Interaction between RNA Polymerase and RapA, a Bacterial Homolog of the SWI/SNF Protein Family*

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EXPERIMENTAL PROCEDURES

Recently, we identified a novel Escherichia coli RNA polymerase (RNAP)-associated protein, an ATPase, called RapA (Sukhodolets, M. V., and Jin, D. J. (1998) J. Biol. Chem. 273, 7018–7023). RapA is a bacterial homolog of SWI2/SNF2. We showed that RapA forms a stable complex with RNAP holoenzyme and that binding to RNAP holoenzyme stimulates the ATPase activity of RapA. We have further analyzed the interactions between purified RapA and the two forms of RNAP: core RNAP and RNAP holoenzyme. We found that RapA interacts with either form of RNAP. However, RapA exhibits its higher affinity for core RNAP than for RNAP holoenzyme. Chemical cross-linking of the RNAP-RapA complex indicated that the RapA-binding sites are located at the interface between the α and β′ subunits of RNAP. Contrary to previously reported results (Muzzin, O., Campbell, E., A., Xia, L., Severinova, E., Darst, S. A., and Severinov, K. (1998) J. Biol. Chem. 273, 15157–15161), our in vivo analysis of a rapA null mutant suggested that RapA is not likely to be directly involved in DNA repair.

In Escherichia coli, RNA polymerase (RNAP) exists in two forms: core RNAP and RNAP holoenzyme. The basic transcription machinery, core RNAP, consisting of subunits α2β′β′, is capable of transcription elongation and termination, but it is unable to initiate transcription; whereas RNAP holoenzyme, consisting of α2β′α′, is capable of initiating transcription from promoters on a DNA template (1, 2). A number of proteins that associate with core RNAP and/or holoenzyme and participate in different aspects of transcription have been identified, such as NusA (3, 4), GreA, and GreB (5–9).

Recently, we described a new RNAP-associated protein named RapA with a molecular mass of 110 kDa (10). Interestingly, the RapA protein is a member of the SWI/SNF protein family (10–16), which has been implicated in eukaryotic nucleosome remodeling and DNA repair (for reviews, see Refs. 16–18). We found that the RapA protein co-purifies with RNAP holoenzyme exclusively and that after purification to homogeneity, RapA is capable of forming a stable complex with RNAP holoenzyme in vitro (10). In addition, the RapA protein is an ATPase, and its ATPase activity is stimulated upon binding to RNAP holoenzyme, indicating that RapA interacts with RNAP holoenzyme both physically and functionally. Independently, Muzzin et al. (19) also reported that the same 110-kDa protein (but named HepA) is associated with RNAP in E. coli. However, they found that this new protein appeared to be associated only with core RNAP but not with RNAP holoenzyme (19). Thus, it is important to address whether and how RapA interacts with the two forms of RNAP. This is not only an unsolved issue but could also shed some light on the function of RapA.

In this study, we further characterized the interaction between RNAP and the RapA protein biochemically. Specifically, we analyzed the conditions that affect the interactions between RapA and either core RNAP or holoenzyme and measured the dissociation constants (Kd) of RapA-RNAP complexes. We also identified the subunits of RNAP that are in close contact with RapA by chemical cross-linking. Furthermore, in an attempt to address the cellular function of RapA, we constructed a rapA null mutation and studied the effect of this mutation in vivo.

Materials and Chemical Reagents—The RapA protein and RNAP were purified from E. coli K12 cells (MG1655) as described previously (10). The protein concentrations were determined using the Bradford assay (20) with bovine serum albumin as a standard. RNAP concentrations were also determined by UV absorbance using the molar extinction coefficient data of Lowe et al. (21). The RapA-specific and α-70-specific polyclonal antibodies were previously described (10). The monoclonal antibodies specific for the α (4RA1), β (NT63), or β′ (NT73) subunits of RNAP were kindly provided by Dr. Richard Burgess (University of Wisconsin, Madison, WI), and the polyclonal antibodies specific for the ω subunit of RNAP were kindly provided by Dr. Dan Gentry (SmithKline Beecham). The bifunctional cross-linker ethylene glycol bis(succinimide N-hydroxysuccinimide ester) (EGS-NHS) was purchased from Sigma.

Reconstitution of RNAP-RapA Complex in Vitro—Stability of the complex of RapA with either core RNAP or holoenzyme was studied by gel filtration using a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech) as described previously (10). All runs were performed in TGED buffer (0.01 mM Tris-HCl, pH 7.9, 5% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol) with the salt concentrations indicated in the legends for Figs. 1 and 2.

Glycerol Gradient Ultracentrifugation Experiments—Purified enzymes were premixed in 100 μl of glycerol gradient centrifugation buffer (10 mM Tris, pH 7.8, 10 mM MgCl2, 0.1 mM EDTA, 0.1 mM dithiothreitol, 200 mM NaCl) and layered on top of 4 ml of 15–30% (top to bottom) linear gradients of glycerol in the above buffer. The samples were then spun in a SW-60 rotor for 2 h at 37,500 rpm (6 °C). Each 4-ml tube was fractionated into 13 fractions using the Beckman Fraction Recovery system. Equal volumes of 2× Laemmli sample buffer were then added to each fraction, and the samples were analyzed on SDS 10% polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue R-250 or silver.

ATPase Assays—The ATPase activities were determined by measuring the amount of [α-32P]ADP released from [α-32P]ATP. The reaction conditions and separation of samples by chromatography using poly-(ethylenimine)-cellulose plates (J.T. Baker Inc.) were as described (10). Plates were autoradiographed using Kodak Bio-Max MR film and scanned on a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA) to quantitate the amount of [α-32P]ATP hydrolyzed. The ATPase activity was expressed as pmol of ATP hydrolyzed/min/μg of RapA.

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* The abbreviations used are: RNAP, RNA polymerase; EGS-NHS, ethylene glycol bis(succinimide N-hydroxysuccinimide ester).

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**RESULTS**

**RapA Forms Complexes with either Core RNAP or RNAP Holoenzyme**—Previously, we showed that RapA forms a stable complex with RNAP holoenzyme, as if it were a subunit of RNAP (10). Using the same conditions (in the presence of 0.1 M NaCl), we determined whether RapA also interacts with core RNAP. We found that a stable core RNAP-RapA complex can be reconstituted with highly purified RapA and core RNAP in vitro (Fig. 1). When the 110-kDa RapA protein and core RNAP were mixed and passed through a Superose 6 HR gel filtration column (Amersham Pharmacia Biotech), they coeluted as a complex (Fig. 1A), whereas each of the two proteins eluted in different fractions when they were run separately (Fig. 1, C and D). These results showed that RapA also forms a stable complex with core RNAP, just as it forms a stable complex with RNAP holoenzyme (Fig. 1B).

Because we found that RapA co-purified only with RNAP holoenzyme and not with core RNAP during our purification procedure and that the fractions containing both RapA and RNAP holoenzyme were eluted at about 0.4 M NaCl in the final step of Mono-Q chromatography (10), we again studied the interaction of RapA with core or holoenzyme by gel filtration at 0.4 M NaCl (Fig. 2). When RapA and the core RNAP were mixed and passed through the gel filtration column at 0.4 M NaCl, a significant fraction of RapA coeluted with RNAP as an RNAP-RapA complex (Fig. 2A). The core RNAP-RapA complex was less stable at 0.4 M NaCl than at 0.1 M NaCl, as shown by the increasing dissociation of RapA from the complex, resulting in more free, unbound RapA at 0.4 M NaCl (compare Fig. 2A with Fig. 1A). However, the fraction of RapA bound to core RNAP at 0.4 M NaCl was still much greater that bound to RNAP holoenzyme (Fig. 2B).

To examine the Coomassie Brilliant Blue R-250-stained gels, we estimated that the fraction of RapA coeluted with core RNAP was approximately 10 times greater than that complexed with RNAP holoenzyme.

**Determination of the Dissociation Constants (Kₐ Values) of RNAP-RapA Complexes**—The stimulation of RapA ATPase activity by RNAP was used to determine the apparent Kₐ values of RNAP-RapA complexes (Ref. 10; this work). Sequential dilutions of purified RapA alone or purified RapA premixed with either purified core RNAP or RNAP holoenzyme were performed in 1 M MgCl₂ buffer (40 mM Tris-HCl, pH 7.4, 4 mM MgCl₂, 1 mM dithiothreitol, 50 μM bovine serum albumin, 50 mM NaCl) were made. It was found necessary to use siliconized tubes and maintain bovine serum albumin in the buffer to eliminate nonspecific binding of proteins to the tube walls, particularly at low protein concentrations. ATPase activity of each dilution was determined and calculated as pmol of ATP hydrolyzed/min. Using the same conditions (in the presence of 0.1 M NaCl), we determined whether RapA also interacts with core RNAP. We found that a stable core RNAP-RapA complex can be reconstituted with highly purified RapA and core RNAP in vitro (Fig. 1). When the 110-kDa RapA protein and core RNAP were mixed and passed through a Superose 6 HR gel filtration column (Amersham Pharmacia Biotech), they coeluted as a complex (Fig. 1A), whereas each of the two proteins eluted in different fractions when they were run separately (Fig. 1, C and D). These results showed that RapA also forms a stable complex with core RNAP, just as it forms a stable complex with RNAP holoenzyme (Fig. 1B).

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Apparently, the RNAP-RapA complexes behaved differently in gel filtration than in Mono-Q chromatography. To further demonstrate that RapA can interact with either core or holoenzyme, we also analyzed the interaction between RapA and the two forms of RNAP by glycerol gradient ultracentrifugation.
Interaction between RNA Polymerase and RapA

**FIG. 1.** Formation of stable complexes of purified RapA with either core RNAP or RNAP holoenzyme. The stability of the RNAP-RapA complex was studied by gel filtration on a Superose 6 HR 10/30 column as described under “Experimental Procedures.” All runs were performed in TGED buffer containing 0.1 M NaCl. SDS-8% polyacrylamide gels stained with Coomassie Brilliant Blue R-250 are shown. The positions of the subunits of RNAP and RapA are indicated. A, 2.5 μM core RNAP plus 0.6 μM RapA. B, 2.5 μM RNAP holoenzyme plus 0.6 μM RapA. C, 0.6 μM RapA. D, 2.5 μM core RNAP. E, 2.5 μM RNAP holoenzyme. (Fig. 3). Consistent with the gel filtration results, we detected complex formation between RapA and either core or holoenzyme. Again, we found that the interaction between RapA and core RNAP was stronger than that between RapA and RNAP holoenzyme. At 0.7 μM RNAP and 0.7 μM RapA, while all RapA remained bound to core RNAP during the course of ultracentrifugation (Fig. 3A), the less stable RNAP holoenzyme-RapA complex showed a trace of RapA dissociating from the complex (Fig. 3B). The differences in RapA binding to core or holoenzyme were even more apparent at 0.06 μM RNAP and 0.12 μM RapA. At this lower concentration of proteins, while a fraction of RapA was still associated with core RNAP (Fig. 3D), almost no RapA was associated with holoenzyme (Fig. 3E).

To determine whether binding to core RNAP can also stimulate the ATPase activity of RapA, we compared the ATPase activities of free RapA and core RNAP-RapA complexes (Fig. 4). The ATPase activity of core RNAP-RapA complex was nearly 4-fold higher than that of RapA alone (compare lane 2 with lane 5), very similar to that of RNAP holoenzyme-RapA complex (compare lane 2 with lane 6). The stimulation of RapA ATPase by binding to either core RNAP or RNAP holoenzyme further confirms that RapA interacts with both forms of RNAP. Moreover, it also provides a basis for determining the dissociation constants of RNAP-RapA complexes.

**Determination of the Affinity of RapA to RNAP Holoenzyme or Core RNAP**—We also determined the relative affinities of holoenzyme and core RNAP-RapA complexes by taking advantage of the stimulatory effect of RNAP on the RapA ATPase activity (Fig. 4). We used a fixed ratio (2:1) of Rap and RNAP in one set of experiments and RapA alone in a parallel set of experiments. We measured ATPase activity (A_{obs}) in sequentially diluted reaction mixtures. We calculated the difference (ΔA_{obs}) between the ATPase activities of RNAP-RapA mixtures and RapA alone at each RapA concentration and determined ΔA_{max} (maximal activation) as described under “Experimental Procedures.” The ratio of ΔA_{obs} and ΔA_{max} was calculated and plotted as a function of RapA concentration (Fig. 5). We found that at high concentrations of proteins, the activation of RapA ATPase was relatively constant (plateau in Fig. 5). Upon sequential dilution, the activation of RapA ATPase decreased, as the RNAP-RapA complexes dissociated. Although this method cannot be used to obtain an explicit K_{d} for the complex, we assumed that at half-maximal activation of RapA ATPase the free and bound RNAP concentrations were equal. Therefore, the K_{d} can be approximated as the concentration of RapA at that point. The apparent K_{d} values, calculated from this method, were 5–10 nm for RapA-core RNAP and 50–80 nm for RapA-RNAP holoenzyme complex. These values show that the RapA protein exhibits higher affinity to core RNAP than to RNAP holoenzyme, in agreement with the gel filtration and glycerol gradient ultracentrifugation studies described above.

**RapA Cross-links to the β’ and α Subunits of RNAP**—We probed the interface between RapA and RNAP using various cross-linking agents. Cross-linking experiments with EGS-
NHS, a 12-carbon atom linker-bifunctional cross-linking reagent capable of modifying amino groups, yielded positive results, although we failed to obtain cross-linked RNAP-RapA complexes using several bifunctional reagents with the linker arm of fewer than 10 carbon atoms.

To simplify the identification of new cross-linked species of the RNAP-RapA complex, we compared the patterns of the cross-linked protein products in the reactions containing RapA and RNAP with those in parallel reactions containing RNAP alone. It has been shown that RNAP subunits are capable of intramolecular cross-linking forming complex patterns (30). After cross-linking, the reaction mixtures were separated on SDS-5% polyacrylamide gels, and the gels were either silver-stained or transferred onto Immobilon P membranes for immunostaining with antibodies specific for RapA or various subunits of RNAP (Fig. 6).

Fig. 6A shows the kinetics of a representative cross-linking reaction. Core RNAP treated with EGS-NHS showed a complex pattern of high molecular weight bands as a result of intramolecular cross-linking of its subunits (Fig. 6A, lanes 2–6). However, new cross-linked species appeared in the reaction containing core RNAP and RapA (Fig. 6A, lanes 8–12). One new cross-linked product with an apparent molecular mass of 260–
270 kDa that reacted with both β'-specific monoclonal antibodies (Fig. 6B, lane 6, arrow) and RapA-specific polyclonal antibodies (Fig. 6B, lane 2, arrow) is the RapA-β' complex. Another new cross-linked product with an apparent molecular mass of about 150 kDa that reacted with both α-specific monoclonal antibodies and RapA-specific polyclonal antibodies (Fig. 6C, lanes 8 and 4, respectively) is therefore the RapA-α complex. Because this cross-linked product co-migrated with the β and β' subunits, it was not apparent by silver staining (Fig. 6A).

In addition, a few new cross-linked complexes were found in the presence of RapA. One example is the product with a molecular mass of about 125 kDa that was apparent even by silver staining (Fig. 6A, marked as RapA(βL)). It reacted with RapA-specific polyclonal antibodies (Fig. 6B), suggesting that the cross-linked product might have an additional mass of about 15 kDa. Since our RNAP preparation contained the ω subunit of RNAP, which has a molecular mass of 10.1 kDa, we investigated whether this 125-kDa product was the RapA-ω complex. However, this cross-linked product did not react with ω-specific polyclonal antibodies (data not shown), confirming that it is not the RapA-ω complex. Since no other small proteins (or small RNAs) were noted in RNAP or RapA preparations (judging from silver-stained SDS-12% polyacrylamide gels), it seems likely that the unidentified cross-linked species are the result of intramolecular cross-linking of RapA itself (Fig. 6A, RapA(βL) and RapA(βL')). The same intramolecular cross-linking of RapA could explain the formation of another new RapA-containing cross-linked complex in Fig. 6C (marked as RapA(βL')-α).

Similarly, we treated either purified RNAP holoenzyme or the mixture containing RNAP holoenzyme and RapA with EGS-NHS in parallel cross-linking experiments (Fig. 7). The patterns of the cross-linked complexes of RNAP holoenzyme were significantly more complicated than that of core RNAP due to the presence of the α-70 subunit, which is known to cross-link to multiple subunits of RNAP (30). We detected no apparent cross-linked species that reacted with both RapA-specific and α-70-specific antibodies in the reactions containing RNAP holoenzyme and RapA (data not shown). However, it is interesting to note that in the presence of RapA the amount of the cross-linked α-α-70 complex (which has a molecular mass of about 130 kDa) and the amount of the cross-linked α-α complex were significantly reduced (Fig. 7, lanes 2 and 4 and lanes 6 and 8). This apparent RapA effect on the cross-linking efficiency indicates that RapA changes the configuration of holoenzyme upon its binding to RNAP.

The rapA Null Mutants Exhibited No Significant UV Sensitivity—Previously, we analyzed the effects of RapA in *in vitro* transcription and detected only a marginal activation of the RNAP transcriptional activity (10). To study the function of the RapA protein in the cell, we constructed a rapA null mutation by combining an internal deletion and an insertion of a cat (Cm') cassette in the gene.

**Fig. 7.** The efficiency of the cross-linking between the α and α-70 subunits of RNAP is greatly reduced in the presence of RapA. Immunoblotting of the cross-linked products separated on a SDS 5% polyacrylamide gel with α-specific monoclonal antibodies and α-70-specific polyclonal antibodies. RNAP holoenzyme (lanes 1, 2, 5, and 6) or 1:1 RNAP holoenzyme-RapA complex (lanes 3, 4, 7, and 8) was incubated with EGS-NHS for 30 min as described under "Experimental Procedures." The positions of the α and α-70 subunits of RNAP and the cross-linked α-α and α-α-70 complexes are indicated.
The *rapA* gene appeared to be nonessential for the bacterial cell, based on the following criteria. 1) The *rapA* null mutation could be introduced into other cells that harbored either a plasmid expressing the wild-type *rapA* gene or only the vector with similar efficiency by phage P1-mediated transduction. 2) The *rapA* null mutant exhibited no detectable difference in growth compared with wild-type cells under a variety of conditions, such as different incubation temperatures and growth media (data not shown). Similar results were also reported in Ref. 19.

Because some eukaryotic SWI/SNF family members have been implicated in DNA repair, we explored the possibility that RapA could be important for DNA damage recovery. Originally, our preliminary results suggested that the *rapA* null mutant was hypersensitive to UV irradiation and the antibiotic mitomycin C (22). However, further studies determined that these phenotypes were caused by the presence of a cryptic phage with *lf*-80 immunity. Thus, the originally constructed *rapA* mutant was a lysogen for the phage; upon UV irradiation or mitomycin C treatment, the repressor of the cryptic phage was inactivated and the lysogenic phage was induced, resulting in increased cell death. After we constructed a phage-free *rapA* null mutant, we observed no UV-hypersensitive or mitomycin C-hypersensitive phenotypes compared with the wild-type isogenic strain (data not shown).

Because it was reported recently by Muzzin et al. (19) that disruption of the same *E. coli* gene (also called *hepA*) caused UV sensitivity, we carefully analyzed this phenotype further (see "Experimental Procedures"). To assure that we could detect even marginal increases in UV sensitivity in our assays, we included the *mfd* mutant in every UV sensitivity assay as a control. The *mfd* gene encodes the transcription-repair coupling factor (26). The *mfd* mutation has been reported to confer either no (27) or only mild increase in UV sensitivity (28) when compared with the isogenic wild-type strains. Initially, we performed the UV sensitivity assays in the JC7623 strain background that contains the *recBC* and *sbcBC* mutations and was used for UV sensitivity assays by Muzzin et al. (19). The *rapA* null mutant behaved somewhat similar to the isogenic wild-type strain in response to UV irradiation, whereas the *mfd* mutant exhibited mild UV sensitivity compared with the wild-type strain (Fig. 8). At low UV doses, there was a very small difference among the wild type and *mfd* and *rapA* mutants. At high UV doses, while the *mfd* mutant became noticeably UV-

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**Fig. 8.** The *rapA* null mutation has no significant effect on UV sensitivity. A, the *rapA* null mutant exhibited only a marginal increase in UV sensitivity in the JC7623 (*recBCsbcBC*) background or in the MG1655 background (either with low UV doses or high UV doses). The survival percentages are plotted as a function of UV dose. UV irradiation assays were performed as described under "Experimental Procedures." Open rectangles, parental strain; closed rectangles, the *rapA* null mutant; open circles, the *mfd* mutant. The data were averaged from at least two independent experiments. B, the *rapA* null mutation was confirmed by Western blotting with RapA-specific antibodies. Cell lysates were prepared from exponentially growing cells (E) or overnight cultures (O), and templates corresponding to 0.2 ml of the culture with an average of 0.4 were loaded on an SDS 8% polyacrylamide gel. The gel was immunostained with RapA-specific antibodies. Lanes 1, 2, 7, and 8, wild type parental strains; lanes 3, 4, 9, and 10, the *rapA* null mutants; lanes 5, 6, 11, and 12, the *mfd* mutants. Lanes 13 and 14 contain 0.15 and 0.6 ng of purified RapA, respectively. The 100–105-kDa protein that cross-reacted with RapA-specific antibodies was identified as alcohol dehydrogenase E (M. V. Sukhodolets and D. J. Jin, unpublished data). However, it shows no significant sequence homology to RapA (E. Koonin, personal communication). Note that the level of RapA is higher in the exponentially growing cells than that in the overnight cultures.

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sensitive, the rapA mutant was only slightly more UV-sensitive than wild type strain. We also determined the UV sensitivity of the fampicin resistance (Rifr) from either wild type cells or the rapA mutant (Fig. 9). We observed very similar spontaneous or UV-induced mutational rates of Rifr mutations from the two strains. With the wild type strain.

We have demonstrated that RapA interacts with both core RNAP and RNAP holoenzyme in a purified system using several different methods. By binding to either form of RNAP, the RapA ATPase activity is stimulated. Under the experimental conditions we used, RapA formed complexes with either core RNAP or RNAP holoenzyme in the nanomolar range, suggesting that the interaction is highly specific. However, the affinity of RapA to core RNAP is about 5–10-fold higher than to RNAP holoenzyme. If core RNAP is indeed the main RapA-binding form of RNAP in vivo, it indicates that the function of RapA is more likely related to transcription elongation/termination than to initiation. We are currently exploring this possibility.

Previously, we found that almost all of the RapA protein molecules in the cell co-purified with RNAP holoenzyme (10). We do not know why RapA was not associated with core RNAP during the Mono Q step of the RNAP purification procedure. Several possibilities could contribute to this apparent difference: 1) some special conditions during the RNAP purification procedure; 2) special chromatographic conditions; and 3) some factors missing in a purified system. On the other hand, Muzzin et al. (19) reported that the same protein interacted only with core RNAP but not with holoenzyme. It is possible that differences in the nature of the protein and in the conditions for the binding assays contributed to these apparently different results.

We have shown that RapA cross-links to the α and β′ subunits of RNAP, suggesting that it lies in the interface of these two subunits. Furthermore, it appeared that upon binding to RapA the RNAP holoenzyme alters its configuration, because the cross-linking efficiency of the α-α-70 and α-α complexes was greatly reduced in the presence of RapA, although we detected no cross-linking between RapA and the α-70 subunit or an α-70 complex. Because RapA is able to bind to RNAP holoenzyme, forming a 1:1 complex (10), it is clear that the binding sites for RapA and the α-70 are distinct. The E. coli RNAP structure has been determined from a combination of x-ray crystallography and electron microscopy (31). The binding site for GreB on RNAP has been proposed (32). The binding sites for RapA on RNAP await future determination.

The rapA null mutant behaved very similar to the isogenic wild-type strain under various laboratory conditions. In the course of extended examination using two different genetic backgrounds, our experiments consistently showed that the rapA null mutant exhibited only a marginal increase in UV sensitivity compared with the wild-type strains (Fig. 8). The observed UV sensitivity of the rapA mutant was significantly less than that of the mfd mutant, which by itself showed only a mild increase in UV sensitivity compared with the wild type strain. Thus, our results were quantitatively different from the results reported by Muzzin et al. (19). This apparent difference could be due to the differences in strain backgrounds or experimental conditions used between the two laboratories. In that report, the authors also claimed that the expression level of the RapA (HepA) protein was substantially higher in the recBC sbcBC (JC7623) strain and speculated that the possible disruption of RecBCD function that is important for DNA repair pathways may be compensated by increased expression of RapA (HepA). However, we detected no substantial difference in the amount of RapA protein between the JC7623 strain and the wild-type MG1655 strain by immunoassays using antibodies against RapA (Fig. 8B). Furthermore, the RapA expression level was not increased upon DNA damage induced by mitomycin C (data not shown). We also detected little or no effect of rapA on spontaneous or UV-induced mutational rates. Based on our data, it seems unlikely that RapA plays a major role in DNA repair, at least for damage induced by UV irradiation or mitomycin C. Currently, we are investigating the regulation of rapA in the hope that it will lead to understanding of the function of this RNAP-associated protein.

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