Characterization of the DNA Damage-inducible Helicase DinG from Escherichia coli*

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The dinG promoter was first isolated in a genetic screen scoring for damage-inducible loci in Escherichia coli (Lewis, L. K., Jenkins, M. E., and Mount, D. W. (1992) J. Bacteriol. 174, 3377–3385). Sequence analysis suggests that the dinG gene encodes a putative helicase related to a group of eukaryotic helicases that includes mammalian XPD (Koonin, E. V. (1993) Nucleic Acids Res. 21, 1497), an enzyme involved in transcription-coupled nucleotide excision repair and basal transcription. We have characterized the dinG gene product from E. coli using genetic and biochemical approaches. Deletion of dinG has no severe phenotype, indicating that it is non-essential for cell viability. Both dinG deletion and overexpression of the DinG protein from a multicopy plasmid result in a slight reduction of UV resistance. DinG, purified as a fusion protein from E. coli cells, behaves as a monomer in solution, as judged from gel filtration experiments. DinG is an ATP-hydrolyzing enzyme; single-stranded (ss) DNA stimulates the ATPase activity 15-fold. Kinetic data yield a Hill coefficient of 1, consistent with one ATP-hydrolyzing site per DinG molecule. DinG possesses a DNA helicase activity; it translocates along ssDNA in a 5′ → 3′ direction, as revealed in experiments with substrates containing non-natural 5′-5′ and 3′-3′ linkages. The ATP-dependent DNA helicase activity of DinG requires divalent cations (Mg²⁺, Ca²⁺, and Mn²⁺) but is not observed in the presence of Zn²⁺. The DinG helicase does not discriminate between ribonucleotide and deoxyribonucleotide triphosphates, and it unwinds duplex DNA with similar efficiency in the presence of ATP or dATP. We discuss the possible involvement of the DinG helicase in DNA replication and repair processes.

The double-stranded (ds)³ oligonucleotide TTGG(N₈)ACAG bound the LexA repressor with high affinity in an electrophoretic mobility shift assay (2). The dinG promoter was also up-regulated upon DNA damage by nalidixic acid (3). dinG, along with lexA and dinI, was isolated in another genetic screen aimed at isolating multicopy suppressors of the cold-sensitive phenotype of the DinD68 mutation. This particular mutation in the DNA damage-inducible dinD gene, which is also regulated by the LexA-RecA system, results in the constitutive expression of the SOS response at lowered temperature (4). Because both dinG and dinI are part of the SOS response (1, 2, 5) and they suppress an SOS phenotype of the dinD68 mutation (6), dinG could also be a negative regulator of the SOS response in a manner similar to dinI (7, 8).

Analysis of the protein sequence of E. coli dinG reveals that it encodes a putative DNA helicase related to yeast DNA helicases Chl1 and Rad3 from Saccharomyces cerevisiae, Rad15 from Schizosaccharomyces pombe, and the human helicases XPD and BACH1 (9, 10). The mutant forms of the last two proteins result in well described human diseases, three human recessive photosensitive syndromes for XPD, and early onset breast cancer for BACH1 (10, 11). DinG and its eukaryotic counterparts have been classified as superfamily II helicases on the basis of the presence of seven canonical helicase motifs in their sequences (9, 12, 13). Still, the presence of the helicase-specific motifs in the protein amino acid sequence per se does not necessarily imply that it is a bona fide helicase. Proteins having helicase motifs but lacking a helicase activity are well known. Among them are the endonuclease (R) subunits of type I and type III restriction-modification enzymes (14), both bacterial and human transcription-repair coupling factors Mfd (15), and CSB/ERCC6 (16), members of SWI2/SNF2 family chromatin remodeling factors (17) and the RAD54 recombinational DNA repair protein (18).

To prove that DinG is a true helicase, we carried out the purification and biochemical characterization of the E. coli DinG protein. In agreement with the prediction (9), DinG possesses DNA-dependent ATPase and helicase activities. We discuss the possible biological role that DinG helicase might play.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids—**Gene deletions were created using a combination of described procedures (19, 20). To construct the metB gene deletion, plasmid pKD3 (19) was used to amplify a deletion cassette containing the cat gene flanked by FLP recognition target sites and 36-nucleotide extensions homologous to regions of DNA directly

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Table I

| Name | Sequence |
|------|----------|
| DinG-5′-pET30 | 5′-GGTTAGGGCTGTCAGGATTTAACCCG3′ |
| DinG-3′-pET30 | 5′-AAGAGGATGGTCAGGCTCTAAAGGCGCG3′ |
| DinG-5′-Xba | 5′-GTTTCTTAGCTAGAAAGGTTTGGC3′ |
| DinG-3′-Xho | 5′-TTTTATCTTCAGGGGTTTACCTGACACTATCA3′ |
| 55B | 5′-CCGTAACACTGATTTCCTGCAAGTAGAGATACAACTACATACGTCGTACCTCAACA3′ |
| BS-S1 | 5′-GCGGCCCTAATTCAGGTTGTCACCTACGTCGTACCTCAACA3′ |
| BS-S2 | 5′-CTGGTTCCAGTCTAGAAAGGTTTGGC3′ |
| TS | 5′-TGTGTTACGTCGTACCTCAACA3′ |
| T8-3′ | 5′-TGTGTTACGTCGTACCTCAACAGGTTTGGC3′ |
| B9 | 5′-GGTACCGACCTGATCTAGCAGGTTCGTTCCAGTACCTCAACA3′ |
| B9-5′ | 5′-GGTACCGACCTGATCTAGCAGGTTCGTTCCAGTACCTCAACA3′ |

The latter PCR fragment was cloned between 5′-Xba and DinG-3′/H11032 and DinG-3′/H11032-pET30 and DinG-3′/H11032-pET30DinG. The latter PCR fragment was cloned between 5′-Xba and DinG-3′/H11032 and DinG-3′/H11032-pET30 and DinG-3′/H11032-pET30DinG. Expression of the multicopy plasmid pDinGPro. Overnight cultures were prepared in LB medium supplemented with 100 μg/ml ampicillin. One hundred microliters of serial dilutions of the cells were plated on LB plates with 100 μg/ml ampicillin and irradiated with different doses of shortwave UV light from a Stratalinker 1800 (Stratagene). The plates were incubated in the dark overnight at 37 °C, and the colonies derived from surviving cells were counted.

Purification of the DinG Protein—Overexpression of the DinG protein was performed in BL21(DE3)/pLysS strain (Fomp T hsdS r (ρ m n) 1 gal dcm Δsrl-reaA306/Tn10(TcR) (DE3) pLysS (CodIV)) transformed with pET30DinG upon induction with IPTG. Four flasks with 1 liter of a bacterial culture in LB medium supplemented with 30 μg/ml kanamycin and 25 μg/ml chloramphenicol were grown at 37 °C to A600 = 0.6, and the protein expression was induced with 1 mM IPTG over a 1.5-h period. The cells were collected by centrifugation at 7,000 rpm for 10 min in a Sorvall SSC-400 rotor, washed with 80 ml of Buffer I (50 mM sodium phosphate at pH 8.0, 300 mM NaCl, 10 mM imidazole, 10% glycerol, and 0.1% Triton X-100), and frozen at −70 °C.

All purification procedures were performed at 4 °C. The cell paste was resuspended in 50 ml of Buffer II (50 mM sodium phosphate at pH 8.0, 1 mM NaCl, 10 mM imidazole, 10 mM β-mercaptoethanol, 0.1% Triton X-100, and 30% glycerol) and sonicated. The lysate was cleared by centrifugation at 18,000 r.p.m. for 1 h in an SS-34 Sorvall rotor. The supernatant was added to 20 ml of drained and equilibrated with Buffer II Ni2+-NTA-agarose (Qiagen). Binding of the His6-tagged protein to the resin was done in batch on a rotcher overnight. The slurry was transferred to a disposable column and washed 6 times with 12 ml of Buffer II. The protein was eluted with 72 ml of Buffer III (50 mM sodium phosphate at pH 6.8, 100 mM NaCl, 10 mM β-mercaptoethanol, 0.1% Triton X-100, and 30% glycerol) on a HiPrep 26/10 desalting column (Amersham Biosciences). The supernatant was run under conditions identical to those used for DinG.

Purification of the DinG Protein—Overexpression of the DinG protein was performed in BL21(DE3)/pLysS strain (Fomp T hsdS r (ρ m n) 1 gal dcm Δsrl-reaA306/Tn10(TcR) (DE3) pLysS (CodIV)) transformed with pET30DinG upon induction with IPTG. Four flasks with 1 liter of a bacterial culture in LB medium supplemented with 30 μg/ml kanamycin and 25 μg/ml chloramphenicol were grown at 37 °C to A600 = 0.6, and the protein expression was induced with 1 mM IPTG over a 1.5-h period. The cells were collected by centrifugation at 7,000 rpm for 10 min in a Sorvall SSC-400 rotor, washed with 80 ml of Buffer I (50 mM sodium phosphate at pH 8.0, 300 mM NaCl, 10 mM imidazole, 10% glycerol, and 0.1% Triton X-100), and frozen at −70 °C.

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Analytical Sizing Chromatography—The molecular mass of DinG in different conditions was determined by gel filtration on a Superdex 200 PC 3.2/30 column (Amersham Biosciences) equilibrated with the running buffer (25 mM Tris-HCl at pH 8.0, 1 mM NaCl, 10 mM β-mercaptoethanol, and 100 μM EDTA). The separation was conducted on a SMART System (Amersham Biosciences) at 40 μl/min. DinG resuspended in the running buffer was injected at concentrations of 0.86 and 20.9 μM. The molecular mass of DinG was estimated as described in the manual of the gel filtration calibration kit (Amersham Biosciences) using a calibration curve obtained with ribonuclease A (13.7 kDa), bovine serum albumin (67 kDa), ferritin A (440 kDa), thyroglobulin (669 kDa), and blue dextran 2000 (~2000 kDa). Molecular weight markers were run under conditions identical to those used for DinG.
ATPase Assay—The steady-state ATPase activity of DinG was measured using a coupled enzymatic system (23) as described (24).

Reaction mixtures contained 50 nM DinG protein, 7.8–1000 μM ATP, 42.5 mM Tris·HCl at pH 7.5, 100 mM NaCl, 5 mM MgCl₂, 1 mM β-mercaptoethanol, 0.1 mM EDTA, 5% glycerol, 50 μg/ml bovine serum albumin, and 2 mM phosphoenolpyruvate, 150 μM β-NADH, 200 units/ml pyruvate kinase, and 100 units/ml lactate dehydrogenase. If DNA was included in the reaction, its concentration was 6 μM, expressed as the concentration of bases for ssDNA or of base pairs for dsDNA. Hydrolysis reactions were performed in a total volume of 100 μl at 37 °C. The time course of the reaction was monitored using the kinetics mode of a Hewlett-Packard 8453 spectrophotometer equipped with an 89090A temperature controller and Peltier thermostated cell holder. V_{max} and Km values were calculated from the initial rates of ATP hydrolysis using the EnzymeKinetics version 1.11 program (Trinity Software).

Helicase Assays—In a typical experiment, 2 nm helicase substrate M1 or 0.5 nm bifurcated substrate was incubated with DinG in 15 μl of the reaction mixture containing 42.5 mM Tris·HCl at pH 7.5, 100 mM NaCl, 5 mM MgCl₂, 1 mM β-mercaptoethanol, 0.1 mM EDTA, 5% glycerol, 50 μg/ml bovine serum albumin, and 2 mM ATP. Unless otherwise specified, the concentration of DinG was 100 nM, and incubations were conducted for 20 min at 30 °C. Every experiment contained a “positive control” in which the helicase substrate was denatured by incubation at 95 °C for 5 min without DinG. We could detect no more than 5% of renaturation of the denatured 2 nm M1 substrate after incubation for 20 min at 30 °C in the standard buffer (data not shown). In some cases, Tris·HCl at pH 8.0 or sodium cacodylate at 60 °C was used instead of Tris·HCl at pH 7.5; ADP or ATP-β-S was used in place of 2 mM ATP; 5 mM MgCl₂ was substituted with 20 mM EDTA, 5 mM ZnCl₂, 5 mM MnCl₂, or 5 mM CaCl₂. After incubation, the reactions were placed on ice and quenched by addition of SDS and EDTA to 1% and 10 mM, respectively.

Deproteinized products were separated in 1% TAE-agarose gels for 1.5 h at 0.25 V/cm (substrates D1, P1, P2, and P3). Gels were dried and quantitated using a BAS 2500 Bio-Imaging Analysis System (Fuji Medical Systems). Percent of the substrate unwind by helicase was calculated using Equation 1:

\[
\% = \frac{100(C_{\text{prod},i} - C_{\text{sub1},i})}{C_{\text{prod},i}} = C_{\text{prod},i} \times \left(1 - \frac{C_{\text{sub1},i}}{C_{\text{prod},i}}\right) \quad (\text{Eq. 1})
\]

where \(C_{\text{prod},i}\) and \(C_{\text{sub1},i}\) are counts of the displaced strand and the remaining substrate, respectively, and \(C_{\text{prod},i}\) is their sum. Equation 1 contains two factors α and β, which correct Equation 1 for helicase-independent melting of the substrate and renaturation of the unwound substrates after termination of the reaction. For each set of experiments, the correction factors α and β were calculated using Equation 2 and Equation 3, respectively.

\[
\alpha = \frac{C_{\text{prod},i}}{C_{\text{sub1},i}} \quad (\text{Eq. 2})
\]

\[
\beta = \frac{C_{\text{prod},i} - C_{\text{sub1},i}}{C_{\text{prod},i}} = \frac{C_{\text{sub1},i}}{C_{\text{prod},i}} \quad (\text{Eq. 3})
\]

where \(C_{\text{prod},i}\) are the counts of the single-stranded oligonucleotide generated during storage of the substrate, and the protein-free incubation in the mock experiment \(C_{\text{sub1},i}\) are the counts of the intact substrate remaining under the same circumstances.

RESULTS

Overexpression and Deletion of dinG Do Not Significantly Impair UV Sensitivity of E. coli Cells—We tested UV sensitivity of the strains lacking and overexpressing dinG. UV resistance of the bacterial cells is one of the manifestations of the SOS response. Fig. 1 shows that both the null mutation of dinG and overexpression of the protein from the multicopy plasmid confer only a mild UV sensitivity to the FV002 strain, which is wild type with respect to recombination, DNA repair, and replication functions. The lack of severe UV sensitivity in a ΔdinG strain is in agreement with the observation of Yasuda et al. (6), who reported that inactivation of dinG by mini-Tn10 did not have a considerable effect on the survival of cells with a dnaA57(Tn) rnhA genetic background after UV irradiation. At the same time, these results are different from those for the dinI gene, where deletion of dinI had virtually no effect (7), and its overexpression led to a significant UV sensitivity (7, 8).

Purification of DinG—The fusion DinG protein was expressed in E. coli cells carrying the pET30 plasmid. Induction with IPTG resulted in appearance of an 86-kDa polypeptide in cell lysates (Fig. 2, compare lanes 2 and 3). Chromatography on Ni²⁺-NTA was the initial step in the purification procedure (Fig. 2, lanes 5 and 6). Next we used a combination of two ion exchange columns, MonoQ and MonoS, to further purify DinG. Because the theoretical pl of the fusion protein, calculated with a ProtParam tool, is 7.66, it should bind to a cation exchange, but not to an anion exchange, column at pH 6.8. Accordingly, we passed the imidazole elution fractions through a MonoQ-MonoS tandem column at pH 6.8. Impurities with pl values below pH 6.8 bound to the MonoQ column, whereas the fusion protein passed through MonoQ was retained on the MonoS column. After the MonoS column was disconnected, DinG was eluted from MonoS with a salt gradient (Fig. 2, lane 7). Further purification was achieved after chromatography on a HiTrap Blue column (Fig. 2, lane 8). We stored purified DinG at pH 8.0 in a buffer containing 1 M NaCl at −80 °C. Prolonged storage of the protein at pH 7.5 in a low salt buffer resulted in its precipitation.

DinG Is a DNA-dependent ATPase—Helicases are molecular motors coupling the ATP hydrolysis to unwinding of the complementary strands of DNA or RNA. NTP binding and hydrolysis is a prerequisite for the strand-separating activity for any of the known helicases. We began the biochemical characterization of DinG with the evaluation of its ATPase activity. As evident from Fig. 3A, DinG possesses an ATPase activity that can be stimulated by DNA. ssDNA and dsDNA differ in their ATP-hydrolysing activity of DinG are summarized in Table II. The ssDNA-stimulated k_{cat} value of 24 s⁻¹ places
although it does not prove, the idea that DinG functions as a monomer during ATP hydrolysis.

**DinG Exists as a Monomer in Solution**—We used gel filtration to estimate the assembly state of purified DinG in solution. Irrespective of the protein concentration (0.86 or 20.9 μM), DinG eluted as a globular protein with a molecular mass of 74–86 kDa (Fig. 4). This indicates that DinG is a monomer in solution, in accord with the value of the Hill coefficient of 1. In order to minimize nonspecific protein associations and prevent protein precipitation, we performed all the gel filtration experiments in 1 M NaCl, conditions quite different from the intracellular environment. Furthermore, the fact that the helicase is present as a monomer in solution does not necessarily imply that the functional form of the enzyme is monomeric. Helicases can oligomerize when bound to nucleotide cofactors and DNA substrates. Models for DNA unwinding that utilize monomeric, dimeric, and hexameric helicases have been proposed (25–28).

**DinG Is a Helicase**—An examination of the amino acid sequence of DinG (9) revealed seven signature helicase motifs placing the DinG protein in the superfamily II of helicases (12). We carried out experiments aimed at demonstrating that the DinG protein is a bona fide helicase. The helicase activity was assayed using substrate M1 that was composed of a 32P-labeled 55-mer oligonucleotide annealed to M13mp18 ssDNA. Indeed, the DinG protein is a DNA helicase, as evident from Fig. 5.

**Incubation of the substrate with increasing amounts of the protein leads to the DinG concentration-dependent release of the oligonucleotide 55B from the M1 complex (Fig. 5, A and B). DinG at concentrations below 50 nM resulted in 50% of product formation, and the amount of the displaced strand reached the plateau at a concentration of the protein between 100 and 200 nM. In the experiments shown in Fig. 5A, the reaction was allowed to proceed for 20 min at 30 °C. To get a better estimation of the rate of DNA unwinding, we incubated 2 nM M1 substrate with 100 nM DinG at 30 °C for different times. As one can see in Fig. 5, C and D, a 55-mer oligonucleotide is displaced nearly quantitatively in 2.5 min. Nevertheless, we would like to point out that the actual rate of unwinding can be much higher because ssDNA displacement is achieved via two consecutive steps: a possible relatively slow binding of the DinG protein, which is the slope of the line in Fig. 5, C and D, to the helicase substrate and the unwinding step itself.

**Reaction Requirements for the DinG Helicase Activity**—Next we characterized the cofactor requirements for the DinG helicase activity. Whereas DinG unwound the M1 substrate at mild alkaline conditions, no helicase was observed at slightly acidic pH (Fig. 6A, lane 3). The lack of helicase activity at a pH below neutral may be attributable to the rapid denaturation or precipitation of DinG. This suggestion is in agreement with the observation that DinG precipitates upon prolonged storage at pH 7.5.

As expected, the ability of DinG to unwind a DNA duplex completely depends on the presence of a nucleotide triphosphate cofactor. Whereas DinG acts as a very efficient helicase when ATP is added to the reaction (Fig. 6B, lane 3), we could not detect any unwinding in the presence of nonhydrolyzable ATP analog ATPγS (Fig. 6B, lane 9) or in the absence of a nucleotide (Fig. 6B, lane 6). Because ATPγS is a competitive...
inhibitor of ATP hydrolysis by DinG (data not shown), these findings suggest that not only binding of ATP but also its hydrolysis is essential for unwinding. ADP did not support unwinding either (Fig. 6B, lane 12). Apparently, the DinG helicase does not discriminate between ribonucleotide and deoxyribonucleotide triphosphates, because dATP is a competent ATP substitute in the helicase reaction. We could see no difference in the DinG-mediated ssDNA displacement when dATP substituted for ATP (Fig. 6D).

All enzymatic reactions involving nucleotide triphosphates require divalent cations. DinG is no exception to this rule. The omission of bivalent metals from the reaction resulted in com-
plete inhibition of the helicase activity (Fig. 6, lane 6). As expected, the DinG helicase was fully active in the presence of Mg$^{2+}$ ions; Mn$^{2+}$ and Ca$^{2+}$ were valid substitutes for Mg$^{2+}$ (Fig. 6C, lanes 12 and 15), but no helicase activity was detected in the presence of Zn$^{2+}$ (Fig. 6C, lane 9). There are several possible explanations for the absence of helicase activity in the presence of zinc ions. The first consists of the inability of DinG to hydrolyze ATP chelated to zinc, hence the absence of the energy needed for DNA unwinding. A greater stability of DNA or much faster reannealing of unwound ssDNAs in the presence of Zn$^{2+}$ is a second possibility. This suggestion is supported by the fact that heat denaturation of the M1 substrate in the presence of Zn$^{2+}$ produced little of the product ssDNA (Fig. 6C, lane 7). Inactivation or denaturation of the enzyme by zinc ions might be another reason for the absence of helicase activity. Finally, it could be a combination of any of these possibilities.

Polarity of DNA Unwinding by DinG—All known helicases translocate along one strand of DNA duplex unidirectionally. The directionality, or polarity, of DNA unwinding is an intrinsic feature of the helicase. It is linked to the biological process in which the protein is mechanistically involved. Hence knowledge of the polarity of unwinding could provide clues about the molecular transaction in which the helicase might be engaged.

To determine the polarity of DNA unwinding by DinG, we used bifurcated substrates D1, P1, P2, and P3 composed of natural and modified oligonucleotides with switched polarity. P-series substrates are derivatives of the D1 substrate, depicted in Fig. 7A. All of the forked substrates share a common feature; they are 34-mer duplexes with unpaired 31-nucleotide extensions at the 3’-end of the top strand and at the 5’-end of the bottom strand. Although D1 is an “all natural” substrate, P1 has a 3’-3’ linkage in the top strand, P2 contains a 5’-5’ linkage in the bottom strand, and P3 combines both modifications. Non-natural linkages are located exactly at the border of the duplex and the single-stranded region.

The prediction is that, depending on their polarity, 3’→5’- and 5’→3’-helicases should unwind different subsets of the bifurcated substrates. Both 3’→5’ and 5’→3’ enzymes should work on D1 substrate, and substrate P3 should be immune to unwinding by either type of the helicase. The polarity of the helicase can be elucidated from its choice between P1 and P2 substrates. A 5’→3’-helicase would unwind P1 but not P2, and a 3’→5’-helicase would be active on P2 but not P1. The above reasoning assumes that DinG is incapable of unwinding DNA from blunt ends; this is the case for DinG.2

The results of the helicase activity of DinG on bifurcated substrates are presented in Fig. 7, B–F. The protein was active on substrates D1 and P1 only, as expected for a 5’→3’-helicase (Fig. 7, B and C). This is in agreement with the directionality of DNA unwinding by the eukaryotic counterparts of DinG (29, 30). Surprisingly, DinG had a much greater activity on the modified P1 substrate compared with the wild type D1 substrate (Fig. 7F). The formation of secondary structures in the 5’ extension of the bottom strand or interaction between non-complementary single-stranded tails could account for the re-

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duced helicase activity observed with D1. Consistent with either of these two possibilities is the fact that the band corresponding to D1 smears during gel electrophoresis (Fig. 7B). Such a behavior is usually indicative of the formation of secondary structures.

To validate use of the substrates with unnatural linkages in assessing the polarity of the DinG-catalyzed DNA unwinding, we also performed control experiments with a more conventional substrate, a blunt-ended duplex with a single-stranded gap. After binding to the single-stranded region, the unidirectionally moving helicase can unwind only one of the two available duplexes. Results obtained with this kind of substrates confirmed that DinG is a 5'-3' helicase (data not shown).

**DISCUSSION**

We characterized biochemical properties of the DNA damage-inducible protein DinG from *E. coli* (1–3). DinG is a protein of great interest because it is a bacterial homolog of two human helicases, XPD and BACH1 (Fig. 8), implicated in human disorders. The human XPD protein is a subunit of the large protein complex TFIH that has a dual role: in basal transcription and in nucleotide excision repair (11, 31). Three human
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 recessive photosensitive syndromes, xeroderma pigmentosum (XP), xeroderma pigmentosum/Cockayne syndrome, and trichothiodystrophy, are associated with different mutations in the XPD gene (32). Totally, 14 mutations in XPD have been described in humans; 7 mutations are associated with XP, 6 mutations associated with trichothiodystrophy, and 1 mutation associated with XP/Cockayne syndrome (33). Of them, 7 mutations were found in, or 10 amino acid residues from, helicase motifs.

BACH1 is a recently described putative helicase that shares a significant homology with the Rad3/XPD family of eukaryotic helicases (10). BACH1 physically interacts with the C terminus of the BRCA1 protein and contributes to its DNA repair function. That the overexpression of a BACH1 gene carrying Lys-52 → Arg mutation in the conservative helicase motif I greatly decreased the ability of the cell to repair double-stranded breaks suggests that the helicase activity of BACH1 is required for DNA repair (10). However, the DNA helicase activity of BACH1 has not yet been demonstrated. The potential involvement of BRCA1 in transcription, replication, and DNA repair opens the possibility that BACH1 could participate in any of these cellular processes as well.

Similarly, the DinG helicase, a prokaryotic counterpart of Rad3/XPD and BACH1, could be involved in different aspects of nucleic acid metabolism. As there are no molecular structures for any of the members of this helicase family, the bacterial DinG protein could pave the way for the elucidation of such a structure. Thus, genetic, mechanistic, and structural studies of DinG, XPD, and BACH1 in the regions of helicase motifs.

The distances between motifs and from the protein termini are indicated with roman numerals. Amino acid residues identical in at least two proteins are shown in red, and similar residues are shown in blue. The distances between motifs and from the protein termini are indicated with arabic numerals. B, the identity (red) and similarity (blue) between DinG, XPD, and BACH1 in the regions of helicase motifs.

Two E. coli helicases, DhpA (36, 37), involved in ribosome maturation, and RhlB (38), a subunit of the E. coli RNA degradosome, have been shown to possess RNA-RNA unwinding activity. The genes kepA (39), hpaA and rhpB (40), rhIE (41), phoH and yfH (42), lhr (43), yfH (34), srmB (44), b1808 (34) and deaD (45) have been predicted to encode DNA and RNA helicases, but the corresponding proteins have never been purified, and their helicase activities have yet to be demonstrated. DinG and UvrD are the only E. coli helicases induced by DNA damage. Currently we can only speculate about the biological function of the DinG protein.

That dinG deletion does not significantly compromise UV resistance of FV004 cells indicates that it is dispensable for induction and maintenance of the SOS response and the repair of photodestabilizations. The absence of a profound UV-sensitive phenotype in FV002 cells carrying the plasmid pDinGPro implies that DinG probably is not involved in the down-regulation of SOS functions. Nevertheless, the slight but reproducible UV sensitivity of cells lacking or overexpressing DinG suggests its possible involvement in the control of the SOS response and/or DNA repair processes; the loss of a DinG activity might be backed up by a redundant compensatory pathway(s).

The SOS response is not the only cellular process triggered by DNA damage. The alternative pathway of DNA replication, inducible stable DNA replication (iSDR), can be initiated at chromosome sites different from oriC. iSDR was discovered as a DNA damage-resistant replication induced in the absence of protein synthesis (46). In fact, there is a striking similarity between the stimuli that induce SOS functions and those that result in stable DNA replication: thymine starvation, UV irradiation, incubation of dnaB(Tc) mutants at the restrictive temperature, and exposure to DNA-damaging agents (47). Moreover, the genetic requirements for the induction of the SOS and iSDR are the same; both processes are inhibited by a lexA(Ind−) mutation and a recA mutation allowing constitutive RecA function. The complete dependence of iSDR on the recombination activity of RecA and the helicase activity of RecBC (48, 49) suggests a D-loop model for the initiation of iSDR.
The d-loop model presumes generation of double-strand breaks (DSBs) at the oriM, the origin for iSDR. The DSB is introduced by a hypothetical oriM-specific endonuclease induced during the SOS response (47). At the same time, DSBs generated at sites of DNA lesions (50) can be converted into substrates for the homologous recombination machinery (tailed duplexes and relatively short fragment of ssDNA) after processing by the helicase and endonuclease activities of the RecBCD enzyme. This would result in the uncontrolled formation of d-loops in numerous illegitimate chromosomal locations. Such “junk” d-loops will interfere with the normal progression of the replication fork and must be removed to ensure efficient replication. A structure-specific helicase recognizing and specifically destroying d-loops could be responsible for clearing the DNA duplex. DinG could play the role of such a “janitor” sweeping the road ahead of a replication fork. Our observations that the absence of a functional DinG did not permit the removal of RNA transcripts hybridized to DNA under conditions of active transcription (6). This speculation is consistent with a role for DinG in the removal of R-loops. The S. cerevisiae RAD3 has been shown to possess DNA-RNA helicase activity (52). In this regard, it would be of interest to test the ability of DinG to unwind DNA-RNA hybrids and R-loop structures.

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