JOURNAL OF BACTERIOLOGY, Dec. 1983, p. 1359–1362
0021-9193/83/121359-04$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 156, No. 3

In Vitro Coupled Transcription-Translation of Linear DNA Fragments in a Lysate Derived from a *recB rna pnp* Strain of *Escherichia coli*

CAROLE LEAVEL BASSETT AND JAMES R. Y. RAWSON*
Botany Department, University of Georgia, Athens, Georgia 30602

Received 8 August 1983/Accepted 27 September 1983

A *recB21* derivative (CLB7) of an *Escherichia coli rna-19 pnp-7* strain (PR7) was constructed for use in examining the in vitro coupled transcription-translation of linear DNA. The expression of linearized DNAs in CLB7 (*recB21 rna-19 pnp-7*) lysates was enhanced significantly when compared with expression of the same DNAs in lysates prepared from the PR7 or the original *recB21* (CF300) strains. In addition, the endogenous incorporation of [^{35}S]methionine into protein was considerably reduced in CLB7 lysates relative to lysates derived from the original *recB21* strain.

In vitro DNA-dependent coupled transcription-translation systems prepared from *Escherichia coli* have proven useful for identifying DNA sequences that have the potential to encode specific polypeptides (3, 12). However, the expression of DNA in commonly used RNase-deficient strains is limited to either circular or relatively large linear DNAs. This problem is largely due to the presence in these lysates, of exonuclease V, which degrades small linear DNA molecules (11). If one eliminates exonuclease V (*recB recC*) activity by using lysates prepared from the strain CF300 (*recB21*), the expression of linear DNAs is greatly enhanced (11). On the other hand, the high level of endogenous incorporation of [^{35}S]methionine into protein in CF300 lysates may obscure the identification of polypeptides encoded by added DNA (11). Since RNase-deficient strains (*rna*) show a much lower level of endogenous incorporation of radioactive amino acids and inactivation of polynucleotide phosphorylase (*pnp*) activity has been shown to enhance the expression of several genes in vivo (4, 5), lysates were prepared from a *recB21 rna-19 pnp-7* strain (CLB7) and were used to examine the in vitro expression of linear plasmid DNAs.

Construction of *E. coli* CLB7 was as follows. A Thy$^-$ derivative of PR7 (*rna-19 pnp-7*), obtained by trimethoprim selection (8), was transduced with P1 phage which had been grown on CF300. The Thy$^+$ transductants were selected on minimal media containing no added thymine and were tested for sensitivity to UV light by replica plating (2). Strain CLB7 was one of the Thy$^+$ UV^s transductants resulting from this cross. Inheritance of the *recB21* mutation was confirmed by marker rescue analysis with *E. coli* JC5422 (*thyA325 argE3 his4 thrl leu6 proA2 thil*) as the recipient for P1 phage grown on CLB7. The cotransduction frequency between *recB* and *thyA* was 46 to 51%, which is in good agreement with previously reported frequencies of 34 to 50% (10).

Cell free lysates were prepared from *E. coli* PR7, CLB7, and CF300 by the method of Zubay et al. (12) as modified by Andrews and Rawson (1). Each lysate was used to examine the expression of several different plasmid DNAs that were either in the supercoiled or linear form. Figure 1 shows the results of an experiment in which the plasmid pBR325 was digested with either *Bam*HI or *Pst*I and expressed in either the PR7, CLB7, or CF300 lysate. Digestion of pBR325 DNA with *Pst*I inactivates the ampicillin resistance gene, resulting in the disappearance of the 32-kilodalton (kd) β-lactamase polypeptide but the continued synthesis in CF300 and CLB7 lysates of chloramphenicol transacetylase (24 kd) from the chloramphenicol determinant and several of the gene products (32, 13.5, and 8.2 kd) from the tetracycline resistance gene (Fig. 1, lanes C and E). When pBR325 DNA digested with *Bam*HI was added to either the CF300 or the CLB7 lysate, the chloramphenicol transacetylase and β-lactamase polypeptides were effectively expressed (Fig. 1, lanes D and F). Lysates derived from PR7 showed a dramatic reduction in the expression of pBR325 DNA linearized with either *Bam*HI or *Pst*I (Fig. 1, lanes A and B). Expression of supercoiled pBR325 DNA was similar in all three lysates and was generally more efficient than expression of linear plasmid DNA (data not shown). Lysates from strain

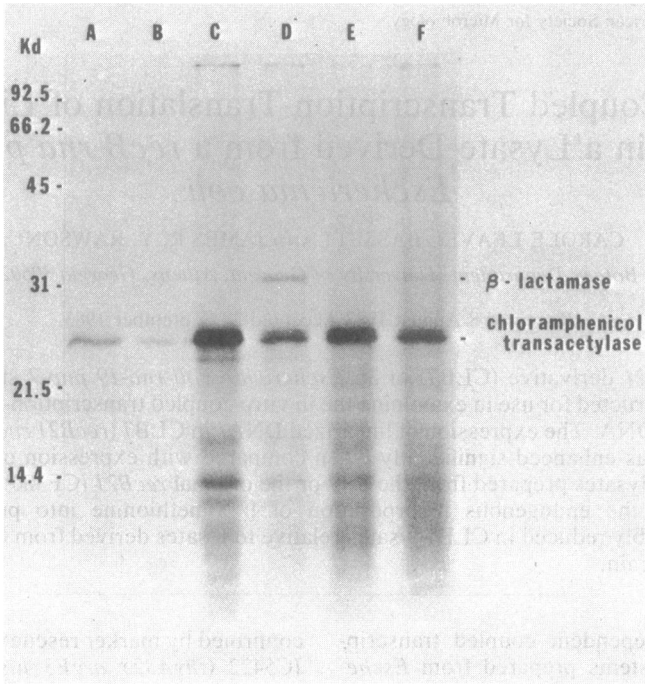

FIG. 1. Autoradiographs of SDS-polyacrylamide gels showing the [^{35}S]methionine-labeled polypeptides synthesized in vitro from linear pBR325 DNA in *E. coli* PR7, CLB7, and CF300 lysates. The pBR325 DNA was digested with either *Bam*HI or *Pst*I. The digested DNAs were precipitated with 2 volumes of ethanol and suspended in TE buffer (50 mM Tris-hydrochloride [pH 8], 1 mM EDTA). The linearized DNAs (12 μg of pBR325 DNA digested with *Pst*I or 3 μg of pBR325 digested with *Bam*HI) were resuspended in 40 μl of translation cocktail (11) containing [^{35}S]methionine (48 μCi, 1,280 Ci/mmol). Lysate (10 μl) prepared from either PR7, CLB7, or CF300 was added to these mixtures and incubated for 1 h at 37°C. The reaction mixtures were then precipitated with 20 volumes of acetone at −20°C for 12 to 16 h. The precipitates were suspended in electrophoresis buffer (6) and equal volumes of each mixture were loaded onto an SDS-polyacrylamide (15% [wt/vol]) gel. Electrophoresis was carried out, and autoradiographs were prepared as described by Andrews and Rawson (1). These autoradiographs show polypeptides made in vitro from: lane A, pBR325 DNA digested with *Pst*I in the PR7 lysate; lane B, pBR325 DNA digested with *Bam*HI in the PR7 lysate; lane C, pBR325 DNA digested with *Pst*I in the CLB7 lysate; lane D, pBR325 DNA digested with *Bam*HI in the CLB7 lysate; lane E, pBR325 DNA digested with *Pst*I in the CF300 lysate; and lane F, pBR325 DNA digested with *Bam*HI in the CF300 lysate.

CF300 routinely showed a three- to sixfold greater endogenous incorporation of [^{35}S]methionine when compared with lysates from strains PR7 and CLB7. When lysates from strains PR7 and CLB7 were examined for endogenous incorporation of [^{35}S]methionine into protein on sodium dodecyl sulfate (SDS)-polyacrylamide gels, a single distinct polypeptide (68 kd) was detected in both lysates (see Fig. 3, lane A), whereas a smear and numerous distinct polypeptides were observed with the lysate from strain CF300 (data not shown).

The expression of two recombinant plasmids containing specific bacterial genes was also examined in these different lysates. The plasmid pDPK15 contained a *Bam*HI-*Eco*RI DNA fragment carrying the *E. coli sbcB* region inserted into the vector pMOB45 (9). Among the products expressed in minicells from this plasmid was a 53-kd polypeptide identified as exonuclease I (*sbcB*) (9). When supercoiled pDPK15 DNA was used to program lysates from strains PR7, CLB7, and CF300, we saw the 53-kd polypeptide in all cases (data not shown). When this plasmid was digested with *Bam*HI and expressed in the CLB7 lysate, the 53-kd product and several unidentified polypeptides were observed (Fig. 2). These same unidentified polypeptides were also observed when supercoiled pDPK15 DNA was expressed in vitro (data not shown). Similar polypeptides were seen when this plasmid was expressed in vivo in *E. coli* minicells (9). When pDPK15 DNA linearized with *Bam*HI was expressed in the PR7 lysate, we did not see the synthesis of the 53-kd polypeptide. The detection of the expression of the 53-kd polypeptide from pDK15 DNA digested with *Bam*HI in the CF300 lysate was variable and seemed to depend on the relative stimulation of protein synthesis in the system due to the

addition of exogenous DNA over that of endogenous protein synthesis.

The second recombinant plasmid examined in these lysates was pVMK52, which carries the *E. coli uvrD* region inserted into the *Pvu*II site of pBR325 (7). The DNA fragment carrying the *uvrD* region contains a *Pst*I and a *Bam*HI site. When this plasmid was expressed in minicells, a 76-kd polypeptide corresponding to the *uvrD* product was observed (7). Expression of supercoiled pVMK52 DNA in CLB7 lysates also yielded a 78-kd polypeptide (Fig. 3, lane B). When pVMK52 DNA was digested with *Pst*I and was used to program the CLB7 lysate, as expected, neither the 78-kd polypeptide nor β-lactamase was synthesized. Instead, a new and significantly smaller polypeptide (40 kd) was observed (Fig. 3, lane C). This new polypeptide was considerably smaller than the expected truncated polypeptide (58 kd) predicted from the restriction endonuclease map of this gene (7). Since the *Pvu*II DNA fragment contained in this plasmid is only 2.9 kilobase pairs, it is unlikely

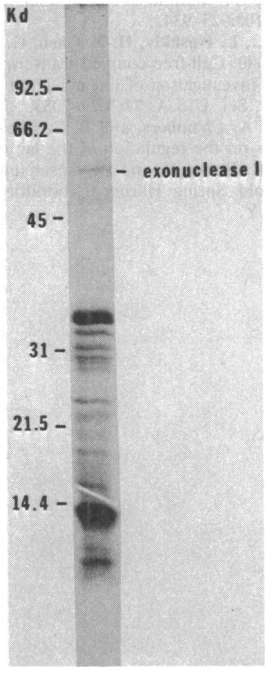

FIG. 2. Autoradiograph of an SDS-polyacrylamide gel showing the [^{35}S]methionine-labeled polypeptides synthesized in vitro from linear pDPK15 DNA in an *E. coli* CLB7 lysate. The in vitro expression in CLB7 lysate of pDPK15 DNA (10 μg) digested with *Bam*HI was the same as that described in the legend to Fig. 1, except that 37 μCi of [^{35}S]methionine (1,100 Ci/mmol) was added. The sample was prepared for electrophoresis as described in the legend to Fig. 1 and 50,000 cpm was loaded onto the gel (12.5% [wt/vol] polyacrylamide).

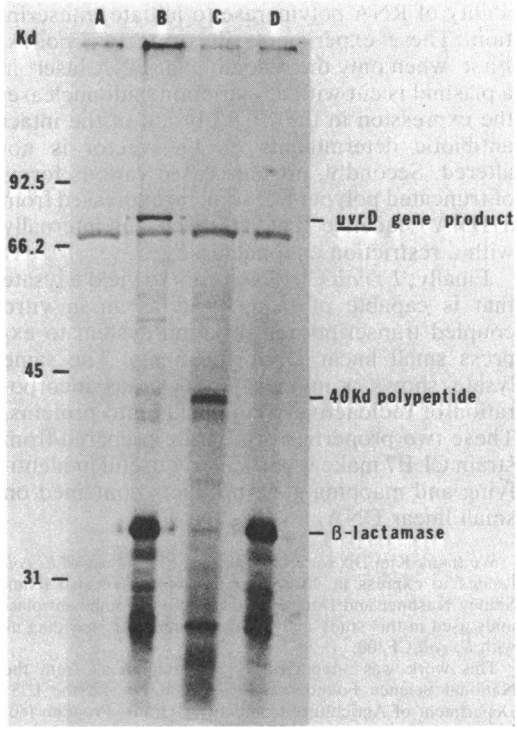

FIG. 3. Autoradiographs of SDS-polyacrylamide gels showing the [^{35}S]methionine-labeled polypeptides synthesized in vitro from linear pVMK52 DNA in an *E. coli* CLB7 lysate. In vitro expression of the DNA (3 μg) in the CLB7 lysate was the same as that described in the legend to Fig. 1, except that 23 μCi of [^{35}S]methionine (1,490 Ci/mmol) was added. The samples were prepared for electrophoresis as previously described (legend to Fig. 1) and 200,000 cpm was loaded per well. The 12.5% (wt/vol) polyacrylamide gel was analyzed as described in the legend to Fig. 1. These autoradiographs show the polypeptides expressed from various forms of pVMK52 DNA in the CLB7 lysate. Lanes: A, No DNA; B, supercoiled pVMK52 DNA; C, pVMK52 DNA digested with *Pst*I; and D, pVMK52 DNA digested with *Bam*HI.

that the 40-kd polypeptide is anything other than some form of an abbreviated truncated polypeptide originating from the *uvrD* gene. When pVMK52 DNA was linearized with *Bam*HI and expressed in the CLB7 lysates, β-lactamase expression was unaffected and neither the synthesis of the 78-kd polypeptide nor the predicted 30-kd truncated polypeptide from this DNA was observed. The absence of the 30-kd truncated polypeptide may be due to one of several reasons. It could have been obscured by the β-lactamase polypeptide (30 to 32 kd) in that region of the gel, or it may have been more susceptible to proteolytic degradation. Alternatively, digesting the DNA with *Bam*HI close to the 5′ end of the gene may have affected the

ability of RNA polymerase to initiate transcription. These experiments illustrate two points. First, when only the recombinant DNA insert in a plasmid is cut with a restriction endonuclease, the expression in the CLB7 lysate of the intact antibiotic determinants on the vector is not altered. Secondly, in some cases various forms of truncated polypeptides can be expressed from a DNA sequence that has been cut internally with a restriction endonuclease.

Finally, *E. coli* CLB7 appears to yield a lysate that is capable of being used as an in vitro coupled transcription-translation system to express small linear DNA fragments. The same lysate shows a minimal endogenous incorporation of radioactive amino acids into proteins. These two properties of a lysate prepared from strain CLB7 make it particularly useful in identifying and mapping gene products contained on small linear DNA.

We thank Kim Dix for technical advice in the use of *E. coli* lysates to express in vitro plasmid DNAs. We also thank Sidney Kushner and Doug Prasher for the recombinant plasmids used in this study and Geoffery Zubay for providing us with *E. coli* CF300.

This work was supported by research grants from the National Science Foundation (PCM-8200949) and the U.S. Department of Agriculture Competitive Grants Program (80-CRCR-1-0489).

LITERATURE CITED

1. **Andrews, W. H., and J. R. Y. Rawson.** 1982. Expression of cloned chloroplast DNA from *Euglena gracilis* in an *in vitro* DNA-dependent transcription-translation system prepared from *E. coli*. Plasmid **8**:148–163.
2. **Clark, A. J., and A. D. Margulies.** 1965. Isolation and characterization of recombination-deficiency mutants of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **53**:451–459.
3. **Collins, J.** 1979. Cell-free synthesis of proteins coding for mobilization functions of ColE1 and transpositon functions of Tn3. Gene **6**:29–42.
4. **Hautala, J. A., C. L. Bassett, N. H. Giles, and S. R. Kushner.** 1979. Increased expression of a eukaryotic gene in *Escherichia coli* through stabilization of its messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **76**:5774–5778.
5. **Kasunic, D. A., and S. R. Kushner.** 1980. Expression of the *HIS3* gene of *Saccharomyces cerevisiae* in polynucleotide phosphorylase-deficient strains of *Escherichia coli* K12. Gene **12**:1–10.
6. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
7. **Maples, V. F., and S. R. Kushner.** 1982. DNA repair in *Escherichia coli*: Identification of the *uvrD* gene product. Proc. Natl. Acad. Sci. U.S.A. **79**:5616–5620.
8. **Miller, J. H.** 1972. Experiments in Molecular Genetics. p. 218–200. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
9. **Prasher, D., D. A. Kasunic, and S. R. Kushner.** 1983. Physical and genetic characterization of the cloned *sbcB* (exonuclease I) region of the *Escherichia coli* genome. J. Bacteriol. **153**:903–908.
10. **Willetts, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thy*A. J. Bacteriol. **100**:923–934.
11. **Yang, H.-L., L. Ivashkiv, H.-Z. Chen, G. Zubay, and M. Chashel.** 1980. Cell-free coupled transcription-translation system for investigation of linear DNA fragments. Proc. Natl. Acad. Sci. U.S.A. **77**:7029–7033.
12. **Zubay, G., A. Chambers, and L. C. Cheong.** 1970. Cell-free studies on the regulation of the lac operon, p. 375–391. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.