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Sequential Folding of UmuC by the Hsp70 and Hsp60 Chaperone Complexes of Escherichia coli*

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Replication-blocking lesions generate a signal in Escherichia coli that leads to the induction of the multigene SOS response. Among the SOS-induced genes are umud and umuc, whose products are necessary for the increased mutation rate induced by induced bacteria. The mutations are likely to result from replication across DNA lesions, and such a bypass event has been reconstituted in vitro (Rajagopalan, M., Lu, C., Woodgate, R., O'Donnell, M., Goodman, M. F., Echols, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10777–10781). In this work, we show that the chaperone proteins promote the proper folding of UmuC protein in vitro. We treated purified and inactive UmuC with Hsp70 and Hsp60. After Hsp70 treatment, the DNA binding activity of UmuC was recovered, but the ability to promote replication across DNA lesions was not. However, lesion bypass activity was recovered upon further treatment with Hsp60. The biological significance of such a folding pathway for UmuC protein is strengthened by in vitro evidence for a role of DnaK in UV-induced mutagenesis.

Among the DNA repair pathways identified in Escherichia coli, the umudc-dependent mutagenic pathway remains a puzzling and intriguing one. The umudc and umud gene products are part of the multigene SOS regulon, whose expression is derepressed in response to replication-blocking lesions that lead to the induction of the SOS regulon. Among the SOS-induced genes are umud and umuc, whose products are necessary for the increased mutation rate in induced bacteria. The mutations are likely to result from replication across DNA lesions, and such a bypass event has been reconstituted in vitro (Rajagopalan, M., Lu, C., Woodgate, R., O’Donnell, M., Goodman, M. F., Echols, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10777–10781). In this work, we show that the chaperone proteins promote the proper folding of UmuC protein in vitro. We treated purified and inactive UmuC with Hsp70 and Hsp60. After Hsp70 treatment, the DNA binding activity of UmuC was recovered, but the ability to promote replication across DNA lesions was not. However, lesion bypass activity was recovered upon further treatment with Hsp60. The biological significance of such a folding pathway for UmuC protein is strengthened by in vitro evidence for a role of DnaK in UV-induced mutagenesis.

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The abbreviations used are: Hsp, heat shock protein; ss, single-stranded; ds, double-stranded; ATPyS, adenosine 5'-O-(3-thiotriphosphate).
**UmuC** is an *E. coli* protein that was found to require the help of a chaperone-like protein (the ribosomal protein S9) to be maintained in a soluble and active form (Woodgate et al., 1989). An additional hint that heat shock proteins might be needed in the folding process of UmuC is that UmuDC-dependent mutagenesis is decreased in groEL and groES mutants and that the stability of UmuC is reduced in these mutants (Donnelly and Walker, 1989). Further evidence of a UmuC-GroEL interaction was obtained by co-immunoprecipitation of these two proteins (Donnelly and Walker, 1992). We investigated whether a Hsp-mediated folding pathway similar to that used by Langer et al. (1992) would allow the recovery of soluble and active UmuC. We treated purified and inactive UmuC with Hsp70 and Hsp60. After Hsp70 treatment, the DNA binding activity of UmuC was recovered, but the ability to promote replication across DNA lesions was not. However, this second activity was recovered upon further treatment with Hsp60. The physiological significance of a role of Hsp70 in UmuC folding was tested in a Hsp-mediated folding pathway similar to that used by Langer et al. (1992). Samples of UmuC used for immunoblotting were purified on Superdex Peptide G-200 (Pharmacia). UmuC was approximately 95% pure as judged by Coomassie staining of the above published protocol up to the phosphocellulose elution step. At this time, an aliquot of the UmuC/Hsp70 mixture was mixed with an equal volume of a GroEL'GroES mixture, which was made immediately prior use, containing 0.4 mM GroEL and 0.8 mM GroES in buffer H. This mixture was diluted 2.5-fold into K Buffer. The dialyzed fraction was then centrifuged at 140,000 g for 1 h. The supernatant, containing most of UmuC in a soluble but inactive form, was stored at −70 °C.

### EXPERIMENTAL PROCEDURES

**Materials**—Sources of the materials were as follows. Protein G-heresardized peroxidase conjugate was from Bio-Rad. ATP and deoxyoligonucleotides were from Pharmacia Biotech, Inc. (DS-PAP) 3000 Cl/mmol) was from Amersham Corp. Ethyl methanesulfonate and N-methyl-N-nitro-N-nitrosoguanidine were from Sigma; single-stranded dX174 DNA (dsDNA) was purified from phage particles (Franke and Ray, 1971), double-stranded dX174 DNA (dsDNA) was purchased from New England Biolabs. The 60-mer oligonucleotide containing an abasic site was prepared as described (Randall et al., 1987).

**Proteins and Antibodies**—The UmuC protein fraction used as a control, which will be referred to as S9-folded UmuC, was purified as described (Woodgate et al., 1989). The fraction of UmuC used throughout this work in the Hsp-mediated folding reactions was purified as described, using the above published protocol up to the phosphocellulose elution step. At this point, UmuC was applied to and eluted from a second phosphocellulose column with a buffer containing 7 M urea and 500 mM KCl. This fraction was dialyzed 2.5-fold into K Buffer (10 mM KPO₄, pH 6.8, 5 mM MgO₄, 50 mM octyl β-D-thioglucosanone, 4 mM glutathione, 4% glycerol) and dialyzed against K Buffer. The dialyzed fraction was then centrifuged at 140,000 g for 1 h and the supernatant, containing most of UmuC in a soluble but inactive form, was stored at −70 °C. This fraction of UmuC was stored at −70 °C. This fraction of UmuC was approximately 95% pure as judged by Coomassie staining of SDS-polyacrylamide gels.

### DNA Binding Activity of UmuC—DNA (175 ng of ss or ds dX174 DNA) was incubated for 5 min at 25 °C in the presence of 50–80 ng of UmuC in buffer B (20 mM Hepes pH 8, 100 mM potassium acetate, 8 mM magnesium acetate, 4% glycerol, 4% glutathione). Nucleoprotein complexes were separated by agarose gel electrophoresis and analyzed as described below.

**Immunoblotting of Protein Complexes and Nucleoprotein Complexes**—Nondenatured protein complexes were separated by agarose gel electrophoresis and then transferred in the presence of SDS to a membrane for immunoblotting as described in Hoffman et al. (1992). Samples (5–10 μl) of reactions were mixed with 4 μl of gel loading solution (0.25% bromphenol blue, 30% glycerol in double distilled H₂O) and loaded onto a 4-mm thick 0.7% agarose gel. Electrophoresis was carried out in LTAE (6.7 mM Tris-Cl, 3.3 mM sodium acetate, 1 mM EDTA, pH 7.5), TGE (25 mM Tris base, 190 mM glycine, 1 mM EDTA, pH 8.5) or TGA (25 mM Tris base, 190 mM glycine, 1 mM ATP, 1 mM magnesium acetate, 1 mM dihydrobiothiophosphate) as indicated in the legends to Figs. 1–3. In the case of low ionic strength buffer (LTAE), recirculation was applied during electrophoresis. When DNA was present, the gel was stained with 0.5 μg/ml of ethidium bromide for 15 min and photographed with UV light to visualize the DNA. In order to probe the same membrane with different antibodies, the membranes were stripped using a procedure adapted from Millipore. The membrane was soaked twice for 1 h in S buffer (0.1 M glycine, 0.1% SDS, 50 °C water bath, using a plastic sealed bag). The membrane was then processed as usual for immunoblotting with the different antibody. In order to estimate the amount of UmuC retained in different complexes and efficiently recovered by the methods used, different amounts of UmuC were deposited directly onto the polyvinylidene fluoride membrane using a slot blot apparatus prior to immunodetection. Spot intensities were compared using a phosphofluorimeter and analyzed as described (Rajagopal et al., 1992).

**Replicative Bypass of Abasic DNA Site**—The replication bypass reaction was performed essentially as described (Rajagopal et al., 1992). Briefly, the DNA template for the assay was a 5.4-kiobase linear DNA with an abasic site located 30 bases from the 5′ end. Replication was carried out by using a 5′-end-labeled primer annealed to the DNA template 65 nucleotides upstream from the abasic site. Hsp-folded UmuC (S9) (50 nM) was preincubated for 2 min at 30 °C in a 10-pl reaction containing 20 mM Tris-Cl, pH 7.5, 8 mM MgCl₂, 5 mM dithiothreitol, 0.1 mM EDTA, 25 mM sodium glutamate, 1 mM ATP, 4% (w/v) glycerol, 40 μg/ml bovine serum albumin, 5% (w/v) PEG 6000, 2.5 mM primed DNA substrate, 3100 nM single-stranded DNA binding protein, 0.1 μM β protein, 2500 nM RecA803, and 1300 nM UmuD′. Replication was then initiated by the addition of dNTPs (80 μM each) and DNA polymerase III holozyme (15 μg) and incubated for 10 min at 37 °C.

**Strain Constructions and UV Mutagenesis Test**—A Thi10kan inserion (thi*:Thi10kan) was introduced by P1 transduction close to the argE3 marker of AB1157 to give strain JC1442. JC1442 was then used as a donor to introduce the argE3 mutation by P1 transduction into strains containing different dnaK alleles. The resulting constructs were MC1061 (araD139 Δ (ara-leu7696 Δ (lacX74 galU galK hisdR2 merB1 rpsL) argE3 thi178:Thi10kan derivative containing wild-type dnaK (JC1443)), dnaK306 (JC1446), dnaK204 (JC1444), or dnaK306 (JC1445). Similar constructions were made for dnaK756 (JC1448) in a C690 derivative, argE3 thi178:Thi10kan (JC1447). The dnaK phenotypes of the strains were determined by UV mutagenesis and thermoadaptation at 42 °C. The dnaK756 mutant was isolated by Georgopoulos (1977), and the other dnaK mutants were obtained by Wild et al. (1992). The dnaK204 mutant corresponds to a glycine to aspartate mutation at amino acid 341 (referred to as “G34D” by Wild et al. (1992)), and dnaK306 corresponds to a glutamate to lysine mutation at amino acid 317 (referred to as “EK317” by Wild et al. (1992)). dnaK306 was a Class 1 mutant, according to Wild et al. (1992) (see “Discussion” for a further description of these mutants). In order to test for UV mutagenesis, exponentially growing cells were pelleted and resuspended in TM (10 mM Tris-Cl, pH 8, 10 mM MgCl₂). Two ml of resuspended cells (corresponding to 10⁶ cells) were UV irradiated at a dose of 45 J/m² in a Petri dish and then incubated in 10 ml of LB at 30 °C for 1 h in the dark, concentrated to 0.4 ml in TM, and spread in triplicate on minimal glucose plates without arginine. D turbidities were plated in triplicate to count viable cells. Results were monitored after 36 h of growth at 30 °C. All strains tested were UV sensitive as described by Calero et al. (1984). Mutagenesis with alkylating agents was performed as described (Miller, 1992), using 0.2 mM ethyl methanesulfonate and 5 μg/ml of N-methyl-N-nitro-N-nitrosoguanidine.
RESULTS

We investigated whether an Hsp-mediated folding pathway adapted from Langer et al. (1992) would result in the recovery of soluble and active UmuC protein. The physiological significance of a role for Hsp70 in UmuC activation is suggested by the in vivo evidence of a defect in UV mutagenesis in some dnaK mutants.

Interaction of UmuC with Hsp70 Complex—An in vitro folding protocol, adapted from Langer et al. (1992) was applied to UmuC. Purified and inactive UmuC was incubated with DnaK and DnaJ as described under “Experimental Procedures.” After 10 min at 25 °C, GrpE was added, and the reaction was incubated further at 25 °C for 20 min. Protein complexes were separated by electrophoresis in a 0.7% agarose gel under non-denaturing conditions, transferred, and detected by immunoblotting. The acidic DnaK and GrpE proteins migrate in such gels toward the anode, whereas the basic DnaJ and UmuC proteins will migrate in the opposite direction (or not at all), unless associated with acidic proteins. We, therefore, considered the presence of basic proteins migrating toward the anode as diagnostic of interaction with the acidic proteins. This technique has been previously used to study the interaction of λ P protein with Hsp70 (Hoffmann et al., 1992).

Results obtained with anti-UmuC antibodies are presented in Fig. 1A. No signal was observed for UmuC protein that had not been treated with heat shock proteins. However, a strong signal was visible when UmuC had been treated with DnaK, DnaJ, and GrpE (Fig. 1A, lane CJK). This signal is designated as complex I. Although the extent of interaction of UmuC with these three heat shock proteins was not precisely quantitated, it appeared to involve most of UmuC added to the reaction, since a similar signal was obtained when the same amount of UmuC was directly applied to the membrane prior to immunodetection (data not shown). A weaker signal was observed when GrpE was omitted from the folding reaction (lane CJK). This indicates that GrpE is required to stabilize the UmuC-Hsp70 complex. When UmuC was incubated with DnaK alone (lane CK), or DnaJ alone (lane CJ), no signal was detected.

In order to determine which proteins interacted with UmuC within complex I, the membrane was stripped and reprobed with different antibodies. The presence of DnaJ, the other basic protein, was investigated with anti-DnaJ (Fig. 1B). A signal corresponding to the position of complex I was observed in the CJK lane. Additionally, a diffuse signal spanning from the position of complex I to the wells was present in the lanes where both DnaK and DnaJ were present (lanes JK and CJK), which may indicate a weak interaction between these proteins. Finally, the position of acidic proteins DnaK and GrpE in the gel were monitored using the corresponding antibodies (data not shown). DnaK migrated at the position of complex I, and its migration was unaffected by the presence or absence of UmuC and DnaJ. GrpE migrated slightly faster than complex I but still overlapped with it, and its migration was unaffected by the presence or absence of UmuC and DnaJ. We, therefore, concluded that UmuC was able to form a complex with DnaK, DnaJ, and GrpE and a weaker one with a combination of DnaK and DnaJ only.

Interaction of UmuC with Hsp60 Requires Prior Treatment with Hsp70—We next addressed whether the UmuC-Hsp70 complexes could interact with GroEL and GroES. These Hsp60 proteins were added to the UmuC-Hsp70 complexes, and the mixture was incubated for 1 h at 25 °C. Results are shown in Fig. 1A, last three lanes. When the UmuC treated with DnaK, DnaJ, and GrpE was incubated in the presence of GroEL and GroES (lanes CJK + ELES), transfer of UmuC from complex I to a faster migrating complex (complex II) was observed. A more diffuse, less intense signal remained at the position of complex I. In the absence of GrpE (lanes CJK + ELES), a weaker signal was observed, as was the case when GrpE was omitted from the Hsp70 folding reaction (lane CJ). Additionally, no UmuC was displaced to the position of complex II. Finally, no signal was observed when UmuC was incubated with GroEL and GroES only without prior treatment with the Hsp70, even when a 5-fold increase in the amount of GroEL and GroES was used (not shown).

Immunoblotting experiment with GroEL antibodies allowed us to detect the position of the GroEL-GroES complex. It corresponded to the position of complex II, and the mobility of this complex was unaffected by the presence or absence of UmuC. No DnaJ (Fig. 1B, lanes CKJE + ELES), GrpE, or DnaK (not shown) was detected in complex II.

Whereas in vivo experiments had already suggested a need for Hsp60 in umuDC-dependent UV mutagenesis (Donnelly and Walker, 1989, 1992), no similar evidence had been previously reported for Hsp70. We, therefore, asked whether the need for a “pretreatment” with Hsp70 could be due to the particular conformation of the UmuC that was used. UmuC protein is purified under denaturing conditions, and the denaturant (urea) is then removed by dialysis. The final conformations of UmuC under such treatments may make them artificially good targets for Hsp70 action. If, however, Hsp70 action is necessary in the biological folding process, we would expect to see a requirement for the action of the Hsp70 proteins in the folding of UmuC starting from a completely unfolded state. A folding reaction was carried out as above, starting with UmuC in 7 M urea. Interaction of UmuC with Hsp60 was monitored as before, with or without pretreatment with Hsp70. We observed a UmuC-Hsp60 interaction only when pretreatment with
Hsp70 had occurred (data not shown).

We, therefore, suggest that UmuC is capable of interacting with Hsp60, but only when UmuC is treated previously with Hsp70. In addition, GrpE appears to be necessary for the transfer of UmuC from the Hsp70 complex to the Hsp60 complex. Further evidence for a role of DnaK in UmuC folding was obtained from genetic experiments (see below).

**UmuC Binds to ssDNA**—The DNA binding ability of UmuC was investigated using the S9-folded UmuC fraction (Woodgate et al., 1989). UmuC was incubated with dX174 ds- or ssDNA for 5 min at 25 °C. Complexes were then separated by agarose gel electrophoresis, stained to visualize DNA molecules, and then subjected to immunoblotting. Following detection with anti-UmuC antibodies, two bands were visible in the lanes containing ssDNA (Fig. 2B, lanes 4–6). The presence of a UmuC band migrating at the position of ssDNA (Fig. 2B, lanes 4–6) suggests that a stable complex is formed between the protein and ssDNA. The presence of a more intense slower migrating band is probably the result of a UmuC-ssDNA complex involving more UmuC but less ssDNA. We were unable to detect an ethidium bromide-stained DNA band migrating at the position of the slower migrating band in the Western blot (Fig. 2A, lanes 4–6) in this particular experiment; however, such a DNA band was visible in other experiments (data not shown). The total amount of UmuC bound to DNA corresponded to the majority of UmuC added to the reaction. The presence or absence of ATP or ATPyS in the incubation buffer did not affect UmuC binding to DNA (Fig. 2B, compare lanes 5 and 6 with lane 4). In contrast to the binding of UmuC to ssDNA, binding of UmuC to dsDNA was not detectable (Fig. 2B, lanes 1–3), even after UV irradiation of the dsDNA (not shown). These data suggest that UmuC binds to ssDNA but does not appear to form a stable complex with dsDNA.

**Recovery of DNA Binding Activity of UmuC upon Hsp Treatment**—The DNA binding ability of UmuC was tested at different steps of the Hsp folding process. Experiments were carried out as above, but ATP was added to 1 mM in the electrophoresis buffer, which resulted in a different mobility of complex I (see Fig. 3). Complex I remained in the well (lanes 5 and 6), and complex II migrated as usual (lanes 7 and 8). We did not further investigate the reason for the change in mobility of complex I. DNA binding activity of UmuC prior to any Hsp treatment was undetectable (lane 2), as was that of UmuC treated with Hsp60 only (lane 4). Upon treatment with Hsp70, however, DNA binding activity of UmuC was recovered. This restoration of activity was observed whether or not the Hsp60 proteins were added (lanes 6 and 8). The amount of UmuC bound to DNA did not exceed 20% of input UmuC, which is significantly less than the amount of S9-folded UmuC that could bind. This may reflect the proportion of UmuC molecules correctly folded by the Hsp. Varying the stoichiometry of the Hsp, as well as incubation times, did not improve the amount of UmuC that could bind. It is possible that the folding reaction is not optimized. In particular, the presence of UmuC still associated with Hsp60 in complex II (Fig. 3, lane 8) suggests that a fraction of UmuC molecules has not been completely folded. We conclude that some (but not all) DNA binding activity of UmuC is recovered upon Hsp70 treatment.

**Recovery of Lesion Bypass Activity of UmuC upon Sequential Hsp Treatment**—The capacity of UmuC to promote replication bypass was investigated using the replication assay described by Rajagopalan et al. (1992). A linear single-stranded DNA substrate containing an abasic site at a defined position was used for in vitro replication with DNA polymerase III. Only nominal replication was possible when the abasic site was located in the absence of UmuC or in the presence of wild-type RecA protein. We used RecA803 protein because the use of this mutant protein resulted in a 2–5-fold increase in bypass efficiency (see discussion).

The results of the bypass reactions with the Hsp-folded UmuC are shown in Fig. 4. As observed by Rajagopalan et al. (1992), significant replication past the abasic site is observed when S9-folded UmuC is present (lane 3) compared with the same reaction without UmuC (lane 1). UmuC protein that had not been treated with S9 nor subjected to Hsp-mediated folding was unable to increase replication bypass (lane 2). The presence of heat shock proteins did not interfere with the replication assay or lead to replication bypass by themselves (lanes 4 and 6). We next tested the replication bypass activity of UmuC treated either with Hsp70, Hsp60, or both. Whereas no significant amount of bypass above background was detected with
The presence of bands above the replication block (visible at the DNA replication. The linear DNA substrate with an abasic DNA le-

sion was replicated by DNA polymerase II in the presence of RecA803, UmuD', and UmuC protein. Replication products were separated by polyacrylamide gel electrophoresis and visualized by autoradiography. The presence of bands above the replication block (visible at the bottom of the gel) reflects translesion replication. Additions to reaction mix-
su.

The signal increased when UmuC was treated successively with Hsp70 and Hsp60, therefore, leads to recovery of lesion bypass activity. A successive treatment of UmuC with Hsp70 and Hsp60

groEL and groES. It may not be coincidental that successive

mutations: those in which UV mutagenesis was as efficient as in wild type (dnaK302 and dnaK756; Table I), and those in which UV mutagenesis was inhibited, to the same extent or even more than the groEL mutant. The dnaK204 mutant showed a 50-fold decrease in mutagenesis relative to the wild type, and dnaK306 a ~1000-fold decrease.

Dnak is involved in many cellular processes; therefore the effects of the dnaK204 and dnaK306 mutations could be indi-

rect. To address this point, two other mutagens, ethyl methanesulfonate, and N-methyl-N-nitro-N-nitrosoguanidine, were tested. These alkylating agents promote mutagenesis in a largely SOS-independent pathway (Schedel et al., 1978); thus, if the mutations in DNAk affect SOS mutagenesis specifically rather than having a more general and indirect effect, they should still be responsive to the mutagenic effect of these agents. Yields of Arg revertants were comparable in strains harboring the wild type and mutant alleles of DNAk with this class of mutants (Table II). One exception was the dnaK204 mutant treated with N-methyl-N-nitro-N-nitrosoguanidine, where mutagenesis was approximately half as high as in the other strains. However, the response to the other agent, ethyl methanesulfonate, was not affected by the dnaK mutation; therefore, the effect of this mutation on N-methyl-N-nitro-N-nitrosoguanidine mutagenesis might not be significant.

In summary, certain dnaK mutations result in a dramatic decrease in UV-induced mutagenesis while having much less effect on non-SOS mutagenesis. These data provide genetic evidence supporting the possible involvement of Hsp70 in umuCD-dependent mutagenesis in vivo.

DISCUSSION

Previous work has suggested the need for chaperone proteins in UmuC folding in vivo (Donnelly and Walker, 1989, 1992). At the same time, an increase in the understanding of chaperone

activities of the Hsp-folded UmuC would be strengthened if Hsp mutants manifested a defect in umuDC-dependent UV mutagenesis. A decrease in UV mutagenesis has been observed in groEL and groES mutants (Donnelly and Walker, 1989). We investigated whether UV mutagenesis was affected in strains harboring different dnaK alleles. Four dnaK mutants were tested, three of which were obtained and characterized by Dr. C. Gross and co-workers (Wild et al., 1992). These three mutations differed slightly in their phenotypes (see "Discussion") and differed from the previously isolated dnaK756 mutation (Georgopoulos, 1975), which was the fourth mutation included in the test. A groEL mutant was also included in the mutagen-

test for comparison. Mutagenesis was assayed by deter-
miming the reversion frequency from Arg to Arg before and after UV irradiation in each strain. Results are presented Table I, showing yields of Arg revertants/10^8 viable cells. The level of revertants prior to UV treatment was generally in the range of 0.2-5/10^6 cells. In the wild type strain, the number of revert-

ants increased to 700 or 1000/10^8 cells upon UV irradiation. Results in the various dnaK strains revealed two groups of

<table>
<thead>
<tr>
<th>Host background</th>
<th>Relevant genotype</th>
<th>Arg^+ mutants/10^8 cells</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before UV</td>
<td>After UV</td>
</tr>
<tr>
<td>MC1061 Wild-type</td>
<td>2.5 (±1.3)</td>
<td>692 (±227)</td>
</tr>
<tr>
<td>MC1061 dnaK204</td>
<td>0.8 (±0.5)</td>
<td>12 (±10)</td>
</tr>
<tr>
<td>MC1061 dnaK306</td>
<td>0.3 (±0.2)</td>
<td>0.4 (±0.1)</td>
</tr>
<tr>
<td>MC1061 dnaK302</td>
<td>3.2 (±0.9)</td>
<td>675 (±280)</td>
</tr>
<tr>
<td>MC1061 groEL</td>
<td>2.0 (±1.7)</td>
<td>58 (±19)</td>
</tr>
<tr>
<td>C600 Wild type</td>
<td>6.8 (±6.5)</td>
<td>1060 (±365)</td>
</tr>
<tr>
<td>C600 dnaK756</td>
<td>2.3 (±1.2)</td>
<td>860 (±540)</td>
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* Each value represents the average of two to three experiments.
domain could be properly folded with Hsp70 chaperones, whereas folding of the “bypass domain” might require further action of the Hsp60 proteins.

Members of the Hsp60 class of chaperones, GroEL and GroES, were unable to fold UmuC into an active form in the absence of prior treatment by DnaK, DnaJ, and GrpE. However, some interaction of UmuC with Hsp60 could be observed (particularly in the absence of ATP), but it did not result in recovery of UmuC activities. Whereas a role for GroEL and GroES was expected from in vivo data (Donnelly and Walker, 1990), to our knowledge, no evidence of a requirement for DnaK, DnaJ, and GrpE proteins in SOS mutagenesis has been reported. We examined umuDC-dependent UV mutagenesis in vivo in various dnaK mutants. In two such mutants, UV mutagenesis was severely inhibited. Mutagenesis by other agents, acting independently from umuDC, was not affected in these dnaK mutants. Bacteria harboring a complete deletion of the dnaK gene grow poorly and accumulate suppressor mutations (Bukau and Walker, 1990). Conversely, most point mutations in dnaK isolated thus far conferred partial loss of function phenotypes, such as several mutants that have been previously characterized and identified by Wld et al. (1992). Class I mutants prevent plasmid minIF replication, but not a λ growth. Class II mutants prevent both plasmid minIF replication and λ growth; they also exhibit negative complementation. The strongest effect on mutagenesis was seen with a class I mutant (dnaK306), where a ~1000-fold decrease in mutagenesis was observed. A 50-fold reduction in mutagenesis was also observed for one class II mutant (dnaK204) but not for another (dnaK302). Finally, the dnaK756 mutant, which does not belong to either of these classes, exhibited no effect on UV mutagenesis. We could not find, therefore, any correlation between these previously designated classes and UV mutagenesis. The absence of mutagenesis phenotype for some dnaK mutants could be due to the necessary leakiness of dnaK alleles. These data are, therefore, compatible with a role of DnaK in UmuC folding in vivo. The need for chaperone assistance in vivo can be considered as an additional regulation of UmuD’C activity, allowing fine tuning of mutagenesis, as has been previously suggested (Donnelly and Walker, 1992).

In the course of this work, we found that in vitro replication bypass was more efficient with the RecA803 protein than with wild type RecA protein. The RecA803 mutant allele was isolated as a recF suppressor, and the purified protein shows an increased rate of association with single-stranded DNA (Madiraju et al., 1988, 1992; Lavery and Kowalczykowski, 1992). RecA803 might be competing better with single-stranded DNA binding protein for binding to the ssDNA. Alternatively, the enhanced bypass observed with RecA803 may reflect a more direct effect of the mutant protein on lesion bypass.

We have found that UmuC binds preferentially to single-stranded over double-stranded DNA. With respect to a stalled DNA polymerase, this could mean that UmuC is loaded on the single-stranded “side” of a DNA lesion. The UmuD’C complex might, therefore, interact with the RecA filament, which is also expected to be positioned on the ssDNA. Indeed, Frank et al. (1993) proposed that the role of the RecA filament could be to target UmuD’C to the single-stranded side of the lesion. The next step, that would presumably involve the interaction of UmuDC and/or RecA with the stalled polymerase in order to rescue it, remains to be investigated. The resumption of DNA polymerization would lead to the cessation of SOS, as has been proposed by Sommer et al. (1993). Clearly, the key step in SOS mutagenesis that remains to be understood concerns the interactions between DNA polymerase III and UmuC, UmuD’, and RecA that allow misincorporation opposite the lesion site and elongation across it. The ability to obtain properly folded UmuC protein by sequential Hsp treatment should allow further understanding of the mechanism of SOS mutagenesis at the molecular level.

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References

| TABLE II | Mutagenesis by alkylating agents in various dnaK strains |
| Host background | Relevant genotype | Arg* mutants/10⁶ cells¹ |
| MC1061 | Wild type | 4.1 (±3.6) |
| MC1061 | dnaK204 | 5.1 (±5.5) |
| MC1061 | dnaK306 | 2.4 (±1.8) |
| MC1061 | groEL | 2.5 (±0.7) |

¹ Each value represents the average of two experiments, except for strain groEL treated with EMS, where the second value was considered as aberrant (575 mutants/10⁶ cells).