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Evidence for RecA Protein Association with the Cell Membrane and for Changes in the Levels of Major Outer Membrane Proteins in SOS-Induced *Escherichia coli* Cells

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Membrane fractions from *Escherichia coli* cells expressing DNA damage-inducible (SOS) functions contain elevated quantities of RecA protein (L. J. Gudas and A. B. Pardee, J. Mol. Biol. 101:459–477, 1976). We used two-dimensional polyacrylamide gel electrophoresis to separate membrane proteins from several strains to determine whether this effect is an artifact due to contamination of membranes during preparation by the large amount of cytoplasmic RecA present in SOS-induced cells. We found that amplification of RecA$^+$ protein without a DNA-damaging treatment does not result in increased RecA-membrane association, whether *recA* is derepressed specifically by an operator-constitutive *recA* allele or coordinately with other SOS genes by a *lexA* mutation that inactivates their common repressor. In contrast, large amounts of RecA appear in membrane fractions from undamaged cells of an SOS-constitutive strain carrying *recA730*, which encodes a spontaneously SOS-activated RecA. We conclude that the increased association of RecA with the membrane fraction requires the presence of the activated form of RecA, and that this association may contribute significantly to the SOS response. We describe also striking effects of SOS expression on the levels of the outer membrane proteins OmpA, OmpC, and OmpF.

Many agents that damage DNA or interrupt its replication trigger the expression of a diverse set of functions in *Escherichia coli* collectively known as the SOS response (for reviews, see references 15 and 26). Considerable genetic and biochemical evidence indicates that RecA protein becomes activated, in response to a metabolic signal generated by the DNA damage, to promote cleavage of LexA protein, the repressor of at least 17 unlinked chromosomal genes, including *recA* itself. The protein products of many of the derepressed genes enhance the survival of the damaged cell or of its phages, and all are synthesized at high levels until the DNA damage is repaired and repression is restored. Several laboratories have measured the amplified levels of RecA protein in SOS-induced cells (6, 11, 14). After a single generation of growth without DNA synthesis, RecA constitutes 3 to 4% of the total protein content of the cell (11). Most (90%) of the protein is located in the cytoplasm, where it presumably participates in DNA repair and recombination.

There have been several reports that RecA protein is also found in increased amounts in the membrane fraction of cells after DNA damage or replication blockage. For instance, thymine starvation (14) or nalidixic acid treatment (10) of wild-type cells results in a halt in DNA synthesis and the concurrent appearance of RecA protein in the membrane fraction. In contrast, *recA* mutants do not accumulate membrane-associated RecA after these treatments, nor do *lexA* (Ind$^-$) strains, in which RecA protein is noninducible. A *dnaB* temperature-sensitive replication mutant also shows a large increase of RecA in its membrane fraction when it is grown at the temperature nonpermissive for DNA synthesis (11).

The significance of the function of RecA, if any, in the membrane of SOS-induced cells has not been vigorously investigated. This may be due, in part, to the difficulty of studying a membrane-associated protein that is also present in large quantities in the cytoplasm. In such cases, it is not easy to rule out the possibility that during sample preparation membranes retain cytoplasmic proteins, accounting for the observed increases. In this study, we offer evidence that the association between RecA protein and the membranes of SOS-induced cells is a genuine one. We have used the technique of two-dimensional polyacrylamide gel electrophoresis to identify RecA in the protein profile of membrane fractions from SOS-induced cells. We also report that alterations in the amounts of three major outer membrane proteins occur in conjunction with SOS induction.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Table 1.

Growth of bacterial cultures. Cells were grown in Vogel and Bonner E medium (24) supplemented with 0.4% glucose and with 1 μg of thiamine per ml and 5 μg of thymine per ml as required. Nonradioactive media were supplemented with 0.4% Casamino Acids. For radioactive labeling experiments, E medium was supplemented with 0.4% glucose, with the required amino acids added individually. Amino acids required by K-12 strains were added to a final concentration of 100 μg/ml, with the exception of proline and arginine, which were added to a final concentration of 200 μg/ml. B and B/K-12 hybrid strains were provided with 20 μg of the required amino acids per ml.

Radioactive labeling of membrane proteins. Cultures of each strain were grown to the exponential phase at 37°C and then left untreated or induced for SOS expression by the addition of 100 μg of nalidixic acid per ml. Cultures were allowed 40 min of growth at 37°C to allow maximum expression of the *recA* gene in the induced cultures. After this time, each culture was given a 15-min pulse of [^{35}S]methionine (10 to 15 μCi/ml; >600 Ci/mmol). During the pulse cells were kept at 37°C. A 100,000-fold excess of unlabeled methionine

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TABLE 1. Bacterial Strains

Strain	Relevant genotype	Source or reference
SC30[a]	*recA730 lexA*$^+$	(28)
SC30-RP1	*recA*$^+$ *lexA*$^+$	This laboratory; AlaS$^+$ revertant of SC30-RP (28)
SC30-SP51	*recA730 lexA 51* (Def)	This laboratory; *lexA51* transductant of SC30
SC30-RP1-SP71	*recA*$^+$ *lexA71*::Tn*5*	This laboratory; *lexA71*::Tn*5* transductant of SC30-RP1
SC30-LM	*recA730 lexA102* (Ind$^-$)	(28)
SC30-OC	*recAo281 lexA*$^+$	This laboratory; *recAo281* transductant of SC30
JM1[b]	*recA*$^+$ *lexA*$^+$	(3)
JM12[b]	*recA441 lexA*$^+$	(3)
JM30[b]	*recA730 lexA*$^+$	This laboratory; *recA730* transductant of GC3217 (9)

[a] SC30 is a B/K-12 recombinant that retains all known parental B/r markers (including *uvrA155* and *trpE56*), has B/r restriction modification, and carries none of the known parental K-12 markers except the selected *thyA*$^+$ and *recA441*, the latter with a new change.

[b] JM1, JM12, and JM30 are K-12 strains.

was added to each culture to stop incorporation of the radioactive amino acid. Cultures were chilled thoroughly on ice, and all subsequent operations were conducted at 0 to 4°C.

Preparation of membrane proteins. Labeled cells combined with similarly treated unlabeled carrier cells were harvested by centrifugation at 5,000 × *g* for 10 min and washed twice with cold buffer A (1 mM Tris-hydrochloride, pH 7.5). Cells were then broken in the presence of bovine pancreatic DNase I and RNase A by either two or three passages through a French pressure cell (18,000 lb/in^2) or by sonication. Cell debris was removed by centrifugation at 3,000 × *g* for 10 min. The total envelope fraction (membrane fraction) was sedimented by centrifugation at 41,000 × *g* for 40 min. The membrane pellet was washed twice in cold buffer A and suspended in buffer A to a protein concentration of approximately 20 to 30 mg/ml. In experiments attempting to remove RecA possibly associated with the membrane fraction via DNA binding, the pellet was suspended and incubated in buffer P (20 mM potassium phosphate, pH 6.8) containing 1 mM ATP (4). Incubation was for 20 min while stirring at 4°C. Membranes were then centrifuged at 18,000 rpm for 40 min and suspended in fresh buffer A. Protein concentration for all samples was determined by the procedure of Lowry et al. (16).

Separation of membrane proteins by two-dimensional polyacrylamide gel electrophoresis. Membrane proteins were solubilized by the procedure of Ames and Nikaido (1). They were then separated by the two-dimensional gel electrophoresis procedure of O'Farrell (19), with the modifications suggested by Ames and Nikaido (1). Nonradioactive second-dimension sodium dodecylsulfate-polyacrylamide gels were fixed overnight in a bath of 25% isopropanol, 10% acetic acid, and 65% water and then stained with Coomassie brilliant blue R-250. Immediately after fixing, second-dimension gels containing radioactively labeled proteins were impregnated with fluor (En^{3}Hance; New England Nuclear Corp., Bedford, Mass.) according to the manufacturer's specifications and dried. Kodak X-Omat AR X-ray film was exposed to the dried gels at −70°C and developed according to film instructions.

RNA and DNA contamination of membrane preparations. For measurement of RNA contamination, exponential cultures were grown in E glucose medium with required supplements and pulse-labeled with [^{3}H]uridine (5 to 10 mCi/ml; 50 mCi/mmol) for 15 min. For determining DNA contamination, an overnight culture grown in E-glucose medium was diluted to a density of 10^7 cells per ml in fresh minimal medium and grown for three generations in the presence of [^{3}H]thymidine (50 μCi/ml; 27 Ci/mmol). The cultures were then chased with a 100,000-fold amount of unlabeled nucleoside to stop incorporation of the radioactive nucleoside. The membrane fraction was collected by the procedures described above. Contamination was determined by measuring the percentage of total radioactivity present in the membrane fraction in a trichloroacetic acid-precipitable form.

Contamination of the membrane fraction with cytoplasmic proteins. Membrane fractions of unlabeled cells were prepared as previously described, except that exponential cultures were induced for SOS expression in the presence of 1 mM isopropyl-β-D-thiogalactopyranoside, which induces the production of β-galactosidase. Final membrane preparations were assayed for β-galactosidase activity by the method of Miller (18).

RESULTS

SOS-dependent association of RecA protein with the membrane fraction. Figures 1a and b compare the patterns of membrane proteins, separated by two-dimensional polyacrylamide gel electrophoresis, from untreated and nalidixic acid-treated cultures of the *recA*$^+$ strain SC30-RP1. Figure 1b shows the induction of wild-type RecA protein (arrow), with a molecular mass of approximately 40,000 daltons and an isoelectric point of 6.0. Additional evidence that this spot is the RecA protein comes from studies of the K-12 strain JM12 carrying the *recA441* allele (data not shown). During growth of this strain at 42°C, the altered RecA protein is produced constitutively. McEntee (17) has demonstrated in two-dimensional gels of whole cell extracts that the *recA441* protein has the same molecular mass, but a more basic isoelectric point (6.5) in comparison with the wild-type protein. Our two-dimensional electropherograms confirm that the membrane fraction of this strain contains increased amounts of this more basic form of RecA, both after growth at 30°C in the presence of nalidixic acid and after growth at 42°C. At 42°C, the membrane fraction of the *recA441* strain, JM12, contains 8.3 times the amount of RecA present in the membrane fraction of uninduced cells grown at 30°C, as measured by the relative amounts of radioactivity present in the isolated protein spots. RecA is not loosely associated with the membrane fraction, since no change in the membrane patterns was seen when the membrane fraction was washed with 0.5 M NH_4Cl.

The *recA730* allele is derived from *recA441* and results in an SOS-constitutive phenotype and in the constitutive production of an altered form of RecA at all growth temperatures. Although the *recA730* and *recA441* proteins differ in other ways, they comigrate at pI 6.5 on two-dimensional gels of whole cell extracts (J. Owen McCall, personal communication). Our examination of the membrane proteins of three *recA730* strains adds to the cumulative evidence that the RecA level is increased in the membrane fraction of SOS-induced cells.

Figure 1c shows the presence of a large amount of RecA protein of the expected electrophoretic variation in strain SC30 (*recA730*), in contrast with its *recA*$^+$ transductant

SC30-RP1 (Fig. 1a), which contains much less RecA in its membrane fraction. Consistent with Gudas' results with *lexA* (Ind$^-$) mutants (10), the accumulation of high levels of RecA in the membrane fraction of SC30 requires the presence of LexA repressor with wild-type sensitivity to cleavage. The membrane fraction of a *recA730 lexA102* (Ind$^-$) strain does not contain a large amount of RecA (Fig. 1e). In contrast, the membrane fraction of the *recA730 lexA51* (Def) (defective LexA repressor) strain, SC30-SP51, has high levels of RecA (Fig. 1d). This result is expected, since the *recA* gene is fully derepressed in this strain and its protein product expresses the SOS-activated state constitutively (28).

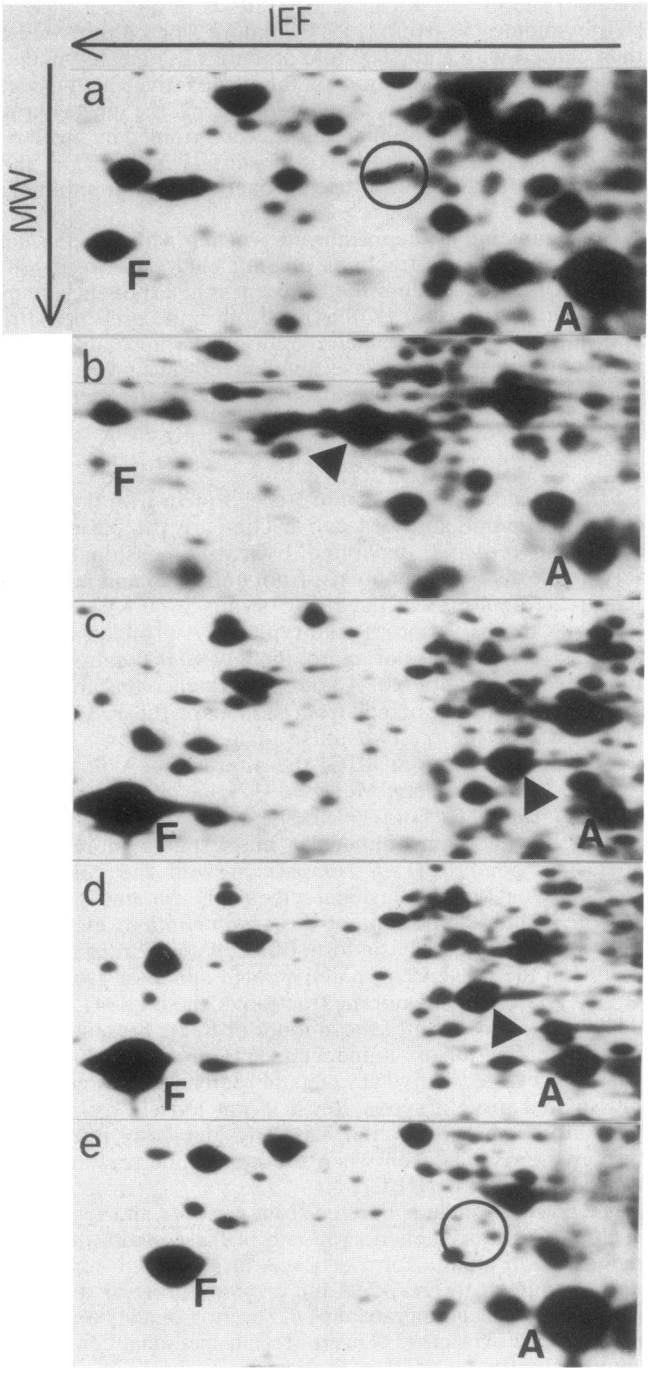

FIG. 1. Portions of autoradiograms of two-dimensional polyacrylamide gels of proteins from the whole membrane fractions of SOS-induced and noninduced *E. coli* cells. Cells were pulsed with [^{35}S]-methionine for 15 min and then incubated in the presence of a large excess of unlabeled methionine. Membrane preparation and gel electrophoresis were performed as described in the text. Panels: (a) SC30-RP1 (*recA*$^+$ *lexA*$^+$), untreated; (b) SC30-RP1 after incubation for 40 min in nalidixic acid; (c) SOS-constitutive strain SC30 (*recA730 lexA*$^+$), untreated; (d) SOS-constitutive strain SC30-SP51 [*recA730 lexA51* (Def)], untreated; (e) SOS-noninducible strain SC30-LM [*recA730 lexA102* (Ind$^-$)], untreated derivative of SC30. Arrows indicate RecA protein, and circles show the expected positions of RecA protein. A and F indicate the proteins OmpA and OmpF, respectively.

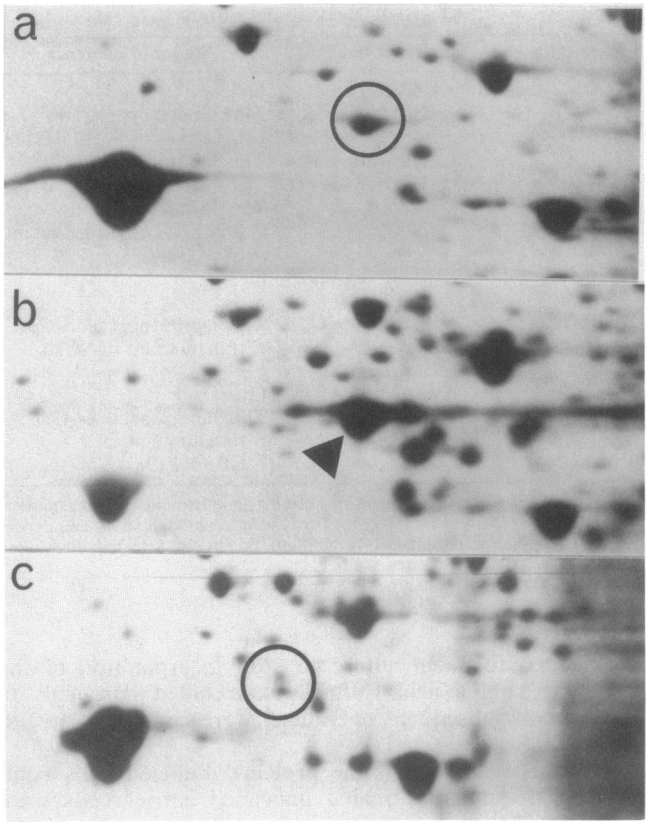

FIG. 2. Portions of autoradiograms of two-dimensional polyacrylamide gels of proteins from whole membrane fractions of (a) strain SC30-OC (*recAo281*), untreated, (b) strain SC30-OC after incubation for 40 min in nalidixic acid, and (c) strain SC30-RP1-SP71 (*recA*$^+$ *lexA71*::Tn*5*, untreated. Symbols, labeling protocol, and gel axes are as given in the Fig. 1 legend.

Failure of large quantities of RecA to associate with membranes in cells not induced for SOS expression. To investigate the role of RecA in the membrane, it is imperative to show that there is a genuine association between the protein and the membrane and not an accidental contamination with cytoplasmic protein during cell breakage and membrane isolation. To this end, we examined the protein profiles from the membranes of a strain carrying the operator constitutive allele, *recAo281*. These cells contain very high levels of wild-type RecA protein, such that no appreciable increase is seen after induction with UV radiation (25). In contrast to strain SC30 (*recA730*), however, the cells do not constitu-

strain SC30 (*recA730*), however, the cells do not constitutively express other SOS genes, since the RecA$^+$ protein in a *recAo281* strain does not cleave the LexA repressor without an SOS-inducing signal. Therefore if the membrane association of RecA results from contamination with cytoplasmic RecA during sample preparation, one would expect that membranes from noninduced cultures of this strain would contain large amounts of RecA in comparison with a noninduced *recA*$^+$ strain. However, Fig. 2a shows that without SOS induction the membrane fraction of the *recAo281* strain SC30-OC does not contain enhanced levels of RecA as compared to the wild-type membrane fraction (Fig. 1a). Upon addition of nalidixic acid to the *recAo281* strain and induction of the proteolytically active form of RecA, association of newly synthesized RecA with the membrane fraction occurs (Fig. 2b).

We have used a second mutant strain to rule out the possibility of cytoplasmic contamination. In this case, we examined RecA content in the membrane fraction of a *recA*$^+$ strain with a transposon insertion in the *lexA* gene, strain SC30-RP1-SP71. The *lexA*::Tn*5* gene product is inactive, so RecA$^+$ protein and other SOS proteins are synthesized at a level equivalent to that found in SOS-induced cells. As in the *recAo281* strain, the RecA$^+$ protein in these cells is not SOS activated, since no induction signal is present. We found no increase in RecA in the membrane fraction of these cells (Fig. 2c).

These results suggest that the RecA observed in the membrane fraction of SOS-induced cells results from a true association between the two components and not from cytoplasmic contamination. However, these experiments do not rule out a greater probability of contamination by activated RecA, which may stick to membranes more persistently than RecA in its nonactivated form. Gudas and Pardee (11) considered this unlikely, because they found that the membrane fractions of noninduced cells do not show increased amounts of RecA when they are mixed with the cytoplasmic fraction from SOS-induced cells (presumably containing activated RecA) before isolation and gel electrophoresis. Another possibility is that SOS induction results in the alteration of the membrane such that it acquires increased stickiness toward cytoplasmic proteins in general or toward RecA in particular. We have found that the membranes in SOS-induced cells are no more sticky than those from noninduced cells toward another copiously synthesized cytoplasmic protein, β-galactosidase. Membrane preparations from both SOS-induced cells and from noninduced cells retained less than 0.05% of the total β-galactosidase activity present in cells in which the synthesis of this protein had been derepressed (see above for details). If increased stickiness of the membrane in SOS-induced cells is responsible for the increased amounts of RecA associated with these membranes, it must act selectively to increase affinity with a subgroup of cytoplasmic proteins or specifically to promote association with the activated form of RecA. The extremely small amount of β-galactosidase activity associated with our membrane preparations indicates that these preparations are virtually cytoplasm-free. The enhanced amount of RecA protein in SOS-induced membrane preparations is also unlikely to result from contamination with free ribosomes in the process of completing translation of *recA* transcripts. We found that membrane preparations contained less than 0.7% of the RNA in the cell, as measured by total incorporation of [^{3}H]uridine (see above for details). In addition, the experiments with the *recAo281* and *recA*$^+$ *lexA71*::Tn*5* strains suggest that ribosomes translating *recA* transcripts do not significantly contaminate the membrane fraction.

Attachment of DNA to the bacterial membrane could account for the association of cytoplasmic RecA to membranes, since the protein binds tightly to double- and single-stranded DNA. To address this possibility, we compared the amounts of radioactivity remaining in the final DNase-treated membrane fractions of induced and noninduced cells whose DNA had been uniformly labeled with radioactive nucleotides (see above for details). Retention of labeled DNA was virtually identical in the two membrane fractions; less than 0.04% of the total radioactivity was incorporated. These results are in excellent agreement with those reported by Inouye and Pardee (14) and suggest that there are no gross changes in levels of membrane-associated DNA as a result of SOS induction.

Although there is not a quantitative difference between the DNA content of membranes fractions of induced and noninduced cells, it is possible that a qualitative difference exists. For example, induced cells may contain more single-stranded DNA, which binds RecA more readily than double-stranded DNA. Cox et al. (4) have found that treatment with 1 mM ATP will efficiently remove RecA bound to a DNA (double or single stranded) column during purification of the protein. We incubated membranes from strain SC30 in 1 mM ATP before protein solubilization in an attempt to dislodge any RecA that may have been bound to DNA in the preparation. This treatment had no detectable effect upon the large amount of RecA protein found in the membrane fraction of strain SC30 (Fig. 3). However, we cannot be certain that the treatment conditions recommended for removal of RecA from a DNA column are appropriate for the removal of the protein from a DNA-membrane complex.

Alterations in other membrane proteins upon SOS induction. During the course of these experiments, we have noticed other differences between the membrane profiles of induced and noninduced cells. The most striking are alterations in the amounts of outer membrane proteins OmpA, OmpF, and OmpC. All strains examined exhibited striking

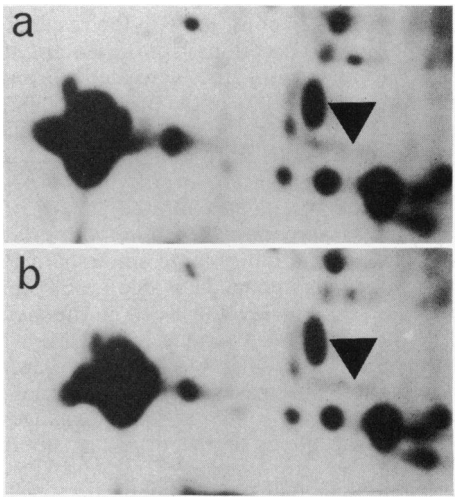

FIG. 3. Portions of autoradiograms of two-dimensional polyacrylamide gels of proteins from whole membrane fractions of *recA730* strain SC30. Membrane pellets were incubated (a) with or (b) without 1 mM ATP before protein solubilization as described in the text. Labeling protocol and gel axes are as given in the Fig. 1 legend.

changes in at least one of these outer membrane proteins coincident with SOS induction.

Figure 1 shows differences in OmpA levels in SOS-induced and noninduced cells. In the *recA*$^+$ strain, SC30-RP1, SOS induction leads to a marked decrease in OmpA synthesis (Fig. 1a and b). The two strains constitutive for SOS induction also show this decrease (Fig. 1c and d) as compared with the noninduced *recA*$^+$ strain. The level of this protein returns to that of the wild type in SC30-LM, the noninducible derivative. The drop in OmpA concurrent with SOS induction was also seen in the K-12 strains examined in this study, including JM1 (*recA*$^+$), JM30 (*recA730*), and JM12 (*recA441*) strains. Changes in the levels of OmpF (and OmpC in K-12 strains) were also seen (Fig. 1a and b). However, the magnitude of the alterations appeared to be different, depending on the genetic background of the strain examined.

DISCUSSION

By examining the two-dimensional membrane protein profiles of *recA* mutant strains, we have strengthened the evidence that the association between RecA protein and the membranes of SOS-induced cells is a genuine one, rather than an artifact of membrane isolation techniques. Inouye and Pardee (14) and Gudas and Pardee (10, 11) have observed the accumulation of RecA in the membrane fractions of a variety of strains induced to express the SOS response. However, their results could be explained as the accidental inclusion of some of the large pool of cytoplasmic RecA protein that is present in SOS-induced cells. Such contamination could result from entrapment of cytoplasmic RecA in membrane vesicles during mechanical cell breakage, from binding of the protein to contaminating DNA, or from binding to the membrane of ribosomes in the process of translating the abundant *recA* message.

Our studies extend and validate the earlier observations. The absence of RecA protein from the membrane fraction of a *recA* operator-constitutive strain shows that the presence of a large quantity of cytoplasmic RecA is not sufficient to account for the accumulation of the protein seen in the membrane fraction of SOS-induced cells. We can thus eliminate the possibility of contamination of membranes by vesicular entrapment. In contrast with the result with SC30-OC, the membrane preparations from a mutant strain constitutive for production of the activated form of RecA protein contain large amounts of the protein. Thus, activated RecA must be present for the association to occur. This could mean that either (i) only activated RecA is able to associate with the membrane, or (ii) in the presence of activated RecA, membranes are altered as part of the SOS response so as to acquire increased affinity for either form of the protein. The association of large amounts of RecA with the membrane depends upon a cleavable LexA repressor, as indicated by the very low level of RecA in the membrane of the *recA730 lexA102* (Ind$^-$) strain, SC30-LM, which confirms that this association is an SOS-inducible event. Moreover, it shows that the increases observed after nalidixic acid treatments cannot be attributed to direct changes in transcription due to the effect of the drug on supercoiling of DNA (20, 22).

It is possible that the RecA-membrane association in SOS-induced cells results from the binding of the protein to DNA either attached to or accidentally contaminating the isolated membranes. Our radioactive labeling experiments have shown that almost identical small amounts of DNA remain in the membrane fractions of SOS-induced and noninduced cells. Therefore, to account for our observations, one could propose that the membrane-associated DNA in the induced cells must either be in a form more conducive to RecA binding (e.g., more single stranded), or that the RecA protein in these cells must have increased affinity for DNA. Both proposals may be required to explain our findings. In strain SC30, the mutant RecA730 protein could have greater affinity for DNA than RecA$^+$, which could account for the increased RecA-membrane association in SC30 compared with its *recA*$^+$ derivative. There is no reason to assume that these two strains differ in the single strandedness of their undamaged DNA. On the other hand, in strain SC30-OC the same RecA$^+$ protein is synthesized before and after treatment with nalidixic acid. In vitro studies (5) suggest that RecA becomes activated only after binding single-stranded DNA. If that is true in vivo, there should be no difference in the affinity of RecA$^+$ for single-stranded DNA before and after inducing treatment. In this case, treatment could increase the amount of membrane-associated single-stranded DNA available for RecA binding. Our finding that exposure of the membrane fraction to ATP does not alter the amount of membrane-associated RecA in the *recA730* strain SC30 does not support a RecA-DNA-membrane interaction as a mechanism for RecA accumulation in the membrane fraction of SOS-induced cells. However, this negative result by no means eliminates the possibility that the membrane-associated RecA in SOS-induced cells is actually bound to DNA.

Even if the RecA found with the membranes of SOS-induced cells should prove to be bound to DNA, rather than intimately associated with or integrated into the membrane itself, its presence in the membrane fraction need not be dismissed as a trivial artifact. If it is bound to DNA that is actually attached to the membrane and not just to DNA contaminating the preparation, such a complex could have major importance in the SOS response. It is possible that only the RecA bound to a DNA-membrane complex becomes activated after SOS induction treatments, making the membrane the site of proteolytic cleavage of LexA and lambda repressor. Activation of only a small amount of RecA protein, e.g., the amount present in undamaged wild-type cells, is sufficient to cause induction of lambda prophage (2). It is likely that no more than a small fraction of the RecA synthesized after SOS induction ever becomes activated; the rest probably participates in DNA repair. The portion of the DNA that has been shown to be bound to the membrane of *E. coli* includes the origin of replication (12). There is considerable evidence that nascent DNA is also membrane associated, in support of models of cell division that explain segregation of genomes by linking the replication fork of the growing chromosome to the cell membrane (8, 23). Although damaged DNA that cannot initiate replication (conjugationally transferred Hfr DNA, certain lambda mutants) can stimulate weak indirect SOS induction when introduced into undamaged bacteria, a much stronger induction signal is generated by damaged plasmids or phages that are replicating or at least attempting to initiate replication (P1 phage, F plasmids, wild-type lambda) (7). Thus, a RecA-DNA-membrane complex may be required for in vivo activation of RecA. In that case, such a complex would play a key role not only in SOS induction, but also in the expression of SOS phenotypes that require activated RecA for a second function distinct from LexA cleavage, such as SOS mutagenesis and stable DNA replication (27). In this connection, it is noteworthy that UmuC, a DNA damage-inducible protein required for bacterial SOS mutagenesis,

has also been found to be membrane associated (L. Marsh, L. Dodson, and G. C. Walker, personal communication).

What might be the nature of the RecA-membrane association if it is directly and intimately bound to the membrane itself rather than to DNA remaining in the membrane fraction? Cotranslational membrane insertion of RecA is highly unlikely since there is no N-terminal amino acid signal sequence for membrane targeting coded within the gene sequence. The association is probably not a weak charge effect, since the protein is not eliminated by salt washing, a treatment that should remove loosely bound charge-associated proteins.

Another way in which RecA could be found in association with membranes is by binding to either a membrane protein or a cytoplasmic protein whose final destination is the membrane. Our observation that RecA does not accumulate in the membranes of the *lexA*::Tn*5* *recA*$^+$ strain SC30-RP1-SP71 rules out the possibility that one or more SOS-inducible LexA-repressed gene products act as a membrane glue to mediate the association between RecA, at least in its nonactivated state, and the membrane. In this strain, all LexA-repressed genes, in addition to *recA*, are derepressed and thus expressed at SOS-induced levels. The lack of increased amounts of RecA in the membrane fraction of this strain (Fig. 2c) shows that the amplification of proteins in the LexA regulon is insufficient for the association to occur. Interaction between RecA and a membrane protein not repressed by LexA remains a viable explanation for the appearance of RecA in the membranes of SOS-induced cells. Recent studies of SulA, the cell division inhibitor induced by DNA damage (13), provide a precedent for interaction of a DNA damage-inducible protein with a membrane protein. Like RecA, SulA (SFiA) has been localized to both the soluble and membrane fractions of the cell (21). C. Jones and I. Holland (Proc. Natl. Acad. Sci. U.S.A., in press) have demonstrated a physical interaction between SulA and its target protein SulB(FtsZ), a membrane-associated protein with an important role in cell division. They have proposed that this interaction, which probably occurs at the cytoplasmic interface of the inner membrane, initiates SOS-induced cell division delay.

The effect of SOS induction on the synthesis of certain major outer membrane proteins raises new questions about the relationship between the SOS system and cell membrane metabolism. The proteins involved not only contribute crucially to the general structure and stability of the outer membrane, but also function specifically in the uptake of cell nutrients and, in the case of OmpA, in the conferring of mating pair stability during conjugation. Our observation of these alterations in cells constitutive for SOS induction in the absence of DNA damage emphasizes that they cannot simply be attributed to the generalized effects of nalidixic acid on levels of gene expression after induction with the drug. The presence of these proteins at wild-type levels in the *lexA102* (Ind$^-$) strain SC30-LM also hints that the SOS response influences the expression or membrane insertion of these proteins. The precise nature of changes in outer membrane proteins during SOS induction, and how they are brought about, await further investigation.

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LITERATURE CITED

1. **Ames, G. F.-L., and K. Nikaido.** 1976. Two-dimensional gel electrophoresis of membrane proteins. Biochemistry **15**:616–623.
2. **Bailone, A., A. Levine, and R. Devoret.** 1979. Inactivation of prophage lambda repressor *in vivo*. J. Mol. Biol. **131**:553–572.
3. **Castellazi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. I. Further characterization of the thermosensitive mutation *tif-1* where expression mimics the effects of UV irradiation. Mol. Gen. Genet. **119**:139–152.
4. **Cox, M. M., K. McEntee, and I. R. Lehman.** 1981. A simple and rapid procedure for the large-scale purification of the recA protein of *Escherichia coli*. J. Biol. Chem. **256**:4676–4678.
5. **Craig, N. L., and J. W. Roberts.** 1980. *E. coli recA* protein-directed cleavage of phage lambda repressor requires polynucleotide. Nature (London) **283**:26–30.
6. **Darby, V., and I. B. Holland.** 1979. A kinetic analysis of cell division, and induction and stability of *recA* protein in UV irradiated *lon*$^+$ and *lon*$^-$ strains of *Escherichia coli* K-12. Mol. Gen. Genet. **176**:121–128.
7. **D'Ari, R., and O. Huisman.** 1982. DNA replication and indirect induction of the SOS response in *Escherichia coli*. Biochimie **64**:623–628.
8. **Ganesan, A. T., and J. Lederberg.** 1965. A cell-membrane bound fraction of bacterial DNA. Biochem. Biophys. Res. Commun. **18**:824–835.
9. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
10. **Gudas, L. J.** 1976. The induction of protein X in DNA repair and cell division mutants of *Escherichia coli*. J. Mol. Biol. **104**:567–587.
11. **Gudas, L. J., and A. B. Pardee.** 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459–477.
12. **Hendrickson, W. G., T. Kusano, H. Yamaki, R. Balakrishnan, M. King, J. Murchie, and M. Schaechter.** 1982. Binding of the origin of replication of *Escherichia coli* to the outer membrane. Cell **30**:915–923.
13. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. U.S.A. **81**:4490–4494.
14. **Inouye, M., and A. B. Pardee.** 1970. Changes of membrane proteins and their relation to deoxyribonucleic acid synthesis and cell division of *Escherichia coli*. J. Biol. Chem. **245**:5813–5819.
15. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **McEntee, K.** 1977. Protein X is the product of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5275–5279.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
20. **B. Sanzey.** 1979. Modulation of gene expression by drugs affecting deoxyribonucleic acid gyrase. J. Bacteriol. **138**:40–47.
21. **Schoemaker, J. M., R. C. Gayda, and A. Markovitz.** 1984. Regulation of cell division in *Escherichia coli*: SOS induction and cellular location of the SulA protein, a key to *lon*-associated filamentation and death. J. Bacteriol. **158**:551–561.
22. **Smith, C. L., M. Kubo, and F. Imamoto.** 1978. Promoter-specific inhibition of transcription by antibiotics which act on DNA gyrase. Nature (London) **275**:420–423.
23. **Smith, D. W., and P. C. Hanawalt.** 1967. Properties of the growing point region in the bacterial chromosome. Biochim. Biophys. Acta **149**:519–531.
24. **Vogel, J. H., and D. M. Bonner.** 1956. Acetylornithinase of

Escherichia coli: partial purification and some properties. J. Biol. Chem. **218**:97–106.
25. **Volkert, M. R., L. J. Margossian, and A. J. Clark.** 1981. Evidence that *rnmB* is the operator of the *Escherichia coli recA* gene. Proc. Natl. Acad. Sci. U.S.A. **78**:1786–1790.
26. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.
27. **Witkin, E. M., and T. Kogoma.** 1984. Involvement of the activated form of RecA protein in SOS mutagenesis and stable DNA replication in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **81**:7539–7543.
28. **Witkin, E. M., J. O. McCall, M. R. Volkert, and I. E. Wermundsen.** 1982. Constitutive expression of SOS functions and modulation of mutagenesis resulting from resolution of genetic instability at or near the *recA* locus of *Escherichia coli*. Mol. Gen. Genet. **185**:43–50.