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Synthesis of Colicin E1 in a Cell-Free System

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Colicin E1 was synthesized in a cell-free system. The in vitro synthesis was found to be dependent on the Col E1 DNA concentration and was not enhanced by the addition of mitomycin C.

Under regular growth conditions, the synthesis of colicin E1 is repressed in *Escherichia coli* cells harboring Col factor E1. Upon ultraviolet irradiation or the addition of chemicals, such as mitomycin C, however, synthesis of colicin E1 is induced (3, 4). As there is only scarce information available as to the mechanism of colicin induction and the regulation of its synthesis in vivo, it seems useful to study these processes in vitro. In a first approach to this problem, we give evidence that colicin E1 can be synthesized in a cell-free system.

Col E1 DNA was isolated by centrifugation in dye-buoyant density gradients (7) of cleared lysates of *E. coli* W3110 (Col E1-K 30) lysozyme spheroplasts which were treated with Brij 58 (1). Plasmid bands obtained from several centrifugations were pooled and examined in the electron microscope by the Kleinschmidt technique (6). The preparation contained only Col E1 DNA. The contour length for the open circle was 2.2 μm, which is in agreement with published data (8). Sixty-eight percent of the molecules were covalently closed circular DNA and 32% were open circles.

From *E. coli* 514 F⁻ (*lac*Δ, *trp*, *tsx*^{r}, *ton*^{r}, and *str*^{r}) a cell extract was prepared as described by Zubay et al. (9) with the exception that the cells were grown in yeast-peptone medium in 2-liter batches at 37 C on a New Brunswick shaker. The cells were harvested in the late log phase by low-speed centrifugation at 4 C and were used immediately for the preparation of the cell extract. The incubation mixture had a volume of 0.4 to 0.5 ml and contained all components in concentrations as given in the original method (9). For each cell extract, Mg^{2+} and Ca^{2+} concentrations had to be optimized. A typical example is given in the legend to Fig. 1.

Since in *E. coli* 514 the *lac* operon is deleted, the efficiency of the cell extract to synthesize protein was tested by determination of the β-galactosidase synthesized after addition of λ *plac* DNA to the cell-free system.

When Col E1 DNA was added to the incubation mixture, a bacteriocidal substance was synthesized. That this substance in fact was colicin E1 was concluded from several observations. (i) Spontaneous *E. coli* mutants resistant against the in vitro-synthesized antibiotic were also resistant against purified colicin E1. (ii) The amount of the antibiotic produced was dependent on the concentration of Col E1 DNA in the system (Fig. 1) and increased linearly in the range of 10 to 80 μg of DNA per ml of

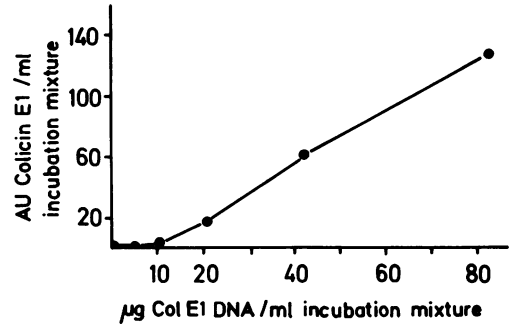

FIG. 1. *Dependence of colicin E1 synthesis on Col E1 DNA concentration. Synthesis was performed under optimized conditions (magnesium acetate, 16.5 mM; calcium acetate, 8.3 mM) with 8.6 mg of protein per ml of cell extract and Col E1 DNA at concentrations as indicated. All other components were used in concentrations as given by Zubay et al. (9) with the only exception being that dithiothreitol was replaced by dithioerythritol at the same molarity. After an incubation period of 70 min at 37 C, colicin E1 activity in the system was assayed. NB-soft agar was seeded with 10^8 cells of E. coli W3110 in stationary phase and layered onto NB-streptomycin agar plates (50 μg of streptomycin per ml). Volumes (0.01 ml) of serial dilutions of the incubation mixture were placed on the agar and the plate was incubated overnight at 37 C. The number of "arbitrary units" (AU) per ml of colicin E1 was defined as the reciprocal of the highest dilution which still prevented macroscopic growth of the indicator lawn.*

incubation mixture. In control experiments, no antibiotic activity was detectable in the Col E1 DNA preparation, nor was there any in the complete incubation mixture when Col E1 DNA was omitted.

Mitomycin C at final concentrations between 0.1 and 0.8 μg/ml, usually sufficient for colicin induction in vivo, had no influence on the amount of colicin E1 synthesized in vitro. Whether this finding means that mitomycin C is not converted to the active state (5) in the cell-free system or whether other interpretations are needed to explain the failure of mitomycin C to stimulate colicin E1 synthesis under these conditions remains to be clarified.

The repression of colicin E1 synthesis may be due either to an active repressor molecule or to the fact that more than 95% of the Col E1 DNA molecules in the cells are supercoiled (8) and may not be transcribed in this conformation. Induction of colicin synthesis may act by inactivating the repressor molecule and/or by activating a nuclease which transfers the supercoiled DNA to an open circle. The Col E1 relaxation complex, consisting of a protein molecule linked to supercoiled DNA (2), may be the natural state of a repressed Col E1 factor. Experiments to determine the plasmid DNA conformation required for the in vitro synthesis of colicin E1 are in progress.

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