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Selective Synthesis of Plasmid-Coded Proteins by *Escherichia coli* During Recovery from Chloramphenicol Treatment

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Protein products of recombinant ColE1 plasmids are preferentially synthesized and can easily be identified in *Escherichia coli* cells recovering from prolonged treatment with chloramphenicol.

We have been interested in using the maxicell method of Sancar et al. (9) to examine the protein products of the hybrid ColE1 plasmids of the Clarke and Carbon (2) colony bank. This method involves destroying chromosomal DNA in an *Escherichia coli* strain that is defective in repairing UV damage. To improve the chances that these recombinant ColE1 plasmids would survive the UV treatment, we incubated cells bearing such a plasmid with chloramphenicol under the conditions described by Clewell (3) to amplify the plasmid DNA. Examining control, unirradiated cultures revealed that cells recovering from prolonged chloramphenicol treatment have only a small capacity for protein synthesis. For approximately 1 h, plasmid-free cells directed most of this synthesis to a selected group of proteins, a few of which are not detectably present in exponentially growing cells. Cells containing plasmid DNA, however, preferentially synthesized proteins coded by the amplified plasmid DNA for over 30 min. Here we describe the characteristics of this system and demonstrate its several advantages for identifying plasmid-coded proteins.

Plasmid pLC1-3 of the Clarke and Carbon bank was introduced into *E. coli* CSR 603, the UV repair-defective strain used by Sancar et al. (9). (Experiments were done under P1-EK1 conditions.) Parallel cultures of CSR 603 and CSR 603(pLC1-3) in the exponential phase of growth were treated with chloramphenicol overnight. The cells were centrifuged and washed to remove the chloramphenicol and were then suspended in a labeling medium containing [^{35}S]-methionine. Samples were taken from each culture at 2, 5, 10, 20, and 40 min for analysis by O'Farrell (6) two-dimensional gel electrophoresis. Figure 1A and B show the proteins revealed by autoradiography of gels made from the 20-min samples. The plasmid-free strain CSR 603 (Fig. 1A) made detectable amounts of only a few dozen of the thousand proteins normally made during exponential growth. Seven of the major ones are labeled a to g; they are proteins found during normal growth, and some have been identified (a, protein synthesis elongation factor G; b, unknown; c, product of the *groE* gene; d, protein synthesis elongation factor Tu; e and f, unknown; g, ribosomal protein L7/L12). In addition, two proteins synthesized in large amounts, labeled x and y, are not detectable in normally growing cells. Use of non-equilibrium gels (7) to display basic proteins revealed only one additional major protein, z (not shown); it also was not seen in normally growing cells. (It is not the shift to the rich, labeling medium after chloramphenicol treatment that induces these new proteins; the same spots were labeled when the cells were grown in medium of the same composition used subsequently for labeling.)

The protein pattern of the strain with the amplified plasmid pLC1-3 (Fig. 1A) was quite different. The new proteins x and y (also z, not shown) were still produced in large amounts, but the normal cellular proteins, including those labeled a to g, were made in noticeably reduced amounts, and instead five new proteins (labeled with arrows in Fig. 1B) were made. The uppermost of these corresponds to the β subunit of glycyl-transfer RNA synthetase (8). Plasmid pLC1-3 is known to carry the *E. coli* genes for glycyl-transfer RNA synthetase as well as genes for xylose utilization (2), so it is reasonable to regard all five of the unique proteins made in large amounts by CSR 603(pLC1-3) as plasmid coded. This view has been confirmed by demonstrating that some of these proteins are synthesized in vitro in a coupled transcription-translation system using plasmid DNA as template (results not shown).

In Fig. 1C and D are the patterns produced during 20 min of labeling two strains taken directly from the Clarke and Carbon bank, JA200(pLC20-30) and JA200(pLC3-33), respectively. In each case proteins a to g were made in

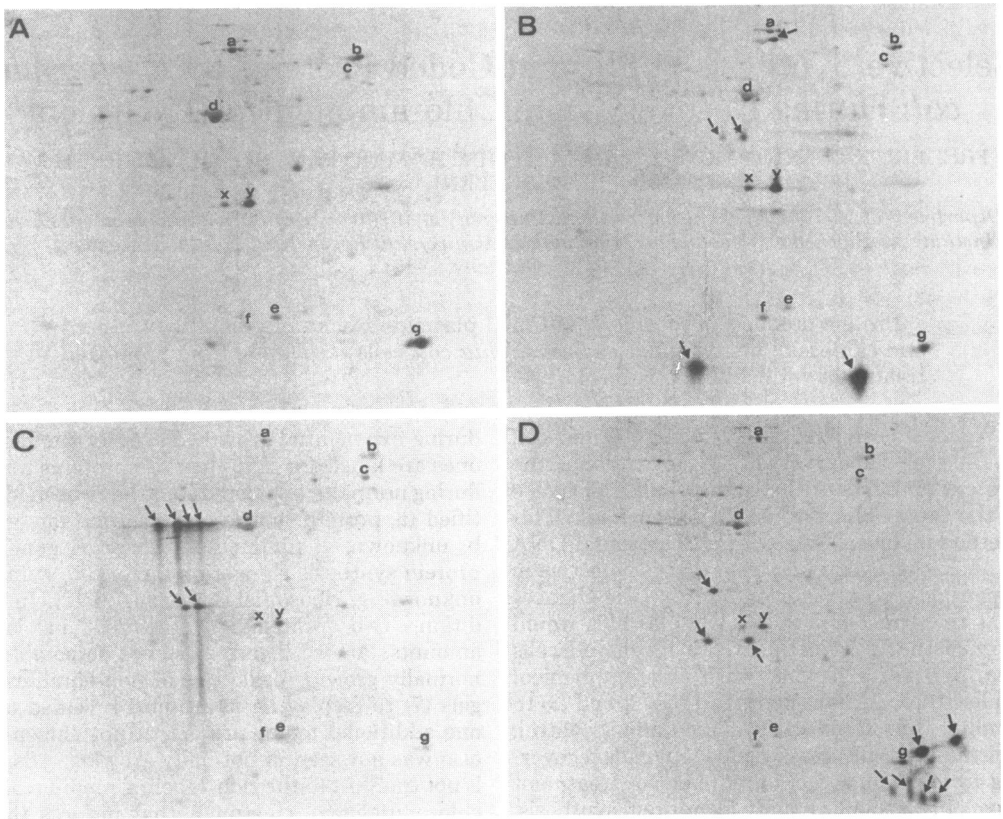

FIG. 1. *Labeling of proteins in E. coli cells with and without plasmids during recovery from chloramphenicol treatment. Cultures (5 ml) were grown in MOPS medium (5) supplemented with 1% Casamino Acids (Difco) and thiamine (1 μg/ml) to an optical density (420 nm) of approximately 2, at which time chloramphenicol (200 μg/ml) was added. After overnight incubation at 37°C with shaking (3), the chloramphenicol was removed by centrifuging and washing the cells with MOPS medium. The cells were suspended in 5 ml of a labeling medium containing MOPS medium supplemented with 2% dehydrated Methionine Assay Medium (Difco) and [^{35}S]methionine (Amersham Buchler, approximately 1,000 Ci/nmol) at a final concentration of 20 μCi/ml together with carrier L-methionine at 0.1 μg/ml. After 20 min of incubation at 37°C with shaking, the samples received non-radioactive methionine (1 mg/ml) and were chilled and centrifuged. Extracts were made for processing by the O'Farrell equilibrium and non-equilibrium systems (6–8). Each gel employed 20 μl of cell extract containing approximately 40 μg of protein. After electrophoresis the gels were dried and subjected to autoradiography for 3 days. Tentative identifications of proteins (given in the text) were made by correspondence of the labeled proteins with stained spots on the gels and comparison of the stained gels with the familiar E. coli O'Farrell pattern (8). (A) CSR 603; (B) CSR 603(pLC1-3); (C) JA200(pLC20-30); (D) JA200(pLC3-33).*

reduced amounts (relative to a plasmid-free strain), proteins x and y (and z, not shown) were labeled prominently, and six to nine plasmid-specific spots were readily observed (labeled with arrows, Fig. 1C and D).

Up to now, identifying the protein products of *E. coli* genes (or other genes) present on recombinant plasmids has had to be done using minicells (4), in vitro labeling (10), or the maxicell method of Sancar et al. (9). In our hands the chloramphenicol method is the simplest technically. It contains no critically sensitive step, at least when used with *E. coli* strains CSR 603, JA200, or NC 3, and presumably can be used with any strain that is sensitive to chloramphenicol but survives prolonged treatment. One hybrid pBR322 plasmid (placed in *E. coli* NC 3) was examined and was found to express preferentially at least one of its known bacterial genes. The full range of usefulness of the technique with plasmids other than those in the Carbon bank remains to be seen.

One feature unique to the chloramphenicol method may enhance its usefulness. Amplification of the plasmid occurs in the absence of protein synthesis, and therefore the copy num-

ber (perhaps as high as 2,000) of certain genes carried by the plasmids may easily outstrip the number of regulatory protein molecules specific for those genes. Though there are a large number of cyclic AMP receptor protein molecules in a cell (1), other regulatory proteins, particularly operon-specific ones, are likely to be incapable of saturating the plasmid DNA. Presumably, proteins under negative, repressor control will be made in this system at least transiently, even in the absence of inducer, whereas proteins under positive, activator control will be made only at low rate. This method might therefore be combined usefully with, for example, the minicell method, to test the mode of control of a particular protein in vivo.

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