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Simple Method for Identification of Plasmid-Coded Proteins

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Proteins encoded by plasmid DNA are specifically labeled in UV-irradiated cells of *Escherichia coli* carrying *recA* and *uvrA* mutations because extensive degradation of the chromosome DNA occurs concurrently with amplification of plasmid DNA.

Because of the widespread study of cloned genes and the biology of plasmids, convenient methods for identifying plasmid-coded proteins are of considerable interest. When heavily irradiated *Escherichia coli* cells are infected with lambda phage (9) or its transducing derivatives (5), the radioactive protein label added to the medium after infection is preferentially incorporated into phage-coded proteins because of the inability of the damaged bacterial DNA to act as a template for transcription. Although this method has been very useful in identification of protein products of several *E. coli* genes incorporated into transducing phages (6, 8, 11), it cannot be applied directly to plasmids because the transformation frequency of *E. coli* with plasmids is at most 10^{-2} (1). Therefore, alternative methods, such as minicells (7) and in vitro labeling (12) techniques, have been used to identify plasmid proteins. These methods, although quite useful, are technically difficult and time consuming. Therefore, we have developed an alternative method to preferentially label and identify plasmid proteins in intact *E. coli* cells.

Our approach is based on two observations: first, when irradiated with UV light (254 nm), *E. coli recA uvrA* cells stop DNA synthesis and chromosomal DNA is extensively degraded so that only a small amount of the chromosomal DNA remains several hours after irradiation (4). Second, if these cells contain a ColE1-like multicopy plasmid, the plasmid molecules that did not receive a UV hit continue to replicate with plasmid DNA levels increasing about 10-fold by 6 h after irradiation in cells where greater than 80% of the chromosonal DNA was degraded (Fox, Sancar, and Rupp, unpublished data). This amplification of plasmid DNA resembles that previously reported for other treatments that preferentially stop chromosome replication (3).

Hence, we reasoned that when chromosome degradation caused by UV light was maximum, the nondividing cells, "maxicells," would contain mostly plasmid DNA and would synthesize almost exclusively plasmid proteins which could be labeled by the [^{35}S]methionine added to the medium. We chose *E. coli* CSR 603 (*recA1 uvrA6 phr-1*) (10) for our studies because the Phr$^-$ (non-photoreactivable) phenotype of this strain allows the experiments to be conducted under ordinary laboratory lighting. This strain and its plasmid-containing derivatives were exposed to UV light, incubated until chromosome degradation was extensive, and then labeled with [^{35}S]methionine. The result is shown in Fig. 1. Although there is no incorporation of label into CSR 603, the strain carrying pBR 322 (2) shows three protein bands of molecular weights of ~37,000, 31,000, and 28,000, respectively. Cells containing pDR 1483 (molecular weight, 4.9×10^6, a pBR 322 derivative constructed in our laboratory which has an insertion in the *amp* gene) have lost the 28- and 31-kilodalton proteins. Cells bearing pDR 3709 (molecular weight, 1.4×10^6, another pBR 322 derivative which has a deletion of the entire *tet* gene) are missing the 37-kilodalton protein. These results show that the *tet* gene of pBR 322 codes for a 37-kilodalton protein, whereas the *amp* gene codes for two proteins of molecular weights of 28,000 and 31,000. Because the success of our method is critically dependent upon the different target size of plasmids and the chromosome, it is important to know the maximum size of plasmids that can be used. Although we have not yet rigorously analyzed this size dependence, it can be seen from the figure that plasmids with a molecular weight of up to 10×10^6 can be used because the cells carrying pBR 322 tetramer (molecular weight, $4 \times 2.6 \times 10^6$) give the same protein band pattern as that of cells harboring pBR 322 monomer. Because many recombinant plasmids constructed in vitro are in this size range, our method should also be useful for identifying proteins synthesized by genes incorporated into recombinant plasmids. We have

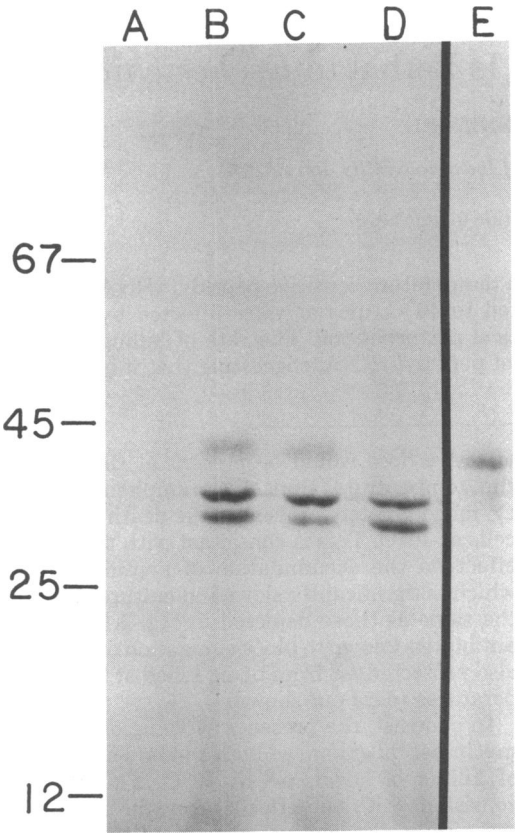

FIG. 1. *Labeling of plasmid proteins in UV-irradiated whole cells ("maxicells"). E. coli CSR 603 and its plasmid-carrying derivatives were grown in M9 medium plus 1% Casamino Acids (Difco) to 2 × 10^8 cells per ml, irradiated with a UV fluence of 50 J m^{-2} from a germicidal lamp at a fluence rate of 0.5 J m^{-2} s^{-1}. Cells were then incubated at 37°C with shaking for 16 h. Ten milliliters of each culture was centrifuged, and the cells were washed with M9 buffer and suspended in 5 ml of minimal medium lacking sulfate. After 1 h of starvation, [^{35}S]methionine (Amersham Searle, 1,050 Ci/mmol) was added to a final concentration of 5 μCi/ml and incubation was continued for another hour. Cells were then collected by centrifugation and suspended in 0.2 ml of sample buffer [2% sodium dodecyl sulfate, 5% mercaptoethanol, 10% glycerol, 0.0005% bromophenol blue, and 0.0625 M tris(hydroxymethyl)aminomethane - hydrochloride, pH 6.8], and heated for 2 min at 100°C. Fifty-microliter samples were loaded onto a polyacrylamide sodium dodecyl sulfate gel (10%). After the run, the gel was dried and subjected to autoradiography for 3 days. Bovine serum albumin (67,000), ovalbumin (45,000), chymotrypsinogen (25,000), and cytochrome c (12,000) were used as molecular-weight standards. (A) CSR 603; (B) CSR 603/pBR 322 monomer; (C) CSR 603/pBR 322 tetramer; (D) CSR 603/pDR 3709 (tet amp$^+$); (E) CSR 603/pDR 1483 (tet$^+$ amp).*

already used this system to identify specific proteins from recombinant plasmids generated in our laboratory, but we do not expect to see the products of those genes that are expressed at very low levels due to the presence of specific repressors, inefficient promoters, or poorly translated mRNA's.

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