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Expression of the Cloned Bacteriophage φX174 A* Gene in *Escherichia coli* Inhibits DNA Replication and Cell Division

JOSEPH COLASANTI AND DAVID T. DENHARDT*

Cancer Research Laboratory and the Department of Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5B7 Canada

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The A* gene of bacteriophage φX174 has been cloned into the inducible expression vector pCQV2 under conditions allowing its lethal action to be controlled by the λcI857 repressor. Upon induction of expression, DNA synthesis in *Escherichia coli* carrying the recombinant plasmid is severely inhibited; however, these same cells permit β-galactosidase induction at a rate similar to that observed in control cells at the inducing (for A*) temperature. Cells in which A* is expressed form filaments and produce more RecA protein, indicating at least a partial induction of the SOS response; however, there is no evidence of damage to the bacterial chromosome. It appears that the A* protein has as one function the inhibition of cell division and DNA replication but not transcription or protein synthesis during phage infection.

The functions of most of the genes encoded by bacteriophage φX174 have been elucidated by genetic experiments, particularly by the isolation of mutations in those genes (see reference 31 for review). For the A* gene, however, this strategy has not been fruitful because of the nature of the relation between A* and the larger A gene. Gene A codes for a polypeptide with an M_r of ca. 58,000 whose essential role as a nickase or ligase in the replication of the phage genome has been well documented (4), whereas the function of the A* protein (M_r, ca. 37,000) in vivo is unknown. Unlike the overlapping genes of φX174, genes A and A* are read in the same reading frame; A* results from a translational restart from a ribosome-binding site (RBS) approximately one-third of the distance from the beginning of the gene A RBS (20). Because of the nested nature of these two genes, any mutation in A* also affects A, making it difficult to differentiate their respective functions during phage infection.

An early study by Lindqvist and Sinsheimer (19) implicated a phage-encoded protein in the phage-induced shutoff of host DNA synthesis during infection. This protein was later found to be a product of the A (or A*) gene (13, 23). These studies, however, did not reach a clear and unambiguous position regarding the role of A* protein (7). A more recent study has shown that A* has the ability to inhibit replication of both replicative form (RF) and DNA single-stranded (ssDNA) φX174 in vitro (11). The purified protein binds strongly to ssDNA and double-stranded DNA (34) and has endonucleolytic activity with some sequence specificity towards ssDNA (16). In the presence of Mn^{2+} or the φX gene A protein, the A* protein may be capable of nicking and binding covalently to φX RF (10, 35). The A* protein bound to the 5′ end of a DNA strand can be displaced under appropriate conditions by the 3′ OH end of the same or another DNA strand, resulting in the formation of a 3′-5′ phosphodiester bond (12, 35, 36).

To elucidate further the function of A*, we used recombinant DNA technology to clone the A* gene to study its effects in vivo on *Escherichia coli*. Initial attempts at this cloning revealed that expression of the A* protein was, as expected, lethal to cells in which it was expressed. Consistent with this, van der Avoort et al. (33) were unable to isolate a clone with the intact A or A* gene in circumstances in which cloned segments of φX174 RF producing other phage products were isolated. For our cloning strategy, we therefore undertook to use plasmid vehicles which allow control of expression of cloned genes. After initial failures with vectors pPLc28 (28), pKC30 (30), and pJL6-8 (18), we succeeded with pCQV2, which contains both the λ P_R promoter and the λ *c*I857 repressor (27). This vector was found to maintain the A* gene in a stable and silent form at 30°C and to allow its expression at 42°C. Upon induction of expression of the A* gene, the rate of DNA synthesis in *E. coli* was drastically reduced, and the cells were killed.

MATERIALS AND METHODS

***E. coli*, phage, and plasmid strains.** The amber mutants φX174*am*16 and φX174*am*3 (lysis defective), deficient in gene B and *E* functions, respectively, were propagated in *E. coli* CR *thy*$^-$ Su$^+$ (6). *E. coli* C Su$^-$ and CR were grown in mT3XD (6). Other strains were grown in L broth or supplemented M9 minimal medium containing 0.2% glucose (22) as indicated. The plasmid pCQV2 (27) was provided by G. A. Chaconas; pPLc28 (28) was obtained from W. Fiers along with its hosts K12ΔH1Δ*trp* and M5219; pKC30 was from M. Rosenberg; pJL6-8 was obtained from D. Court.

DNA purification and manipulation. Plasmid DNA was purified from a lysozyme-Brij 58 cleared lysate by isopycnic centrifugation in cesium chloride-ethidium bromide followed by extraction with *n*-butanol, dialysis, phenol-chloroform extraction, and a final dialysis and alcohol precipitation. Phage RF DNA was purified from *E. coli* CR infected with φX174*am*16 at a high multiplicity (2 to 3) when the cells had reached a density of 2×10^8/ml; chloramphenicol was added 12 min after infection to a concentration of 20 μg/ml, and aeration of the cultures was continued at 37°C for 5 h. RF DNA was isolated by the same method used to isolate plasmid DNA.

All enzymes were obtained from Bethesda Research Laboratories, Boehringer-Mannheim, or New England Biolabs and were used as recommended by the suppliers unless indicated otherwise. To fill in recessed 3′ ends, restricted duplex DNA was incubated with 65 mM Tris-hydrochloride (pH 7.6)–40 mM KCl–25 mM $MgCl_2$ in the presence of the

* Corresponding author.

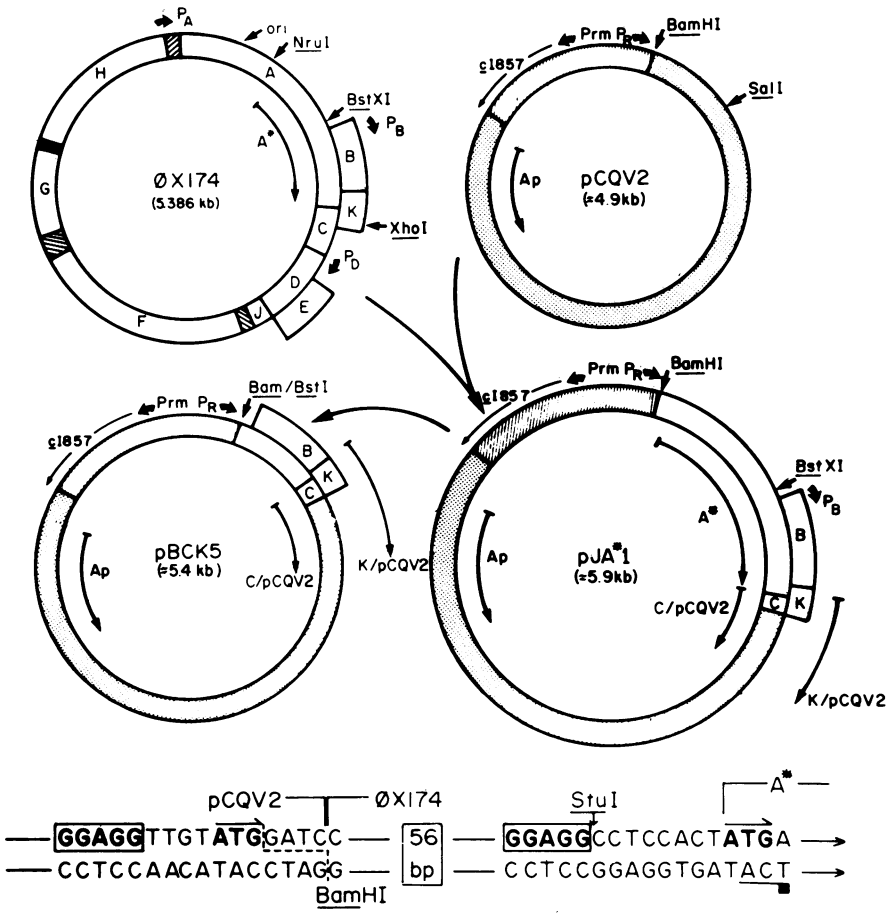

FIG. 1. Construction of pJA*1 and pBCK5. The recombinant pJA*1 was constructed by inserting the fragment containing the A* gene of ϕX174 into the cloning vehicle pCQV2 (see the text). This construction results in placement of the A* gene in the correct orientation for transcription from the λ P_R promoter. Repressor protein (*c*I857) prevents transcription from the P_R promoter at 30°C; at 42°C, the temperature-sensitive repressor is rendered nonfunctional, and transcription occurs, allowing the A* protein to be synthesized starting from its own RBS. The plasmid pBCK5 was constructed from pJA*1 by deleting the *Bam*HI-*Bst*XI fragment, blunting with T4 DNA polymerase, and religating. The deletion eliminates A* expression but leaves the rest of the plasmid intact, thereby allowing the effects of A* to be separated from those of the *am*16 gene B product and the gene C- and gene K-pCQV2 fusions. The predicted sequence of the pCQV2-ϕX174 joint at the A* N terminus is shown in detail. A peptide initiated from the *cro* RBS and AUG codon terminates near the A* start codon.

four deoxynucleoside triphosphates (0.5 mM) and the large (Klenow) fragment of DNA polymerase I (60 to 100 U/ml) for 30 to 60 min at 25°C. DNA fragments were purified from 0.7% low-melting-point agarose gels by electroelution onto a dialysis membrane placed in a trough in the gel (22). Recombinant DNA was used to transform *E. coli* RR1 cells to ampicillin resistance by a modified Hanahan procedure for cells other than χ_{1776} (22). Cells were freeze-shocked in a −70°C ethanol-dry ice bath before addition of dimethyl sulfoxide, which was added only once. Heat shock was performed at 36°C, and the transformation mixture was incubated at 30°C.

Determination of the rate of DNA synthesis. Cells with various plasmids were grown to mid-log phase in L broth in the presence of carbenicillin (100 μg/ml) at 30°C. When the cell density reached 10^8/ml, half of each culture was transferred to 42°C. Portions (1 ml) were taken at various times before and after the shift and added to 10 μCi of [^{3}H]thymidine and sufficient deoxyguanosine to produce a 50 mM solution. The cultures were incubated for 2 min, and then incorporation was terminated with 2 ml of cold 10% trichloroacetic acid. Trichloroacetic acid-precipitable material was collected on glass fiber filters and washed, and its radioactivity was determined.

β-Galactosidase assay. *E. coli* RR1 cells carrying plasmids were grown at 30°C in M9 minimal medium supplemented with 0.2% Casamino Acids and 100 μg of carbenicillin per ml to a density of 5×10^7 cells per ml, at which point half of each culture was shifted to 42°C; 6 min later, isopropyl β-D-thiogalactopyranoside was added to a final concentration of 2 mM. During the course of growth, aliquots (0.25 ml) were taken and added to an equal volume of Z-buffer (25), and the cells were permeabilized with a drop of toluene. The level of β-galactosidase production was determined as described by Miller (25). The apparent A_{600} of the cultures was also determined at various times during growth.

Electroblotting of proteins and immunodetection. Cultures of *E. coli* RR1 harboring plasmids were grown in L broth with carbenicillin (100 μg/ml) at 30°C to a density of $\approx 10^8$ cells per ml. The cultures were divided, one half was shifted to 42°C, and the incubations were continued for another 2 h. Aliquots were taken and centrifuged, and the pellets were suspended in Laemmli sample buffer and boiled for 5 min. Equivalent amounts of each sample (determined by the

apparent A_{600} of the cultures) were then run on a sodium dodecyl sulfate-polyacrylamide gel (12.5% acrylamide, 2.25% *N,N'*-diallyltartardiamide) with a 4.5% acrylamide stacking gel. Immunoblotting was performed essentially as described by Towbin et al. (32) with the following modifications. After electrophoresis, proteins were transferred from the gel onto nitrocellulose paper (Schleicher & Schuell, Inc.) in Laemmli running buffer containing 20% methanol with an E-C Electroblot system. The paper was stained with 0.1% amido black, destained for 1 min with 10% acetic acid–10% methanol, washed three times for 5 min each in distilled water, and incubated overnight with Tris-buffered saline (TBS; 20 mM Tris-hydrochloride [pH 7.5], 150 mM NaCl). Nonspecific adsorption was blocked by incubating the paper with a solution of TBS containing 0.05% Tween 20 (Sigma) for 2 h at room temperature (2). Rabbit antiserum (provided by Shlomo Eisenberg) raised against the φX gene A protein was diluted 1:750 in TBS containing 0.05% Tween 20 and incubated with the blot in a volume of 20 ml for 2 h at room temperature. Rabbit antiserum (provided by Jeff Roberts) raised against *E. coli* RecA protein was diluted 1:4,000 in the same buffer and incubated similarly. The blots were washed six or seven times for 10 min each in TBS plus Tween 20 and then incubated with ^{125}I-labeled protein A (New England Nuclear Corp.), ca. 30,000 cpm per lane, for 2 to 3 h. After extensive washing with TBS plus Tween 20, blots were subjected to autoradiography.

RESULTS

Controlled expression of a lethal gene. Phage λ regulatory elements have been used to control the expression of cloned genes in several systems (3, 24, 29). Shimatake and Rosenberg (30), for example, used the λ P_L promoter-*c*I857 thermolabile repressor system of pKC30 to control expression of the lethal λ *c*II gene. In attempting to clone the A* gene of φX174, however, we discovered that several vectors bearing the P_L promoter apparently exerted insufficient repression of this gene at 30°C (the restrictive temperature for expression) to permit the isolation of stable clones. This was possibly due to an inadequate concentration of repressor, since the *c*I857 gene was located on the host chromosome in a defective prophage in the systems we tried.

This prompted a search for temperature-inducible expression vectors that used either the λ P_L or P_R promoter and encoded the λ *c*I857 repressor. The plasmid pCQV2 was found to be satisfactory (see Fig. 1). It contains the λ *c*I857 repressor gene transcribed from the P_{RM} promoter (which can be stimulated by *c*I) and the P_R rightward promoter (which is repressed by the *c*I or *c*I857 protein) just upstream from the RBS and the AUG start codon of the λ *cro* gene. (These latter were not essential in cloning the A* fragment since it carried its own RBS and start codon).

Construction, diagrammed in Fig. 1, of a recombinant with the A* gene in the proper orientation relative to the P_R promoter involved cleaving pCQV2 with *Bam*HI, creating blunt ends on the duplex with the Klenow fragment of DNA polymerase I, and then cutting with *Sal*I. The digestion of φX174 double-stranded RF DNA with *Nru*I, which cleaves 74 base pairs upstream from the A* start codon, and *Xho*I, which cleaves 32 base pairs after the A-A* stop codon, yields a 1,142-base-pair fragment containing the A* gene. The normal promoter for the A* gene is the P_A promoter, which is just before the start of the A gene and, thus, not present. Consequently, transcription of this fragment is dependent on transcription from the P_R promoter.

Ligation of the prepared pCQV2 DNA with the φX174 A* fragment, followed by transformation of *E. coli* RR1 to ampicillin resistance, permitted the isolation of the plasmid pJA*1. The flush-ended *Bam*HI-*Nru*I joining results in regeneration of the *Bam*HI site, whereas the *Sal*I-*Xho*I cohesive end joining does not regenerate either site. Given transcription from the P_R promoter, the nature of this construction allows synthesis of a peptide from the *cro* RBS-start codon into φX174 DNA but out of frame with the A* gene and resulting in termination of translation upon reaching the A* start region. (Expression of this peptide does not affect *E. coli* metabolism because clones have been constructed in which it is absent [by cloning the *Stu*I-*Xho*I fragment of φX174 into pCQV2, see Fig. 1] and they behave as pJA*1 does [data not shown]. These recombinants were not used in this study because the A* produced was altered in that it had five additional amino acids fused in its N terminus.) Since the cloned segment of φX DNA in pJA*1 carried the RBS and initiation codon for A*, it was possible to obtain expression of A* protein. Expression was initially evidenced by the inability of the cells to form colonies at 42°C. The presence of φX174 DNA was confirmed by colony hybridization with labeled (by nick translation) φX174 RF DNA or fragments containing the A* gene.

Properties of pBCK5, a deletion mutant of A*. Because of the presence of overlapping reading frames in this segment of φX174 DNA, it is impossible to clone the A* gene without cloning all or parts of others, in this case the entire B gene and the 5′ ends of genes C and K. To minimize interference

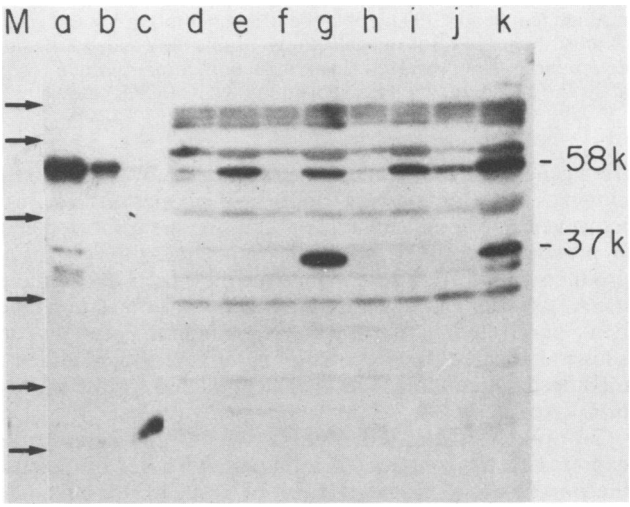

FIG. 2. Electroblotting and immunodetection of gene A* protein. Equivalent amounts of cell extracts (*E. coli* RR1) were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel, electroblotted onto nitrocellulose paper, and probed first with antibody to φX gene A protein and then with ^{125}I-labeled protein A as described in the text. Lanes a through c contain 2, 0.4, and 0.04 μg, respectively, of a purified preparation of φX174 gene A protein provided by S. Eisenberg. Lanes d and e are extracts of strain RR1(pCQV2) at 30 and 42°C (2 h), respectively; lanes f and g are extracts of strain RR1(pJA*1) at 30 and 42°C (2 h), respectively; and lanes h and i are extracts of strain RR1(pBCK5) at 30 and 42°C (2 h), respectively. Lanes j and k are extracts of *E. coli* C uninfected (lane j) and infected (lane k) with φX174*am*3 for 1.5 h at 37°C. Molecular weight markers (Bio-Rad Laboratories) on the left are phosphorylase *b* (M_r, 92,500), bovine serum albumin (M_r, 66,200), ovalbumin (M_r, 45,000), carbonic anhydrase (M_r, 31,000), soybean trypsin inhibitor (M_r, 21,500) and lysozyme (M_r, 14,400).

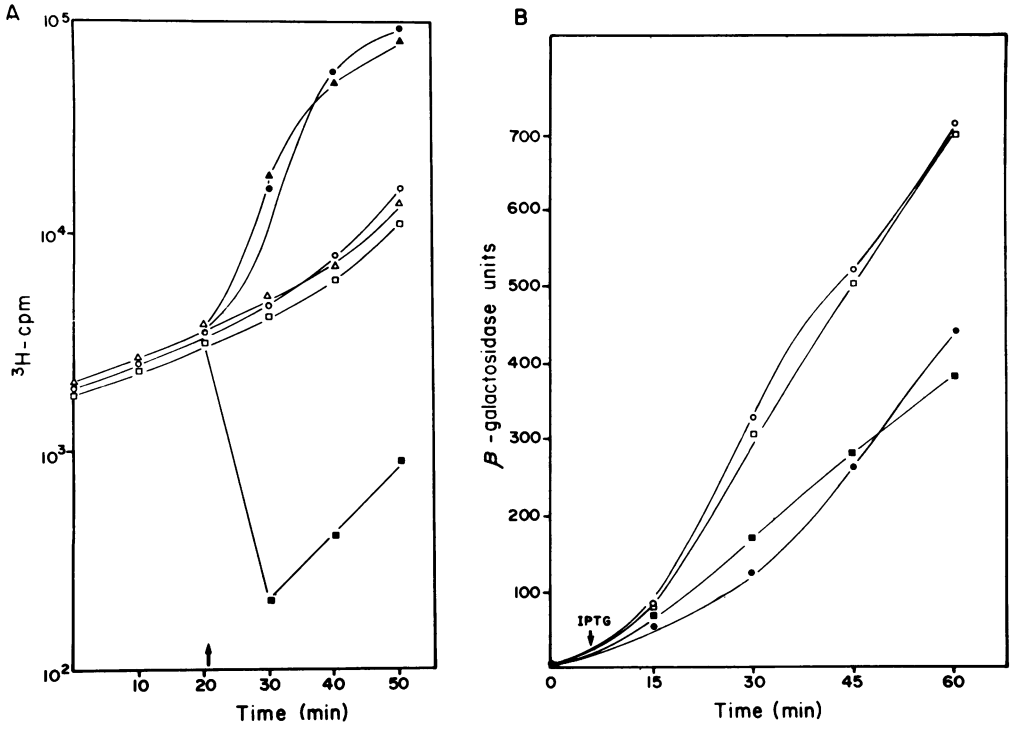

FIG. 3. Effect of A* protein on DNA synthesis and β-galactosidase induction in *E. coli*. (A) DNA synthesis was determined by measurement of [^{3}H]thymidine uptake into acid-precipitable material. Cells were grown in L broth (containing 100 μg of carbenicillin per ml) to 10^8 cells per ml at 30°C, and 1-ml portions were taken (0 min) and added to 10 μCi of [^{3}H]thymidine for 2 min (see the text). At 20 min after the first sampling, half of each culture was transferred to 42°C, and the measurements were continued. (B) Cells were grown in minimal medium (containing 100 μg of carbenicillin per ml) to 5×10^7 cells per ml at 30°C, at which point half of each culture was transferred to 42°C (0 min). Isopropyl β-D-thiogalactopyranoside was added six min later to a 2 mM final concentration, and 0.25-ml samples were taken to determine levels of β-galactosidase induction. Strains used: *E. coli* RR1(pCQV2) grown at 30°C (○) and at 42°C (●), *E. coli* RR1(pJA*1) grown at 30°C (□) and at 42°C (■), and *E. coli* RR1(pBCKR) grown at 30°C (△) and at 42°C (▲).

from gene B, an amber mutant, φX174*am*16, was used in the cloning, and studies were done in a nonpermissive host. However, one could still argue that it was the truncated gene B product or gene C-pCQV22 or gene K-pCQV2 fusion products which were responsible for the effects attributed to the A* protein. DNA-directed transcription-translation reactions performed in vitro with recombinant plasmids (not shown) indicated the presence of putative fusion peptides of the approximate molecular weight predicted for the φX174-pCQV2 construct.

To prove that the lethal effects were indeed due to A* expression, we constructed a mutant, pBCK5, by deleting the *Bam*HI-*Bst*XI fragment from pJA*1 (Fig. 1). This deletion removes the A* start site along with the first half of the gene but still allows transcription of gene B and the gene C and K regions as before. The *cro* start site is joined to the remaining φX174 sequence such that the peptide produced is in a different reading frame from A* and terminates almost immediately after it starts. This is borne out by immunoblotting results (see Fig. 2). The ability of cells bearing this plasmid to form colonies at 42°C indicates that these other polypeptides did not kill the cell and that expression of the A* protein was necessary for the lethal effect.

An additional argument that gene B, gene C, and gene K products are not lethal to the cell can be based on the existence of the P_B promoter. This promoter precedes gene B (Fig. 1) and presumably permits constitutive transcription of this region in the cell. Since the cells survived at 30°C, this transcription was not lethal. In addition, van der Avoort et al. (33) showed that cells that were expressing the B and C genes (and, hence, able to complement φX gene B and C mutations) grew well.

Detection of the A* protein. In cases in which genes are put under control of λ P_L or P_R promoters and *c*I857, induction at 42°C often results in the production of a large amount of protein (27, 30). Unfortunately, this was not observed with induction of *E. coli* carrying pJA*1. The A* protein (M_r, ca. 37,000) did not stand out on Coomassie blue-stained polyacrylamide gels or in autoradiographs of gels of [^{35}S]methionine-labeled cell extracts induced at 42°C (not shown). However, major *E. coli* proteins migrated in this region and could have obscured moderate amounts of an induced protein.

The availability of an anti-A antiserum made it possible to use the more sensitive immunoblotting procedure to detect the A* protein. Figure 2 shows the presence of the A* protein after induction of expression at 42°C, whereas none at all was detectable at 30°C. Cells carrying pBCK5 produced neither A* protein nor a truncated product after induction.

Interestingly, the anti-A antiserum seemed to react specifically with another *E. coli* protein in the region of the gel indicating an M_r of ca. 56,000. The nature of this bacterial protein is unknown; however, since at 42°C heat shock increases its expression, it could be the B56.5 protein (also called the *A* protein and the *gro*E protein [26]). The cross-reaction with anti-φX174 gene *A* protein serum may indicate a shared epitope. The other (less intense) bands on the gel

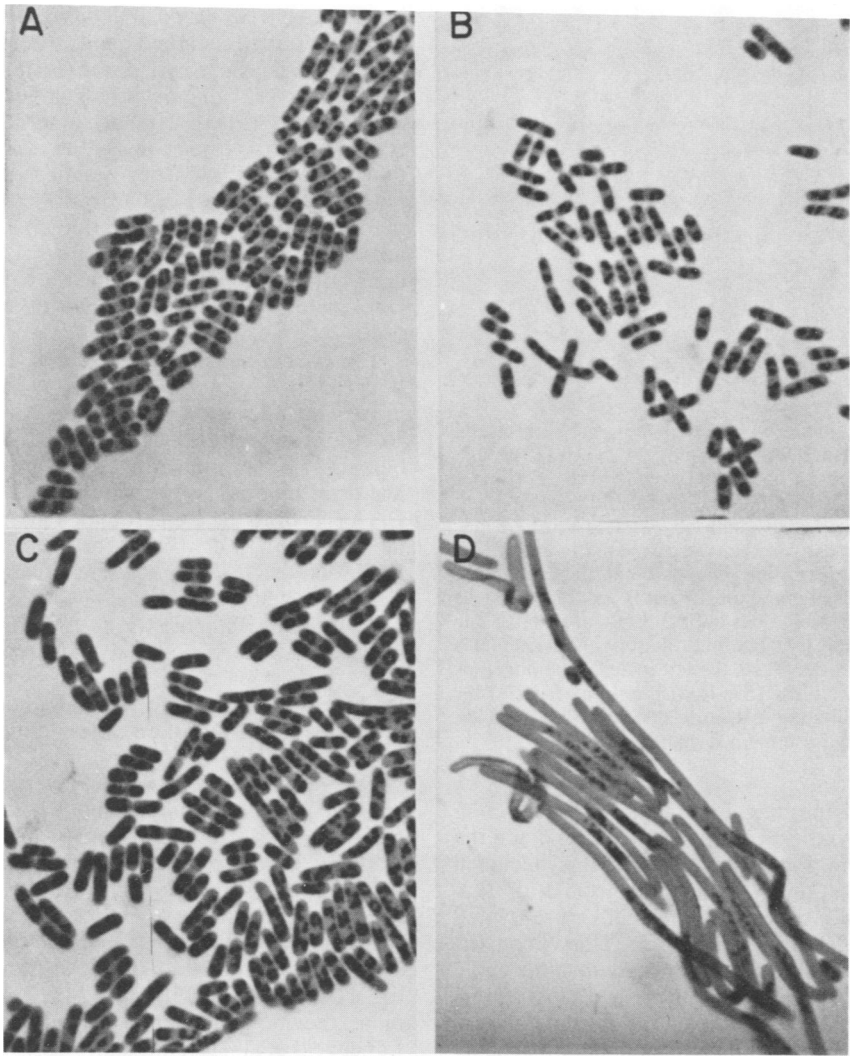

FIG. 4. Induction of filaments by A* protein. *E. coli* RR1 cells harboring either pCQV2 or pJA*1 were grown to mid-log phase in L broth (with 100 μg of carbenicillin per ml) at 30°C, transferred to L agar plates (with carbenicillin), and allowed to grow at either 30 or 42°C for 1.5 h. Blocks of agar containing cells were then fixed with 1% OsO_4; the cells were transferred to glass slides and then stained with HCl-Giemsa. This procedure stains the nuclear regions of *E. coli* darkly. Strains shown: *E. coli* RR1(pCQV2) at 30°C (A) and 42°C (B) and *E. coli* RR1(pJA*1) at 30°C (C) and 42°C (D).

appeared with nonimmune rabbit serum also (data not shown).

Effect of A* protein on *E. coli* DNA synthesis. One of the effects of A* protein in vitro is inhibition of φX174 DNA synthesis (11). Induction of A* expression in vivo allows one to determine its effects on *E. coli* replication directly. Measurement of the rate of DNA synthesis via [^{3}H]thymidine uptake revealed a marked decrease in synthesis in cells in which A* expression had been induced (Fig. 3A), whereas cells at 30°C and those carrying the parent plasmid at both temperatures continued synthesizing DNA. Similarly, cells harboring pBCK5 showed no inhibition of DNA synthesis at 42°C, providing further evidence that A* protein directly inhibits *E. coli* DNA replication. The residual DNA synthesis in the *E. coli* RR1/(pJA*1) culture at 42°C may be due to a subpopulation of cells lacking the ability to express A* protein.

Effect of A* on β-galactosidase induction in *E. coli*. To ascertain the effect of the A* protein on cellular transcription and translation, we monitored β-galactosidase production. Cells carrying either pCQV2 or pJA*1 were grown to early log phase at 30°C, shifted to 42°C, and then induced to produce β-galactosidase with isopropyl β,D-thiogalactopyranoside. The effect of A* protein on enzyme production was assayed colorimetrically. There was not a noticeable difference in β-galactosidase levels in cells carrying either of the plasmids at either temperature (Fig. 3B). This may indicate that A* acts specifically to inhibit host DNA synthesis and does not significantly affect transcription or translation. Consistent with this conclusion is the fact that the cells bearing pJA*1 form filaments (Fig. 4) and continue to increase in mass at 42°C several hours after induction of A* synthesis.

Are SOS functions induced? It is usually the case that when cellular DNA is damaged or cell DNA replication is inhibited or both, the cell responds by expressing a set of genes under the control of the *lex*A repressor (21). The phenomenon is called the SOS response and in large part is directed at facilitating repair of the damaged DNA. We have investigated whether this response occurs after induction of

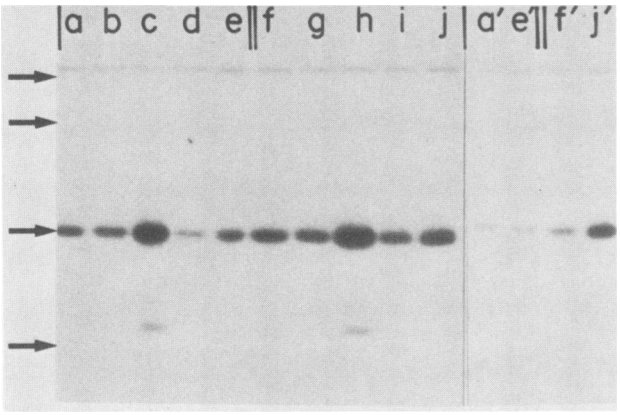

FIG. 5. Quantification of RecA protein by immunoblotting. Equivalent amounts of *E. coli* RR1 carrying pCQV2 (lanes a through e) or *E coli* RR1 carrying pJA*1 (lanes f through j) were electrophoresed on an sodium dodecyl sulfate-polyacrylamide gel and immunoblotted with antiserum raised against *E. coli* RecA protein (see the text). Cells in early log phase either were exposed to mitomycin C (1.5 μg/ml) for 30 min (lanes b and g) and 90 min (lanes c and h) or were shifted to 42°C for 30 min (lanes d and i) and 90 min (lanes e and j). Lanes a and f represent cells kept at 30°C for 90 min. The four lanes on the right represent twofold dilutions of the samples seen in lanes a, e, f, and j. Arrows on the left indicate the positions of the molecular weight markers (Bio-Rad) phosphorylase *b* (M_r, 92,500), bovine serum albumin (M_r, 66,2000), ovalbumin (M_r, 45,000), and carbonic anhydrase (M_r, 31,000).

A* expression. One of the diagnostic features of the SOS response is increased synthesis of the RecA protein, and the experiment presented in Fig. 5 reveals that this occurred when A* was induced, although the augmentation of synthesis was not as great as was found when the cells were treated with mitomycin C, a DNA-damaging agent. This is consistent with a partial turn-on of the SOS function and could account for the filamentation observed, since LexA repression of the *sfiA* gene, whose product inhibits septation, would be lifted. However, the complete explanation is likely more complex, since A*-induced filamentation was also observed in *recA56* and *lexA3*(Ind$^-$) strains in which the SOS response can not be induced, and in which mitomycin C treatment did not cause filamentation (data not shown).

DISCUSSION

Early attempts by our laboratory and others (33) indicated that successful cloning of gene A* required that its lethal action be repressed. To accomplish this, we used the vector pCQV2 (27), which uses the λ P_R and *c*I857 controlling elements to regulate the expression of cloned genes. Several P_L-containing vectors (pPLc24, pKC30, and pJL6-8) used in earlier experiments apparently provided insufficient repression of A* synthesis to allow isolation of stable clones; this may be because these vectors rely on *c*I857 repressor provided by a single-copy defective lysogen, whereas pCQV2 carries a copy of the repressor gene itself, thus increasing the gene copy number of the repressor gene.

When expression of A* protein in cells carrying pJA*1 was induced by shifting the cells to 42°C, large amounts of the protein were not made, raising the possibility of autoregulation of synthesis. Detection of expression required the immunoblotting technique (Fig. 2) to visualize the A* protein, which was otherwise obscured by *E. coli* proteins. Upon expression of A*, cellular DNA synthesis was inhibited, and the cells stopped dividing. However they increased in mass, formed filaments, and, when induced to synthesize a new protein (here by isopropyl β,D-thiogalactopyranoside induction of β-galactosidase synthesis), responded as did control cells (Fig. 3). Ghosh and Poddar (14) found that φX174 infection caused inhibition of β-galactosidase production in *E. coli*, and they speculated it was caused by a phage-encoded protein. Our results suggest that it is not A* that is reponsible for this shutdown. Apparently, the mechanism that A* uses to shut down *E. coli* DNA replication does not result in a generalized breakdown of the bacterial DNA, and does not interfere with transcription and translation.

The greater sensitivity of ssDNA to cleavage by the gene A* protein (12, 16, 17) encourages the thought that the A* protein may act specifically at single-stranded regions in the *E. coli* growing fork, breaking the DNA at that point and blocking further replication. Other possibilities include physical obstruction merely as the result of A* binding to duplex DNA (11) or to the Rep protein, a helicase normally involved in separating the strands of the bacterial chromosome. Inhibition of DNA replication causes induction of the SOS response, which includes the increased production of the SfiA protein, an inhibitor of septation (9, 15). However, since septation is inhibited under conditions in which the LexA repression of the *sfiA* gene is not lifted (in *recA56* or *lexA3* mutants), it may be that inhibition of cell division occurs as the result of an SOS-independent coupling of cell division to DNA replication (5).

The role of the φX174 gene A* protein in the viral life cycle is unknown. That it is made by all the isometric phages examined and that it seemingly has a counterpart in the filamentous phages (8) implies that it is advantageous if not necessary for some aspect of the viral life cycle. Its actions on ssDNA suggest a role in superinfection exclusion (7). Other possible roles include shutting off host cell DNA synthesis and facilitating the transition from RF replication to DNA synthesis (10, 11, 13, 17, 23). Earlier research, the more recent studies of Eisenberg and Ascarelli (11) and Langeveld et al. (16), and our own data argue strongly for an effect on host DNA synthesis; presumably a cessation of host DNA synthesis increases the pool of nucleotide precursors available for phage DNA synthesis.

Evidence against a direct role for the A* protein in φX174 ssDNA synthesis comes from in vitro studies indicating that it is not required for RF replication or ssDNA synthesis in vitro (1). One explanation for the inferred essential function of the A* protein could be the existence of a host cell protein, required for replication of the bacterial DNA, that interferes with viral ssDNA synthesis (1). The role of the A* protein would be to prevent this interference. Oligonucleotide-directed in vitro mutagenesis of the A* coding sequence in plasmid pJA*1 will make possible incisive experiments into A* function.

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