The DinG Protein from *Escherichia coli* Is a Structure-specific Helicase*

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**The Escherichia coli** DinG protein is a DNA damage-inducible member of the helicase superfamily 2. Using a panel of synthetic substrates, we have systematically investigated structural requirements for DNA unwinding by DinG. We have found that the helicase does not unwind blunt-ended DNAs or substrates with 3'-ss tails. On the other hand, the 5’-ss tails of 11–15 nucleotides are sufficient to initiate DNA duplex unwinding: bifurcated substrates further facilitate helicase activity. DinG is active on 5’-flap structures; however, it is unable to unwind 3’-flaps. Similarly to the homologous *Saccharomyces cerevisiae* Rad3 helicase, DinG unwinds DNA-RNA duplexes. DinG is active on synthetic D-loops and R-loops. The ability of the enzyme to unwind D-loops formed on superhelical plasmid DNA by the *E. coli* recombinase RecA suggests that D-loops may be natural substrates for DinG. Although the availability of 5’-ssDNA tails is a strict requirement for duplex unwinding by DinG, the unwinding of D-loops can be initiated on substrates without any ss tails. Since DinG is DNA damage-inducible and is active on D-loops and forked structures, which mimic intermediates of homologous recombination and replication, we conclude that this helicase may be involved in recombinational DNA repair and the resumption of replication after DNA damage.

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DinG is a damage-inducible (1–5) SOS-regulated (1) gene encoding for a superfamily 2 DNA helicase (6, 7) related to DNA helicases Chl1 and Rad3 from *Saccharomyces cerevisiae*, Rad15 from *Schizosaccharomyces pombe*, and the human helicases XPD and BACH1 (6, 8–11). XPD, a 5’ → 3’ helicase, is a part of the multisubunit complex TFIIH that plays a dual role in the initiation of transcription from RNA polymerase II promoters and in nucleotide excision repair (NER) (12). TFIIH participates in both subpathways of NER: global genome NER and transcription-coupled NER (13). Mutations interfering with the proper function of XPD helicase in humans result in three rare recessive photosensitive syndromes: xeroderma pigmentosum, xeroderma pigmentosum/Cockayne syndrome, and trichothiodystrophy (13). Mutations in BACH1, the protein interacting with the breast cancer susceptibility factor BRCA1, cause an early onset familial breast cancer (9); one of those mutations was shown to abrogate the ATPase and helicase activity of BACH1 *in vitro* (8). BACH1, also known as BRIPI, was recently identified as the gene defective in the J complementation group of Fanconi anemia, FANCI (14).

Although the functions of XPD and its complexes are intensely studied and fairly well understood, much more has to be learned about BACH1/BRP1/FANCJ and its role in the Fanconi anemia pathway and in cancer susceptibility. As related helicases, BACH1 and DinG proteins could be potentially involved in similar aspects of nucleic acids metabolism. Thus, genetic, mechanistic, and structural studies of the quite tractable bacterial DinG protein could shed some light on the function of human BACH1.

Recently, we purified the *Escherichia coli* DinG protein and carried out its biochemical characterization (7). The DinG protein possesses DNA-dependent ATPase and helicase activities and, like its eukaryotic counterparts, unwinds DNA duplex with a 5’ → 3’-polarity. In doing so, DinG presumably acts as a monomer (7). Both deletion and overexpression of dinG gene result in a mild but measurable and reproducible effect on cell survival after UV irradiation (7).

In the current study, we investigated structural requirements for DinG helicase substrates. We found that DinG is capable of unwinding not only DNA-DNA duplexes but also DNA-RNA hybrids. It prefers branched substrates over 5’-tailed duplexes and can unwind structures that model intermediates of replication and homologous recombination. Based on our findings, we discuss possible biological roles of DinG.

**EXPERIMENTAL PROCEDURES**

**DNAs and Proteins**—All oligodeoxynucleotides were prepared on an Applied Biosystems 380A synthesizer and purified by urea-denaturing PAGE. Synthesis, deprotection, isolation, and handling of 2’-O-methyl oligonucleotides were identical to the procedures for unmodified oligodeoxynucleotides. All the synthetic oligonucleotides exploited in this work are listed in Table 1. The T1 oligonucleotide was obtained by ligation of 55T and chemically 5’-phosphorylated 45T in the presence of the Splint T oligonucleotide. The oligonucleotides to be joined and the splint 20-mer were annealed in T4 DNA ligase buffer and ligated with T4 DNA ligase (*New England Biolabs*). Similarly, B1 was created by ligation of 55B and 5’-phosphorylated 45B in the presence of Splint B. Both the T1 and the B1 strands were PAGE-purified after ligation. T1 and B1 are identical to the + and − strands, correspondingly, of the bacteriophage M13 mp18 genome at

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2 The abbreviations used are: NER, nucleotide excision repair; ATPγS, adenosine 5’-O-(thiotriphosphate); ss, single-stranded; ds, double-stranded; 2’-O-Me, 2’-O-methyl.
positions 1741–1840. The 2′-O-methyl-RNA, biotin, and DNA β-cyanethyl phosphoramidites for preparation of both natural and modified oligonucleotides, as well as chemical phosphorylation reagent II, were purchased from Glen Research. The DNA concentrations are expressed in terms of molecules.

The DinG protein was purified as described previously (7). The protein was expressed in the pET30 Xa/LIC vector and contained His6 and S tags (peptide sequence KETAAAK -FRQHMDS) at the N terminus. This fusion protein was used throughout all the experiments and is referred to as DinG. The purification of the E. coli RecA protein was described in
Human Dmc1 protein was a kind gift from Dr. R. Pezza and was purified as described (16).

**Substrate Preparation**—Oligonucleotide substrates for the unwinding reaction were prepared by thermal annealing. 5 μM 5'-32P-phosphorylated and non-phosphorylated oligonucleotides were premixed in 25 μl of annealing buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM MgCl2), heated at 85 °C for 5 min, and cooled to room temperature over a 1–1.5-h period. The annealed substrates were purified on a 8% (19:1) non-denaturing Tris/taurine/EDTA polyacrylamide gel. To assure that the correct band was excised from the gel, substrates composed of three or more strands were separated along with all the possible intermediates of annealing. DNA was extracted from the gel slices by passive diffusion overnight into the elution buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% SDS, 10 mM EDTA) at room temperature with rigorous shaking, precipitated and reprecipitated with ethanol, and washed with 80% ethanol. Substrates were dissolved in annealing buffer and stored at -20 °C. The integrity of the purified substrates was confirmed by rerunning aliquots of the purified material on a gel. Concentrations were determined after counting aliquots of purified radioactive substrates in a scintillation counter and comparing counts with those for the oligonucleotides with known specific activity that were used for substrate annealing in that experiment.

D-loops stabilized by superhelicity were prepared after deproteinization of the RecA protein-mediated synaptic complexes formed on supercoiled plasmid DNA. 1 μM oligonucleotide (R3, R4, or R5) was preincubated for 10 min at 37 °C with 10.4 μM (for oligonucleotide R3) or 25 μM (for R4 and R5) RecA in 50 μl of the solution containing 50 μg/ml bovine serum albumin, 40 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 0.5 mM ATPγS. After 0.25 μM supercoiled pUC19 DNA was added, the reaction mixture was incubated for an additional 30 min. RecA was removed by treating the reaction mixture twice with buffer-saturated phenol and with phenol-chloroform. Unincorporated single-stranded oligonucleotides were separated from the D-loops by gel filtration through Chromaspin 400 columns (BD Biosciences), equilibrated with annealing buffer. The deproteinization procedure was performed at 4 °C, and the D-loops were stored at -20 °C. All the substrates used in this study are shown in Fig. 1. In the description of D- and R-loops, the strand annealed to the bubble region is referred to as “invading” or “incom-
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The same terms are used for the oligonucleotide strands forming D-loops on supercoiled plasmid DNA.

**Helicase Assay**—Typically, 2 nM helicase substrate was incubated with DinG in 10 μl of the reaction mixture containing 67.5 mM Tris-HCl at pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 2.5 mM ATP, 50 μg/ml bovine serum albumin, 1 mM β-mercaptoethanol, 0.01 mM EDTA, and 5% glycerol. DinG concentrations are indicated in the figures. When a single concentration of DinG was used through the experiment, it was 15 nM in experiments shown in Figs. 3, 6A, and 8 and 50 nM in experiments shown in Figs. 2C and 6B. Unwinding reactions were allowed to proceed for 15 min (or the time indicated in the figure) at 30 °C and were deproteinized by the addition of SDS and EDTA to 1% and 10 mM, respectively. Each set of experiments contained a “positive control” in which the helicase substrate was heat-denatured by incubation for 5 min at 95 °C followed by quick chilling in an ice water bath. Products of the reaction performed with oligonucleotide substrates were separated in 0.4-mm-thick 8% (19:1) Tris/taurine/EDTA-polyacrylamide gels for 1.5–2 h at 3 V/cm. Unwinding of plasmid-based substrates (DR5, DR6, and DR7) was monitored by separating the products in a 1% Tris/acetate/EDTA agarose gel with 3 mM MgCl₂ for 1.5 h at 3 V/cm. Agarose gels were fixed with 5% trichloroacetic acid, blot-dried, and exposed to phosphorimaging screens. Polyacrylamide gels were exposed without prior fixation and drying. Images of the gels were obtained with a BAS 2500 phosphorimaging analysis system (Fuji Medical Systems).

**Surface Plasmon Resonance Biosensor Analysis**—All the experiments were performed on a Biacore 3000 instrument (Biacore) at 25 °C. The continuous flow buffer contained 20 mM Tris-HCl at pH 8.0, 150 mM NaCl, 10 mM MgCl₂, 1 mM β-mercaptoethanol, and 0.005% surfactant P20 (purchased from Biacore). All DNA binding substrates were constructed based on the Bio-T1 oligonucleotide. Bio-T1 was immobilized on the surface of sensor chip SA to give a signal of about 100 response units. Substrates containing two DNA strands (S1, D3, and F1) were made by annealing the second strand to Bio-T1 on the chip surface. The second oligonucleotide (B1, B11, or B8) was injected at the concentration of 400 nM in flow buffer at a flow rate of 2 μl/min over a 20–30-min period at 25 °C. Resonance signals increased and reached the plateau levels expected for the molecular mass of the added oligonucleotide. Substrates F2, F3, and D6 were created by a two-step annealing approach. To prove that this procedure yields completely annealed duplexes and is a valid way to prepare complex immobilized substrates, we made a chip containing immobilized single-stranded oligonucleotides and corresponding duplexes prepared in two different ways. In one case, duplex was prepared by surface annealing; in the second instance, it was preformed in solution and then was immobilized on the chip to the same response units level. We used this chip to investigate the DNA binding activity of human Dmc1 protein that demonstrates clearly different binding properties with respect to ss- and dsDNA. Although different from ssDNA, dsDNA binding by Dmc1 was very similar on both duplexes, proving that the two helices have similar properties (data not shown). Both DinG-DNA complex formation and dissociation were conducted in flow buffer supplemented with 1 mM ATPγS using the “co-inject” mode of the autoinjector and a flow rate of 10 μl/min. DinG was injected at concentrations of 0, 7.8, 15.6, 31.2, 62.5, 125, and 250 nM. Each experiment contained an “empty” control surface used as a measure for nonspecific binding. The surfaces were regenerated by consecutive injections, 20 μl each, of 0.5% SDS in flow buffer and 2 mM NaCl. After baseline normalization, we used BIAevaluation software, version 4.0.1, to fit the experimental sensorgrams to the Langmuir model, which presumes 1:1 binding of the analyte to the ligand. The extracted rate and equilibrium constants of DinG interactions with all the DNA substrates tested are presented in Table 2.

**RESULTS**

The DinG Helicase Prefers Bifurcated Substrates—Initial characterization of the DinG helicase activity (7) suggested that the enzyme might have a preference for branched substrates. Here we investigate the specificity of this helicase for different DNA structures using a variety of synthetic substrates depicted in Fig. 1. First, we tested the ability of DinG to unwind blunt-ended dsDNA. Fig. 2A shows that no unwinding of the 100-mer duplex S1 was observed, even when the DinG concentration was as high as 200 nM. To exclude the possibility that the inability to unwind this 100-mer is a consequence of the low proces-
DinG Requires an 11–15-nucleotide 5'-Extension for Efficient DNA Duplex Unwinding—Fig. 3 shows that, like blunt-ended S14, substrates S2 and S3, having none or 5'-nucleotide 5'-tails, are not unwound by DinG. There is very little, if any, unwinding of substrate S4 carrying a 10-nucleotide 5'-ss extension. Increasing the 5'-overhang to 15 nucleotides in substrate S5 results in efficient unwinding that is similar to that for substrates S6 and S7. The above result suggests that DinG is capable of binding a stretch of ssDNA as short as 11–15 nucleotides. This finding is in agreement with the observation that DinG is capable of displacing a DNA strand after binding to a duplex with a 15-nucleotide gap (data not shown).

The DinG Helicase Is Active on DNA-RNA Hybrid Duplexes—Since the S. cerevisiae homolog of E. coli DinG, Rad3, possesses a DNA-RNA helicase activity (17), we tested whether DinG is capable of unwinding DNA-RNA duplexes. For these experiments, we used a 2'-OMe modified RNA, commonly used as a functional analog of natural RNA (for review, see Ref. 18). RNA composed of 2'-OMe ribonucleotides is conformationally more rigid and chemically more stable than natural RNAs (19, 20). The 2'-OMe sugar modification does not alter ribose sugar pucker and preserves the canonical right-handed A-form conformation in both RNA-RNA and DNA-RNA duplexes (21, 22). Nuclease resistance of 2'-OMe RNA (21, 22) allows much easier manipulations of 2'-OMe RNA-containing oligonucleotides without taking any special precautions.

The ability of DinG to unwind a DNA-RNA hybrid duplex was tested on substrate D4-RNA, which is identical to substrate D4, except for the fact that the labeled strand, R4-RNA, consists of 2'-OMe-RNA. Fig. 4 shows that despite the fact that, as a rule, 2'-OMe-RNA-DNA duplexes are more stable than DNA-DNA duplexes, it is proficiently unwound by DinG.

Helicase Activity of DinG on Flap Structures—That DinG demonstrates an enhanced DNA helicase activity on bifurcated substrates opens up the possibility that it could be active on another type of branched substrates, flap structures. Flap structure can be viewed as a bifurcated structure with one ss and one ds branch. The ability of DinG to unwind both 5'-flaps and 3'-flaps would argue that it can translocate on both ssDNA and dsDNA. Fig. 5 shows that whereas DinG helicase is active on a bifurcated F1 structure, a 3'-flap structure (substrate F2) is unquestionably resistant to unwinding by the DinG protein. At the same time, the 5'-flap (substrate F3) is easily dismantled by DinG, presumably by removing oligonucleotide B8 first and then unwinding the resulting 5'-tailed duplex. Since flap structures can be considered a model for a replication fork, our
results suggest that DinG can use the lagging strand arm to translocate along DNA and unwind duplex ahead of a stalled (or collapsed) replication fork.

**The Invading Strand Can Be Easily Removed from Model D-loops by the DinG Helicase**—D-loops formed upon invasion of ssDNA molecule into the homologous region of a DNA duplex constitute intermediates of RecA-mediated homologous recombination and double-stranded break repair. The DNA damage inducibility of *dinG* (2, 3) suggests its possible role in recombinational DNA repair, and thus, its potential interaction with (or an unwinding activity on) D-loops. Such a possibility was tested, and the results are presented in Fig. 6. We found that the bubble structure D3 serving as a basic building block for synthetic D- and R-loops remained intact after treatment with DinG. The inability of DinG to unwind a bubble duplex cannot be accounted for by weak binding of this substrate by DinG (see below). We can offer two explanations for the lack of helicase activity on substrate D3. First, if only one helicase molecule at a time can be loaded on the bubble region of the substrate, only one duplex can be unwound. The presence of the second intact duplex would significantly facilitate reannealing of the unwound duplex. If the rate of reannealing, which would follow first order reaction kinetics in this case, is higher than the rate of helicase-induced unwinding, DinG would never be able to separate the top and bottom strands from each other. Alternatively, the bubble region can be collapsed and unsuitable for DinG loading. Interestingly, helicases PcrA, PriA, and RecG do not unwind bubble substrates either (23, 24). However, oligonucleotides complementary to the unpaired region in the bubble, the incoming strands, were efficiently released after the addition of DinG. Since DinG is a 5′→3′-helicase that requires an ss tail for its unwinding activity (Fig. 2), we anticipated that only substrate D6 would be unwound. To our surprise, DinG was very proficient in removing the incoming strand from both D5 (without any extension of the invading strand beyond a bubble region) and D7 (having only a 3′-non-homologous extension). Although all D-loop substrates (D5, D6, and D6) were almost completely unwound in 15 min, the possibility exists that the presence of the free 5′-ss tail in substrate D6 would result in its faster unwinding when compared with D5 and D7. Interestingly, we could not see any significant kinetic difference in the unwinding of substrates D1 and D5 (Fig. 6B). Finally, that oligonucleotide R3 could not be displaced from substrate D2 (Fig. 6B) argues against the possibility that a discontinuity in one strand of the DNA duplex is a feature that is responsible for unwinding of substrate D5 by DinG.

**DinG Binds Different DNA Substrates with Similar Affinity**—The lack of helicase activity on blunt-ended dsDNA, a 3′-flap, and a bubble structure could be due to poor DinG binding to those substrates. To examine such a possibility, we measured the rate and equilibrium constants of DinG binding to some of the DNA substrates used in the helicase assays. The binding constants were obtained using surface plasmon resonance technology on a Biacore 3000 instrument. Examples of sensorgrams used to quantify interactions are shown in Fig. 7, and the binding constants for seven representative substrates are presented in Table 2. These data prove that the absence of the helicase activity cannot be accounted for by the instability of DNA-DinG complexes. DinG binds dsDNA, 3′-flap, and bifurcated structures with an affinity similar to that for ssDNA with dissociation equilibrium constants close to 200 nM. Nevertheless, substrates S1 and F2, in contrast to F1, cannot be unwound. Moreover, a preferable helicase substrate F3 has the lowest affinity to DinG, whereas the best DinG binder, bubble D3, cannot be unwound at all. Thus, we could not observe a correlation between the stability of DNA-DinG complexes and the inability of DinG to unwind some of the substrates.

**2′-OMe-RNA Oligonucleotides Can Be Displaced from R-loop Structures**—Since DinG is capable of dismantling synthetic D-loops and unwinds DNA-RNA duplexes, we examined whether it is active on R-loops. The results presented in Fig. 8 demonstrate that the unwinding activity of the helicase on R-loops is very close to that on D-loops. As with the D-loop counterparts, DinG was active on R-loops containing 2′-OMe-RNA without an 5′-extension (Fig. 8, substrates D5-RNA and D7-RNA).
DinG Unwinds D-loops Formed on Superhelical Plasmid DNA—In the experiments described in Fig. 6, the two strands in the D-loop-forming region are not complementary to prevent dissociation of the invading strand via spontaneous branch migration (25, 26). However, the behavior of such model D-loops may be different from the natural structures in which the displaced strand is identical to the invading strand. To form stable bona fide D-loops, we targeted ssDNA to a homologous region of dsDNA in the superhelical plasmid with the E. coli recombinase, the RecA protein. After treatment with SDS, RecA-mediated synaptic complexes are converted to protein-free D-loops that are stabilized by superhelical stress (Fig. 9A).

**DISCUSSION**

Since only a limited amount of information about the biochemical properties of the DinG protein and the phenotypes of mutant dinG genes in various genetic environments is currently available, the biological role of the DinG protein remains elu-
Sive. That DnG is a DNA damage-inducible helicase (1–5), regulated by the SOS circuit (1, 4, 32), suggests its possible involvement in post-DNA damage events, including DNA repair and the resumption of replication. Still, the sole fact that DnG is a LexA-regulated SOS protein does not assure its participation in DNA repair processes. Some of the LexA-regulated proteins (e.g., SulA and FtsK) have no direct role in DNA repair, but rather, are a part of the cellular machinery that regulates cell division and coordinates septation with chromosome segregation (33, 34). On the other hand, to be involved in DNA repair, it is not necessary that a protein should be DNA damage-inducible. In addition to DnG, there are two other DNA damage-inducible helicases in E. coli, UvrD and RuvAB, which are actively involved in different aspects of DNA repair and are part of the SOS response. However, two other helicases, RecG and RecQ, despite taking part in recombinational DNA repair, contain no lexA boxes and were not identified as DNA damage-inducible in two unrelated microarray screening studies of genes induced by DNA damage (5, 32). The DNA damage inducibility of DnG, combined with its ability to unwind model intermediates of homologous recombination and replication, strongly argues for its role in DNA repair and/or replication fork restart pathway(s).

That deletion of dng does not significantly increase UV sensitivity of wild type E. coli cells (7) indicates that the DnG protein is not indispensable in the repair of DNA photolesions or restoration of replication disrupted by UV irradiation. At the same time, it does not exclude DnG from participation in DNA repair and replication re-establishment processes that are, most likely, backed up by multiple layers of redundant pathways. As an example, recQ, a gene that is extremely important in the induction of the SOS response and resumption of DNA replication (30), has a minimal effect on cell survival after UV irradiation when deleted in a wild type background. However, a recQ mutation introduced in a recBCsbcBC background

| Substrate | $k_a$ (M$^{-1}$s$^{-1}$) | $k_d$ (s$^{-1}$) | $K_a$ (M$^{-1}$) | $K_d$ (nM) |
|-----------|-------------------------|-----------------|-----------------|-------------|
| T1        | 6.37 $\times$ 10$^4$    | 1.16 $\times$ 10$^4$ | 5.31 $\times$ 10$^4$ | 193         |
| S1        | 5.81 $\times$ 10$^4$    | 1.27 $\times$ 10$^4$ | 4.59 $\times$ 10$^4$ | 218         |
| F3        | 1.45 $\times$ 10$^4$    | 9.05 $\times$ 10$^4$ | 1.61 $\times$ 10$^4$ | 622         |
| D3        | 5.75 $\times$ 10$^4$    | 6.45 $\times$ 10$^4$ | 1.02 $\times$ 10$^4$ | 98          |
| D6        | 4.96 $\times$ 10$^4$    | 6.44 $\times$ 10$^4$ | 7.71 $\times$ 10$^4$ | 130         |
| F1        | 8.86 $\times$ 10$^4$    | 1.59 $\times$ 10$^4$ | 5.58 $\times$ 10$^4$ | 179         |
| F2        | 9.00 $\times$ 10$^4$    | 1.90 $\times$ 10$^4$ | 4.73 $\times$ 10$^4$ | 212         |
increases UV sensitivity 20-fold (35, 36). Future genetic experiments combining a dinG deletion with mutations in genes whose products are involved in different aspect of DNA metabolism would help to shed a light on the biological role of DinG. At the present, based on our biochemical results, we can only speculate what that role(s) might be.

DinG is not unique in its ability to unwind a variety of DNA substrates different from a plain B-DNA duplex; several structure-specific helicases have been described in viruses, bacteria, fungi, and mammals. Among them are T4 UvsW (37), SV40 large T-antigen (38, 39), Staphylococcus aureus PcrA (23), E. coli RecQ (40), PriA (24, 41), RecG (24), UvrD (42), archaeal Pyrococcus furiosus Hjm (43), S. cerevisiae Sgs1 (44, 45), mitochondrial Hmu1p (46), Drosophila RECQ5 (47), human RECQ5B (48), BLM (45, 49, 50), WRN (50, 51), and BACH1 (10). Over several recent years, many of the helicases listed above have been recognized as important factors that, either alone or in collaboration with other proteins, are responsible for DNA transactions on stalled, damaged, or collapsed replication forks and thus contributing to the maintenance of genome integrity (10, 30, 48, 51–56). DNA damage-induced blockage or spontaneous collapse of the replication fork presents a serious threat to cell viability. Depending on the nature of the DNA lesion and on its location on either the leading or the lagging strand, multiple pathways coping with these damages may be involved (for review, see Refs. 57–63). In E. coli, several helicases (RecBCD, RuvABC, RecG, PriA, and UvrD) have been implicated in overcoming replication arrest (61, 64).

One of the pathways for resumption of replication is replication fork reversal. It involves the annealing of newly synthesized leading and lagging strands to form a four-way Holliday junction, or “chicken-foot,” that is further processed by the recombination machinery (for review, see Ref. 62). Although the first model proposing the chicken-foot is over 30 years old (65), the genetic requirements for this recA-independent pathway of replication fork repair were characterized in detail only recently by Bénédicte Michel et al. (62). They found that UvrD (a 3′ → 5′-helicase) and RuvAB (the branch migration motor complex) are essential for replication fork reversal in certain replication mutants (64, 66); however, inactivation of RecQ, PriA, helicase IV, and DinG did not affect replication fork reversal (64, 67). That DinG is not engaged in this particular pathway for replication fork repair does not exclude its participation in other pathways that might utilize the ability of DinG to unwind the replication fork after binding to a small (10–15-nucleotides) gap in the lagging arm. It is worth mentioning that RecQ has a similar activity (30); however, because of its opposite 3′-→ 5′-polarity, it unwinds the replication fork translocating along the leading strand.

Remarkably, DinG shares many biochemical properties with respect to substrate specificity with its human homolog, BACH1 (10). First of all, both enzymes are inactive on blunt-ended duplexes and require a 5′-ssDNA tail for their unwinding activity. The minimal length of the 5′-ss tail is 15 nucleotides for BACH1 and 11–15 nucleotides for DinG. Although both helicases unwind duplexes with 5′-ss extensions, they are more active on forked substrates, either bifurcated duplexes with ss tails or 5′-flap structures. Neither is active on 3′-flap structures; this suggests that they are not able to translocate on dsDNA. Furthermore, both DinG and BACH1 can remove an incoming or invading strand from model D-loop structures. Amazingly, despite the fact that the unwinding activity of DinG and BACH1 on duplexes DNA have a strict requirement for a 5′-ss tail, both proteins are capable of dismantling D-loops without any protruding ss tails at the incoming strand. Finally, both DinG and BACH1 fail to unwind synthetic Holliday junction structures. The similarity in substrate specificity suggests that DinG and BACH1 may fulfill similar roles, which yet have to be established, during DNA repair or/and recombination.

The enzymatic activity of DinG on D- and R-loops opens the possibility that it might modulate inducible stable DNA replication and constitutive stable DNA replication, two alternative pathways of replication that start at the non-conventional origins, oriK and oriM, respectively. As the formation of D-loops at oriK and R-loops at oriM was proposed to be required for initiation of inducible stable DNA replication and constitutive stable DNA replication, respectively, DinG might inhibit these pathways by dismantling initiation complexes. We have discussed these possibilities in our previous publication (7). Additionally, we proposed that DinG could be responsible for
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Removing D- and R-loops and other aberrant DNA structures ahead of the replication machinery (7). Furthermore, removal of non-productive “junk” D-loops in numerous chromosome locations by DinG would prevent incidents of illegitimate recombination.

Recent work by Rudolf et al. (68) showed that Sulfolobus acidocaldarius XPD helicase is an iron-sulfur (Fe-S) containing protein. They identified four conservative cysteines that are responsible for positioning the Fe-S cluster between the Walker A and B boxes. Moreover, purified E. coli DinG proteins (supplemental Fig. 6 in Ref. 68) demonstrates the D-loop structure by the DinG helicase. The arrowhead next to the gel depicts the top of the gel.

chemical and structural data demonstrate that the iron-sulfur cluster participates in DNA binding and that DNA binding facilitates oxidation of the [4Fe-4S]2+ cluater (reviewed in Ref. 69). This opens the exciting possibility that the E. coli DinG protein might be involved in the binding to and removal of lesions caused by DNA damage.

In conclusion, the biological function of DinG in E. coli cells has yet to be established. Our results on substrate specificity of this helicase strongly argue for its role in recombinational DNA repair or/and re-establishment of replication fork after DNA damage.

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