The Mutagenesis Protein MucB Interacts with Single Strand DNA Binding Protein and Induces a Major Conformational Change in Its Complex with Single-stranded DNA*

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The MucA and MucB proteins are plasmid-encoded homologues of the *Escherichia coli* UmuD and UmuC proteins, respectively. These proteins are required for SOS mutagenesis, although their mechanism of action is unknown. By using the yeast two-hybrid system we have discovered that MucB interacts with SSB, the single strand DNA binding protein (SSB) of *E. coli*. To examine the interaction at the protein level, the MucA, MucA’, and MucB proteins were overproduced, purified in denatured state, and refolded. Purified MucA and MucA’ each formed homodimers, whereas MucB was a monomer under native conditions. RecA promoted the cleavage of MucA to MucA’, and MucB was found to bind single-stranded DNA (ssDNA), similarly to the properties of the homologous UmuD and UmuC proteins. Purified MucB caused a shift in the mobility of SSB in a sucrose density gradient, consistent with an interaction between these proteins. Addition of MucB to SSB-coated ssDNA caused increased electrophoretic mobility of the nucleoprotein complex and increased staining of the DNA by ethidium bromide. Analysis of radiolabeled SSB in the complexes revealed that only a marginal release of SSB occurred upon addition of MucB. These results suggest that MucB induces a major conformational change in the SSB-ssDNA complex but does not promote massive release of SSB from the DNA. The interaction with SSB might be related to the role of MucB in SOS-regulated mutagenesis.

UV mutagenesis in *Escherichia coli* is a regulated process, controlled by the SOS stress response through its two global regulators, RecA and LexA. The mechanism underlying this process is trans-lesion replication by a DNA polymerase, most likely DNA polymerase III (for reviews see Refs. 1–3). This process requires specifically two SOS-induced proteins, UmuD and UmuC (4–6), whose mechanism of action is unknown. A prevailing hypothesis is that UmuD’, the active form of UmuD (7–9), along with UmuC are required to assist the DNA polymerase in replicating the damaged site (10). However, the nature of this assistance is not clear because purified DNA polymerases can bypass DNA lesions unassisted (11–19). Moreover, in an *in vitro* system for UV mutagenesis carried out with crude protein extracts (20, 21) or with purified proteins (22), we have found that UV mutations were effectively produced in the absence of UmuD’ and UmuC.

Homologues of UmuD and UmuC have been identified in other bacteria, and some of them are encoded by conjugal plasmids (23, 24). The most well-studied of these are the mucA and mucB genes, encoding homologues of UmuD and UmuC, respectively (25, 26). An approach that has proven useful in the study of many proteins is to examine their interactions with other proteins. Employing this approach we present here *in vivo* and *in vitro* data that show, for the first time, that MucB interacts with SSB and greatly changes the structure of the SSB-ssDNA complex.

**EXPERIMENTAL PROCEDURES**

**Materials**—The sources for the materials used in this study were as follows: isopropyl-1-thio-β-D-galactopyranoside, bovine serum albumin, and DTT, Boehringer Mannheim; Tris base, spermidine, sodium deoxycholate, Tween 20, lithium acetate, polyethylene glycol (molecular weight 3350), Sigma; acrylamide, BDH; glycerol, U. S. Biochemical Corp.; dialysis bags, Spectrum.

**Media**—The media used in this study were M9, LB, and minimal A medium (27). Medium M9ZB was used to grow *E. coli* BL21 (DE3) for overproduction of the MucB proteins (28). Antibiotics used were ampicillin (100 mg/ml) and kanamycin (70 mg/ml). Isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.4 mM.

**Bacterial Strains**—The *E. coli* strains used in this study are as follows: *E. coli* K12 AB1157XL argE3 his4 leuB6 proA2 thi1 ara14 galK2 lacY1 thi1 tsx33 rpsL31 supE44 Tn10 lacZM15 proAB, MC4100 ΔargF-lac205 araD139 rpsL50 thiAI relA1 bfi301 decoC1 ptsF25 rbsR, and BL21 F- ompT r P B (ΔDE3). JM105 endA1 thi, hsdR17, supE44, relA1, Δ(pro-lac), F′ lacD16, lacF(lacZ ΔM15, proAB); JM109, same as JM103 but also recA1. The yeast strain used was *S. cerevisiae* CEN.PK113-7D (ΔADE2). The yeast strain used was Y190 MATa gal4 gal8 his3 trp1-901 ade2-101 ura3-52 leu2-3, 112 + URA3::GAL → lacZ LYS2::GAL(UAS) → HIS3 cyh2.

**Plasmids and Other DNAs**—The plasmids used in this study are presented in Table I. Plasmid p1-66 is a *pBluescript SK* derivative in which a 1.1-kilobase pair HindIII-HindII fragment containing mucA and a part of mucB (Fig. 1) from pWW1700 was inserted to the ApEl site in *pBluescript SK* under of the T7 RNA polymerase promoter. Plasmid p2 contains the same fragment in the opposite orientation (under the lac promoter). Plasmid p1-66-3 contains the entire mucAB operon in plasmid *pBluescript SK* under the T7 promoter. It was constructed by ligating the 1.2-kilobase pair BglII-AceI fragment containing the 3‘-terminal part of mucB (Fig. 1) into plasmid p1-66. Plasmid p1-66MucAI is plasmid p1-66 with an NdeI site in front of the first ATG of mucA.

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1 The abbreviations used are: SSB, single strand DNA binding protein; ssDNA, single-stranded DNA; DTT, dithiothreitol; PCR, polymerase chain reaction; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; ATPS, adenosine 5’-O-(thiotriphosphate).
Plasmid p1–66MucA/B is similar to p1–66MucA1, except that the missing 3′-terminal fragment of mucA was added, to reconstitute the entire mucA operon, with an NdeI site in front of its first ATG. Plasmid p1–66MucB1 is plasmid p1–66 with an NdeI site in front of the first ATG of mucB. Plasmid p1–66MucB is similar to p1–66MucB1, except that the 1.2-kilobase pair BglII-AvaiI fragment containing the 3′-terminal portion of mucB was added, to reconstitute the entire mucB gene, with the NdeI site in front of it. Plasmid pETpMuCA is the overproducer of MucA. It was constructed by ligating the Ndel-BamHI fragment from p1-66MucA1 to plasmid pET3a that was digested with NdeI and BamHI, putting mucA under the T7 610 promoter. pETpMuCA is the overproducer of mucB. It was prepared by inserting the Ndel-BamHI fragment from p1-66MucB into Ndel- and BamHI-cleaved pET3a. Plasmid pETpMuCA is the mucA overproducer. It was constructed by PCR amplification of mucA from plasmid p1-66MucAB1 by using primers 70 and 71 (see Table IV), followed by cleavage of the PCR fragment with NdeI and BamHI, and ligation to Ndel- and BamHI-cleaved pET3a. The plasmids used in the two-hybrid system are presented in Table II. They were constructed by cloning PCR-amplified open reading frame of the tested genes into plasmids pACTII and pAS-CYH2 as described in Table III, using the primers described in Table IV, which contained the restriction sites needed for cloning. Single-stranded DNA of phage M13mp8 was isolated as described (29).

**Tease Methods—** Yeast transformation was done using the lithium acetate method (30). Detection of β-galactosidase activity was done on filters as described (31).

**Purification of MucA and MucB**—E. coli BL21(DE3) cells harboring plasmid pETpMuCA (1 liter) were grown at 37 °C in a 5-liter flask containing 50 mM Tris HCl, pH 7, and 15% sucrose, and frozen in liquid nitrogen. The cell suspension was thawed at 15 °C, and its volume was adjusted with Tris HCl, pH 7, and 15% sucrose, and frozen in liquid nitrogen. The cell suspension was thawed at 15 °C, and its volume was adjusted with Tris HCl, pH 7.5, 10 mM DTT, and 1 mM EDTA. The suspension was centrifuged at 10,000 rpm for 1 h at 4 °C, and the supernatant was used.

**Construction of plasmids used in the two-hybrid system**

| Name     | Gene source | Primers | Restriction sites | Insert size | Source |
|----------|-------------|---------|-------------------|-------------|--------|
| pACTMuCA | p1–66MucA/B | NdeI, BamHI | 796 | bp |
| pACTMuCA’ | pETpMuCA’ | NdeI, BamHI | 700 | bp |
| pACTMuB | p1–66MucB | NdeI, BamHI | 1462 | bp |
| pACTSSB | pRS773 | 160, 125 | NdeI, BamHI | 537 | bp |
| pACTRecA | pGE226 | 161, 123 | NdeI, BamHI | 1062 | bp |
| pACTDnaB | genomic DNA | 172, 173 | BamHI, BamHI | 1416 | bp |
| pASMuCA | p1–66MucA/B | NdeI, BamHI | 796 | bp |
| pASMuCA’ | pETpMuCA’ | NdeI, BamHI | 700 | bp |
| pASMuB | p1–66MucB | NdeI, BamHI | 1462 | bp |
| pASMuB1 | p1–66MucB | NdeI, BamHI | 1250 | bp |
| pASSSBB | pRS773 | 124, 125 | NdeI, BamHI | 537 | bp |
| pASSRecA | pGE226 | 122, 123 | NdeI, BamHI | 1062 | bp |
| pASDnaB | genomic DNA | 172, 173 | BamHI, BamHI | 1416 | bp |

**Fusion to the transcription activation domain of GAL4 in plasmid pACT**

| Plasmid | Fused gene | Source |
|---------|------------|--------|
| pACTMuCA | mucA | This study |
| pACTMuCA’ | mucA’ | This study |
| pACTMuB | mucB | This study |
| pACTSSB | ssb | This study |
| pACTRecA | recA | This study |
| pACTDnaB | dnaB | This study |

**Table III**

| Name | Gene source | Primers | Restriction sites | Insert size | Source |
|------|-------------|---------|-------------------|-------------|--------|
| pACTMuCA | p1–66MucA/B | NdeI, BamHI | 796 | bp |
| pACTMuCA’ | pETpMuCA’ | NdeI, BamHI | 700 | bp |
| pACTMuB | p1–66MucB | NdeI, BamHI | 1462 | bp |
| pACTSSB | pRS773 | 160, 125 | NdeI, BamHI | 537 | bp |
| pACTRecA | pGE226 | 161, 123 | NdeI, BamHI | 1062 | bp |
| pACTDnaB | genomic DNA | 172, 173 | BamHI, BamHI | 1416 | bp |
| pASMuCA | p1–66MucA/B | NdeI, BamHI | 796 | bp |
| pASMuCA’ | pETpMuCA’ | NdeI, BamHI | 700 | bp |
| pASMuB | p1–66MucB | NdeI, BamHI | 1462 | bp |
| pASMuB1 | p1–66MucB | NdeI, BamHI | 1250 | bp |
| pASSSBB | pRS773 | 124, 125 | NdeI, BamHI | 537 | bp |
| pASSRecA | pGE226 | 122, 123 | NdeI, BamHI | 1062 | bp |
| pASDnaB | genomic DNA | 172, 173 | BamHI, BamHI | 1416 | bp |

**Purification of MucB—** E. coli BL21(DE3) cells harboring plasmid pETpMuCB were grown and induced like the MucA overproducing cells. The overproduced protein formed inclusion bodies, and those were purified like the MucA inclusion bodies, except that no lysozyme was used. The inclusion bodies were denatured in a buffer containing 8 M urea, 50 mM Tris-HCl, pH 7.4, 10 mM DTT, and 1 mM EDTA. The solution of denatured protein was incubated for 2 h at 4 °C with constant stirring. The solution was then cleared at 40,000 rpm for 2 h in a Ti45 rotor at 4 °C. The denatured MucB was dialyzed against a buffer containing 50 mM Tris-HCl, 0.1 mM EDTA, 10 mM β-mercaptoethanol, and 10% glycerol (buffer C). After dialysis the solution was cleared by a 2-h spin at 200,000 × g. The supernatant contained the soluble MucA (80%; 13 mg). The purity was greater than 95% as estimated by Coomassie Blue staining. MucA was purified from BL21(DE3) cells carrying plasmid pETpMuCA using the same procedure.

**MucB Interacts with ssDNA-bound SSB**

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from New England Biolabs. Polynucleotide kinase was from U. S. Biochemical Corp.

Analysis of Native Size of MucA and MucA'—MucA and MucA' were analyzed by HPLC size exclusion chromatography on a TSK250 column equilibrated with 0.1 M NaCl, 25 mm Tris-HCl, pH 7.5, and 10 mm DTT at a flow rate of 1 ml/min. The protein solution containing the purified MucA or MucA' was injected in a volume of 50 μl. The elution profile was monitored with a UV detector at 214 nm.

RecA-mediated Cleavage of MucA and LexA—Two μg of MucA were incubated with 1 μg of RecA in a mixture containing 20 mm Tris-HCl, pH 7.5, 5 mm DTT, 40 ng M13mp8 ssDNA, 1 mm ATP, and various concentrations of MgCl₂, in a total volume of 25 μl, at 37 °C for 2 h. Cleavage product were analyzed by SDS-PAGE.

Gels containing radiolabeled SSB were treated with amplifier (Amerham Corp.) was incubated at room temperature for 25 min, after which the reaction was terminated by the addition of 100 μl of Tris-HCl (50 mm, pH 7.9). The radiolabeled SSB was separated from the unreacted NaB³H₄ by a brief spin at 480 g in a 0.8-ml Sephadex G-50 mini-column, followed by dialysis against 25 mM Tris HCl, pH 7.5, 5 mM DTT, 40 ng M13mp8 ssDNA, 1 mM ATP, and 4 °C. Fractions of 200 μl were collected from the top and analyzed by scintillation counting.

Gel Mobility Shift Assay (31)—Purified MucB and MucA' proteins (2.7 and 27 pmol, respectively) were incubated with 32P5' labeled SSB and 5 μg of bovine serum albumin or 5 μg of MucB were loaded on a 5-ml 5–20% sucrose gradient containing 2 mm MgCl₂, 2 mm DTT, and 25 mm Tris-HCl, pH 7.5. The gradient was run for 8 h at 49,000 rpm in a SW 50.1 rotor at 4 °C. Fractions of 200 μl were collected from the top and analyzed by autoradiography of the gradient.

Autoradiography of Radiolabeled Nucleic Acids and Proteins— Autoradiography of radiolabeled nucleic acids and proteins was performed by loading on a 1% agarose gel containing ethidium bromide and run in a 2.7 and 27 pmol, respectively) were incubated with SSD (100 μm), H₂O₂, NaOH (0.2 M, pH 9.0), formaldehyde (5 mM), and NaB³H₄ (170 μM, 24 Ci/mmol; Amersham Corp.) was incubated at room temperature for 25 min, after which the reaction was terminated by the addition of 100 μl of Tris-HCl (50 mm, pH 7.9). The radiolabeled SSB was separated from the unreacted NaB³H₄ by a brief spin at 480 g in a 0.8-ml Sephadex G-50 mini-column, followed by dialysis against 25 mm Tris-HCl, pH 8.0, 1 mm EDTA, and 0.3 M NaCl.

Sucrose Gradients—Fifteen μg of ‘H-labeled SSB and 5 μg of bovine serum albumin or 5 μg of MucB were loaded on a 5-ml 5–20% sucrose gradient containing 2 mm MgCl₂, 2 mm DTT, and 25 mm Tris-HCl, pH 7.5. The gradient was run for 8 h at 49,000 rpm in a SW 50.1 rotor at 4 °C. Fractions of 200 μl were collected from the top and analyzed by autoradiography of the gradient.

RESULTS

Protein-Protein Interactions of the Muc Proteins in Vivo—To gain a molecular insight into the mechanism of action of the mutagenesis proteins MucA and MucB, we sought to identify their involvement in novel protein-protein interactions using the yeast two-hybrid system. First, we examined self-interactions of the Muc proteins fused to the two-hybrid system reporter proteins. Each of the muc genes was fused to the transcriptional activation domain or to the DNA binding domain of the yeast GALA gene, and pairs of the fused genes were introduced into yeast. The interactions among various combinations of the fused Muc proteins were tested using the filter assay for β-galactosidase (31). The results shown in Table V suggest that MucA and MucA' interact with themselves and with each other. MucB did not show self-interaction, but it did interact with both MucA and MucA'. These interactions parallel those of the homologous UmuD and UmuD' proteins (35, 36). A truncated form of MucB (MucB₃) lacking 30 amino acid residues from its C terminus was also fused to the GAL4 activation domain. This truncated protein did not interact with either MucA or MucA' in the two-hybrid system (Table V), suggesting that the C-terminal 30 amino acids are required for the interaction of MucB with MucA or MucA'.

We looked for interactions between RecA and the Muc proteins in the two-hybrid system (Table VI). When RecA was fused to the transcription activation domain of GAL4, we found that it interacted with MucA and MucA'. No interactions were found between RecA and MucB. RecA did not interact with MucA when the former was fused to the DNA binding domain of GAL4. This result is most likely due to an altered structure of RecA within the fusion protein that prevents MucA recognition and/or binding. These results demonstrated that the known interactions of Muc proteins can be detected in the two-hybrid system (37–39), rendering it a useful tool for the detection of novel interactions of the Muc proteins.

In Vivo Evidence for the Interactions of Muc Proteins with SSD—Since Umu/Muc-dependent mutagenesis requires DNA synthesis, we tested additional proteins, associated with DNA synthesis, for interactions with the Muc proteins. We tested SSB and DnaB, the major replicative DNA helicase. MucA, MucA', and MucB were found to interact with SSB (Table VI).

| Primer sequence | Gene | Site | ID no. |
|-----------------|------|------|-------|
| CTCAAATCCTCAATGAATAGGGTTCCCAGGCCGGG | mucA' sense | NdeI | 70 |
| GCGGATCCGCAATGCAACGAGCAACACAC | mucA anti | BamHI | 71 |
| TCAATATCCTGTCCTGTTGCC | recA sense | NdeI | 122 |
| GCGGATCCGACATGATATGGAAC | recA anti | BamHI | 123 |
| TCAATCTCCCATTGCTGACGAAAACAAACAGGAGG | ssb sense | NdeI | 124 |
| GCGGATCCGACATGACAGGAGCAACACAC | ssb anti | BamHI | 125 |
| CATGACATGCAAGAGGCTGTAAC | ssb sense | NcoI | 160 |
| GCGGATCCGACATGCAAGAGGCTGTAAC | dnasB sense | BamHI | 172 |
| GCGGATCCGACATGCAAGAGGCTGTAAC | dnaB anti | BamHI | 173 |
TABLE VI

Interactions of the Muc proteins fused to the DNA binding domain with different proteins assayed by the yeast two-hybrid system

| Fusion to transcription activation domain | RecA | SSB | DnaB | BETa2 |
|----------------------------------------|------|-----|-----|-------|
| Fusion to DNA binding domain            |      |     |     |       |
| MucA                                   | +    | -   | -   |       |
| MucA'                                  | +    | -   | -   |       |
| MucB                                   | +    | -   | -   |       |
| MucBΔa                                 | -    | -   | +   |       |
| E2A                                    | -    | -   | -   |       |
| p53                                    | -    | -   | -   |       |

*p53* represents a truncated protein lacking 30 amino acid residues from its C terminus. BETa2 (β-cell E-box transactivator 2) and E2A (helix-loop-helix transcription factor E12 (60)) were used as controls. They interact with each other but not with any of the Muc proteins.

No interactions were found between the Muc proteins and DnaB. Similarly, no interactions were found between the p53 protein and any of the other proteins that were examined. Interestingly, MucBΔa, which did not interact with MucA and MucA', showed a strong interaction with SSB. The finding that SSB interacted with the Muc proteins suggested it as a novel target for the action of the Muc proteins. In addition, the binding of MucBΔa to SSB suggested that MucB has at least two domains. One domain, located at the C terminus, binds MucA or MucA', and the other domain binds SSB. The interactions observed in the yeast two-hybrid system need to be verified by biochemical experiments with purified proteins. To that end we have overproduced the Muc proteins, purified them, and examined their interaction with SSB.

**Overproduction of the Muc Proteins**—The Muc proteins were overproduced using the phage T7 expression system as follows. 1) An Apal-HincII fragment from plasmid pGW1700 (25) containing mucA and a part of mucB was cloned into the Apal site of plasmid Bluescript SK. 2) NdeI sites were created in front of the initiation ATG codons of mucA and mucB by site-directed mutagenesis. 3) A BglII-AceI fragment containing the 3' end of mucB (Fig. 1) was ligated into both constructs, and the in vivo activity of the cloned and mutated muc genes was verified by demonstrating their ability to restore UV mutability in a ΔmutDC strain. 4) Finally, each gene was ligated separately to the pET3a vector to generate pETMucA and pETMucB that overproduce the Muc proteins. The mucA' gene fragment was amplified by PCR and cloned into pET3a to yield plasmid pETMucA', which overexpresses MucA'. The mucA genes in the three final overexpression plasmids were sequenced to ensure overproduction of the wild-type proteins.

**Purification and Analysis of MucA**—The overproduced MucA protein had the expected size of 16.5 kDa in SDS-PAGE (Fig. 2). N-terminal amino acid sequence analysis revealed that the first 10 amino acids of the overproduced protein were identical to the sequence of MucA', as predicted from the cleavage site of MucA (26). The overproduced MucA protein precipitated into inclusion bodies. Therefore, the purification procedure included the isolation of inclusion bodies, solubilization of the protein in 7 M urea, followed by chromatography in a denatured form on phosphocellulose, DEAE-Sephalcel, and Sephacid G-100 columns. Finally the protein was refolded by dialyzing out the urea. Typically, 15 mg of MucA were obtained from 1 liter of culture (Fig. 2).

**Purification of MucA'**—The overproduced MucA' protein had an apparent mass of 14 kDa on SDS-PAGE (Fig. 2). N-terminal amino acid sequence analysis revealed that the first 10 amino acids of the overproduced protein were identical to the sequence of MucA', as predicted from the cleavage site of MucA by RecA (40). In addition we noted that the overproduced MucA' did not contain an N-terminal methionine, as expected from the additional ATG codon that we have engineered into the gene. It was probably removed in the cell. The overproduced MucA' precipitated into inclusion bodies. We have purified and refolded it using a procedure similar to that used for the purification of MucA. At this point our only indication that MucA' was folded properly was that it formed homodimers under native conditions (see below).

**Determination of the Molecular Weight of Native MucA and MucA' Proteins**—The molecular weight of native MucA was analyzed by HPLC size exclusion chromatography, using a TSK2500 column. The elution of MucA was monitored by an UV detector at 214 nm. Fig. 4 (top) shows the elution profile of MucA from the HPLC column (retention time is 17.6 min). Based on calibration with HPLC protein standards, native MucA has a molecular mass of 25.7 kDa, representing most likely a dimer (the molecular mass of the monomer is 16.5 kDa). When resolved under the same conditions, MucA' (monomer size of 14.5 kDa) appeared to have an apparent molecular mass of 27.1 kDa (retention time of 17.5 min, Fig. 4, bottom). Therefore, MucA' is also, most likely, a dimer.
The finding that MucA migrates slightly faster than MucA on a size exclusion column can be attributed to a change in the shape of MucA as compared with MucA. This is supported by information derived from the crystal structure of UmuD, the MucA homologue showing that UmuD assumes an extended structure as compared with the intact UmuD (41). The finding that MucA' and MucA are dimers under native conditions suggests that these proteins were correctly refolded.

Purification and Analysis of MucB—The overproduced MucB protein had the expected size of 45 kDa on SDS-PAGE (Fig. 2). N-terminal amino acid sequence analysis revealed that the first 20 amino acids were identical to those predicted from the nucleotide sequence of the mucB gene (26). Like the other Muc proteins, the overproduced MucB precipitated into inclusion bodies. The protein was purified in the denatured state and was refolded. Total yield was 0.5 mg of MucB from 1 liter of culture. To our knowledge, this is the first report of the purification of MucB. Previously UmuC has been shown to bind ssDNA (42, 43). Based on its homology to UmuC, MucB was expected to bind ssDNA as well. This was examined using the gel mobility shift assay. Incubation of purified MucB protein with a single-stranded 40-mer oligonucleotide resulted in the appearance of a slower migrating band (Fig. 5). This band represented most likely the MucB-bound oligonucleotide. Purified MucA' or MucA did not demonstrate any DNA binding activity and had no effect on the binding activity of MucB in this assay (Fig. 5). The ability of MucB to bind ssDNA suggested that it was folded correctly.

Analysis of MucB-SSB Interactions on Sucrose Gradients—To test whether MucB physically interacts with SSB, we sedimented 3H-labeled SSB in a sucrose gradient in the presence or absence of MucB (Fig. 6). When MucB and SSB were run together in the sucrose gradient the migration rate of SSB in the gradient was slightly enhanced. No change in the migration rate of SSB was observed when bovine serum albumin or MucA was added (not shown). This suggests that the alteration in the sedimentation rate of SSB caused by MucB, although modest, resulted from specific binding of MucB to SSB, consistent with the results obtained with the two-hybrid system (Table IV). The small effect may be due to a low affinity of MucB to SSB. Alternatively, MucB may recognize preferentially the DNA-bound form of SSB, or it may form a ternary DNA<sub>z</sub>SSB<sub>z</sub>MucB complex.

MucB Induces a Major Conformational Change in SSB-ssDNA Complexes—To characterize further the interactions of MucB and SSB in the presence of ssDNA, we used a gel mobility shift assay. Binding of SSB to M13mp8 ssDNA retarded the migration of the DNA when run in a neutral agarose gel (Fig. 7). SSB has been shown to possess at least two modes of binding to ssDNA (34, 44). In the fully cooperative mode, the entire DNA molecule is covered with SSB (each SSB tetramer binding 35 nucleotides), leading to a strong retardation (e.g., the 3 µm lane in Fig. 7). In the non-cooperative mode, only part of the DNA is covered with SSB leading to a milder retardation which increases with the amount bound to the DNA (the 0.8-2 µm lanes in Fig. 7).
FIG. 6. MucB alters the migration of SSB in a sucrose gradient. 

\[ ^{3}H \text{-Labeled SSB, either alone or together with MucB, were sedimented in a 5–20\% sucrose gradient as described under "Experimental Procedures." Fractions were collected from the top of the tube and analyzed in a 5–20\% sucrose gradient as described under "Experimental Procedures." The results represent the average of five experiments.} \]

\[ \text{BSA} \quad \text{Fraction no.} \quad \text{Catalse} \]

\[ \text{SBB conc.} \mu\text{M} \]

\[ \text{Origin} \quad \text{SSB-DNA complex} \quad \text{MucB} \]

\[ \text{MucB conc.} \mu\text{M} \]

\[ \text{Origin} \quad \text{SSB-DNA complex} \quad \text{MucB} \]

\[ \text{MucB changes the mobility and ethidium bromide staining of the SSB-ssDNA complex. SSB and M13mp8 ssDNA were incubated with the indicated concentrations of MucB, after which the reaction mixtures were fractionated by agarose gel electrophoresis. The details are presented under "Experimental Procedures."} \]

\[ \text{FIG. 7. Gel mobility shift assay of SSB binding to ssDNA. The reaction mixtures contained M13mp8 ssDNA and the indicated concentrations of SSB. The analysis was done by electrophoresis in an 1\% agarose gel containing ethidium bromide. The details are presented under "Experimental Procedures." The gel was photographed under UV light.} \]

\[ \mu\text{M} \text{ lanes} \text{ in Fig. 7 (34). At lower SSB concentrations both modes of binding were observed. The binding of SSB seems to inhibit the binding of ethidium bromide to the ssDNA, since the fully shifted bands were stained to a lesser extent than the partially covered DNA molecules (Fig. 7 (34)).} \]

When increasing amounts of MucB were added to a mixture of SSB and ssDNA, an increase in the intensity of the staining of the ssDNA by ethidium bromide was observed, along with an enhanced mobility of some of the DNA (Fig. 8). The addition of MucB in the absence of SSB, both with or without MucA and MucA', did not have any affect on the migration of ssDNA (not shown). The effect of MucB on the migration of ssDNA in the presence of SSB might have resulted from the removal of SSB from the DNA by MucB. Such a displacement can explain the observed increased mobility of the DNA in the presence of MucB and the enhanced binding of ethidium bromide to DNA (Fig. 8). An alternative explanation is that MucB interacted with SSB, therefore changing its binding mode to the ssDNA without displacing it. In the latter scenario, the conformational change in the SSB-ssDNA complex induced by the binding of MucB would increase the mobility of the nucleoprotein complex in the gel and enhance the staining of the ssDNA by ethidium bromide. To distinguish between these two possibilities we repeated the gel mobility shift assay using this time radiolabeled SSB. The distribution of the radiolabeled SSB along the gel followed that of the ssDNA, as revealed when the fluorogram and the ethidium bromide-stained picture of the gel are compared (Fig. 9). Quantitation of the autoradiogram by densitometry showed a marginal 10–15\% decrease in the amount of radiolabeled SSB bound to ssDNA in the presence of MucB, which is within the range of the experimental error (Fig. 9). Similar results were obtained when the complex was formed at 30 or 0 °C (Fig. 9). This result suggests that MucB alters the conformation of the SSB-ssDNA complex, without causing a massive release of SSB from the DNA.

\[ \text{DISCUSSION} \]

The yeast two-hybrid system had proven useful in identifying interactions of the Muc proteins. It was previously shown that the UmuD' and UmuD proteins form both homo- and heterodimers when assayed biochemically (35, 36, 45) and in the yeast two-hybrid system (45). Here we show that MucA' and MucA behave similarly and exhibit both self-interactions and an interaction with each other. Each of these proteins was found to interact also with MucB. The C-terminal 30 amino acids of MucB were critical in mediating this interaction, since their deletion abolished the interaction of MucB with both MucA and MucA'. The C terminus of the UmuC protein had been previously reported to be essential for UV mutagenesis (46). Based on the homology between UmuC and MucB, our results suggest that the interaction between MucB and MucA' is crucial for the activity of these proteins in UV mutagenesis, consistent with the current view that the active species in mutagenesis is a complex of MucA' and MucB (or UmuD' and UmuD) (reviewed in Ref. 47). Interestingly, it has been reported that UmuC interacts with UmuD'; but not with UmuD, in the yeast two-hybrid system (45). In our case, MucB was found to interact both with MucA and MucA'. At this stage it is not clear whether these differences are real or whether they reflect differences in the assay systems.

The interaction between MucA and RecA as revealed in the two-hybrid system was expected based on the finding that RecA promotes the cleavage of MucA to MucA' (37, 38), similarly to the cleavage of UmuD to UmuD' (7–9). Interactions between RecA and MucA' or UmuD' were proposed previously based on chemical cross-linking experiments (39). Taken together with our in vivo results, it seems plausible that the MucA'-RecA interactions fulfill a role additional to mediating the cleavage of MucA, possibly the recruitment of MucA' to
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DNA, as previously suggested (39). We found no interaction between MucB and RecA. A related binding interaction was the retention of UmuC on activated RecA immobilized on a column, when an extract of E. coli cells was passed through the column (48). However, in this reported case, since an extract (rather than purified proteins) was used, the possibility that a third protein had mediated the interaction between RecA and UmuC hasn’t been ruled out.

SSB, reported here to interact with MucB, is an essential protein, which is involved in DNA replication, recombination, and repair (49–51). It is a homotetramer that binds ssDNA specifically and in a cooperative manner. Each SSB monomer contains a DNA-binding site interacting with 16 nucleotides. The extent of DNA bound by SSB strongly depends on salt and Mg
concentrations. With the increase in their concentration, the number of subunits that interact with DNA increases from 2 (SSB
mode) to 4 (SSB
mode). Electron microscopic analysis revealed that at low SSB to DNA ratio, a beaded structure is observed, with the DNA wrapped around beads of single or double tetramers of SSB, leading to a reduction in the contour length of DNA. This binding is of limited cooperativity and represents most likely the SSB
mode. At higher SSB to DNA ratios, the binding to DNA is cooperative leading to the formation of a smooth SSB-DNA filament, where SSB is presumably in the 35 mode (reviewed in Refs. 44, 50, and 52).

Our results suggest that the interaction with MucB affects the cooperative mode of SSB binding (presumably the 35 mode), which is the initial binding state of the SSB under our conditions. The exact nature of the MucB-SSB interaction is not clear yet, but it causes a major change in the SSB-ssDNA complex, without causing massive release of the SSB from DNA. This is evident from the increase in the mobility of the nucleoprotein complex and its increased staining with ethidium bromide. The quantitation of radiolabeled SSB is accurate within 10–15%. Thus, while not causing a massive displacement from DNA, it is possible that MucB does replace some SSB molecules. This, however, must have a major effect on the conformation of the DNA, as evident from its increased mobility. Several attempts to examine whether the nucleoprotein complex contains the MucB protein, using anti-MucB antibodies, were unsuccessful. At this point it is not clear whether this is a technical problem or whether MucB is released after rearranging the SSB-ssDNA complex. The increased staining can be explained by at least the following two mechanisms: 1) an increase in the accessibility of the DNA bases to ethidium bromide and 2) a change in the stacking of the DNA bases, which enables better intercalation of the dye. Noteworthy, although MucB bound a ssDNA 40 nucleotides long, we found no effect of MucB alone, or in combination with MucA or MucA’, on the migration of naked M13mp8 ssDNA in agarose gel electrophoresis. The reasons for this result are not clear yet.

What are the consequences of the changes in the structure of the nucleoprotein complex caused by MucB? It is possible that such changes allow easier bypass of DNA lesions by a DNA polymerase or else they provide binding sites for other proteins required for the bypass reaction. A natural candidate for being involved in this reaction is the RecA protein. It has been shown to compete with SSB for binding to ssDNA and under certain conditions can form a ternary SSB-RecA-ssDNA complex (reviewed in Refs. 53 and 54). MucB interacted with SSB but not with RecA (Table IV). On the other hand, MucA’ did interact with RecA. This may mean that a MucA’B complex interacts with both RecA and SSB. The interactions of MucB with SSB and with MucA’ must occur via different regions of the protein. The C terminus of MucB is involved in the interaction with MucA’, whereas an additional domain is involved in the interaction with SSB. Thus, a putative 4-protein RecA-MucA’-MucB-SSB complex can be imagined, in which RecA is bound to MucA’B via MucA’, and SSB is bound via MucB. So far there is no evidence for such a complex. An alternative possibility is that the various interactions appear transiently during the mutagenic bypass reaction.

One of the paradoxes in the field of SOS mutagenesis is that in vitro bypass of DNA lesions requires the UmuD’ and UmuC proteins (or their homologues) (reviewed in Ref. 3), whereas in vitro UV mutagenesis (20–22) can occur without these proteins. The report on umuC-independent UV mutagenesis in phage S13 (55) and the finding that umuC-independent UV mutagenesis is observed when a screening rather than selection procedure is used (56) support the view that UmuD’C stimulates the mutagenic reaction, rather than being absolutely required. An obvious difference between the in vivo and in vitro situations is that in the former there exist many more proteins that may affect bypass. One such family includes DNA damage binding proteins, which usually function in error-free DNA repair (3).

We have recently shown that DNA damage binding proteins regulate induced mutagenesis via a mechanism that does not involve the removal of DNA damage. We found that DNA damage binding proteins directly inhibit trans-lesion replication by binding to lesions present on ssDNA (57). The current study points toward SSB as another candidate that might affect SOS mutagenesis. In vivo studies have shown that E. coli strains carrying the sshl mutation, encoding a temperature-sensitive SSB, had reduced UV mutagenesis at the non-permissive temperature (58, 59). However, this was attributed to the inability...
of the mutant to fully induce the SOS response. Of course, this analysis does not rule out the possibility of a direct involvement of SSB in the mutagenic reaction. We have previously reported that SSB facilitates unassisted bypass of UV lesions by DNA polymerase III holoenzyme (29). It is possible that the MucB-SSB complex increases bypass, especially when additional proteins are present. The exact mechanism of the involvement of SSB in SOS UV mutagenesis needs further biochemical and in vivo investigations.

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