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Amplification of the *uvrA* gene product of *Escherichia coli* to 7% of cellular protein by linkage to the p_L promoter of pKC30

(DNA repair/incision/gene amplification/cloning/gene expression)

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ABSTRACT **We have constructed a hybrid pKC30-*uvrA* plasmid (pGHY5003) in which transcription of the *uvrA* gene can be induced under p_L control to amplify the *uvrA* gene product to 7% of cellular protein. To construct pGHY5003, we developed a genetic selection using the basal level of expression (30°C) from p_L in thermosensitive cI857 lysogens to isolate appropriately tailored repair genes inserted at the *Hpa* I site of pKC30 from recombinant DNA mixtures with a variety of products. In addition, a post-UV-irradiation radiolabeling method was adapted to screen inserts for temperature-inducible polypeptide synthesis directed by transcription under p_L control rapidly. This should prove generally useful for isolating genes inserted at the *Hpa* I site of plasmid pKC30 with the following characteristics: (*i*) genetically functional hybrid plasmids selected from a large population of exonucleolytically tailored fragments ligated into *Hpa* I of pKC30 and (*ii*) production of high-level amplification for the gene product of interest by screening for post-UV-irradiation temperature inducibility of polypeptides synthesized from hybrid plasmids. The level of amplification obtained for the *uvrA* gene product from pGHY5003 is ≈10,000-fold higher than estimates of the level of uvrA protein in logarithmic phase *Escherichia coli*.**

The excision of pyrimidine dimers from DNA is a major mechanism of repair involving an initial endonucleolytic step followed by excision, resynthesis, and ligation (1). The enzymatic mechanism for endonucleolytic cleavage of UV-damaged DNA by *Micrococcus luteus* (2) and *Escherichia coli* bacteriophage T4 (3, 4) dimer-specific endonucleases involves two steps: (*i*) N-glycosylic cleavage of the 5′ moiety of the pyrimidine dimer followed by (*ii*) cleavage of the phosphodiester bond 3′ to the apyrimidinic site produced by the first step. In contrast, the *E. coli* UV endonuclease system requires functional *uvrA*, *uvrB*, and *uvrC* genes (5) to cleave UV-damaged DNA, also acts on a variety of unrelated types of DNA damage (6–9), and requires the concerted action of at least three proteins to enzymatically cleave damaged DNA (10–13). These differences have created a significant challenge to determine the mechanism of *E. coli* UV endonuclease. However, attempts to isolate quantities of uvr proteins sufficient for studies of their enzymatic and physical properties have proven difficult since logarithmic phase *E. coli* produce only 10–20 polypeptide chains from the *uvr* genes per cell (14, 15). Although the *uvrA* (14, 16), *uvrB* (17, 18), and *uvrC* (15, 16) genes have been isolated on multicopy plasmids, the mechanisms regulating these genes (19, 20) apparently obviate any significant amplification of the gene products as a result of the increase in gene copy number.

The utility of plasmid pKC30 for amplification of potentially lethal gene products has been demonstrated by the amplification of λ phage cII protein (21). To obtain high levels of uvrA protein, we have bypassed *uvrA* regulatory elements that might interfere with expression from p_L by gene tailoring and linkage downstream from the regulable λ phage promoter p_L on plasmid pKC30. Transcription from p_L can be regulated by λ cI repressor, a product that is autogenously regulated in λ lysogens (22) but temperature inducible in λ lysogens carrying the thermosensitive cI857 repressor (21, 22).

The expression of *uvr* genes is controlled to maintain low intracellular levels of uvr proteins (14, 15). Therefore, we were concerned that high levels of uvrA protein might be lethal or damaging to cells. To circumvent these problems and allow genetic selection of pKC30-*uvrA* hybrid plasmids, we isolated λ lysogens of *uvrA6 recA1* strains carrying (*i*) a thermosensitive cI857 repressor and (*ii*) a wild-type cI repressor for transformation with hybrid plasmids. Since low levels of uvr proteins are required to complement the *uvrA6* mutation, we assumed that the thermosensitive cI857 repressor would allow sufficient levels of p_L expression at 30°C (repressed condition) for complementation of *uvrA6* while prohibiting lethal or damaging levels of *uvrA* expression. To couple the *uvrA* gene in transcriptive phase with the p_L promoter of pKC30, we trimmed a mixture of *uvrA*$^+$ pBR322 *Pst* I fragments with mixed nuclease BAL-31 (21) to remove potentially limiting regulatory elements. This mixture was joined with pKC30 by ligation at the *Hpa* I site downstream from p_L. To select pKC30-*uvrA* hybrid plasmids, transformants of the *uvrA6 recA1* λ cI857 lysogen were irradiated with 254-nm light and plated to select for ampicillin-resistant (Ap^R) colonies.

The resultant UV-resistant (UV^R) Ap^R colonies were screened for induction of uvrA protein synthesis by post-UV-irradiation labeling after shifting cultures to 42°C. In this fashion, we isolated pGHY5003, which can be induced to amplify the *uvrA* gene product to 7% of cellular protein. This has facilitated purification of large (milligram) quantities of uvrA protein for enzymatic and physical characterization. The approach used here to express the *uvrA* gene on a high-level regulable transcript should be useful for isolating similar plasmids for other genes ordinarily expressed at low levels.

MATERIALS AND METHODS

Construction of pKC30-*uvrA* Hybrid Plasmid pGHY5003. The procedures for cell growth in Luria (L) broth or K medium (23) and transformation with plasmid DNA (24) have been described. Strain CSR603 (*recA1 uvrA6 phr*) (D. Rupp) was used to isolate λ lysogens carrying (*i*) a thermosensitive cI repressor (cI857Sam7) (GHY3600) and (*ii*) a wild-type cI repressor, λ *Kan* (GHY3700) (phage source, R. McMacken). Strain GHY3600 was

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Abbreviations: Ap^R, ampicillin resistant; UV^R, UV resistant; kb, kilobase(s); kbp, kilobase pair(s); bp, base pair(s).

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used for initial transformation with recombinant DNA mixtures for selection of pKC30-*uvrA* hybrid plasmids.

Plasmid pATY1103 (25) carries a 4.5-kilobase (kb) *Pst* I fragment with the *uvrA* gene separated from the nearby *ssb* gene. It was obtained by subcloning the *uvrA* gene from the γ-terminal *Pst* I site in a γδ insert that inactivated the *ssb* gene. Digestion of pATY1103 with *Pst* I was followed by treatment with mixed nuclease BAL-31 (22). The BAL-31 digests were selected to yield products ranging from 10 to 800 nucleotides removed per molecule. After phenol extraction and ethanol precipitation, the BAL-31 reaction products were used to form recombinant DNA mixtures with *Hpa* I-digested pKC30 by blunt-end ligation with T4 ligase (26) (Boehringer Mannheim). Ligation reactions were terminated by heating to 70°C for 15 min. Recombinant DNA mixtures were used to transform (24) GHY3600 (*recA1 uvrA6 c*I857Sam7). After 90 min of incubation at 30°C to express plasmid genes in transformed cells, cultures were chilled on ice, pelleted by centrifugation, and suspended in M9 buffer (16) for selection treatment with 0.1 J/m^2 of 254-nm light. Irradiated cultures were pelleted by centrifugation, suspended in 0.5 ml of L broth, plated on L plates supplemented with ampicillin at 100 μg/ml, and incubated at 30°C for 48 hr. Colonies were screened for UV^R and Ap^R at 30°C.

Small amounts of plasmid DNA were isolated (27) from 2-ml L-broth cultures (ampicillin at 100 μg/ml) after screening for UV^R and Ap^R. Isolated plasmid DNA was tested by restriction with *Hpa* I to reveal recircularized pKC30, and selected DNAs were transformed (24) into GHY3600 (*recA1 uvrA6 c*I857) and GHY3700 (*recA1 uvrA6 c*I wild type) to test for *uvrA*$^+$ hybrid plasmids. Large quantities of pGHY5003 were purified by the method of Vapnek *et al.* (23) from GHY3633 (*recA1 uvrA6 c*I857/pGHY5003), which is UV^R and Ap^R. Isolated pGHY5003 DNA was analyzed by restriction with (*i*) *Sal* I and (*ii*) *Bgl* II/*Kpn* I. UV-survival tests of stationary phase cultures of *recA1 uvrA6* λ lysogens, comparing the effects of the thermosensitive *c*I857 and wild-type *c*I repressors on the ability of pGHY5003 to complement the *uvrA6* mutation, were conducted as described (16).

Determining the Inducibility of *uvrA* Polypeptide Synthesis. The maxicell (28) method of labeling plasmid-encoded products was adapted to study the heat induction of uvrA polypeptide synthesis from pGHY5003. The maxicell method uses a phenotype of recA mutants that results in hydrolysis of chromosomal DNA after low doses of UV light to minimize the expression of host genes during labeling experiments. This procedure calls for overnight incubation (12–18 hr) after UV treatment of the cell culture (28). To study the induction of plasmid genes downstream from the p_L promoter, a 2-hour post-UV-irradiation incubation at 30°C was used. Attempts to measure induction of uvrA polypeptide synthesis after overnight post-UV-irradiation incubation were unsuccessful; we attribute this to the loss of factors important to regulation of transcription from p_L.

Cell cultures (GHY3633) were irradiated to 50 J/m^2, suspended in K medium/casamino acids, and incubated for 1.5 hr at 30°C. Irradiated cultures were washed and suspended in K labeling medium (15), which lacks methionine. After an additional 30-min incubation at 30°C, the culture was shifted to 42°C and 1.0-ml samples were removed after various times for labeling. Each sample was placed in a tube with 0.1 ml of K labeling medium supplemented with 50 μCi of [^{35}S]methionine (Amersham; 1500 μCi/mmol; 1 Ci = 3.7×10^{10} becquerels), and incubated at 42°C for 45 sec. Labeling was terminated by addition of 0.1 ml of 1.0% methionine and placing the culture on ice. [^{35}S]Methionine-labeled cells were pelleted by centrifugation, washed, and lysed by boiling for 2 min in 75 mM Tris·HCl, pH 6.8/2% $NaDodSO_4$/5% 2-mercaptoethanol. Aliquots assayed by electrophoresis on 10–20% polyacrylamide/$NaDodSO_4$ gels (29). After electrophoresis, the polypeptide products synthesized during pulse labeling were visualized by fluorography as described (15).

RESULTS AND DISCUSSION

The expression of *uvr* genes is controlled to maintain low intracellular levels of uvr proteins (14, 15). Therefore, we were concerned that high levels of uvrA protein, which we expected from pKC30-*uvrA* hybrid plasmids, might be lethal or damaging to cells. To circumvent these problems and allow genetic selection of *uvrA*$^+$/pKC30 plasmids, *recA1 uvrA6* strains carrying thermosensitive *c*I857Sam7 were used for transformation with hybrid plasmid recombinant DNA mixtures. Hybrid plasmids carrying the *uvrA* gene linked in proper orientation downstream from the p_L promoter of pKC30 were selected by screening Ap^R colonies for UV sensitivity at 30°C (repressed temperature). To enrich for pKC30-*uvrA* plasmids, transformation cultures were UV irradiated to 0.1 J/m^2 before plating on L plates supplemented with ampicillin at 100 μg/ml. Screening of ≈400 Ap^R colonies gave 2 colonies carrying plasmids that complemented the *uvrA6* mutation. Both of these carried hybrid plasmids with the *uvrA* gene in transcriptive phase with the p_L promoter (downstream in the proper orientation) of pKC30. DNA isolated from 10 randomly selected Ap^R UV-sensitive colonies gave recircularized pKC30 susceptible to digestion by *Hpa* I. This suggests that similar selection methods will prove useful for isolation of hybrid plasmids carrying other genes that code for proteins required at relatively low levels, permitting rapid construction of plasmids carrying genes under p_L-promoter control from recombinant DNAs consisting of a mixture of blunt-end fragments and *Hpa* I-digested pKC30. Although two similar pKC30-*uvrA* plasmids were isolated by this procedure, we selected pGHY5003 for additional study (Fig. 1).

The orientation of the *uvrA* gene fragment in pGHY5003 is consistent with the orientation of *uvrA* previously identified by the size of truncated polypeptides resulting from γδ-insertion mapping of *uvrA*/pBR322 plasmids (14, 16). Digestion of pGHY5003 with *Bgl* II/*Kpn* I indicates that the *uvrA* structural gene is located ≈1.1 kbp from the start site of transcription of the p_L promoter (Fig. 1). The size of the *Bgl* II/*Kpn* I fragment indicates that 80–120 bp have been removed from the p_L-promoter proximal end of the *uvrA* fragment, and the product of *Sal*HI digestion of pGHY5003 indicates removal of 250–300 bp from the distal end of the *uvrA* fragment. Enough genetic information remains to include a small portion of the *ssb* gene and the entire *uvrA* structural gene, including the region between *uvrA* and *ssb* (25).

Comparison of the UV survival of stationary phase cultures carrying pGHY5003 in a thermosensitive λ *c*I repressor with a wild-type *c*I lysogen indicates that the *uvrA* fragment in pGHY5003 does not contain elements that interfere with expression from p_L. The level of *uvrA* gene products made from pGHY5003 at 30°C is sufficient to completely complement the *uvrA6* mutation (Fig. 2). However, the wild-type *c*I repressor significantly reduces the ability of pGHY5003 to complement the *uvrA6* mutation in the same genetic background. The reduction of UV complementation caused by wild-type λ repressor for pGHY5003 suggests that expression of *uvrA* is under p_L control and that the region between the site of blunt-end ligation and the start site of the *uvrA* structural gene does not interfere with expression from p_L. This is consistent with previous studies indicating that uvrA protein produced in maxicell labeling experiments is significantly reduced by removing the upstream *ssb* gene (25). This is somewhat different, however,

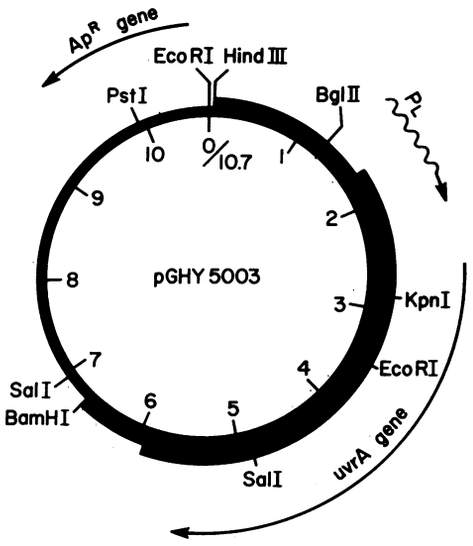

FIG. 1. Hybrid plasmid pGHY5003 consists of pKC30 containing a 4.2-kilobase pair (kbp) insert with the *uvrA* gene of *E. coli* in transcriptive phase with the p_L promoter. The location and orientation of the *uvrA* gene were deduced from the size of the fragment produced by *Bgl* II/*Kpn* I digestion of pGHY5003 DNA. The segment of the map from 1.7 to 5.9 kbp shows the fragment of *E. coli* DNA blunt-end ligated at the *Hpa* I site of pKC30. The segments from the site of ligation to the *Hin*dIII and *Bam*HI sites are segments of λ DNA, and the region from 6.7 to 10.7 kbp is the pBR322 portion of pKC30. *Bgl* II/*Kpn* I digestion indicates that 80–150 base pairs (bp) have been deleted by BAL-31 nuclease treatment from this segment of DNA. The location of the start site for the structural gene is placed relative to the *Eco*RI and *Kpn* I sites as mapped in other studies (14, 25). The distance between the *uvrA* structural gene and the start site of p_L transcription is ≈1.1 kbp. This region is large enough to include 50–100 bp of the *ssb* gene (14, 25). Thus, the region between the 5′ terminus of the *uvrA* gene and the site of blunt-end ligation into pGHY5003 includes the region between *ssb* and *uvrA*. The size of the *Sal*HI fragment after digestion of pGHY5003 was used to determine that 250–350 bp were deleted from this end of the fragment before blunt-end ligation into pGHY5003. Therefore, we estimate that the site of blunt-end ligation is within ≈350 bp of the 3′ terminus of the *uvrA* structural gene. The positions of the p_L promoter and the *uvrA* gene indicate that a 5.0-kbp transcript from the p_L start site will include the entire *uvrA* message.

from observations involving expression of those genes whose promoters are linked in the same orientation downstream from p_L (30).

To determine whether the *uvrA* gene on pGHY5003 can be expressed as a temperature-inducible gene from p_L transcripts, the maxicell method of labeling plasmid-encoded proteins was adapted to study the induction of uvrA polypeptide synthesis. To study the induction of plasmid genes downstream from the p_L promoter a 2-hr post-UV incubation at 30°C preceded shifting the culture to 42°C for induction of expression from p_L. Aliquots were removed after various times of incubation at 42°C, pulse labeled with [^{35}S]methionine, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography. The *uvrA* gene product is induced to a maximal rate of synthesis in GHY3633 (*uvrA6 recA1* cI857Sam7/pGHY5003) after 45–60 min of incubation at 42°C (Fig. 3*A*). Although uvrA synthesis is extremely low before induction at 42°C, we estimate that the induced rate of synthesis is ≈100 times the preinduction rate of accumulation of uvrA polypeptide.

To determine the maximal level of uvrA protein obtainable, we transformed pGHY5003 into a lysogen with cI857 repressor and mutations affecting the replication, excision, and lethal functions of λ (λ N99) (source, M. Rosenberg). Growth of this strain carrying pGHY5003 (GHY8533) to ≈5 × 10^8 cells/ml before shifting the culture to 42°C yields optimal amplification of uvrA protein after 4 hr of incubation (Fig. 3*B*). Although a number of other proteins are also induced by shifting the culture to 42°C, the difference in the uvrA band is immediately obvious. To estimate the level of amplification reached after 4 hr at 42°C, a sample was subjected to electrophoresis on a 7.5% polyacrylamide/$NaDodSO_4$ gel, stained with Coomassie blue, and scanned by densitometry to estimate the percentage of uvrA protein present (Fig. 4). The densitometric scan indicates that the uvrA band is ≈7% of total cellular protein. Thus, uvrA protein is amplified under these conditions to ≈10,000 times the estimated level in logarithmic phase *E. coli*.

The amplification of uvrA protein to 7% of cellular protein will facilitate study of the enzymatic and physical properties of this important protein. Although uvrA protein is essential for the incision step of repair (5, 11) and its size is known to be 114,000 daltons on $NaDodSO_4$/polyacrylamide gels (13, 14), little is known about its enzymatic and physical properties. This is attributable to the fact that a nearly 30,000-fold purification is required to obtain pure uvrA protein from an unamplified source. The observation that stringently regulated proteins involved in *E. coli* DNA metabolism can be amplified to high levels by transcriptive linkage to the p_L promoter of pKC30 sug-

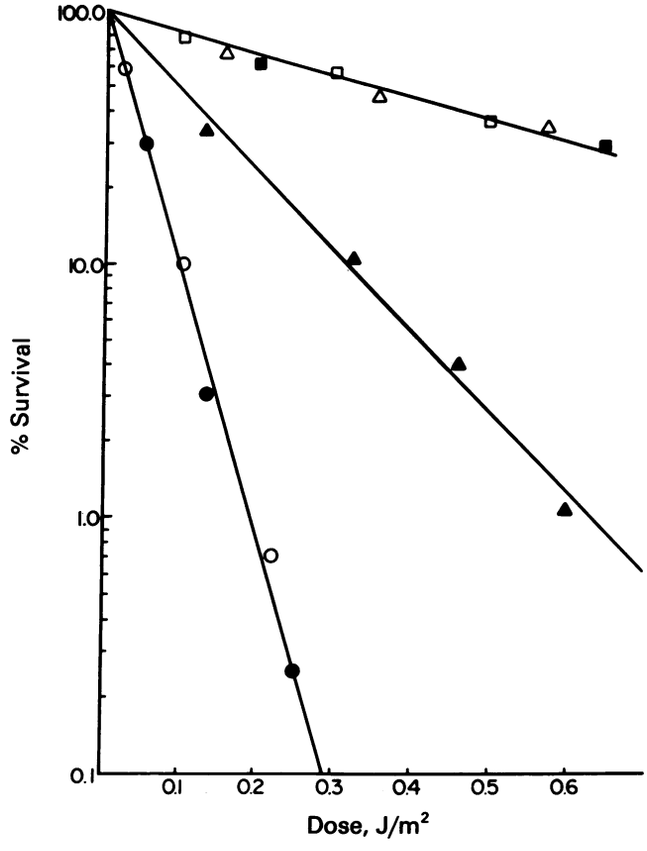

FIG. 2. UV survival of stationary phase cultures of *recA1 uvrA6* λ lysogens, comparing the effects of thermosensitive cI857 and wild-type cI repressors on the ability of pGHY5003 to complement the *uvrA6* mutation. A *recA1* strain lysogenized with λ cI857Sam7 carrying plasmid pKC30 (GHY2951) (■) is compared with a λ *kan* (wild-type cI repressor) GHY3951, carrying pKC30 (□). A λ cI857Sam7 lysogen of *recA1 uvrA6*, carrying pKC30 (GHY3631) (●) is compared with a λ *kan* lysogen of *recA1 uvrA6*, carrying pKC30 (GHY3731) (○). The ability of plasmid pGHY5003 to complement the *uvrA6* mutation in λ lysogens carrying (1) the temperature-sensitive cI857 repressor (GHY3633) (△) is compared in the same genetic background to a lysogen of λ *kan* that has a wild-type cI repressor (GHY3733) (▲).

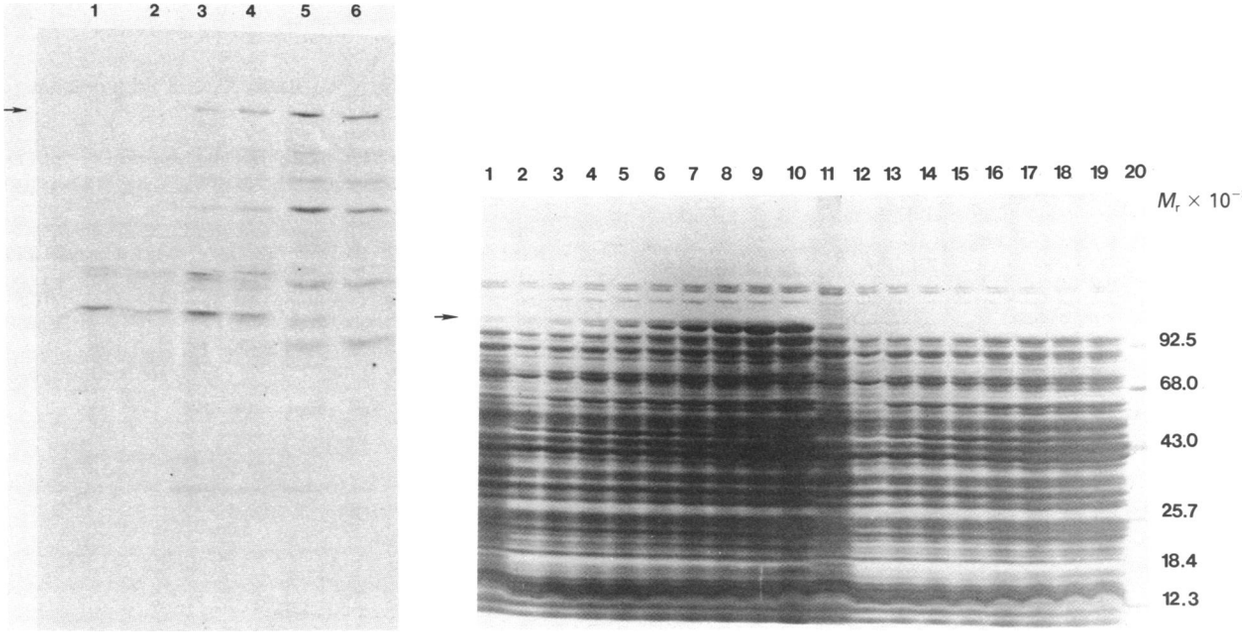

FIG. 3. (*A*) Post-UV-irradiation induction of uvrA synthesis after shifting the incubation temperature of GHY3633/pGHY5003 (*recA1 uvrA6* λ *cI857Sam7*) to 42°C. After 2 hr of post-UV-irradiation incubation at 30°C to eliminate host gene expression, the culture was shifted to 42°C. Induction of uvrA synthesis was measured by pulse labeling 1.0-ml samples with [^{35}S]methionine, and samples were analyzed by electrophoresis on 10–20% polyacrylamide/NaDodSO$_4$ gels followed by fluorography. →, uvrA. Identification of this band was confirmed by comigration of the sample in lane 5 with [^{35}S]methionine-labeled uvrA from a maxicell labeling experiment with *uvrA*$^+$ pGHY3243 (14) (data not shown). Time of incubation at 42°C: lane 1, zero min; lane 2, 15 min; lane 3, 30 min; lane 4, 45 min; lane 5, 60 min; lane 6, 75 min. (*B*) Optimum conditions for amplification of uvrA from pGHY5003. Plasmid pGHY5003 was transformed into a λ lysogen producing thermosensitive cI857 repressor, with defects in the excision and lethal functions of λ (GHY8533). To compare the protein profile of GHY8533 with pKC30 in the same genetic background (GHY8531), culture samples of each were removed after various times at 42°C, resolved by electrophoresis in 7.5% polyacrylamide/NaDodSO$_4$ gels, and stained with Coomassie blue. Profiles of total cell protein were indexed against fluorographs of samples containing [^{35}S]methionine-labeled uvrA from previous experiments (25) (lanes 1 and 11). →, uvrA, determined by superimposing the fluorograph (data not shown) over the Coomassie blue-stained gel. Lanes: 2–10, protein profiles after induction at 42°C of GHY8533/pGHY5003; 3, 15 min; 4, 30 min; 5, 1 hr; 6, 1.5 hr; 7, 2 hr; 8, 3 hr; 9, 4 hr; 10, 6 hr; 12–19, samples from induction (0–4 hr) of pKC30 in the genetic background GHY8531/pKC30 at 42°C; 20, marker standards (BRL). Cellular levels of proteins other than uvrA increase during induction; determination of the number of viable cells and OD_{550} measurements indicate that the cell number increases during the 4-hr incubation by a factor of <2. Many of these proteins may be λ proteins induced during the 42°C incubation.

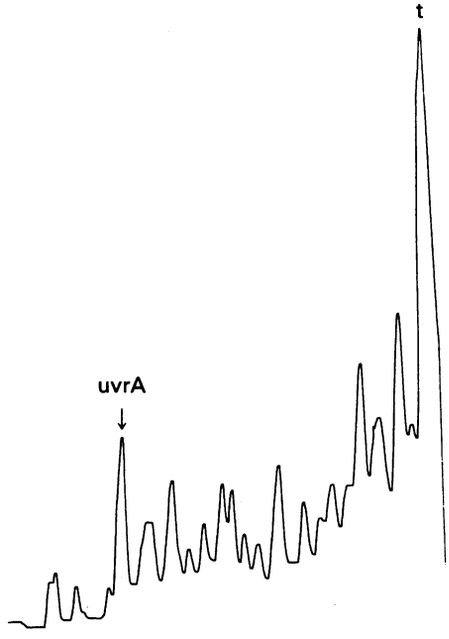

FIG. 4. Densitometric scan of the protein profile of GHY8533 after 4 hr of incubation at 42°C, cell lysis in NaDodSO$_4$ and electrophoresis on a 7.5% NaDodSO$_4$/polyacrylamide gel that was stained with Coomassie blue. ↓, uvrA; *t*, tracking dye. Quantitation of the area under the uvrA peak indicates that uvrA is ≈7% of total cell protein.

gests that similar plasmids could be constructed to obtain large amounts of other DNA metabolic proteins.

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1. Grossman, L. (1981) *Arch. Biochem. Biophys.* **211**, 511–522.
2. Haseltine, W. A., Gordon, L. K., Lindan, C. P., Grafstrom, R. H., Shaper, N. L. & Grossman, L. (1980) *Nature (London)* **285**, 634–641.
3. Radany, E. H. & Friedberg, E. C. (1980) *Nature (London)* **286**, 182–185.
4. Demple, B. & Linn, S. (1980) *Nature (London)* **287**, 203–208.
5. Howard-Flanders, P., Boyce, R. P. & Theriot, L. (1966) *Genetics* **53**, 1119–1136.
6. Murray, M. L. (1979) *Environ. Mutag.* **1**, 347–352.
7. Lawley, P. D. & Brooks, P. (1965) *Nature (London)* **206**, 480–483.
8. Kohn, K., Steigbigel, N. & Spears, G. (1965) *Proc. Natl. Acad. Sci. USA* **53**, 1154–1159.
9. Yoakum, G. H. & Cole, R. S. (1977) *J. Biol. Chem.* **252**, 7023–7030.
10. Braun, A. & Grossman, L. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1838–1842.
11. Seeberg, E., Nissen-Meyer, J. & Strike, P. (1976) *Nature (London)* **263**, 524–526.
12. Seeberg, E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2569–2573.
13. Seeberg, E. (1981) in *Prog. Nucleic Acid Res. Mol. Biol.* **26**, 217–226.

14. Sancar, A., Wharton, R. P., Seltzer, S., Kacinski, B. M., Clark, N. D. & Rupp, W. D. (1981) *J. Mol. Biol.* **148**, 45–62.
15. Yoakum, G. H. & Grossman, L. (1981) *Nature (London)* **229**, 171–173.
16. Yoakum, G. H., Kushner, S. R. & Grossman, L. (1980) *Gene* **12**, 243–248.
17. Pannekoek, H., Noordermeer, I. A., van Sluis, C. A. & van de Putte, P. (1978) *J. Bacteriol.* **133**, 884–896.
18. Sancar, A., Clark, N. D., Griswold, J., Kennedy, W. J. & Rupp, W. D. (1981) *J. Mol. Biol.* **148**, 63–76.
19. Kenyon, C. J. & Walker, G. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2819–2823.
20. Kenyon, C. J. & Walker, G. C. (1981) *Nature (London)* **289**, 808–810.
21. Shimatake, H. & Rosenberg, M. (1981) *Nature (London)* **292**, 128–132.
22. Ptashne, M., Backman, K., Humayun, M. Z., Jeffrey, A., Maurer, R., Meyer, B. & Sauer, R. T. (1976) *Science* **194**, 156–161.
23. Vapnek, D., Alton, N. K., Basset, C. L. & Kushner, S. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3508–3512.
24. Kushner, S. R. (1978) in *Genetic Engineering*, eds. Boyer, H. W. & Nicosia, S. (Elsevier/North–Holland, Amsterdam), pp. 17–23.
25. Yeung, A. T., Yoakum, G. H., Mattes, W. B. & Grossman, L. *Gene* (in press).
26. Sugino, A. *et al.* (1977) *J. Biol. Chem.* **252**, 3987–3991.
27. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523.
28. Sancar, A., Hack, A. & Rupp, W. D. (1979) *J. Bacteriol.* **137**, 692–693.
29. Laemmli, U. (1970) *Nature (London)* **227**, 680–685.
30. Bernard, H.-U. & Helinski, D. R. (1980) *Methods Enzymol.* **68**, 482–493.