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Is Abortive Infection by Bacteriophage BF23 of *Escherichia coli* Harboring ColIb Plasmids due to Cell Killing by Internally Liberated Colicin Ib?

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Infection of *Escherichia coli* harboring ColIb$^+$ plasmids with bacteriophage BF23$^+$ is abortive and resulted in changes of membrane permeability as measured by efflux of nucleotides and K$^+$. A single pre-early gene product of BF23$^+$ was necessary and sufficient to elicit the abortive response. Appropriate mutations in this pre-early gene allowed a productive infection in ColIb$^+$ cells. Appropriate mutations in the ColIb plasmid also allowed a productive infection with BF23$^+$. A comparison of changes occurring during abortive infection and during killing of sensitive cells by external colicin Ib or Ia, together with certain genetic data, has led to the conclusion that membrane changes accompanying the two phenomena are the result of a common mechanism, namely, the interaction of free colicin with the cytoplasmic membrane.

Bacteriophage BF23 and its close relative bacteriophage T5 do not productively infect hosts that carry ColIb plasmids (31). Adsorption of phage particles and transfer of phage DNA proceed normally in such infections, but soon after the pre-early genes, which comprise the initially transferred 8% segment of DNA, are expressed, further gene expression ceases (24) despite the persisting intactness of the phage DNA in the cell (31). This sequence of events has led to the suggestion that a ColIb-specified product interferes with transcription of phage DNA (25, 32). However, the occurrence of early abortive lysis in such infections (27) suggests that changes more severe than arrest of transcription are occurring in the infected cells. We reported preliminary data at the phage meetings in Cold Spring Harbor, N.Y., during 1974 (A. F. Morgan and D. J. McCorquodale, Abstr. Annu. Bacteriophage Meet. 1974, p. 117) and 1976 (A. R. Shaw and D. J. McCorquodale, Abstr. Annu. Bacteriophage Meet. 1976, p. 86) that suggested that the arrest of phage development was due to a decline in the integrity of infected cells, because low-molecular-weight components leak from them into the surrounding medium. Here we present those data plus additional data that support an arrest mechanism that involves interference with the integrity of membranes of infected cells by liberated intracellular colicin Ib.

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MATERIALS AND METHODS

Bacteria and bacteriophages. Bacterial strains used in this study are listed in Table 1. *Escherichia coli* W3110 and CR63 are permissive for growth of both BF23$^+$ and BF23h$^-$, whereas *E. coli* W3110(ColIb$^+$) and CR63(ColIb$^+$) are nonpermissive for BF23$^+$ but permissive for BF23h$^-$. BF23h$^-$ was isolated as a spontaneous mutant of BF23$^+$ and carries a missense mutation in gene A3. BF23*am*M57 carries an amber mutation in gene A1, so that in a *su*$^-$ host it injects only the initial 8% segment of its DNA (called the first-step-transfer DNA, or FST DNA) because the product of gene A1 along with that of gene A2 is required for completion of phage DNA transfer (18). BF23*am*M57h$^-$ is a double mutant that will grow only in the presence of an amber suppressor, but that can productively infect ColIb$^+$ cells that are *su*$^+$. The construction of bacterial strains carrying the ColIb plasmid and the isolation of BF23 mutants have been described previously (23).

Isolation of *pha*$^-$ mutants. A strain of *E. coli* that carries a ColIb plasmid with a mutation that permits growth of BF23$^+$ was isolated as follows. The parental plasmid was the *uvp-2* mutant of a wild-type ColIb plasmid in *Salmonella typhimurium* LT2, which we designated MUT201. The *uvp-2* mutation eliminates resistance to UV irradiation (14), which in turn ensured efficient mutagenesis. An overnight culture of MUT201 in nutrient broth was diluted in fresh broth and grown with aeration at 37°C for 2 h. A 5-ml

TABLE 1. *Bacterial strains*

Strain	Genetic markers	Reference
E. coli K-12		
W3110	su^-	*23*
CR63	su^+	*23*
W3110(ColIb$^+$)	su^-	*23*
CR63(ColIb$^+$)	su^+	*23*
MUT20	*nalA tonB* F$^-$(ColIb$^-$)	This paper
MUT26	*nalA tonB* F$^-$(ColIb$^+$ *uvp-2* *phaA2*)	This paper
S. typhimurium LT2		
MUT201	*trpD1*(ColIb$^+$ *uvp-2*)	14

portion of this exponentially growing culture (approximate cell concentration, 5×10^8/ml) was sedimented by low-speed centrifugation, washed once, and suspended in an equal volume of 0.1 M sodium citrate, pH 5.0, containing *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) at a final concentration of 100 μg/ml. The bacteria were incubated for 15 min at 37°C in the presence of NTG, centrifuged, washed twice with 0.85% saline to remove the NTG, and mated overnight in broth with *E. coli* K-12 strain 594 [*nalA tonB* F$^-$(ColIb$^-$)], which we designated MUT20. The mating mixture was diluted and plated for isolated colonies on nutrient agar containing 100 μg of nalidixic acid per ml. The colonies appearing after incubation overnight at 37°C were replica plated onto nutrient agar plates previously seeded with 10^9 PFU each of bacteriophages T5 and BF23. The use of both phages reduced the likelihood of interference by singly phage-resistant mutants. Triplicate replicas were incubated overnight at 30, 37, and 42.5°C. Under these conditions, bacteria with the wild-type genotype (pha^+) appeared as resistant colonies, whereas the permissive bacterial mutants (pha^-) appeared as sensitive, i.e., "eaten" colonies. Three such pha^- mutants were isolated and purified, and their characteristics were verified. The pha^- mutant used in these studies was designated MUT26.

Growth of bacteria and preparation of phage. Bacteria were grown in nutrient broth (1), tryptone (8 g of tryptone [Difco Laboratories] plus 5 g of NaCl per liter), morpholinepropanesulfonic acid (MOPS) medium (26), or modified MOPS medium containing one-third the standard amount of sodium phosphate, all containing 1 mM $CaCl_2$. Phage were prepared from plate lysates (1) and purified by differential centrifugation. The phage suspension eluted from plates was centrifuged at low speed (12,000 × *g* for 10 min at 4°C), and the resulting supernatant was centrifuged at high speed (27,000 × *g* for 2 h) to sediment the phage into a pellet. The pelleted phage were suspended in phage suspension buffer (0.1 M Tris, 0.05 M sodium maleate, 27 mM KCl, 0.8 mM Na_2SO_4, 1.28 mM sodium phosphate, 1 mM $CaCl_2$, 0.1 mM $MgSO_4$, '.8 μM $FeSO_4$, and 0.01% gelatin, all at pH 7.3). Bacteria and phage titers were determined by the soft-agar overlay method of Adams (1).

Determination of nucleoside triphosphate levels. Nucleoside triphosphate levels were measured by the method of Britton and Haselkorn (3). Bacteria were grown to a concentration of 2.5×10^7/ml in MOPS medium with one-third the concentration of phosphate, at which time $^{32}P_i$ (Schwarz/Mann 006-22) was added to give a final concentration of 10 μCi/ml. Growth of the culture was continued until the concentration of cells reached 2×10^8/ml, at which time phage were added at an input ratio of 10 phage per bacterium. Just before and at various times after the addition of phage, a 1.0-ml volume was removed from the culture, filtered through a nitrocellulose membrane (Schleicher and Schuell no. BA85), and washed with 1.0 ml of MOPS medium. Both the filtrate and the wash were collected in a tube containing 0.1 ml of 92% formic acid. Norit A was added to a concentration of 2%. The Norit was pelleted and washed once with 1 mM HCl, and the nucleotides were eluted with 2 ml of 1 M ammonium hydroxide in 50% ethanol. The filters were soaked overnight in 2 ml of cold 1 M formic acid. The nucleoside triphosphate content of the filter suspension and of the extracted filtrate was analyzed by thin-layer chromatography (5). A 50-μl amount of each sample was spotted onto a polyethyleneimine (PEI)-cellulose plate (Brinkman EM no. 5758/0001) and developed with 1.0 M sodium phosphate, pH 3.4. The plates were dried, and the nucleoside triphosphates were located by autoradiography for 10 days on Kodak DF-85 dental X-ray film. Relative quantities of nucleoside triphosphates were determined by scraping the PEI resin within 13-mm circles centered over the spots on the autoradiogram into scintillation vials and counting with toluene-Permafluor (Packard no. 6003004). Unlabeled nucleoside triphosphates were chromatographed as reference standards and located under UV light.

Radioactive labeling of proteins after infection. Radioactive labeling of proteins after phage infection was followed concurrently with the determination of nucleoside triphosphate levels. At 30 s before each portion was removed for nucleotide analysis, a 1.0-ml volume was taken from the ^{32}P-labeled culture, added to a tube containing 1 ml of MOPS medium and 0.4 μCi of ^{3}H-labeled amino acid mixture (Schwarz/Mann no. 3130), and incubated with aeration at 37°C for 1 min. The labeling was terminated by adding 2.0 ml of 10% trichloroacetic acid, and the tube was then placed in an ice bath. The tube was next placed in a boiling-water bath for 30 min to solubilize most of the ^{32}P-labeled material, and the precipitates were collected and washed on nitrocellulose filters. The filters were dried and counted in toluene-Permafluor in a Beckman LS-250 scintillation counter, and the counts were corrected for ^{32}P by recounting 14.5 days later. The difference in counts per minute, attributable to ^{32}P decay, was subtracted from the "14.5-day" counts per minute to yield the ^{3}H counts per minute.

Measurement of potassium levels. Levels of potassium inside the cell before and during infection were measured by the method of Silver et al. (30). Bacteria were grown in tryptone plus 1 mM $CaCl_2$ and 0.3 μCi of ^{42}K (New England Nuclear Corp. no. 951964) per ml to a concentration of 3×10^8/ml and pelleted by centrifugation (8,500 rpm at 20°C for 10 min in a Sorvall GSA rotor). The cells were washed once in

tryptone, resuspended at a concentration of 6×10^8/ml in fresh tryptone, and placed in a metabolic shaking incubator at 30°C. Phage were added at various multiplicities, and 1.0-ml samples were taken at various times before and after the addition of phage. In some experiments chloramphenicol (50 μg/ml) was added 8 min before the addition of phage. The 1.0-ml samples were filtered through nitrocellulose membranes, and the filtrates were collected. Both the filters and a 0.5-ml volume of each filtrate were placed in Aquasol (New England Nuclear Corp.) and counted in a Beckman LS-250 scintillation counter. Appropriate corrections were made for the short half-life of ^{42}K.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed by the method of Laemmli (17). Gels contained 15% acrylamide, 0.4% bisacrylamide, 0.1% sodium dodecyl sulfate (SDS), and 0.375 M Tris, pH 8.8. Stacking gels contained 2.5% acrylamide, 0.064% bisacrylamide, 0.63 M Tris at pH 6.8, and 0.1% SDS. Polymerization of acrylamide solutions was catalyzed by 0.03% *N,N,N',N'*-tetramethylethylenediamine (TEMED) plus 0.03% ammonium persulfate for 15% acrylamide gels and 0.05% TEMED plus 0.1% ammonium persulfate for the 2.5% acrylamide stacking gels. Electrode buffer was 0.383 M glycine, 0.05 M Tris, and 0.1% SDS, pH 8.3. Electrophoresis was performed in a refrigerator at 4°C with a constant current of 17 mA for 5 h. After electrophoresis, the gels were fixed in 10% trichloroacetic acid for 30 min, stained in Coomassie brilliant blue R250 (Eastman no. 14013; 0.123% of Coomassie brilliant blue in 7.5% ml of glacial acetic acid–50% methanol) for 2 h, destained in 7.5% acetic acid, and dried on Whatman 3MM filter paper for autoradiography. Autoradiography was performed with Kodak DF85 dental X-ray film.

Samples were prepared for electrophoresis by several methods depending on the type of experiment. For analysis of pre-early phage proteins, bacteria were grown in MOPS medium plus 1 mM $CaCl_2$ to a concentration of 2×10^8/ml, centrifuged at 12,000 × *g* (8,500 rpm at 4°C for 10 min in a Sorvall GSA rotor), resuspended at 2×10^9/ml in cold MOPS buffer (MOPS medium without glucose) plus 1 mM $CaCl_2$, and irradiated at 0°C for 20 min with three Westinghouse Sterilamps (G157B). A 0.5-ml volume of irradiated cells was added to 4.5 ml of MOPS medium plus 1 mM $CaCl_2$ containing 7×10^9 phage and 0.4 μCi of a ^{14}C-labeled amino acid mixture (Schwarz/Mann no. 0562) per ml, incubated for 5 min at 37°C with gentle aeration, quickly cooled, and placed in an ice bath. The labeled culture was centrifuged at 12,000 × *g*, and the pellet was resuspended in the appropriate sample preparation buffer. The sample preparation buffer for SDS-polyacrylamide gel electrophoresis consisted of 0.05 M Tris at pH 6.8, 1% SDS, 1% 2-mercaptoethanol, 10% glycerol, and 0.003% bromophenol blue. The pellet resulting from a 5-ml labeled culture was resuspended in 0.5 ml of SDS sample preparation buffer.

For analysis of phage proteins during the course of an infection, bacteria were grown to a concentration of 2×10^8/ml in MOPS medium plus 1 mM $CaCl_2$, and phage were added at an input ratio of seven phage per cell and mixed rapidly. Immediately thereafter, a 5-ml sample of infected cells was removed and added to a tube containing 0.5 ml of MOPS medium and 2.2 μCi of ^{14}C-labeled amino acid mixture. This mixture was incubated for 5 min at 37°C and then quickly cooled in an ice bath. Additional samples of 5 ml were removed from the infected culture at various other times after infection and treated in the same manner. Samples were centrifuged, and the pellet cells were resuspended in SDS sample preparation buffer to a concentration of 2×10^9/ml.

RESULTS

Patterns of protein synthesis in W3110 and W3110(ColIb) after BF23 infection. We have shown previously that pre-early protein synthesis proceeds normally after infection of *E. coli* W3110(ColIb) by BF23h⁺ (24). Early protein synthesis either does not occur or is initiated but soon shut off. These previous results were obtained by separating the various temporal classes of phage-induced proteins by electrophoresis in polyacrylamide gels under nondenaturing conditions, which do not reveal all proteins. Figure 1 shows which temporal classes of proteins were synthesized after infection of *E. coli* W3110(ColIb) by BF23h⁺, as revealed by electrophoresis in polyacrylamide gels under denaturing conditions. Synthesis of pre-early proteins proceeded normally, and synthesis of early proteins was initiated at the usual time (about 6 min after infection) but virtually ceased soon thereafter (by 12 to 15 min). No late proteins were detectable by the present methods. This pattern was confirmed by measurement of total acid-insoluble ^3H-labeled proteins synthesized at various times after infection (Fig. 2). In comparison, all temporal classes of proteins were syn-

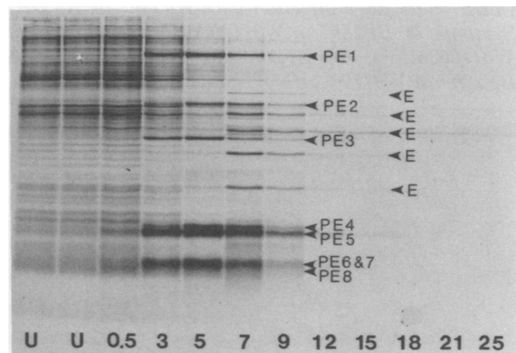

FIG. 1. *Autoradiogram of electrophoretically separated ^{14}C-labeled polypeptides synthesized in W3110(ColIb⁺) after infection with BF23h⁺. The number below each pattern specifies the time after infection at which a 5-min labeling period was begun. The autoradiographic pattern of ^{14}C-labeled polypeptides synthesized in uninfected cells is designated U. Pre-early polypeptides are designated PE1 through 8, and early polypeptides are designated E. No late polypeptides are detectable in this autoradiogram.*

thesized after infection of *E. coli* W3110(ColIb) with BF23h$^-$ (Fig. 2 and 3), and a productive infection resulted. Both BF23h$^+$ and BF23h$^-$ productively infected W3110 that lacked a ColIb plasmid (autoradiographic data are not shown, but ^{3}H-labeled proteins synthesized after these infections are shown in Fig. 2).

Efflux of low-molecular-weight phosphorylated compounds from infected cells. W3110(ColIb) cells leaked low-molecular-weight cellular constituents into the medium after infection with BF23h$^+$. Cells were incubated with $^{32}P_i$ for three generations to uniformly label nucleotide pools. Intracellular and extracellular levels of nucleotides were measured before and during infection of W3110 or W3110(ColIb) by BF23h$^+$ or BF23h$^-$. Nucleotides were separated by thin-layer chromatography, located by autoradiography, and quantified by scraping the nucleotide-containing region of the chromatography resin into a scintillation vial and counting in toluene-Permafluor. Figure 4 shows the autoradiographic pattern produced by thin-layer chromatography of intracellular and extracellular ^{32}P-labeled nucleotides extracted at various times from *E. coli* W3110(ColIb$^+$) infected with BF23h$^+$. Intracellular levels of UTP, CTP, ATP, and GTP fell after infection, whereas extracellular levels of ATP increased rapidly starting at about 7 min after infection. In contrast, when W3110 was infected with BF23h$^+$, intracellular levels of nucleoside triphosphates did not fall but instead slowly rose, and no extracellular nucleotides were detectable (Fig. 5). The visual estimations of nucleotides from the thin-layer chromatography patterns were supported by quantitative determinations of [^{32}P]ATP in each spot (Fig. 6 and 7). In W3110 cells infected with BF23h$^+$, levels of intracellular [^{32}P]ATP tended to decrease initially but then increased biphasically to levels almost three times higher than the level at the time of infection. In contrast, after W3110(ColIb$^+$) cells were infected with BF23h$^+$, levels of intracellular ^{32}P decreased fairly rapidly to a level of about one-fifth that at the time of infection, with only a transient rise at 5 min postinfection. If the intracellular level of [^{32}P]ATP at the time of infection was taken as 650 cpm/50 μl spotted on the PEI plate and if the plateau level of [^{32}P]ATP after 10 min of infection was taken as 150 cpm/50 μl (Fig. 6), the decrease in intracellular ATP would be approximately 77%. However, 130 cpm of [^{32}P]ATP per 50 μl appeared in the medium (Fig. 7), making the overall decrease in the ATP levels about 57%.

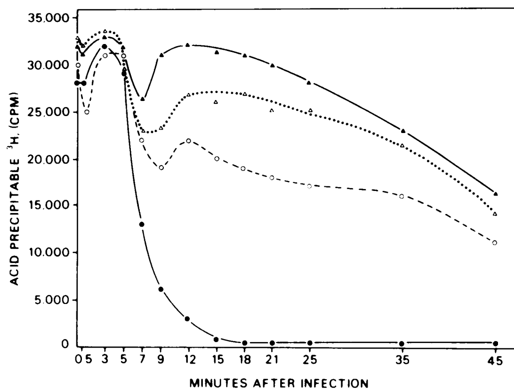

FIG. 2. *Incorporation of ^{3}H-labeled amino acids into acid-insoluble proteins synthesized in ColIb$^+$ or ColIb$^-$ cells after infection with BF23h$^+$ or BF23h$^-$. Symbols:* ●, *BF23h$^+$ in W3110(ColIb$^+$);* ▲, *BF23h$^+$ in W3110(ColIb$^-$);* ○, *BF23h$^-$ in W3110(ColIb$^+$);* △, *BF23h$^-$ in W3110(ColIb$^-$).*

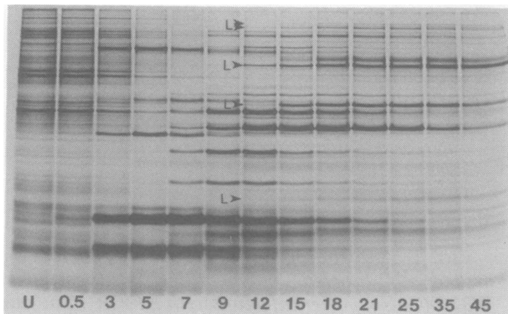

FIG. 3. *Autoradiogram of electrophoretically separated ^{14}C-labeled polypeptides synthesized in W3110(ColIb$^+$) after infection with BF23h$^-$. The number or letter below each gel pattern has the same meaning as in the legend to Fig. 1. Pre-early and early polypeptides are comparable to those in Fig. 1. Late polypeptides are designated L.*

If BF23h$^-$, rather than BF23h$^+$, was used to infect W3110(ColIb), the level of intracellular nucleotides increased with time after infection in a manner similar to that for W3110 infected with BF23h$^+$, and no extracellular nucleotides were detectable (Fig. 8). Infection of W3110 by BF23h$^-$ gave similar results (data not shown). Again, [^{32}P]ATP spots were counted to confirm the visual impression (Fig. 6 and 7).

These results showed that the mechanism by which the growth of BF23h$^+$ was arrested in ColIb$^+$ cells involved gross changes in permeability of the membranes of the infected cell. Furthermore, the permeability changes induced by infection by BF23h$^+$ could be prevented by a mutation in pre-early gene A3 that yielded the BF23h$^-$ phenotype.

Since the cause of abortive infection in ColIb$^+$ cells is the presence of the ColIb plasmid, it is reasonable to expect that appropriate mutations in the plasmid should prevent abortive infec-

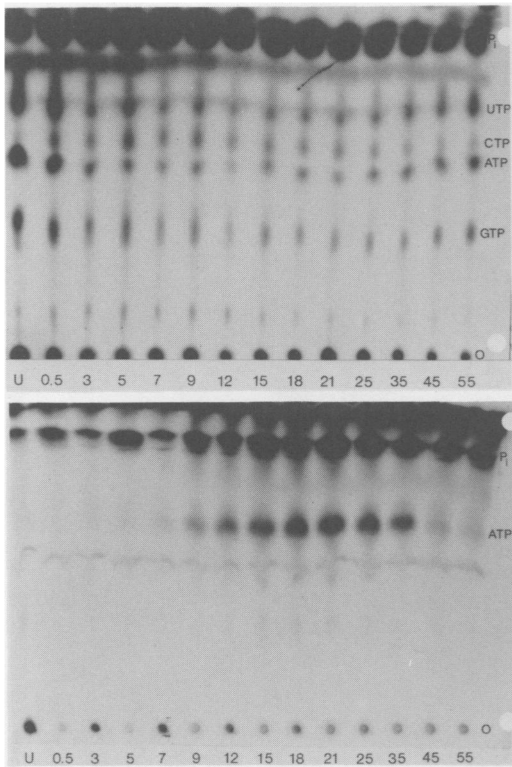

FIG. 4. *Autoradiogram of intracellular (upper) and extracellular (lower) ${}^{32}P$-labeled nucleotides extracted from W3110(ColIb⁺) at various times after infection with BF23h⁺ and after separation by thin-layer chromatography on PEI-cellulose. The number below each pattern specifies the time after infection at which the sample was taken for extraction of ${}^{32}P$-labeled nucleotides. U designates the autoradiographic pattern of ${}^{32}P$-labeled nucleotides extracted from cells before infection.*

tions. MUT26 is a strain of *E. coli* that harbors a ColIb plasmid that is mutated in a manner that allows a productive infection by BF23⁺ or T5⁺ (see above). Infection of MUT26 by BF23h⁺ caused the level of intracellular nucleotides to rise in a manner similar to that caused by a normal productive infection (i.e., BF23h⁺ in ColIb⁻ cells). Furthermore, no extracellular nucleotides could be detected (Fig. 9). Thus, the phage arrest mechanism could be prevented not only by a mutation in the phage genome (gene A3 specifically) but also by a mutation in the plasmid.

Since it has been proposed that early, as well as pre-early, gene products participate in the phage arrest mechanism (13), we infected ColIb⁺ and ColIb⁻ cells with BF23*am*M57h⁺ and BF23*am*M57h⁻. Both of these phage have an amber mutation in gene A1, and they therefore are able to transfer to *su*⁻ host cells only their FST DNA, which is composed of pre-early genes only. Infection of *su*⁻ ColIb⁻ cells with BF23*am*M57h⁺ caused the intracellular levels of ATP and GTP to decrease rapidly (Fig. 10A), as

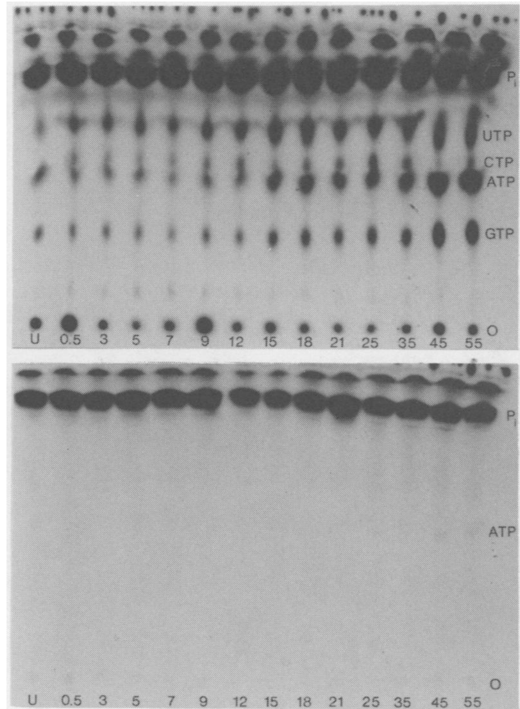

FIG. 5. *Autoradiogram of intracellular (upper) and extracellular (lower) ${}^{32}P$-labeled nucleotides extracted from W3110 at various times after infection with BF23h⁺ and after separation by thin-layer chromatography on PEI-cellulose. The number or letter below each pattern has the same meaning as in the legend to Fig. 4.*

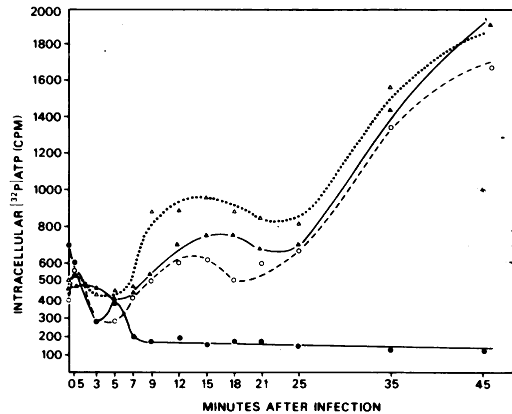

FIG. 6. *Quantitative determination of intracellular [${}^{32}P$]ATP at various times after infection of W3110 with BF23h⁺ (▲), W3110(ColIb⁺) with BF23h⁺ (●), W3110 with BF23h⁻ (△), and W3110(ColIb⁺) with BF23h⁻ (○).*

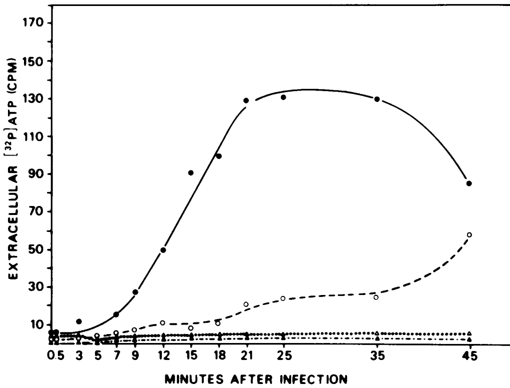

FIG. 7. *Quantitative determination of extracellular [^{32}P]ATP at various times after infection of W3110 with BF23h$^+$ (▲), W3110(ColIb$^+$) with BF23h$^+$ (●), W3110 with BF23h$^-$ (△), and W3110(ColIb$^+$) with BF23h$^-$ (○).*

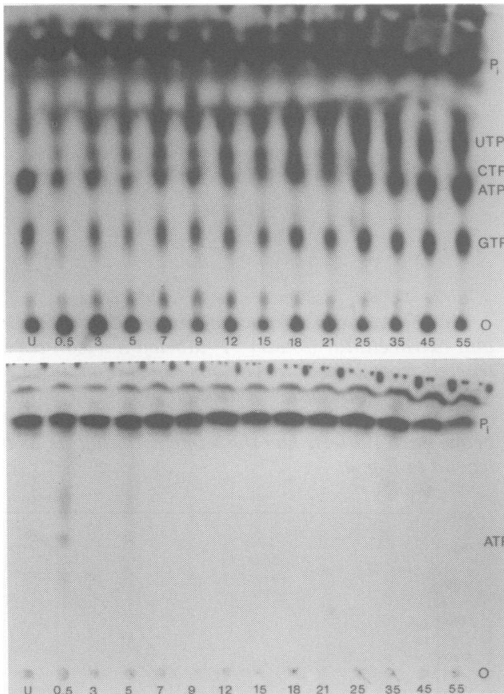

FIG. 8. *Autoradiogram of intracellular (upper) and extracellular (lower) ^{32}P-labeled nucleotides extracted from W3110(ColIb$^+$) at various times after infection with BF23h$^-$ and after separation by thin-layer chromatography on PEI-cellulose. The number or letter below each pattern has the same meaning as in the legend to Fig. 4.*

did infection of ColIb$^+$ cells with BF23h$^+$ (Fig. 4). Furthermore, some ATP appeared extracellularly (Fig. 11A). Infection of *su*$^-$ ColIb$^+$ cells with BF23*am*M57h$^+$ did not result in decreased intracellular levels of ATP and GTP or in the appearance of extracellular nucleotides (Fig. 10B and 11B). Infection of either *su*$^-$ ColIb$^+$ cells or *su*$^-$ ColIb$^-$ cells with BF23*am*M57h$^-$ also did

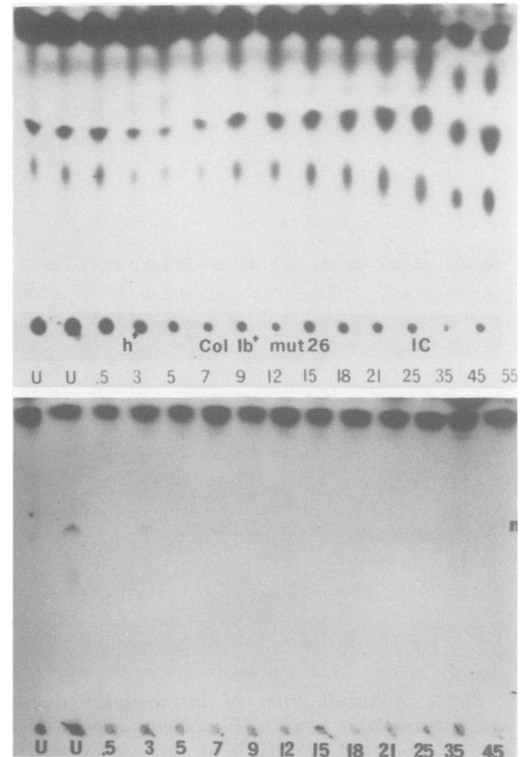

FIG. 9. *Autoradiogram of intracellular (upper) and extracellular (lower) ^{32}P-labeled nucleotides extracted from MUT26 at various times after infection with BF23h$^+$ and after separation by thin-layer chromatography on PEI-cellulose. The number or letter below each pattern has the same meaning as in the legend to Fig. 4.*

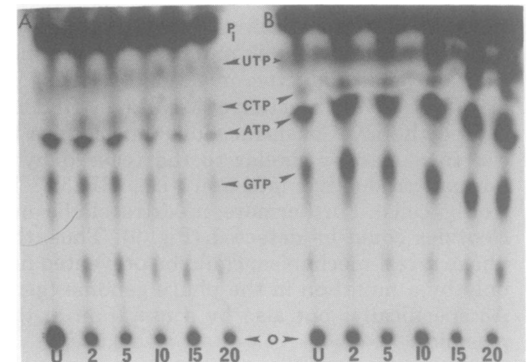

FIG. 10. *Autoradiogram of intracellular ^{32}P-labeled nucleotides extracted from W3110(ColIb$^+$) at various times after infection with BF23amM57h$^+$ (A) or with BF23amM57h$^-$ (B) and after separation by thin-layer chromatography on PEI-cellulose. The number or letter below each pattern has the same meaning as in the legend to Fig. 4.*

not result in decreased levels of ATP and GTP or in the appearance of extracellular nucleotides (Fig. 12 and 13).

Efflux of ^{42}K from cells not only is considered to be a sensitive measure of cell membrane integrity (30), but also may be indicative of the "energized state" of bacterial membrane structures involved in production of ATP (12). We therefore examined ^{42}K efflux from infected cells to extend our findings concerning efflux of ATP. When ColIb$^-$ cells were infected with either BF23h$^+$ or BF23h$^-$, the rate of efflux of ^{42}K increased over that from uninfected cells, but only for about 10 min. After 10 min, the rate of efflux was about the same as or less than that from uninfected cells (Fig. 14A). A similar transient efflux or ^{42}K occurred when ColIb$^+$ cells were infected with BF23h$^-$. However, when ColIb$^+$ cells were infected with BF23h$^+$, the transient increased rate of ^{42}K efflux did not stop but continued unabated until by 25 min only 10% of the initial ^{42}K remained in the infected cells (Fig. 14B). If chloramphenicol was present before infection of ColIb$^+$ cells with BF23h$^+$, continued efflux of ^{42}K was prevented (Fig. 14C).

The ^{42}K efflux data support the ^{32}P-nucleotide efflux data and indicate that the increased rate of ^{42}K efflux, which was transient in productive infections, continued unabated in abortive infections. The transient increased rate of ^{42}K efflux was stopped at about 9 to 10 min after infections that were productive, but continued past this time after infections that were abortive. This time was when protein synthesis started to be inhibited in abortive infections (Fig. 1 and 2).

Only pre-early, and thus no early, genes of the phage are required to induce the unabated ^{42}K efflux from infected cells. When ColIb$^+$ cells were infected with BF23*am*M57h$^+$, which can transfer only its FST DNA and thus only pre-early genes to host cells, increased ^{42}K efflux continued well past 9 to 10 min postinfection

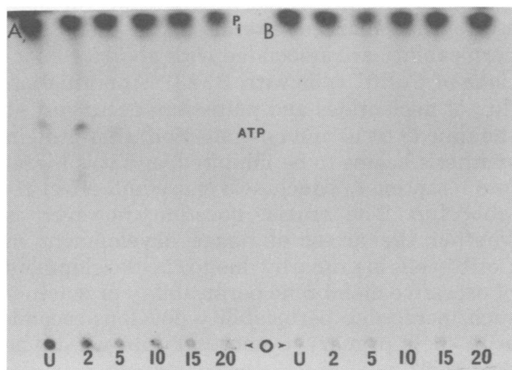

FIG. 11. *Same as for Fig. 10, but for extracellular* ^{32}*P-labeled nucleotides.*

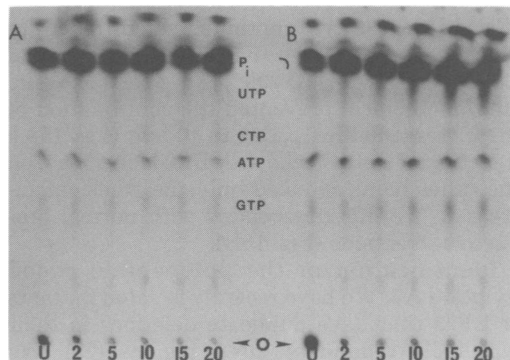

FIG. 12. *Autoradiogram of intracellular* ^{32}*P-labeled nucleotides extracted at various times after infection of W3110(ColIb$^+$) (A) or W3110 (B) with BF23amM57h$^-$ and after separation by thin-layer chromatography on PEI-cellulose. The number or letter below each pattern has the same meaning as in the legend to Fig. 4.*

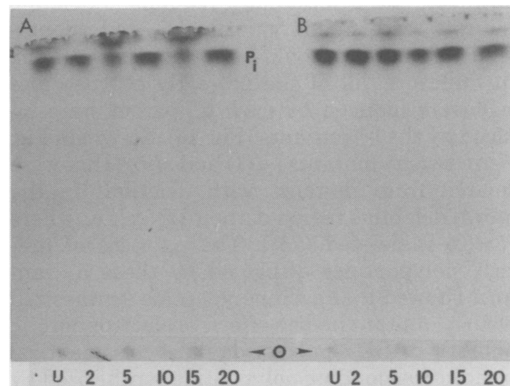

FIG. 13. *Same as for Fig. 12, but for extracellular* ^{32}*P-labeled nucleotides.*

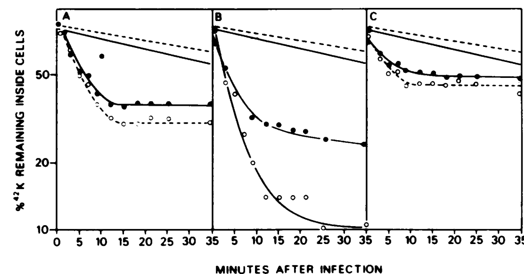

FIG. 14. *Efflux of* $^{42}K^+$ *from uninfected and infected cells as a function of time and the effect of chloramphenicol. Efflux from uninfected W3110 is shown by the solid line without symbols, whereas efflux from uninfected W3110(ColIb$^+$) is shown by the dashed line without symbols. (A) Efflux from W3110 after infection with BF23h$^+$* (○) *or BF23h$^-$* (●)*; (B) efflux from W3110(ColIb$^+$) after infection with BF23h$^+$* (○) *or BF23h$^-$* (●)*; (C) efflux from W3110(ColIb$^+$) in the presence of chloramphenicol after infection with BF23h$^+$* (○) *or BF23h$^-$* (●).

(Fig. 15A), just as it continued when the entire phage genome entered the cell (i.e., infection of ColIb$^+$ cells with BF23h$^+$ [Fig. 14B]). Again, chloramphenicol prevented the continuation of the increased efflux past 9 to 10 min (Fig. 15A). Infection of ColIb$^+$ cells with BF23*am*M57h$^-$, on the other hand, induced only the transient increased ^{42}K efflux associated with normal productive infections (Fig. 15B).

Identification of the polypeptide coded by gene A3. We have recently isolated mutants of BF23 that have duplicate deletions in their terminally redundant DNA regions (29). We considered that the deletions in these mutants may have included gene A3, particularly in view of the report that h$^-$ mutants of T5 do not synthesize one small pre-early polypeptide (31). If these mutants had gene A3 deleted, either they should be phenotypically h$^-$ or they should not be able to mutate to the h$^-$ phenotype. However, these terminally redundant deletion mutants were phenotypically h$^+$, and spontaneous h$^-$ mutants could be isolated from them. Furthermore, all of the pre-early polypeptides that were induced by each h$^+$ parent were induced by the h$^-$ mutants (Fig. 16). Shown in Fig. 16 are two h$^-$ mutants [*st*(1) and *st*(5)] that were isolated from mutants with deletions in the known deletable region of their DNA [i.e., where T5*st*(0) is deleted (28)]. The patterns of pre-early polypeptides displayed by these h$^-$ mutants showed that each polypeptide synthesized by an h$^-$ mutant had an effective electrophoretic mobility in SDS-polyacrylamide gels identical to the corresponding polypeptide synthesized by its h$^+$ parent, except polypeptide PE5 (Fig. 16; see McCorquodale et al. [22] for designations of pre-early polypeptides). Whereas PE5 from each h$^+$ parent migrated identically, PE5 from BF23*st*$^+$h$^-$, BF23*st*(1)h$^-$, and BF23*st*(2)h$^-$ migrated slightly faster, PE5 from BF23*st*(3)h$^-$ and BF23*st*(5)h$^-$ migrated slightly slower, and PE5 from BF23*st*(4)h$^-$ migrated at the same rate as PE5 from its h$^+$ parent. The migration of PE5 from BF23*st*(3)h$^-$ had slowed to such an extent that it coincided with PE4, making it appear that PE5 was totally missing. The changes in electrophoretic mobility of PE5 could be due to missense mutations that replace one amino acid with another of a different charge. Dunker and Rueckert (7) have shown that polypeptides with the same molecular weight but a different net intrinsic charge migrate differently in SDS-gels. Since PE5 is the only pre-early polypeptide whose effective electrophoretic mobility is variably affected by different spontaneous mutations that yield the h$^-$ phenotype, we conclude that PE5 is the product of gene A3 (gpA3).

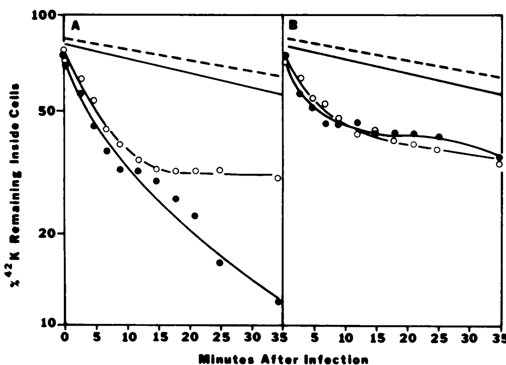

FIG. 15. *Effect of chloramphenicol on efflux of $^{42}K^+$ from W3110(ColIb$^+$) after infection with BF23amM57h$^+$ (A) or with BF23amM57h$^-$ (B). Symbols: ○, plus chloramphenicol; ●, minus chloramphenicol.*

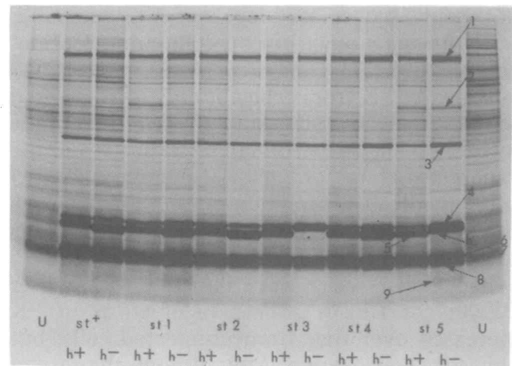

FIG. 16. *Autoradiogram of electrophoretically separated ^{14}C-labeled polypeptides synthesized in W3110 after infection with spontaneous h$^-$ mutants of the deletion mutants st(1), st(2), st(3), st(4), and st(5) and with BF23h$^+$ and BF23h$^-$. U designates the pattern from uninfected cells. Pre-early polypeptides are designated 1 through 9.*

DISCUSSION

The results presented in this paper demonstrate that marked alterations in membrane permeability are associated with abortive infections of ColIb$^+$ cells with BF23$^+$. Abnormal efflux of nucleotides and potassium occur just at the time (9 to 10 min postinfection) that protein synthesis begins to be inhibited, and it is therefore tempting to conclude a cause-and-effect relationship. The crucial question, however, is whether the arrest of phage development in ColIb$^+$ cells is primarily due to the development of excessive membrane permeability or whether such membrane permeability develops secondarily to a primary event that has yet to be detected.

From the data presented in this paper, it is

clear that the phage arrest mechanism requires the product of gene A3 (gpA3) of the phage and the product of one or more genes of the ColIb plasmid. Hull and Moody (15) have shown additionally that the product of at least two genes in the host genome, *cmrA* and *cmrB*, are also required for the phage arrest mechanism. Hence, we are faced with a mechanism that involves the interaction of gene products from three sources: host, phage, and plasmid.

How these gene products interact is suggested by a comparison of the events that occur during killing of sensitive cells by external colicin Ib and during abortive infection of ColIb$^+$ cells with BF23$^+$ (Table 2). This comparison very strongly suggests that the two phenomena are identical. The considerations which have led to this suggestion bear a striking resemblance to the pattern of thought that has led to our current understanding of the mechanism of cell killing by colicins E1, K, and I. Initially, it was thought that killing by these colicins was due to a reduction of intracellular ATP, just as we had thought that abortive infection of ColIb$^+$ cells was due to

TABLE 2. *Comparison of events that occur during killing of sensitive cells by external colicin Ib or Ia and during abortive infection of ColIb$^+$ cells with BF23$^+$ or T5$^+$*

Function or metabolic pool affected	Effect during killing of sensitive cells by external colicin Ib or Ia	Reference	Effect during abortive infection with BF23$^+$ or T5$^+$ in ColIb$^+$ cells	Reference
O_2 uptake	5 to 50% stimulation with glucose as substrate (Ib or Ia)	10, 19	Not yet measured	
	70% inhibition with glycerol as substrate (Ia)	10	70% inhibition of glycerol uptake (T5)	6
DNA synthesis	>98% inhibition (Ib)	19	Almost complete inhibition (BF23)	24
Degradation of DNA	No degradation (Ib)	19	No degradation (BF23)	27
RNA synthesis	>98% inhibition (Ib)	19	Almost complete inhibition (T5)	—[a]
Protein synthesis	Almost complete inhibition (Ib)	19	Almost complete inhibition (BF23)	24
Phospholipid synthesis	84% inhibition (Ib)	19	Not yet measured	
ATP synthesis	60% inhibition (Ib)	19	60% inhibition (BF23)	This paper
Non-nucleotide organic phosphates	Twofold increase (Ib)	19	Not yet measured	
Efflux of nucleotides	Not yet measured		Considerable efflux (BF23)	This paper, 6
Proline uptake	85% inhibition (Ia)	10	80% inhibition (T5)	—[a]
Proline efflux	Considerable efflux (Ia)	10	Not yet measured	
Succinate uptake	Strongly inhibited (Ia)	10	Not yet measured	
Thiomethyl-β-D-galactoside uptake	50% inhibition (Ia)	10	50% inhibition (T5)	6
Thiomethyl-β-D-galactoside efflux	Considerable efflux (Ia)	10	Not yet measured	
α-Methyl glucoside uptake	Tenfold stimulation (Ia)	10	Three- to fourfold stimulation (T5)	—[a]
Glutamine uptake	Not yet measured		Strongly inhibited (T5)	—[a]
K^+ efflux	Massive efflux (Ia)	10	Massive efflux (BF23)	This paper
Immunity breakdown in ColIb$^+$ cells	Occurs with high concentrations of external colicin Ib	19	Proposed to occur in BF23$^+$ and T5$^+$ infections	This paper

[a] J. Glenn and D. H. Duckworth, J. Virol., in press.

such a reduction. Later it became apparent that modification of the cytoplasmic membrane was the likely primary event (20).

Since colicins do not require outer membrane receptors to interact with the cytoplasmic membrane (33) and since an interaction of external colicin Ib with the cytoplasmic membrane of sensitive cells is the event that leads to killing (10), an identical interaction of liberated internal colicin Ib with the cytoplasmic membrane could be the event that leads to abortive infection of ColIb$^+$ cells with BF23$^+$. We therefore propose the following mechanism for the abortive response.

Phage protein, gpA3, inactivates the immunity protein which protects ColIb$^+$ cells from the killing action of the colicin Ib that they themselves produce. Killing of the infected cells by internal colicin Ib then occurs. If gpA3 is so altered by a mutation in gene A3 that it cannot inactivate the immunity protein, abortive infection is prevented. This is just what is shown by h$^-$ mutants of the phage. If the ColIb plasmid is mutated so that it cannot make normal colicin Ib or so that it produces an altered immunity protein that cannot be inactivated by gpA3 but that can still inactivate internal colicin Ib, abortive infection is also prevented. This may be how *pha*$^-$ mutants of the ColIb plasmid prevent abortive infections. Finally, if the internal cellular targets for colicin Ib, which are coded by the host genome, are altered, the internal colicin Ib that is activated because the immunity protein is inactivated cannot kill the cells because either the colicin Ib cannot recognize the cellular targets or, if they are recognized, they no longer transmit the influence of the colicin Ib to other molecules that bring about deterioration and death of the cell. This is what is shown by *cmr* mutants of the host (15).

Since our present data show that a massive efflux of K$^+$ is characteristic of abortive infections in ColIb$^+$ cells and since killing of sensitive cells by colicin Ia, the killing action of which is identical to that of colicin Ib, results in a massive efflux of cellular K$^+$ (10), we are led to suspect that the internal target for colicin Ib is the K$^+$ transport system in the cytoplasmic membrane. This suspicion is converted to a hypothesis by the report that two genetic loci for K$^+$ transport, *trkA* and *trkB* (8), map in positions strikingly similar to the genetic loci for *cmrA* and *cmrB* (15), the products of which, when mutated, allow a productive infection of ColIb$^+$ cells by BF23$^+$ (15). Furthermore, the *tolI* gene in the host chromosome, which when mutated confers tolerance to colicin Ib (4), maps in a region which includes another K$^+$ transport marker, namely, *trkC* (between 0 and 1 min [2]). We therefore feel that the evidence thus far available is so strongly in favor of a membrane target, namely, the K$^+$ transport system, both for external killing by colicin Ib and for the primary lesion in abortive infections of ColIb$^+$ cells by BF23$^+$ and its relatives, that the unifying hypothesis described above is tenable.

An essential component of our proposed mechanism is the liberation of free intracellular colicin Ib by the action of gpA3. The mechanism by which gpA3 liberates free colicin Ib is not yet known, but we have preliminary evidence that supports a mechanism by which the immunity protein is complexed by gpA3 (see also reference 24). At least two types of mutations in the ColIb plasmid should prevent the abortive response, one in the gene coding for the immunity protein and the other in the gene coding for the colicin Ib itself. Since our MUT26 mutant produces colicin Ib (data not shown), we conclude that its ColIb plasmid is mutated in the gene for the immunity protein. Mutations affecting the gene coding for the colicin Ib itself have been isolated by Tung, a student of E. E. Moody. The effects of such mutations on the infective process of T5 constitutes the strongest evidence in support of our mechanism. ColIb$^+$ cells, which give an abortive infection with T5, were mutated to *pha*$^-$ with phage Mu-1 so that infection with T5$^+$ was productive. All such *pha*$^-$ mutants still carried the ColIb plasmid, but produced either no colicin Ib or a colicin Ib that could no longer kill sensitive cells (S. S. Tung, M. S. thesis, University of Texas Health Science Center, San Antonio, 1975). Another component of the mechanism is that free intracellular colicin Ib or free external colicin Ib kills cells by interaction with inner membrane components that comprise the K$^+$ transport system. Since five genes are known to contribute to the K$^+$ transport system (8), it will be helpful for the finalization of the mechanism to know whether mutations in all five *trk* genes confer tolerance to external colicin Ib and allow a productive infection by BF23$^+$ in ColIb$^+$ hosts.

The proposed mechanism suggests that there are certain ionic conditions that may allow a productive infection of ColIb$^+$, colicin-intolerant cells by BF23$^+$, just as in the case of sensitive cells treated with colicin K (16). We are currently testing a number of ionic conditions that might prevent the abortive response due to the ColIb$^+$ plasmid.

We feel that our proposed mechanism may well apply to other abortive systems in which phage growth is arrested in cells that carry another colicinogenic plasmid (11), a sex plasmid (21), or a lysogen (9). As emphasized by Hardy (12), bacteriophage genomes, transmissible plasmids, and Col factors code for a specific immu-

nity to the effects of proteins for which they, themselves, code. A breakdown of this immunity system due to an infecting or a superinfecting phage could lead to an abortive infection.

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LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages, p. 445, 450–451, 456. Interscience Publishers, Inc., New York.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
3. **Britton, J. R., and R. Haselkorn.** 1975. Permeability lesions in male *Escherichia coli* infected with bacteriophage T7. Proc. Natl. Acad. Sci. U.S.A. **72**:2222–2226.
4. **Cardelli, J., and J. Konisky.** 1974. Isolation and characterization of an *Escherichia coli* mutant tolerant to colicins Ia and Ib. J. Bacteriol. **119**:379–385.
5. **Cashel, M., R. A. Lazzarini, and B. Kalbacher.** 1969. An improved method for thin-layer chromatography of nucleotide mixtures containing ^{32}P-labeled orthophosphate. J. Chromatogr. **40**:103–109.
6. **Cheung, A. K.-M., and D. H. Duckworth.** 1977. Membrane damage in abortive infections of colicin Ib-containing *Escherichia coli* by bacteriophage T5. J. Virol. **23**:98–105.
7. **Dunker, A. K., and R. R. Rueckert.** 1969. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. **244**:5074–5080.
8. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
9. **Garen, A.** 1961. Physiological effects of rII mutations in bacteriophage T4. Virology **14**:151–163.
10. **Gilchrist, M. J. R., and J. Konisky.** 1975. Effects of colicin Ia on transport and respiration in *Escherichia coli*. J. Biol. Chem. **250**:2457–2462.
11. **Gomez, B., and L. Nualart.** 1977. Requirement of the bacteriophage T7 0.7 gene for phage growth in the presence of the ColIb factor. J. Gen. Virol. **35**:99–106.
12. **Hardy, K. G.** 1975. Colicinogeny and related phenomena. Bacteriol. Rev. **39**:464–515.
13. **Herman, R. C., and R. W. Moyer.** 1975. *In vivo* repair of bacteriophage T5 DNA: an assay for viral growth control. Virology **66**:393–407.
14. **Howarth-Thomson, S.** 1969. Colicine I factors defective for UV protection. Proc. Univ. Otago Med. Sch. **47**:16.
15. **Hull, R., and E. E. Moody.** 1976. Isolation and genetic characterization of *Escherichia coli* K-12 mutations affecting bacteriophage T5 restriction by the ColIb plasmid. J. Bacteriol. **127**:229–236.
16. **Kopecky, A. L., D. P. Copeland, and J. E. Lusk.** 1975. Viability of *Escherichia coli* treated with colicin K. Proc. Natl. Acad. Sci. U.S.A. **72**:4631–4634.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Lanni, Y. T.** 1969. Functions of two genes in the first-step-transfer DNA of bacteriophage T5. J. Mol. Biol. **44**:173–183.
19. **Levisohn, R., J. Konisky, and M. Nomura.** 1967. Interaction of colicins with bacterial cells. IV. Immunity breakdown studies with colicins Ia and Ib. J. Bacteriol. **96**:811–821.
20. **Luria, S. E.** 1975. Colicins and the energetics of cell membranes. Sci. Am. **233**:30–37.
21. **Mäkelä, O. P., P. H. Mäkelä, and S. Soikkeli.** 1964. Sex specificity of bacteriophage T7. Ann. Med. Exp. Biol. Fenn. **42**:188–195.
22. **McCorquodale, D. J., A. R. Shaw, P. K. Shaw, and G. Chinnadurai.** 1977. Pre-early polypeptides of bacteriophages T5 and BF23. J. Virol. **22**:480–488.
23. **Mizobuchi, K., G. C. Anderson, and D. J. McCorquodale.** 1971. Abortive infection by bacteriophage BF23 due to the colicin Ib factor. I. Genetic studies of non-restricted and amber mutants of bacteriophage BF23. Genetics **68**:323–340.
24. **Mizobuchi, K., and D. J. McCorquodale.** 1974. Abortive infection by bacteriophage BF23 due to the colicin Ib factor. II. Involvement of pre-early proteins. J. Mol. Biol. **85**:67–74.
25. **Moyer, R. W., A. S. Fu, and C. Szabo.** 1972. Regulation of bacteriophage T5 development by ColI factors. J. Virol. **9**:804–812.
26. **Neidhardt, F. C., P. L. Block, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**: 736–747.
27. **Nisioka, T., and H. Ozeki.** 1968. Early abortive lysis by phage BF23 in *Escherichia coli* K-12 carrying the colicin Ib factor. J. Virol. **2**:1249–1254.
28. **Scheible, P. P., and M. Rhoades.** 1975. Heteroduplex mapping of heat-resistant deletion mutants of bacteriophage T5. J. Virol. **15**:1276–1280.
29. **Shaw, A. R., D. Lang, and D. J. McCorquodale.** 1979. Terminally redundant deletion mutants of bacteriophage BF23. J. Virol. **29**:220–231.
30. **Silver, S., E. Levine, and P. M. Spielman.** 1968. Cation effluxes and permeability changes accompanying bacteriophage infection of *Escherichia coli*. J. Virol. **2**:763–771.
31. **Strobel, M., and M. Nomura.** 1966. Restriction of bacteriophage BF23 by a colicin I (Col I-P9) factor. Virology **28**:763–765.
32. **Szabo, C., B. Dharmgrongartama, and R. W. Moyer.** 1975. The regulation of transcription in bacteriophage T5-infected *Escherichia coli*. Biochemistry **14**:989–997.
33. **Tokuda, H., and J. Konisky.** 1978. *In vitro* depolarization of *Escherichia coli* membrane vesicles by colicin Ia. J. Biol. Chem. **253**:7731–7737.