Mitomycin C and Temperature Induction of Colicin B in the Absence of Deoxyribonucleic Acid Synthesis

CELMA HAUSMANN* AND ROYSTON C. CLOWES
Division of Biology, The University of Texas at Dallas, Dallas, Texas 75230
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An Escherichia coli K-12 strain and its mutant, temperature-sensitive in deoxyribonucleic acid (DNA) synthesis, were used as hosts for two different ColB factors (ColB3-K166 and ColB4-K98). Induction of either colicin occurred in both hosts in the presence of mitomycin C at 42 C. Induction of the temperature-sensitive colicogenic hosts also occurred without mitomycin C at 42 C at which temperature no DNA synthesis was observed. Colicin synthesis was a nonlethal event in ColB4-K98+ cultures, whereas in cultures of ColB3-K166+ induction led to death of a large fraction of the population.

Synthesis of colicins by cells of Escherichia coli and related species is coded by plasmids, the colicin (Col) factors. This synthesis can be enhanced (induced) by agents such as ultraviolet radiation (12) or mitomycin C (11) and in temperature-sensitive (ts) strains by elevated temperatures (10). Colicin induction by mitomycin C of several colicin factors was studied by Herschmann and Helinski (9) who found that those colicin factors endowed with sex factor activity (ColV2-K94 and ColBb-P9) were not susceptible to mitomycin C induction, whereas others without sex factor activity (ColE1-K30, ColE2-P9, ColE3-CA38) were mitomycin C inducible. Ozeki et al. (13), analyzing individual cells of Salmonella typhimurium carrying a ColE2 factor, demonstrated that those cells which actively produced colicin did not undergo further cell division. The authors suggested therefore that colicin synthesis was a lethal event. Induced synthesis of colicin has in some cases been suggested to be related to increased replication of the colicin factor (1). However, in other cases (10), colicin E1 induction independent of normal deoxyribonucleic acid (DNA) replication was shown by using a mutant strain ts in DNA synthesis (ts-DNA). However, under these conditions, about 3% normal synthesis still occurred, and, since the size of the ColE1 factor is only about 0.2% that of the chromosome (2), a 15-fold replication of E1 cannot be excluded.

In this work two ColB factors, both endowed with sex factor activity (6), were used. As host, a strain whose DNA synthesis at the nonpermissive temperature of 42 C is reduced to less than 1% normal was used (3). Under these conditions, the inducing action of mitomycin C was tested, and the culture was analyzed with regard to DNA synthesis and cell survival.

MATERIALS AND METHODS

E. coli K-12 strains 165 (HfrH thy- str-r λ-) and its ts-DNA derivative 166 (3), kindly provided by F. Bonhoeffer, were each infected with ColB3-K166 or ColB4-K98 (8) (herein after referred to as ColB3 and ColB4) by mixed overnight culture with an E. coli K-12 58-161 strain harboring the appropriate colicin factor. Cultures of these strains grown at 30 C in 2.5% Oxoid nutrient broth no. 2 to exponential phase were transferred to a shaking water bath and further incubated at 42 C. Mitomycin C (Calbiochem) was added to a final concentration of 0.2 μg/ml, and samples were withdrawn at intervals thereafter. The cells were killed by chloroform and the free colicin was determined by spot-testing serial dilutions on a lawn of colicin B-sensitive indicator bacteria. The highest dilution inhibiting growth of the indicator lawn was used as a measure of the amount of free colicin. DNA synthesis was measured by adding to exponential cultures 3H-thymidine and deoxyadenosine to 1 μCi/ml and 200 μg/ml final concentrations, respectively. After further incubation, samples were taken, treated with an equal volume of cold 10% trichloracetic acid, and filtered through membrane filters; acid-insoluble radioactivity was measured on the filters in a scintillation counter (Beckman Instruments, Inc.)

RESULTS

Table 1 shows that incubation of the ts host strain at 42 C induced colicin production in both the ColB3 and ColB4 derivatives, whereas no induction was seen with wild-type host strains.

* Present address: Institut für Genetik, Universität Freiburg, West Germany.
† To whom all requests for reprints should be addressed.
Addition of mitomycin C induced colicin production in the wild-type cultures and increased the amount of induction in the ts-DNA cultures. Figure 1 shows cell survival under these conditions.

**Table 1. Induction of colicins B3 and B4**

<table>
<thead>
<tr>
<th>Strains (coli factor)</th>
<th>Colicin titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No mitomycin C</td>
</tr>
<tr>
<td>165 ColB3+</td>
<td>1</td>
</tr>
<tr>
<td>166 ColB3+</td>
<td>150</td>
</tr>
<tr>
<td>165 ColB4+</td>
<td>1</td>
</tr>
<tr>
<td>166 ColB4+</td>
<td>250</td>
</tr>
</tbody>
</table>

In Fig. 1A, it can be seen that there was no increase in the killing effect of temperature on the ColB4+ host strain compared to its ts-DNA parental noncolicinogenic strain, whereas the survival of the ColB3+ host strain was proportionately reduced to about 1%. These killing effects were enhanced in the presence of mitomycin C. Again no increase in lethality could be shown associated with the induction of colicin B4, whereas the survival of ColB3+ cells was about 1% of the ts noncolicinogenic parent strain. DNA synthesis in these strains at 42°C is shown in Fig. 2 where it can be seen that, although synthesis continued uninterruptedly in the wild-type strain, it was reduced to less than 1% in its ts mutant and in the two colicinogenic derivatives of this mutant.

Amati (1) suggested that replication of colicin factors is necessary for colicin synthesis and Dewitt and Helinski (7) later demonstrated a 30- to 100-fold increase in ColEl-specific DNA after mitomycin C treatment of a Proteus mirabilis ColEl+ strain which also increased colicin titers to comparable levels. Other workers have shown that induction of colicin E2 occurs under conditions where DNA synthesis is reduced to about 3% of the normal level (10). However, this level would still permit about a 15-fold increase in E1 molecules (2). In the case of the two ColB factors studied here, induction of colicin-B occurred...
at 42 C, a condition in which DNA synthesis of the hosts was reduced to less than 1% normal.

The molecular sizes of the ColB factors and of the F factor that is associated with the ColB3 factor (6) are each approximately 2% the bacterial genome (Clowes, unpublished data). Thus, a simple doubling of the plasmid DNA would have resulted in about a 2% increase, if most cells had been induced. In the case of ColB4, the number of cells induced was not determined, but in the ColB3+ culture, death of cells could be taken as a measure of the extent of induction since the fraction of killed cells is proportional to the extent of colicin induction. On this assumption, it appears that more than 99% of the cells are involved in colicin synthesis, and thus, if DNA replication of the Col factor were necessary for induction, it should have resulted in a higher value than the 1% measured.

However, although increase in colicin synthesis may be thus assumed not to require Col factor replication, colicin induction does occur under those conditions which affect DNA metabolism, such as elevated temperatures in ts-DNA hosts or addition of mitomycin C. Since it has been previously demonstrated (7) that replication of ColE1 DNA occurs under inducing conditions, it may be assumed that the two phenomena, although usually occurring simultaneously, are at least in some instances separate events. Induction thus appears to result in simultaneous but independent stimulation of replication and transcription of the colicin factor genomes.

Although in some instances death of colicin E2-producing cells has been observed (13), it has been concluded that the two events may be separated (9). Colicin synthesis by ColB4 does not obviously depend upon a killing step; although when ColB3 in induced, the host cells are lethally affected. It seems reasonable to assume that the two colicin factors, although producing similar colicins, judged by cross resistance, are physiologically different (8) as indeed they are different genetically (6).

The original observations restricting induction to those colicin factors without sex factor activity (9) appear to be fortuitous, and these two properties are apparently not related. More importantly perhaps, those factors with sex factor activity (ColIb and ColV2) investigated by Helinski appear to be stringently controlled in their replication, about one copy of the plasmid being present for every copy of chromosome (reference 5 and unpublished data), whereas those without sex factor activity are more relaxed in their replication and are present as multiple copies (4). A correlation between relaxed replication and ability to be induced might therefore also be assumed. However, since ColB2 is also a large plasmid (70 x 10^4 atomic mass units), stringently controlled in its replication (reference 5 and unpublished data), this conclusion would not appear to be warranted.

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