# TRANSCRIPTION OF THE COL E1 GENOME IN COLICINOGENIC *E. coli* STRAINS DURING INDUCTION WITH MITOMYCIN C

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## 1. Introduction

The extrachromosomal colicinogenic factor E1 (Col E1) determines the production of the antibiotic protein colicin E1. However, only few *Escherichia coli* cells carrying the colicinogenic factor spontaneously produce this protein. Treatment with mitomycin C induces a high proportion of the cells to produce colicin E1 [1]. Addition of cyclic 3′:5′-adenosine monophosphate (cAMP) results in an additional stimulation of colicin E1 synthesis [2].

The induction of colicin E1 has been reported to depend on an increased amount of plasmid DNA [3]. The positive effect of cAMP, however, points towards a participation of the cAMP/crp system of catabolite repression [4,5], i.e. a regulation on the transcriptional level. The additional stimulation of colicin E1 synthesis by cAMP could also be a secondary effect, as glucose and cAMP are cooperative in colicin production [2].

In this communication it is shown that the regulation of colicin E1 production primarily takes place on the transcriptional level, with a possible additional regulation occurring at translation of the colicin specific mRNA that seems to be cAMP/crp dependent.

## 2. Materials and methods

*E. coli* JC411 (Col E1) has been described previously [6]. Col E1 DNA was isolated by the lysozyme–brij technique which has been described in various instances [7,8]. Isolation of $^{3}H$ pulse-labelled RNA was performed according to Okamoto et al. with slight modifications [9,10]. The DNA–RNA hybridization experiments were carried out as described by Denhardt [11] and Gillespie [12]. In vitro Col E1 RNA was synthesized by RNA polymerase (Boehringer) on purified Col E1 DNA as described by Westphal and Dulbecco [13]. Modifications and other experimental details are given in the legends to the figures.

## 3. Results

Treatment of a culture of *E. coli* JC411 (Col E1) with mitomycin C (3 μg/ml) or mitomycin C (3 μg/ml) and cAMP (350 μg/ml) results in a considerable induction of colicin E1 synthesis without stimulating the plasmid DNA replication. As shown in table 1, there is no increase in the plasmid content (relative to the chromosomal DNA) of the cells even under conditions where high levels of colicin E1 are synthesized. This indicates that the induction of colicin E1 synthesis is not dependent on an enhanced plasmid DNA replication. This result is in agreement with the data of other workers [14,15].

Hybridization of cellular RNA from isogenic Col E1$^{-}$ and Col E1$^{+}$ strains, which have been pulse-labelled with [$^{3}H$]uridine for 3 min, shows a plasmid-specific RNA fraction in the Col E1$^{+}$ strain (fig. 1a). At saturation, when an increase in immobilized plasmid DNA no longer results in an increased binding of RNA, 0.65% of the pulse-labelled RNA isolated from the plasmid containing strain is bound. The



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Table 1
Amount of Col E1 DNA synthesized under induction conditions

| Treatment | Time after treatment when label added (min) | Duration of label (min) | Supercoiled plasmid DNA (%) | Colicin E1 (units) |
|---|---|---|---|---|
| – | 5 | 30 | 1,3 | 5 |
| Mitomycin C | 5 | 30 | 1,7 | 160 |
| Mitomycin C | 50 | 30 | 1,4 | 320 |
| Mitomycin C cAMP | 50 | 30 | 1,4 | 640 |

A logarithmically growing culture of *E. coli* JC411 (Col E1) was divided in 4 equal parts and treated with 3 μg/ml mitomycin C and 350 μg/ml cAMP. Colicin titers were estimated by lacunae tests [17]. The DNA was labelled with 10 μCi/ml [$^3$H]thymidine (spec. act. 17 Ci/mmol) for 30 min. The cells were lysed and Col E1 DNA was partially separated from the bulk of chromosomal DNA by the lysozyme-brij procedure [7,8]. The supercoiled Col E1 DNA was isolated bij the dye buoyant method of Radloff et al. [18]. The plasmid containing fractions were pooled and centrifuged in 5–20% neutral sucrose gradients for 120 min at 45 000 rev/min in a SW65 type rotor at 20°C. The plasmid content is expressed as percent of label in supercoiled Col E1 DNA related to the total cellular DNA.

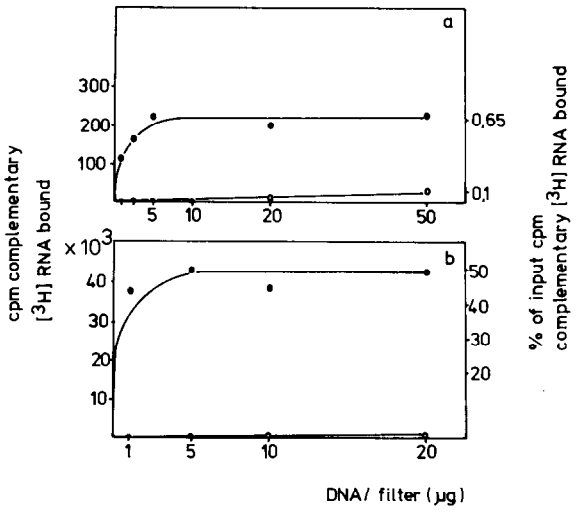


Fig. 1a. Hybridization of pulse-labelled RNA isolated from Col E1$^-$ and Col E1$^+$ strains with increasing amounts of denatured Col E1 DNA immobilized on nitrocellulose membrane filters [11,12]. (o–o–o) RNA isolated from a Col E1$^-$ strain; input counts were constant at 600 000 cpm. (•–•–•) RNA isolated from a Col E1$^+$ strain; input counts were constant at 30 000 cpm. 1b. Hybridization of $^3$H-labelled RNA synthesized in vitro by *E. coli* RNA polymerase on Col E1 DNA with increasing amounts of denatured plasmid DNA immobilized on nitrocellulose membrane filters (•–•–•); input counts were constant at 80 000 cpm. Control with increasing amounts of denatured calf thymus DNA immobilized on nitrocellulose filters (o–o–o); input counts were constant at 80 000 cpm. Hybridization was carried out in vials containing 1 ml of RNA solution at 66°C. The salt concentration was 6 × SSC. After 20 hr the vials were chilled in ice and the filters were removed and washed with 6 × SSC. After treatment with 20 μg/ml pancreatic RNase for 45 min at 25°C in 2 × SSC and extensive washing the filters were dried and counted.

pulse-labelled RNA isolated from the plasmid-negative strain shows only slight binding to Col E1 DNA and probably is unspecific. Under these conditions, the hybridization efficiency as estimated by hybridization of pure $^3$H-labelled Col E1-specific RNA synthesized in vitro by *E. coli* RNA polymerase on a Col E1 DNA template was 50% (fig. 1b). Thus, the amount of plasmid-specific RNA in total pulse-labelled RNA from plasmid-containing cells can be calculated to be 1.3%.

In plasmid-containing cells which are treated with 3 μg/ml mitomycin C for 60–120 min prior to the addition of [$^3$H]uridine a higher level of plasmid-specific RNA is observed (fig. 2). About 8% of the pulse-labelled RNA (corrected value) isolated from such induced cells is now plasmid-specific. The

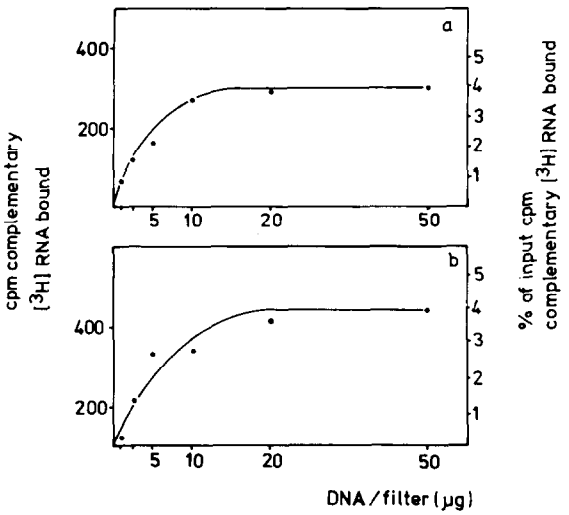


Fig. 2. Hybridization of pulse-labelled RNA isolated from induced Col E1-containing cells with increasing amounts of denatured Col E1-DNA immobilized on nitrocellulose filters. a. RNA isolated from cells induced with 3 μg/ml mitomycin C; input counts were constant at 10 000 cpm, b. RNA from cells induced with 3 μg/ml mitomycin C and 350 μg/ml cAMP, input counts were constant at 15 000 cpm. Hybridization was carried out as described in fig. 1.

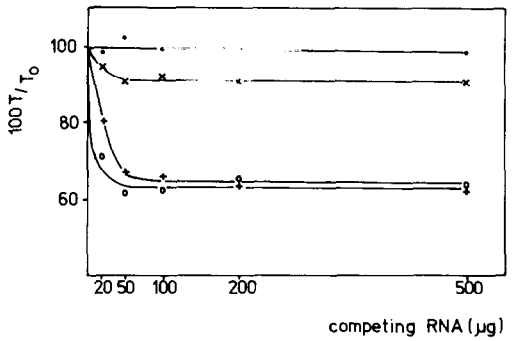


Fig. 3. Competition hybridization between unlabelled RNA of induced and uninduced colicinogenic cultures and $^3$H-labelled RNA synthesized in vitro by *E. coli* RNA polymerase on Col E1 DNA. In the first hybridization step increasing amounts of unlabelled RNA isolated from (X–X–X) uninduced cells, (○–○–○) cells induced with 3 μg/ml mitomycin C, (+–+–+) cells induced with 3 μg/ml mitomycin C and 350 μg/ml cAMP were hybridized to a constant amount (2 μg) of pure denatured plasmid DNA (66°C, 20 hr); (●–●–●) control with soluble yeast RNA. After treatment with 20 μg/ml pancreatic RNase for 45 min at 25°C in 2 × SSC and extensive washing, constant amounts (80 000 cpm) of the $^3$H RNA were hybridized in the second hybridization step (55°C, 20 hr) to the filters. The filters were then treated with RNase, washed dried and counted. T is the amount of radioactivity (in cpm) hybridized to Col E1 DNA in the second hybridization step, $T_0$ the equivalent cpm without prehybridization.

enhanced transcription of the Col E1 DNA reaches a plateau level (8%) about 50 min after addition of mitomycin C and remains constant for at least a further 60 min (results not shown). Additional treatment of mitomycin C-induced Col E1 cells with 350 μg/ml cAMP gives no higher level of Col E1-specific RNA. No increase at all in Col-specific RNA is observed when Col E1$^+$ cells are treated with 350 μg/ml without addition of mitomycin C.

Competition hybridization experiments [12] using labelled RNA from plasmid-containing cells and $^3$H-labelled RNA synthesized in vitro by *E. coli* RNA polymerase with pure Col E1 DNA as template have been performed to determine the extent of transcription of the Col E1 genome under induced and uninduced conditions (fig. 3). The results show that an excess of unlabelled RNA from an uninduced Col E1$^+$ strain reduces hybridization of in vitro synthesized Col E1-specific RNA with Col E1 DNA to 90%, whereas an excess of unlabelled RNA from colicinogenic cells induced with mitomycin C or mitomycin C and cAMP reduces hybridization of in vitro Col E1 RNA with Col E1 DNA to 60%. If one makes the reasonable assumption that in vitro transcription of Col E1 DNA lacks strand specificity, the competition hybridization data indicate that in non-induced Col E1$^+$ cells only about 20% of the plasmid genome, is transcribed, whereas in induced Col E1$^+$ cells roughly 80% of the entire genetic information of the plasmid is expressed.

## 4. Discussion

The mechanism of colicin E1 induction in colicinogenic *E. coli* cells is not connected with a stimulated replication of plasmid DNA, but as shown by the DNA–RNA hybridization data, appears to involve a stimulated transcription of the plasmid genome. Whereas in uninduced colicinogenic *E. coli* cells only little Col E1 specific RNA can be detected, after the

addition of mitomycin C, the amount of this RNA increases considerably. Addition of cAMP, which further stimulates colicin E1 synthesis, does not result in a further stimulation of the transcription of Col E1 DNA. The competition hybridization data indicate that in uninduced Col E1 cells only part of the plasmid genome is transcribed. Taking into consideration that the Col E1 plasmid determines about 8–10 proteins (unpublished results), this transcript contains enough information for 2–3 proteins, which may be engaged in the replication of plasmid DNA (unpublished results). Upon induction with mitomycin C almost the entire genome seems to be transcribed. Induced production of colicin E1 in addition requires cAMP and the cAMP receptor protein (crp) ([15] and unpublished results). Thus the induced transcription of the Col E1 genome following treatment of the cells with mitomycin C is not sufficient for the induced synthesis of colicin E1. This seems to indicate that the translation of the colicin E1-specific RNA needs an intact cAMP/crp system.

We assume that cAMP together with cAMP receptor protein (crp) may be required for the synthesis of another factor necessary for the translation of the colicin E1 specific mRNA and that this factor may be a chromosome-coded protein. Evidence for the involvement of a chromosomal factor in colicin E1 synthesis is provided from studies on plasmid containing minicells of *E. coli* [16]. In these minicells the plasmid DNA is transcribed and several Col E1 DNA specific proteins are translated but no biologically active colicin E1 is synthesized (manuscript in preparation).

The nature of this factor is entirely speculative at the moment. It may be a regulator substance allowing the translation of colicin E1 specific mRNA or a ribonuclease converting an immature precursor RNA to mature colicin E1 mRNA.

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