SYNTHESIS OF COLICIN E1 IN A CELL-FREE COUPLED TRANSCRIPTION–TRANSLATION SYSTEM

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Protein was synthesized in a cell-free coupled transcription–translation (S30) system on plasmid Col E1 and phage T2 and Sd DNA templates. Phage T2 DNA and closed plasmid DNA proved to be the most active templates. The latter controlled the synthesis of eight individual proteins *in vitro*, including the biologically active protein antibiotic colicin. The titer of colicin synthesized in this cell-free system reached 1024 units/ml, two orders of magnitude higher than the titer of the antibiotic in populations of colicinogenic bacteria.

KEY WORDS: *Cell-free transcription–translation system; colicin; plasmid Col E1 DNA.*

In connection with the widespread use of plasmid DNAs in genetic engineering research in the last 2 years interest has risen sharply in the biochemistry and genetics of these factors of cytoplasmic inheritance [9, 13]. To identify the products of expression of the plasmid genome a promising method is to use a cell-free coupled transciption–translation system. Such systems are widely used to study the expression of virus genomes [5, 8], but they have been used only occasionally to study plasmid DNAs and then only to study the synthesis of the resistance factor to colicin E3 [11].

In this investigation coupled transcription–translation was carried out on a Col E1 DNA template isolated from cells of a colicinogenic strain of *Escherichia coli*, with a molecular weight of $4.2 \cdot 10^6$ daltons, as a result of which a number of specific proteins were synthesized, including the biologically active protein antibiotic colicin.

EXPERIMENTAL METHOD

An S30 cell-free system was obtained from *E. coli* Q13, defective for RNase. The cells were disintegrated with the aid of lysozyme and the S30 system was prepared by the method of Zubay and Chambers [15]. Incubation was carried out for 1 h at 37°C and the final volume of the reaction mixture was 0.05 ml.

Col E1 DNA was isolated from *E. coli* P678-54 (Col E1), obtained from J. Inselburg, by the method of Clewell and Helinski [4], followed by purification by centrifugation in a CsCl density gradient with ethidium bromide. The methods of cultivation and purification of phages T2 and Sd and their extraction from DNA were described previously [3].

Colicin was titrated by Tirdo's method [1] on an indicator strain of *E. coli* phage and in derivative resistant to colicin E1. Electrophoresis of the proteins in polyacrylamide in the presence of sodium dodecylsulfate, autoradiography, and determination of the molecular weight were all described previously [2, 7, 12].

EXPERIMENTAL RESULTS AND DISCUSSION

Data on total protein synthesis in the S30 system, based on incorporation of ^{14}C-amino acids into the acid-insoluble fraction, and also on synthesis of the specific protein coli-

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TABLE 1. Synthesis of Total Protein and Biologically Active Colicin in an Intracellular S30 Transcription–Translation System*

DNA template and condition	Incorporation of ^{14}C-amino acids, counts/min/μg DNA × 10^{-3}†	Colicin titer, units/ml
Complete S30 system + Col E1 DNA	33.5	1024
The same without incubation	0.7	0
The same after trypsin treatment	—	0
Complete S30 system + phage T2 DNA	37.8	0
The same + phage Sd DNA	5.1	0
The same without DNA	0.7	0

*Results of a typical experiment.
†Concentration of ^{14}C-amino acid mixture (Radiochemical Centre, Amersham, England) was 2 μCi per sample.

TABLE 2. Characteristics of Polypeptide Material Synthesized in Cell-Free System under Control of Col E1 DNA

No. of polypeptide	Molecular weight (× 10^3 daltons)
1	60*
2	53
3	44
4	40
5	29
6	19
7	16
8	11
Total	272

*Judging by the degree of blackening of the film this protein was synthesized in the greatest amount.

cin, determined by titration on *E. coli*, are given in Table 1. Plasmid Col E1 DNA and DNA of phages T2 and Sd were added to the system as templates controlling protein synthesis. The addition of DNA to the system clearly increased the synthesis of polypeptide material by 10-50 times; DNA of phage T2 and Col E1 plasmid were found to be the most active templates. The protein synthesized on the Col E1 template had the specific ability to inhibit reproduction of the colicin-sensitive strain *E. coli* phage, but had no action on reproduction of the isogenic colicin-resistant strain. The protein nature of the synthesized product was confirmed by experiments with trypsin, incubation with which prevented the inhibitory effect. The titer of colicin synthesized in the S30 cell-free system was extremely high and reached 1024 units/ml, or two orders of magnitude higher on average than the titer of this antibiotic obtained under ordinary conditions in a suspension of colicinogenic cells. This result was due to the presence of the corresponding repression of antibiotic synthesis in the cells by the cell genome, which is absent in the system for synthesis *in vitro*. Although a complete kinetic analysis of the operation of the coupled transcription–translation system was not undertaken, it was found that biologically active colicin was present in the system as early as 6 min after the beginning of incubation.

Meanwhile *in vitro* colicin production begins not earlier than 20 min after induction by mitomycin C [6]. The total quantity of proteins synthesized in the coupled system of synthesis was determined by electrophoresis in polyacrylamide gel followed by autoradiography. In the autoradiographs obtained in the case of Col E1 DNA eight clear bands representing the main polypeptide chains were found (Table 2). These eight basic proteins with molecular weights of between 60 · 10^3 and 11 · 10^3 daltons were not synthesized in the S30 system on T2 DNA and Sd DNA templates or in the control without DNA. The total molecular weight of these proteins was about 280 kilodaltons, or rather more than the coding capacity

of Col E1 DNA (about 230 kilodaltons). This discrepancy can be explained on the grounds that some of the polypeptides found were in fact either abortive translation products or products of a single gene, the mRNA of which later underwent processing by the mechanism described for phage Qβ [14].

Colicin is evidently the largest polypeptide synthesized in a cell-free system in an amount greater than that of all other proteins. According to the results of these experiments it had a molecular weight of 60 kilodaltons, very close to the figure obtained for colicin by Schwartz and Helinski [10], namely 56 kilodaltons. The results open up definite prospects for mapping the genome of the Col E1 plasmid and of various hybrid plasmids created on its basis.

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