Comparative Study of the Events Associated with Colicin Induction

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Colicinogenic factors ColI and ColV, which have been shown to behave as sex factors, could not be induced with mitomycin C. In contrast, the ColE1, ColE2, and ColE3 factors, which do not exhibit any fertility factor characteristics, are inducible by this agent. The induced production of colicins E1, E2, and E3 was accompanied by a loss in viability at a concentration of mitomycin C which was bacteriostatic to noncolicinogenic cells or to cells carrying the ColV or ColII factors. The loss in viability accompanying the mitomycin C induction of the ColE1, ColE2, or ColE3 factors also occurred when colicin synthesis was blocked by chloramphenicol or amino acid starvation. However, chloramphenicol was able to block the loss of viability of a recipient cell after mitomycin C induction of a newly acquired Col factor if the antibiotic was present throughout the mating period. No detectable internal colicin or colicin precursor could be demonstrated during the lag period prior to the appearance of colicin outside the cell 20 to 30 min after the addition of mitomycin C. If chloramphenicol was present during the lag period following the addition of mitomycin C, colicin synthesis began immediately after the removal of these antibiotics. The synthesis of tryptophan synthetase and induced β-galactosidase proceeded normally throughout the lag period and well into the period of colicin production. Regulation of β-galactosidase synthesis did not seem to be profoundly affected during the lag period subsequent to mitomycin C addition. Induced colicin synthesis, like bacterial or induced prophage protein synthesis, was subject to inhibition by virulent phage infection.

Colicins are antibiotic proteins, produced by certain strains of Enterobacteriaceae, which are lethal for other related enteric bacteria. The ability to synthesize colicin depends upon the presence of a colicinogenic factor (Col factor). Certain Col factors are stable, nonessential genetic elements of plasmid nature (12) composed of deoxyribonucleic acid (4). Colicinogeny confers immunity to the host towards the homologous colicin in addition to the potential to synthesize colicin.

Col factors have been postulated to be defective fertility factors (16), defective lysogenic- phage genomes (15), and defective virulent-phage genomes (6), with little consideration given to the existence of physiological differences among various Col factors. Col factors differ in their ability to determine fertility properties (9), in their phage exclusion characteristics (21), and in their molecular weights (Roth and Helinski, Proc. Natl. Acad. Sci. U.S., in press). Colicins have historically been categorized on the basis of cross-immunity, serological cross-reactivity, and common cell-receptor sites as determined by the mutation to multiple resistance to colicins (5). They have also been shown to have markedly different modes of action (14). Thus, there appears to be an extensive spectrum in the genetic, physiological, and physical characteristics of different Col factors and in the structure and function of various colicins.

The synthesis of certain colicins may be induced by agents such as ultraviolet light (15) and mitomycin C (8), as well as by thymine starvation (18). No extensive comparison of the potential or physiology of induction of various colicins has been carried out, however. In the present study, colicinogenic factors of varying fertility characteristics were examined in Escherichia coli for inducibility by bacteriostatic levels of mitomycin C, and some of the physiological events accompanying the induction of inducible colicins were established.

Materials and Methods

Organisms. The bacterial strains employed in this study and their relevant characteristics are described in Table 1. In all cases, control experiments were per-
Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Requirements</th>
<th>Source of Co factor</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>YS40</td>
<td>His, Pro</td>
<td>ColE1 from K-30</td>
<td>Sm</td>
</tr>
<tr>
<td>YS40V</td>
<td>His, Pro</td>
<td>ColE1 from K-30</td>
<td>Sm</td>
</tr>
<tr>
<td>YS40V-T6</td>
<td>His, Pro</td>
<td>ColE2 from Shigella sp. P9</td>
<td>Sm, E1, E2, E3</td>
</tr>
<tr>
<td>YS40Az'</td>
<td>His, Pro</td>
<td>ColE2 from Shigella sp. P9</td>
<td>Sm</td>
</tr>
<tr>
<td>YS40(ColE1)</td>
<td>His, Pro</td>
<td>ColE2 from Shigella sp. P9</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110</td>
<td></td>
<td>ColV from K-94</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110(Sm')</td>
<td></td>
<td>ColV from K-94</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110(ColE1)</td>
<td></td>
<td>ColV from K-94</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110(ColE2)</td>
<td></td>
<td>ColV from K-94</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110SmE'(ColI)</td>
<td></td>
<td>ColV from K-94</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110SmE'(ColE)</td>
<td></td>
<td>ColV from K-94</td>
<td>Sm</td>
</tr>
<tr>
<td>W3110Sm'(ColI)</td>
<td>Leu, His, Arg</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>W3110Sm'(ColV)</td>
<td>Arg, Met</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>JC411(ColE3)</td>
<td></td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>Salmonella typhimurium LT2 (cysD36 ColI)</td>
<td></td>
<td>Cys</td>
<td></td>
</tr>
</tbody>
</table>

*Unless designated otherwise all strains are Escherichia coli. Abbreviations: His, histidine; Pro, proline; Leu, leucine; Arg, arginine; Met, methionine; Cys, cysteine; Sm, streptomycin; Az, azide; T6, phage T6; I, colicin I; V, colicin V; E1, colicin E1; E2, colicin E2; and E3, colicin E3.

formed with strains isogenic for all chromosomal characteristics and differing only in the absence of the colicinogenic factor. Strain W3110SmE'(ColE2) was prepared by transferring ColE2 from CA38(ColI, ColE2) (obtained from P. Frederiq) and removing ColI by acridine orange treatment as previously described (9). Salmonella typhimurium LT2 cysD36(ColI) was kindly provided by M. Nomura.

Media. All induction experiments were performed with Casamino Acids-supplemented minimal medium containing (per liter): Na2HPO4, 7 g; KH2PO4, 3 g; NH4Cl, 1 g; dextrose, 4 g; MgSO4, 10-4 M; FeCl3, 10-4 M; and Casamino Acids (Difco), 3 g. Nutrient broth was Antibiotic Medium 3 (Difco). Hard nutrient agar was prepared with Antibiotic Medium 3 supplemented with 1.5% agar. Soft nutrient agar contained 0.65% agar. When necessary, streptomycin was added at a final concentration of 250 μg/ml.

Lacunae counts and colicin assays. Lacunae counts, a measure of the number of individual cells producing colicin, were performed according to the procedure of Ozeki et al. (15), with E. coli YS40V as the indicator strain. The quantitation of colicin was carried out by treating a colicinogenic culture with chloroform, serially diluting the culture in nutrient broth, and spotting a drop of each dilution on a nutrient agar plate freshly seeded with 108 indicator bacteria (E. coli YS40V) in 5 ml of soft nutrient agar. The number of colicin units per milliliter was defined as the highest dilution which gave a clear zone of inhibition of growth of the indicator bacteria. The double-layer technique of Frederiq (5) was used to determine the percentage of colicinogenic cells in a bacterial population.

Standard growth and induction conditions. Cultures were inoculated and grown overnight in minimal Casamino Acids medium in a water bath (37°C). To initiate an induction experiment, the overnight culture was diluted to approximately 106 cells/ml with the supplemented minimal medium; 25 ml of the diluted culture was transferred to a 125-ml flask and placed in a rotary shaker (37°C). When the culture reached a bacterial concentration of approximately 5 x 108 cells/ml, mitomycin C (Nutritional Biochemicals Corp., Cleveland, Ohio) was added and incubation was continued at 37°C for the time required in each experiment.

Cell lysis. The lysis technique used was a modification of the lysozyme-ethylenediaminetetraacetic acid (EDTA)-sodium dodecyl sulfate technique. To 2.0 ml of culture to be lysed were added 1.8 ml of water, 0.2 ml of 0.07 M EDTA, and 0.08 ml of a 10 mg/ml lysozyme solution (Nutritional Biochemicals). After incubation for 5 min at 0°C, 0.04 ml of a 25% solution of sodium dodecyl sulfate was added. The preparation was diluted for assay immediately upon lysis, to minimize denaturation of colicin.

Enzyme assays. The tryptophan synthetase assay used was a modification of the method of Yanofsky (22), which measures the disappearance of indole due to conversion to tryptophan. Of the cells to be assayed, 1 ml was treated with a drop of toluene, stirred on a Vortex mixer, incubated for 10 min at 37°C, and allowed to settle in an ice bath; 0.5 ml of a reaction mixture containing 5 x 10-4 M glutathione, 8 x 10-4 M serine, 1.6 x 10-4 M pyridoxal phosphate, and 5 x 10-4 M indole in 0.1 M potassium phosphate buffer (pH 7.8) was added to 0.5 ml of toluene-treated cells. After incubation for 1 hr at 37°C, the reaction was stopped with 0.2 ml of 1 N NaOH, and the remaining indole was extracted in 2.0 ml of toluene. The amount of indole present in 1 ml of the toluene extract was determined by the p-dimethylaminobenzaldehyde color reaction described by Yanofsky (22).
**RESULTS**

**Inducibility of Col factors of varying fertility properties.** Strains possessing Col factors of varying degrees of fertility were grown to \(5 \times 10^8\) cells per ml in the minimal Casamino Acids medium, and one-half of each culture was exposed to mitomycin C (0.2 \(\mu\)g/ml). Under these conditions, there was little or no stimulation of colicin I or colicin V synthesis due to the presence of mitomycin C (Table 2). In contrast, the production of colicins \(E_1\), \(E_2\), and \(E_3\) was increased 100 to 1,000 times by the inducing conditions. In a separate experiment, colicin \(E_2\) was shown to induce to the same level from strains W3110-(ColE2) and W3110(ColI, ColE2).

**Mitomycin C induction as a lethal event for ColE2.** Ultraviolet light and temperature-sensitive induction of colicin \(E_2\) have both been reported to be lethal phenomena (10, 15).

As illustrated in Fig. 1, concentrations of mitomycin C bacteriostatic for noncolicinogenic cells caused lethal induction of colicin \(E_2\). Colicinogenic and noncolicinogenic cultures were grown to \(5 \times 10^8\) cells/ml and exposed to mitomycin C (0.2 \(\mu\)g/ml). At the times shown, the cultures were assayed for viable cells and lacunae. At the same time, samples of the induced W3110(ColE2) culture were removed, washed on a membrane filter, resuspended in growth medium at 37°C, and assayed for colicin by the dilution assay 2 hr after the addition of mitomycin C. The majority of colicin was bound to the cells under these conditions, and less than 10% of the colicin activity was removed by filtration. A 2-hr period following mitomycin C addition before assay was chosen to ensure maximal colicin production for each sample. Bacteria carrying ColE2 were committed to the inability to give rise to a colony with first-order kinetics when exposed to mitomycin C at a concentration bacteriostatic to the control noncolicinogenic bacteria. The decrease in viable cells was accompanied by a directly proportional increase in lacunae, or individual cells producing colicin, indicating that each cell committed to death became induced for the synthesis of colicin. The potential to produce lacunae and colicin increased throughout the induction period (Table 3).

**Correlation of induction of colicin synthesis and lethality for various Col factors.** ColE1, ColE2, and ColE3 strains produced relatively large amounts of their respective colicins after exposure to mitomycin C, whereas ColI and ColV cultures were not induced under the conditions employed in these experiments. To deter-

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**Table 2. Inducibility of various colicins**

<table>
<thead>
<tr>
<th>Escherichia coli strain</th>
<th>Without mitomycin C</th>
<th>With mitomycin C (0.2 (\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W3110(ColV)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>W3110(ColI)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>W3110(ColE2)</td>
<td>20</td>
<td>15,000</td>
</tr>
<tr>
<td>W3110(ColE2)</td>
<td>800</td>
<td>80,000</td>
</tr>
<tr>
<td>W3110(ColI, ColE2)</td>
<td>2,000(^b)</td>
<td>200,000(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Each culture was divided into two portions, one of which was exposed to mitomycin C. Both were then incubated for an additional 2 hr at 37°C and then assayed for colicin production (units per milliliter).

\(^b\) Assay of W3110(ColI, ColE2) on a colicin E-resistant indicator strain showed less than 1 unit of colicin I in both uninduced and induced cultures.
mine whether lethality is a common consequence of some aspect of the induction process for all of the inducible Col factors, colicinogenic and noncolicinogenic cultures were exposed to mitomycin C, and viable counts were carried out to determine the kinetics of commitment to death of colicinogenic bacteria. The viability curves (Fig. 2) confirm that the inducibility of the various colicins examined (Table 2) is reflected in the rates of kill upon exposure to a concentration of mitomycin C which is bacteriostatic to noncolicinogenic control cultures. In a separate experiment, the loss of viability of W3110(ColI, ColE3) and W3110(ColE2) due to mitomycin C induction was shown to be identical.

Separation of loss of viability and induced colicin production. Ozeki et al. (15) suggested that the production of colicin was responsible for the death of the induced cell. If this were the case, exposure of inducible colicinogenic bacteria to mitomycin C in the presence of chloramphenicol should protect the host from death. Figure 3 shows that cultures carrying ColE4, ColE3, or ColE2 died in the presence of chloramphenicol after the addition of mitomycin C. Under these conditions, there was no detectable increase in colicin titer. Mitomycin C in the presence of chloramphenicol did not cause the death of noncolicinogenic bacteria. The synthesis of colicin, therefore, is not responsible for the loss of viability. Some other aspect of Col factor induction that is independent of colicin production must cause the commitment to death of the induced cell.

**TABLE 3. Relationship between lacunae production and potential for colicin production after mitomycin C induction of the ColE2 factor**

<table>
<thead>
<tr>
<th>Time of removal of mitomycin C (min)</th>
<th>No. of lacunae/ml</th>
<th>Colicin activity (units/ml) after addition of mitomycin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$0.14 \times 10^8$</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>$0.40 \times 10^8$</td>
<td>1,500</td>
</tr>
<tr>
<td>60</td>
<td>$1.24 \times 10^8$</td>
<td>3,000</td>
</tr>
<tr>
<td>120</td>
<td>$3.60 \times 10^8$</td>
<td>4,000</td>
</tr>
</tbody>
</table>

*At the indicated times after addition of mitomycin C (0.2 μg/ml), 3.0-ml samples were washed on a membrane filter and suspended in fresh medium. Lacunae were assayed on 1-ml portions. The remaining 2-ml portion was placed in a water bath (37°C) and assayed for colicin production 120 min after the addition of mitomycin C.*

**FIG. 2.** Induced commitment to death of cells carrying different Col factors. Cultures were induced with MC (0.2 μg/ml): W3110 (○); W3110(ColI) (△); W3110(ColV) (■); W3110(ColE2) (□); W3110(ColI, ColE3) (▲); W3110(ColI, ColE2) (▲); W3110(ColI, ColE3) (▲).**

**FIG. 3.** Induction of colicinogenic bacteria in the absence of protein synthesis. Chloramphenicol (CAP; 50 μg/ml) was added at the time indicated, and the cultures were then treated with MC (0.1 μg/ml). Control, W3110 (○); YS40(ColE3) (□); W3110(ColE3) (△); W3110(ColI, ColE3) (▲).
genic, streptomycin-resistant recipient in the presence of chloramphenicol, by use of the ColI-promoted ColE2-transfer system of Stocker et al. (20). The source of ColE2 was W3110(ColE3). S. typhimurium LT2 cysD36(ColI) provided the ColI factor for promotion of transfer of ColE2 to the W3110Sm* recipient. The mating mixture was then exposed to mitomycin C in the presence of chloramphenicol, and the viability of the recipients was determined by plating on streptomycin-containing plates after vortexing and diluting the mating mixture. As seen in Fig. 4, if protein synthesis was allowed to occur during and subsequent to transfer of the factor to its new host, the newly acquired factor was inducible, causing a commitment to death of the recipient cell. When chloramphenicol was present throughout mating and during the period of exposure to mitomycin C, cell death did not occur, despite the fact that more than 90% of the recipient cells received the ColE2 factor. Transfer of the ColE2 factor under these conditions was measured after separating the mating pairs by vortexing, dilution, and counterselecting the donor of the ColE2 factor with streptomycin. Although protein synthesis is not necessary for induction (as defined by commitment to death) of a stable Col factor, a chloramphenicol-sensitive step is necessary for the susceptibility of a newly acquired Col factor to mitomycin C induction.

Lack of a detectable pool of internal colicin or colicin precursor during the lag period. Following induction of colicins E2 and E3 by mitomycin C, there is a lag period of 30 and 20 min, respectively, before the titer of colicin as measured in the dilution assay begins to increase. The assay technique employed, however, measures only the appearance of extracellular colicin. The time lag in appearance of colicin following induction may represent the time required for colicin synthesis, the time required for activation of an inactive colicin precursor, the time required for transport of newly synthesized colicin through the cell membrane, or some combination of these factors.

To determine whether an increase in internal colicin occurs during the lag period, cultures singly colicinogenic for ColE2 and ColE3 were induced with mitomycin C and assayed at various times to determine external colicin. At the same time, samples were lysed and assayed to determine internal plus external colicin. The lack of any detectable increase in internal colicin prior to the appearance of colicin activity on the chloroform-extracted cells is illustrated in Fig. 5. Despite the loss in activity due to the lysis procedure, it is evident that a significantly large pool of active colicin is not built up during the lag period.

In the early period following induction, an inactive precursor of colicin may be synthesized and converted to an active form at the end of the apparent lag period. To test this possibility, mitomycin C was added to cultures of ColE2+ and ColE3+ cells, and then chloramphenicol was

![Fig. 4. Protein-synthesis requirement for the induction of a newly acquired colicinogenic factor. Transfer of the ColE2 factor from Escherichia coli W3110(ColE2) to W3110Sm* was allowed to proceed for 20 min in the presence and absence of chloramphenicol (CAP; 50 μg/ml). CAP was added 20 sec after mixing the donor and recipient cells in order to allow effective mating pairs to form. Mitomycin C (0.1 μg/ml) was then added to both mating mixtures, and the viability of recipient cells was followed by plating in the presence of streptomycin. Salmonella typhimurium LT2 cysD36(ColI) × W3110(ColE2) × W3110Sm* + CAP (●); LT2 cysD36(ColI) × W3110(ColE2) × W3110Sm* (○).

![Fig. 5. Lack of an internal pool of colicin after induction. Cultures of Escherichia coli W3110(ColE2) and W3110(ColI, ColE2) were induced with mitomycin C (0.2 μg/ml). At the times indicated, one portion was chloroformed and assayed for external colicin production, and another portion was lysed and assayed to determine internal and external colicin. W3110(ColE2) (○); lysed W3110(ColE2) (□); W3110(ColI, ColE2) (●); lysed W3110(ColI, ColE2) (■).]
added at various times to samples of each culture. The samples were allowed to incubate for a total of 90 min after mitomycin C addition before assay. There was no increase in colicin titer for either colicin E₃ or E₄ after the addition of chloramphenicol, regardless of the time of addition during the lag period. These results indicate that either a large pool of inactive precursor did not accumulate, or its conversion to an active colicin molecule was prevented by blocking protein synthesis.

**Effect of inhibition of protein synthesis on the lag period.** As shown above, induction as defined by loss of viability in ColE₃⁺ and ColE₄⁺ cultures occurs under conditions in which colicin synthesis is prevented. It was of interest to determine whether the events occurring during the lag period leading to colicin production require protein synthesis. Mitomycin C induction of colicinogenic cultures was allowed to proceed in the presence of chloramphenicol. Mitomycin C and chloramphenicol were removed by filtration, the cells were resuspended in fresh medium, and the kinetics of colicin production were followed by assaying for colicin activity at 5-min intervals after removal of these agents. Control colicinogenic cultures were induced in the same fashion without chloramphenicol. Both colicin E₃ and E₄ synthesis began with little or no apparent lag after the removal of chloramphenicol (Fig. 6), whereas control cultures showed the characteristic lag previously described.

In a similar experiment, an amino acid-requiring ColE₄⁺ strain was induced under conditions of amino acid starvation to prevent protein synthesis. The kinetics of colicin production following restoration of the required amino acid were similar to those of the previously described experiment, again indicating that protein synthesis is not involved in the events occurring early in the lag period which lead to the subsequent production of colicin.

**Synthesis of bacterial enzymes during the colicin lag period.** ColE₃⁻ and ColE₄⁻ containing cultures and a noncolicinogenic strain were grown for two generations in the minimal Casamino Acids medium supplemented with 2 × 10⁻³ M isopropylthigalactoside (IPTG) to derepress β-galactosidase synthesis, exposed to mitomycin C, and assayed for colicin, tryptophan synthetase, and β-galactosidase production at the times indicated in Fig. 7. The synthesis of both enzymes continued at a rate similar to the control noncolicinogenic culture throughout the lag period and well into the period of colicin production, diminishing when the rate of synthesis of colicin also began to decrease.

Although the induction of ColE₃⁺ and ColE₄⁺ cultures does not prevent synthesis of at least certain host enzymes during the lag period, it could interfere with the regulation of protein synthesis in the host. To examine this question, the colicinogenic cultures, grown with glucose as a carbon source, were assayed for β-galactosidase activity following colicin induction, and were also tested for their ability to respond to IPTG during the period after exposure of the cultures to mitomycin C. β-Galactosidase synthesis could be induced by IPTG during the lag period of both colicin E₃ and E₄ induction. In addition, induction of either colicin by mitomycin C did not result in a release of β-galactosidase repression in the absence of its specific inducer.

**Inhibition of colicin production by virulent phage.** It is well established that bacterial protein synthesis stops almost immediately after infection with T-even phages. Similarly, lysogenic phage induction can be prevented by infection with a T-even phage (19). To determine whether colicin induction is susceptible to virulent phage shut-off, ColE₃⁺ and ColE₄⁺ cultures were induced with mitomycin C, and samples were infected at various times with phage T6 at a

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**Fig. 6. Kinetics of induced colicin production after removal of chloramphenicol.** Cultures of Escherichia coli W3110(ColE₃⁻) and W3110(ColI, ColE₄⁻) were prepared for induction in the usual fashion, and were divided into two portions. The first was induced with mitomycin C (0.2 µg/ml) at time zero, and colicin assays were performed as indicated. Chloramphenicol (50 µg/ml) and mitomycin C (0.2 µg/ml) were added to the second portion at time -50 min (ColE₃⁺) and -30 min (ColE₄⁺). These cultures were washed on membrane filters and suspended in fresh medium at time zero, and the synthesis of colicin was determined at various times.

W3110(ColE₃⁻) + MC (○); W3110(ColE₄⁻) + MC + CAP (□); W3110(ColI, ColE₃⁻) + MC (●); W3110(ColI, ColE₄⁻) + MC + CAP (■).
Fig. 7. Synthesis of bacterial enzymes during induction. Mitomycin C (0.2 μg/ml) was added to cultures of W3110, W3110(CoIE2), and W3110(CoIE1, CoIE3) as indicated by the arrows. Culture turbidity (○); β-galactosidase activity (△); tryptophan synthetase activity (□); colicin E2 activity (●); colicin E3 activity (■).

Discussion

The experiments described above clearly indicate that the colicinogenic factors CoIV and CoII employed in these studies are physiologically quite distinct from Col factors CoIE1, CoIE2, and CoIE3. CoIV and CoII are not induced with respect to colicin production after exposure to mitomycin C. Colicinogenic factors CoIE1, CoIE2, and CoIE3, however, are significantly induced by this agent. Genetic analysis has shown that CoIV is intimately associated with a fertility factor (9). CoII has been demonstrated to be a highly efficient fertility factor following recent entry into a new host (3). In contrast to the marked ability of CoIV and CoII to act as fertility agents in E. coli, CoIE1 is not capable of promoting either its own transfer or the transfer of chromosomal markers to noncolicinogenic recipients (9, 13). Its transfer to a noncolicinogenic cell is dependent upon the presence of fertility factors such as the F factor, CoIV, or CoII. CoIE2 is also unable to promote its own transfer or the transfer of any other genetic material (unpublished data). Transfer of CoIE2 can be carried out with high efficiency, as described above, by a modification of the CoII transfer system of Stocker et al. (20). However, Hfr- or F+-promoted transfer of CoIE2 is quite low, in contrast to the very effective transfer of CoIE1 in the presence of these fertility factors (12, 13). CoIE3 is similarly unable to promote its own transfer and appears to be transferred only at a very low frequency by fertility factors present in the same cell (unpublished data). Thus, those Col factors (CoIV, CoII) which behave as sex factors appear to be inducible by mitomycin C under the conditions used in this study, and do not render a cell significantly more sensitive to mitomycin C induced-killing than noncolicinogenic bacteria. CoIE1, CoIE2, and CoIE3, which cannot serve as effective fertility agents, are highly inducible. In addition, cells possessing these factors are readily killed by concentrations of mitomycin C that do not kill noncolicinogenic bacteria. The difference in response to mitomycin C of these two classes of Col factors may well reflect fundamental differences in the processes responsible for the regulation of the replication and phenotypic expression of these distinct nonchromosomal genetic elements. It should be pointed out that, although the results described here with mitomycin C distinguish between the Col factors...
CoII and CoIV, on the one hand, and CoE1, CoE2, and CoE3, on the other, ultraviolet light induction of an E. coli strain carrying CoII has been reported to produce an increase in both the number of lacunae and the colicin I titer (3).

Ozeki et al. (15) demonstrated that ultraviolet induction of an S. typhimurium strain carrying CoE2 produces an increase in the number of lacunae present in the cell population. They also demonstrated by micromanipulation techniques that single cells of S. typhimurium that have produced colicin after ultraviolet-light induction are not able to divide, and on this basis concluded that induction is a lethal event. Ben Gurion (2) reported that, despite the increase in lacunae after irradiation, CoE2+ cells grown in minimal medium do not show an increased sensitivity to ultraviolet-induced killing. In her studies, colicinogenic cells grown in broth, in contrast, showed a marked increase in sensitivity to ultraviolet-light irradiation, although there did not appear to be any significant difference in lacunae production after irradiation of colicinogenic cells grown in poor and rich media. In contrast to the results of Ben Gurion, Amati (1) reported that cells carrying colicinogenic factors CoII, CoE1, and CoE2, when grown in broth, did not show an increased sensitivity to ultraviolet light. Kohiyma and Nomura (10) isolated a temperature-sensitive chromosomal mutant of an E. coli CoE2+ strain in which increased temperatures caused colicin induction and a loss of viability. They did not report, however, the effect of elevated temperatures on the loss of viability of the mutant strain after curing of the CoE2 factor. With a system in which the question of lethality due to induction is not obscured by death of the host due to the action of the inducing agent on the cell, the experiments described above indicate that mitomycin C induction of strains possessing CoE1, CoE3, or CoE3 is accompanied by death of the host bacterium.

Ozeki et al. (15) suggested that the production of colicin was the cause of death of the host bacterium. It is evident from the above data, however, that chloramphenicol is able to prevent the production of colicins E1, E3, and E3 but not the loss of viability following the addition of mitomycin C to colicinogenic bacteria. Commitment to death is, therefore, not dependent on the synthesis of colicin, or presumably any other protein, after the addition of mitomycin C. The commitment to cell death must result from some event in the induction sequence not requiring protein synthesis. This result is quite similar to the results described by Lieb (11), demonstrating that heat induction of certain temperature-inducible λ phage mutants could cause death of the host, despite inhibition of protein synthesis by chloramphenicol.

The induction of colicinogenic factors does not cause a sudden cessation of host function. Bacterial enzyme synthesis appears to proceed quite normally during the lag period, and even during much of the period of colicin synthesis. Therefore, the death of the host does not appear to be caused by a Col factor-induced shut-off of host-specific protein synthesis during the lag period. The lack of any significant effect of induced colicin production on β-galactosidase repression or induction during the lag period suggests that regulation of the synthesis of at least certain host proteins is not affected during the lag period and is likewise probably not the cause of cell death.

Our observation that a cell containing a newly acquired Col factor could not be induced if deprived of the opportunity to synthesize protein prior to exposure to mitomycin C is surprising, since it suggests that Col factor-directed synthesis of protein may be required for the initiation of induction. The presence of the noninduced Col factor confers immunity on the host cell, indicating that the noninduced Col factor is certainly not completely dormant physiologically. It is not unlikely that the noninduced factor directs the synthesis of a protein whose activity is required to initiate induction, while not being transcribed in the noninduced state for postinduction proteins such as colicin. Such a protein normally may play a role in the replication or maintenance of the Col factor. Green (7) proposed that cells lysogenic for the temperature inducible λ prophage described above must synthesize an "inducing protein" in the repressed state to permit prophage susceptibility to thermal induction. It is also possible that acquisition of a Col factor induces the synthesis of a protein determined by the bacterial chromosome and required for the maintenance of the factor and also for the initiation of induction.

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