concerted reaction more efficiently than BLM, which occasionally fails to pair the daughter strands in association with unwinding.

Specificity of the Regression Reaction for WRN and BLM. Most RecQ helicases including WRN and BLM use energy from ATP hydrolysis to unwind DNA duplexes. To determine if ATPase and helicase activities were required for these fork regression events, reactions with our replication fork substrate were performed with WRN or BLM in the presence or absence of ATP or the poorly hydrolyzable analog, ATPγS. These experiments again show that WRN- or BLM-mediated fork regression, as evidenced by daughter duplex formation, occurs in the presence of ATP; in contrast, production of daughter or parental duplex is not detected when nucleotide is omitted or when ATPγS is present (Figure 3A,B). Furthermore, the ATPase- and helicase-deficient WRN-K577M and BLM-D795A mutants fail to generate daughter duplex in the presence of ATP (Supporting Information, Supplementary Figure 1A), although the 3′ to 5′ exonuclease activity retained in WRN-K577M can act on fork substrates to digest the leading daughter strand and occasionally generate DNA products not attributable to fork regression. When other riboucleotides were substituted for ATP, only CTP supported (albeit weaker) formation of daughter duplex by WRN (Supporting Information, Supplementary Figure 1B), in agreement with studies examining WRN-mediated unwinding of partial duplex substrates (*40*). These results not only demonstrate that ATP hydrolysis and DNA unwinding are required for fork regression, but also confirm that these events are catalyzed specifically by WRN and BLM.

Regarding specificity, it was also informative to examine how other DNA helicases behaved with respect to our replication fork substrate. Several other unwinding enzymes, including the *Escherichia coli* UvrD, PriA, Rep, and RecQ proteins suggested to participate in regression or resolution of stalled replication forks (*36*, *41*, *42*), were tested to

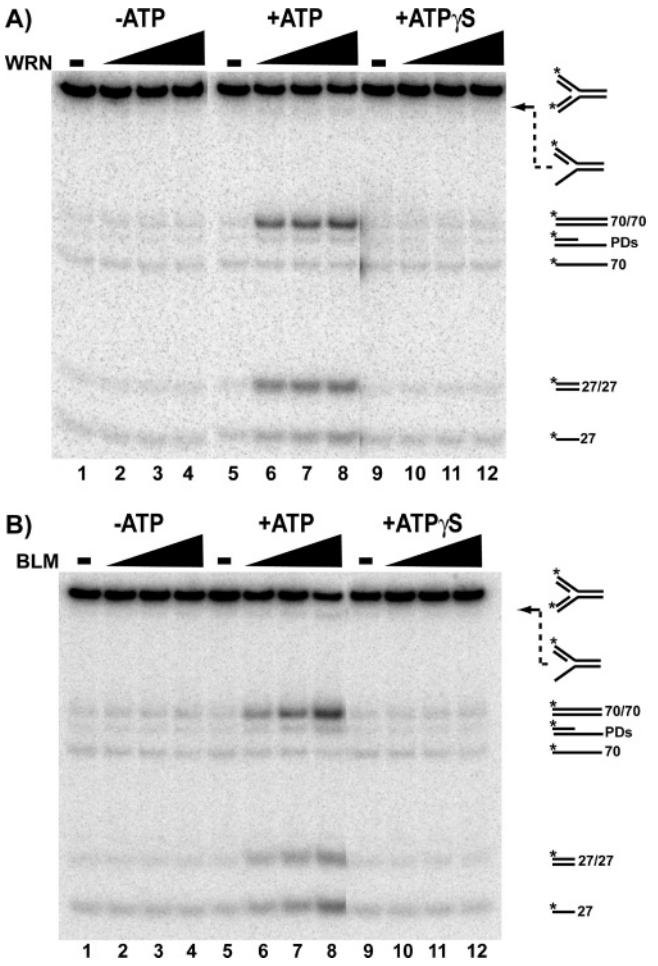

FIGURE 3: ATP hydrolysis and DNA unwinding is required for daughter duplex formation. (A) Regression assays performed on replication fork substrate (100 pM) and WRN-E84A (0, 60, 120, and 240 pM) with or without ATP or ATPγS (1 mM) as indicated were incubated for 5 min at 37 °C. (B) Fork regression assays as described above were performed with BLM (0, 180, 360, and 720 pM) plus or minus ATP or ATPγS as indicated. Markers representing migration of specific DNA structures are depicted at right of panels A and B.

determine whether regression was relatively unique to WRN and BLM. However, none of these helicases produced significant amounts of daughter duplex that reflects fork regression capability. UvrD and Rep generated the leading daughter strand as well as the parental duplex, indicating unwinding of both parental−daughter duplexes without daughter strand pairing (Figure 4, lanes 1−14). In contrast, PriA catalyzed either unwinding of only the leading daughter strand with concomitant production of a three-stranded fork or forward unwinding to generate parental−daughter duplexes (Figure 4, lanes 15−20). Although from the same helicase family as WRN and BLM, RecQ also did not detectably regress our replication fork substrate but primarily catalyzed forward unwinding of the fork to generate parental−daughter partial duplexes that were sometimes further unwound to single-stranded products (Figure 4, lanes 21−26). These experiments also indicated that, in contrast to the near equimolar amounts of WRN, UvrD, and RecQ that could readily act on our fork substrate, significant molar excesses of Rep and PriA were needed to generate new DNA products. In kinetic experiments, UvrD again generated parental duplex and leading daughter strand, indicating

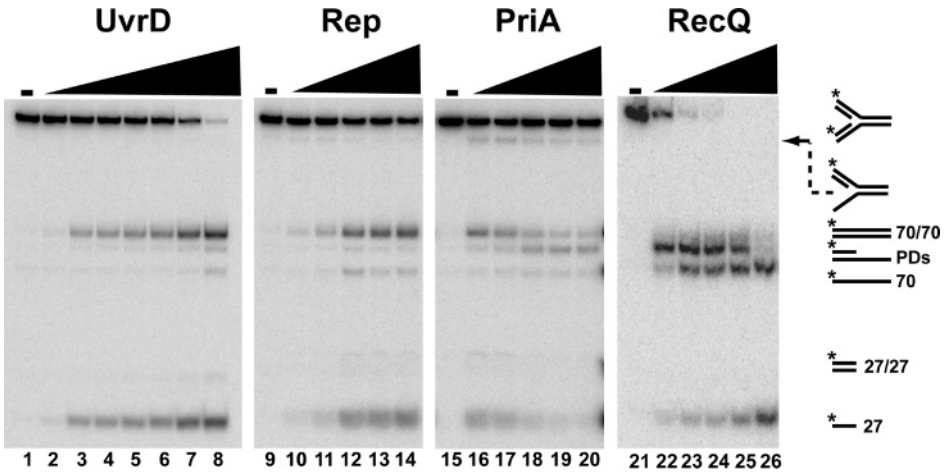

FIGURE 4: Activities of UvrD, Rep, PriA, and RecQ helicases on the replication fork substrate. (A) Regression assays were performed on replication fork substrate (100 pM) with UvrD (lanes 1−8; 0, 25, 50, 100, 200, 400, 600, and 1200 pM), Rep (lanes 9−14; 0, 1, 5, 25, 45, and 90 nM), PriA (lanes 15−20; 0, 1, 5, 15, 45, and 90 nM), or RecQ (lanes 21−26; 0, 0.2, 0.6, 2, 6, 20 nM) for 5 min at 37 °C. Positions of specific DNA structures are depicted at right.

unwinding of both daughter strands without their pairing; throughout the time course, significant amounts of other DNA products are not observed, ruling out the possibility that daughter duplex was formed and then subsequently unwound by UvrD (data not shown). These results demonstrate that fork regression is not a general property of helicases or even all RecQ family members but is specific to a minor subset that includes at least WRN and BLM.

DISCUSSION

Our results show that WRN and BLM can coordinate their unwinding and annealing activities on replication fork substrates, resulting in displacement and pairing of daughter strands and repairing of parental strands in reactions mimicking fork regression. This fork regression capability, as evidenced by daughter duplex formation, appears relatively specific to WRN and BLM and requires their inherent ATPase, DNA unwinding, and strand pairing capabilities, as several other DNA helicases do not perform this function even though they unwind this substrate in other ways. However, a similar fork regression function has also been ascribed to *Escherichia coli* RecG (*43*) that we confirmed using our replication fork substrate (Supporting Information, Supplementary Figure 2). Importantly, the inability of UvrD or Rep to generate daughter duplex even though they displaced both daughter strands indicates that daughter duplex formation is not just a fortuitous consequence of daughter strand unwinding. Instead, WRN and BLM appear to be structurally evolved to coordinate their unwinding and annealing activities, as observed with other DNA structures (*9*). In this regard, WRN, at near equimolar concentrations with respect to DNA substrate, catalyzes exclusively regression instead of forward fork unwinding, even when the parental duplex region is a mere 38 bp (and lacking any obstructing lesion). Notably, WRN appears more adept at performing this multi-faceted task in comparison to simple unwinding or annealing. BLM also prefers to unwind the parental−daughter arms instead of the parental duplex; however, daughter duplex formation is less efficient, apparently due to some uncoupling between unwinding and annealing of daughter strands. Thus, WRN may be more likely than BLM to perform this function in DNA metabolism. Alternatively, the higher effectiveness of WRN might be due to specific properties of this replication fork substrate. However, DNase I protection assays show that both WRN and BLM bind to our replication fork substrates in the vicinity of the fork junction with no dramatic differences in binding affinity (data not shown). Since there is no significant release of free daughter strands at limiting WRN concentrations, unwinding of both parental−daughter duplexes probably occurs concurrently and is directly linked to daughter strand pairing. Moreover, daughter strand pairing is likely initiated while unwinding is ongoing, as WRN cannot pair the isolated short daughter strands derived from our fork substrate. Coordinated unwinding of both parental−daughter arms of the replication fork would suggest that two helicase active sites are involved, implying that WRN acts here as perhaps a dimer or higher order oligomer, an inference that should also apply to BLM.

Regression to form a Holliday junction or chicken foot structure has been proposed as the initial step in dealing with replication forks stalled by DNA damage, dNTP shortages, or other circumstances that inhibit forward progression of the replication apparatus (*29*−*31*). Fork regression might permit (1) access to and removal of the obstacle followed by resumption of replication, (2) use of the lagging daughter strand as template for leading strand synthesis and subsequent bypass of lesions in the leading template strand (the so-called strand switching mechanism), or (3) resolution of Holliday junctions to yield double-strand breaks, followed by replication restart using recombinational repair pathways. The inability to properly deal with blocked replication forks may trigger unregulated recombination resulting in large-scale chromosomal changes. Notably, RecQ helicases have often been proposed to function in response to replication blockage, including the specific act of fork regression (*4*, *5*, *44*). Phenotypes caused by loss of WRN function are consistent with a possible role in regressing blocked replication forks. In this regard, WS cells show replication abnormalities including asymmetric progression of replication forks (*15*, *17*). They are also hypersensitive to HU, a compound that depletes dNTP pools, and selected DNA damaging agents including interstrand crosslinkers that prevent unwinding of the DNA template (*18*−*20*). Immunofluorescence studies on

normal cells indicate that, following treatment with DNA damaging agents or HU, WRN migrates quickly to nuclear foci that coincide with sites of replication (*11*, *24*, *25*). Thus, a role for WRN as a replication fork regression factor is consistent with not only its biochemical activity but also the properties of normal and WS cells. Although the WRN exonuclease function has not been studied here, this activity could potentially be involved in fork regression by attacking the leading daughter strand, an Okazaki fragment on the lagging daughter strand, or the free end of the Holliday junction generated during this process. As BS cells also show replication abnormalities, BLM may also act to regress replication forks under certain circumstances or potentially serve as a back-up for WRN. Importantly, during the preparation of this manuscript, another group also demonstrated fork regression activity associated with BLM (*45*). Thus, WRN, BLM, and perhaps other RecQ family members may be structurally designed to catalyze fork regression, even though *E. coli* RecQ appears not to perform this function. However, the lower effectiveness of BLM in performing fork regression may suggest that its primary function is elsewhere, such as in the processing and resolution of double Holliday junction structures in cooperation with topoisomerase III alpha (*46*).

In summary, the helicase and annealing activities of WRN act in concert on a replication fork substrate resulting in daughter strand pairing reflective of a fork regression reaction. These activities are tightly linked in time and space, as the annealing step cannot occur independently from parental−daughter duplex unwinding and single-stranded intermediates are not observed. BLM also performs fork regression, albeit less effectively than WRN. With the exception of RecG, other helicases tested from within and outside the RecQ family simply unwind duplex regions of the fork substrate with no detectable regression activity. A role for WRN or BLM in replication fork regression would be consistent with the genomic instability and cancer-prone phenotypes of WS or BS patients, respectively.

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SUPPORTING INFORMATION AVAILABLE

Experimental results examining (1) ATPase- and helicase-deficient WRN and BLM mutant proteins or the effects of various ribonucleotide cofactors on WRN-mediated activity that further support specificity of our fork regression reaction and (2) RecG regression activity on our replication fork substrate. This material is available free of charge via the Internet at http://pubs.acs.org.

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