Aberrant Regulation of Synthesis and Degradation of Viral Proteins in Coliphage λ -Infected UV-Irradiated Cells and in Minicells

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The patterns of bacteriophage λ proteins synthesized in UV-irradiated *Escherichia coli* cells and in anucleate minicells are significantly different; both systems exhibit aberrations of regulation in λ gene expression. In unirradiated cells or cells irradiated with low UV doses (<600 J/m²), regulation of λ protein synthesis is controlled by the regulatory proteins CI, N, CII, CIII, Cro, and Q. As the UV dose increases, activation of transcription of the *cI*, *rexA*, and *int* genes by CII and CIII proteins fails to occur and early protein synthesis, normally inhibited by the action of Cro, continues. After high UV doses ($>2,000$ J/m²), late λ protein synthesis does not occur. Progression through the sequence of regulatory steps in λ gene expression is slower in infected minicells. In minicells, there is no detectable cII- and cIII-dependent synthesis of CI, RexA, or Int proteins and inhibition of early protein synthesis by Cro activity is always incomplete. The synthesis of early *b* region proteins is not subject to control by CI, N, or Cro proteins, and evidence is presented suggesting that, in minicells, transcription of the early *b* region is initiated at a promoter(s) within the *b* region. Proteolytic cleavage of the regulatory proteins O and N and of the capsid proteins C, B, and Nu3 is much reduced in infected minicells. Exposure of minicells to very high UV doses before infection does not completely inhibit late λ protein synthesis.

Much of our knowledge of viral gene expression in bacteria is based on analyses of patterns of proteins synthesized in infected cells. Polyacrylamide gel electrophoresis (PAGE) and autoradiography have been used to resolve and quantitate radioactively labeled, virus-encoded polypeptides synthesized in infected cells incubated in the presence of radioactively labeled amino acids. The kinetics and relative quantities of synthesis of many viral proteins, including those for which no biological activity assay exists, can be determined. The utility and sensitivity of this experimental approach is limited if host protein synthesis continues after infection. This problem has been circumvented by irradiating host cells with UV light before infection (28, 52, 54, 59) or by infecting anucleate minicells which lack DNA (26, 63-65). These systems have the attraction of being *in vivo*, but the results obtained might be misleading, as UV treatment severely damages cells and minicells have an intracellular composition different from that of nucleated cells.

Protein synthesis is inhibited by UV irradiation primarily because transcription terminates at the sites of UV damage in DNA (51). Irradiation with UV light, however, also inactivates host proteins (47, 76), cross-links proteins to DNA (46), lowers the effective concentration of ribosomes and RNA polymerase (11, 51), and affects transcription termination (17), cyclic AMP levels, respiration, cell division, and cell permeability (80).

Minicells, being devoid of DNA, have reduced concentrations of DNA-binding proteins, including RNA polymerase, and contain abnormally high levels of proteins normally localized in the polar regions of *Escherichia coli* cells (2, 18, 44, 65, 71, 87). Previous studies of bacteriophage infections of minicells have shown that whereas T7, Mu, D108, ϕ 29,

and SPP1 (22, 50, 58, 63) express their entire genomes, T5, N4, SPO1, and SP82G (63, 68) do not proceed past the early stages of their development in infected minicells. Although repair of viral DNA (4) and synthesis of the replicative form of ϕ X174 DNA (66) have been detected in infected minicells, replication of double-stranded phage DNAs has not been demonstrated (4, 26, 67, 83, 87).

In this report, we provide a detailed analysis of the regulation of expression of bacteriophage λ genes in UV-irradiated cells and in minicells. The kinetics of phage development, the stability of the phage proteins, and the fidelity of the genetic controls which regulate phage gene expression have been analyzed and are compared with the patterns of λ gene expression in normal cells.

MATERIALS AND METHODS

Phage and bacterial strains. The phage and bacterial strains used are shown in Table 1.

Preparation of minicells. Cultures of the minicell-producing strains *E. coli* DS410 or DS410(λ cI⁺) were grown at 32°C in TY medium (8) to stationary phase. Minicells, isolated as previously described (65), were stored submerged in liquid nitrogen, as suspensions of 2×10^{10} minicells per ml in M9 minimal salts medium containing 30% (vol/vol) glycerol.

Preparation of bacteriophages. Phages were grown either by infection of permissive hosts or by thermal induction of lysogens. Lysates were treated with chloroform, and cell debris was removed by centrifugation. For infection of UV-irradiated cells, phages were concentrated by precipitation with 10% (wt/vol) polyethylene glycol-0.5 M NaCl (86) and then dialyzed against 10 mM Tris hydrochloride-10 mM MgSO₄ at pH 7.4. For infection of minicells, phages were concentrated by centrifugation, purified by CsCl equilibrium

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TABLE 1. Bacteriophages and bacterial strains

Bacteria or bacteriophage	Genotype	Source	Reference
Bacteria			
159	<i>uvrA galK2 rpsL</i>	P. Ray	59
159(λ c1857ind1)	<i>uvrA galK2 rpsL</i> (λ c1857ind1)	P. Ray	
DS410	<i>minA minB thi rpsL</i>	D. Sherratt	65
DS410(λ c1 ⁺)	<i>minA minB thi rpsL</i> (λ c1 ⁺)	K. von Meyenberg	
Bacteriophage			
λ c1857Sam7		P. Ray	28, 79
λ c1857Nam7am53		P. Ray	23, 32
λ b2		P. Ray	40
λ c1857bio256Nam53		P. Ray	40
λ c1am34		N. Kleckner	1, 78
λ c1857cro27		H. Murialdo	59
λ cIIam611		I. Herskowitz	16
λ v1v3		M. Belfort	78
λ v2		H. Murialdo	34
λ c1857cIII67		H. Murialdo	34
λ c1857		I. Herskowitz	38
λ c1857cIII67can10		I. Herskowitz	79
λ rexQam		I. Herskowitz	38
λ c1857intam29		G. Gussin	27
λ cIIam60am41		L. Enquist	5
λ c1857Qam117		M. Belfort	6
λ bio10Nam7		H. Murialdo	12
λ c1857Qam73am501Sam7		This work	
		H. Murialdo	32

density gradient centrifugation and dialyzed against 10 mM Tris hydrochloride–10 mM MgSO₄ at pH 7.4.

Preparation of ³⁵S-labeled hydrolysate. ³⁵S-labeled hydrolysate was prepared from *E. coli* cells grown in the presence of H₂³⁵SO₄ as described by Crawford and Gesteland (13). There were no detectable differences in the patterns of proteins obtained after sodium dodecyl sulfate (SDS)-PAGE of lysates labeled with ³⁵S-hydrolysate, [³⁵S]methionine purified from hydrolysates (13), or L-[³⁵S]methionine purchased from commercial sources (77).

Preparation of labeled extracts. (i) UV-irradiated cells. Cultures of *E. coli* were grown to a cell density of 4 × 10⁸ cells per ml at 37°C in RM medium containing 0.1 mM CaCl₂ (52), centrifuged at 8,000 × g for 10 min, and suspended in fresh RM-Mg (the medium mentioned above with 10 mM MgSO₄) at a density of 2 × 10⁹ cells per ml. Aliquots (4 ml) of cell suspension in petri dishes (60 by 22 mm) were agitated continuously while being irradiated with UV light (254 nm). Cells and phages were mixed at an input multiplicity of infection (MOI) of 5, and the mixture was incubated on ice for 30 min to allow phage adsorption, diluted fivefold with prewarmed RM-Mg medium, and labeled at 37°C with ³⁵S-hydrolysate, L-[³⁵S]methionine (400 Ci/mmol), or a mixture of L-¹⁴C-amino acids, as indicated in the figure legends. Incorporation was terminated by adding one-seventh of the volume of 21% (wt/vol) SDS–35% (vol/vol) 2-mercaptoethanol, boiling the mixtures for 1 min, and then freezing them in a dry ice-ethanol bath.

(ii) Minicells. Minicells (2 × 10¹⁰ minicells per ml) suspended in RM-Mg medium (52) containing 0.1 mM CaCl₂, 0.2% (wt/vol) glucose, and 20 µg of D-cycloserine per ml (65) were infected with an MOI of 5 by incubating with λ phage at 4°C for 20 min. Aliquots (100 µl) were placed at 37°C, and the polypeptides synthesized in minicells were labeled by addition of 5 µl of methionine assay medium (Difco) containing 50 µCi L-[³⁵S]methionine (65). Labeled minicells were pelleted, suspended in electrophoresis sample buffer (50 µl), and disaggregated by boiling for 3 min.

SDS-PAGE. SDS-PAGE and fluorography techniques have been described previously (65, 69, 77). The relative amounts of radioactivity incorporated into different polypeptides were measured by a Gilford 2400 spectrophotometer to quantitate exposures of autoradiograms.

FIG. 1. Rates of phage and host protein synthesis in UV-irradiated cells. Aliquots of cultures of *E. coli* 159 were irradiated with incident doses of 0, 200, 600, 1,000, 1,400, and 2,000 J/m² of UV light and infected with λ c1857Sam7 at an MOI of 10. At 6 min after infection, infected cells were labeled for 1 min with 100 µCi of ³⁵S-labeled hydrolysate per ml of culture. Ten minutes after being placed at 37°C, uninfected cells were labeled for 1 min. Incorporation was terminated by rapid lysis of the cells by addition of SDS. (A) Radioactivity incorporated (incorp.) into trichloroacetic acid-precipitable material. (B) Relative amounts of radioactivity incorporated into the phage protein, Ea22, and a host protein, h21, (21,000 molecular weight), as determined by scanning densitometry.

FIG. 2. Kinetics of λ protein synthesis in cells irradiated with increasing doses of UV light. *E. coli* 159 was irradiated with the indicated UV doses and infected with λ c1857Sam7 at an MOI of 10. Cells were pulse-labeled at 37°C for 1 min with ^{35}S -labeled hydrolysate (100 $\mu\text{Ci}/\text{ml}$), commencing at the indicated times after infection. The pulse was terminated by addition of SDS. Labeled proteins were separated by SDS-PAGE through 15% polyacrylamide gels. The gels were dried, impregnated with PPO (2,5-diphenyloxazole), and exposed to Kodak SB-5 X-ray film for 24 (a), 48 (b and c), 58 (d and e), and 144 h (f). uninfect., Uninfected λ proteins are identified at the right of panels a through e. MW (K), Molecular size markers in kilodaltons.

RESULTS

UV-irradiated cells. (i) Protein synthesis. The effects of increasing the dose of UV irradiation before infection on the rates of host and λ protein synthesis in *E. coli* 159 are shown in Fig. 1A. Incorporation of label in both uninfected and infected cells decreased as the UV dose was increased (28, 52). At doses of $>600 \text{ J}/\text{m}^2$, phage infection stimulated net incorporation. The effects on the synthesis of individual host and phage proteins shown in Fig. 2 are quantitated for the synthesis of a representative host protein (h21) and a phage protein (Ea22) in Fig. 1B. Heavy doses of UV irradiation severely inhibited both host protein synthesis in uninfected cells and the synthesis of phage proteins in such cells when subsequently infected.

(ii) Kinetics of λ protein synthesis. The effects of increasing

irradiation on the temporal sequence of λ gene expression are shown in Fig. 2. Rates of synthesis of proteins representative of the early p_L and p_R operons, of the cII - and $cIII$ -dependent delayed-early proteins, and of the late proteins are shown in Fig. 3A, B, C, and D, respectively. Cells preirradiated with UV doses of $<1,400 \text{ J}/\text{m}^2$ initiated synthesis of phage early proteins almost immediately after infection, and the maximal rates of synthesis of early proteins were observed approximately 6 min after infection (Fig. 3A and B). Under these conditions, the synthesis of early proteins follows a time course very similar to that observed for early gene transcription and protein synthesis in unirradiated cells (42, 49). As the UV dose was increased, the time at which the maximal rates of p_L - and p_R -dependent early protein synthesis occurred was delayed and early protein synthesis continued beyond the time at which it would

FIG. 3. Kinetics of synthesis of representative λ proteins in UV-irradiated cells. Autoradiograms from experiments as described in the legend to Fig. 2 were quantitated by scanning densitometry. The rates of synthesis are plotted as percentages of maximal rates of synthesis after each UV dose over time for (A) Ea22, an early protein encoded in the p_L operon, and (B) O protein, an early protein encoded in the p_R operon. At each UV dose, the rates of synthesis after infection relative to the maximal rate of synthesis of Ea22 are plotted for (C) Int protein and (D) the late head protein E.

normally have been terminated. The cII- and cIII-dependent synthesis of CI, RexA, and Int proteins was found to be very sensitive to UV irradiation, such that synthesis of these proteins could not be detected at UV doses of $>1,400 \text{ J/m}^2$. When detectable, the rates of synthesis of CI, RexA, and Int proteins increased coordinately reaching maxima approximately 15 min after infection (Fig. 3C). The maximal rates of synthesis of these proteins have been shown to occur at approximately the same time after infection of nonirradiated cells (70).

The rates of synthesis of late proteins, illustrated in Fig. 3D by the major head protein E, increased 10 to 15 min after infection of unirradiated cells and continued to increase for at least 30 min (49). As the UV dose was increased before infection, the onset of late protein synthesis was increasingly

delayed and the rates of synthesis of late proteins relative to those of early proteins were progressively reduced. Preinfection UV doses of $>2,000 \text{ J/m}^2$ always resulted in a very reduced synthesis of late proteins, and in some experiments, there was essentially no late protein synthesis after these doses.

(iii) **Genetic control of λ protein synthesis.** UV-irradiated cells of *E. coli* 159 and *E. coli* 159 ($\lambda\text{CI857ind1}$) were infected with mutants of λ to analyze the function of regulatory proteins in irradiated cells (Fig. 4). The p_L - and p_R -controlled operons were not expressed in irradiated, uninduced cells of the lysogen (Fig. 4A). As expected, the CI protein remained active after irradiation. Irradiated, lysogenic cells infected with phages carrying the $v2$ mutation (34), in which the o_L operator is less sensitive to repression by CI protein, synthesized Ea8.5, Ea22, Bet, and Ssb proteins encoded in the p_L operon, showing that the specificity of the CI repressor- o_L interaction remained intact. Similarly, mutations $v1v3$ in the right operator, o_R , allowed the synthesis of proteins O and P from the p_R operon in irradiated lysogenic cells (Fig. 4A). In addition to repressing o_L and o_R , the CI protein retained its ability after irradiation to stimulate transcription of the *cI* and *rexA* (60) genes from the p_{RM} promoter after infection of cells of the lysogen with $\lambda\text{CI857Sam7}$ or with $\lambda\text{CI857Nam7}$ am53 (Fig. 4A).

Synthesis of λ proteins in irradiated cells was found to be largely dependent upon the function of N protein (75) (Fig. 4B). In the absence of N protein, there was reduced synthesis of proteins encoded by promoter-proximal genes, such as *bet*, *ssb*, *O*, and *P*, and virtually no synthesis of the products of promoter-distal genes, such as *Ea22*, *Ea8.5*, and *bEa59*. Some transcription through the transcription termination signals t_{L1} , t_{L2} , and t_{R1} must, however, have occurred in irradiated cells in the absence of N function to give the pattern of protein synthesis observed. There is evidence for limited transcription through t_{L1} and t_{R1} in the absence of N activity in unirradiated cells (72, 74); however, transcription through t_{L1} , t_{L2} , and t_{R1} is probably increased in irradiated cells, reflecting a reduction in the activity of transcription termination factor rho after UV irradiation (17).

The decline in the rate of early protein synthesis 6 to 10 min after infection of UV-irradiated cells was shown to result from the action of Cro protein (61) and to occur at approximately the same time as in unirradiated cells (42). Irradiated cells infected with λ carrying mutations in *cro*, namely, *can10* and *cro27* (38), continued to synthesize the early proteins O, Ssb, and CII at times when these syntheses had terminated in λcro^+ -infected, irradiated cells (Fig. 4C). Reduced Cro activity also resulted, as expected (54), in a failure to turn on the synthesis of late proteins in UV-irradiated cells.

In cells irradiated with low UV doses, CII and CIII proteins functioned to stimulate the synthesis of the CI, RexA, and Int proteins (39, 70), presumably by directing transcription from the p_{RE} and p_I promoters (Fig. 4D). In contrast, in cells irradiated with high UV doses ($>1,400 \text{ J/m}^2$), there was no detectable synthesis of the CI, RexA, and Int proteins after infections with wild-type phage (Fig. 2 and 3).

The Q -dependent stimulation of transcription from $p_{R'}$ was also found to operate in cells irradiated with low UV doses. Much higher rates of synthesis of late proteins, such as J, H, E, G, U, and D, occurred in such cells infected with λQ^+ compared with λQ mutant phages (Fig. 4E). The low levels of expression of late genes observed in λQ mutant-

FIG. 4. Proteins synthesized in UV-irradiated cells infected with mutant λ phages. (A) *E. coli* 159(λ cl857ind1) was UV irradiated ($1,400 \text{ J/m}^2$), infected at an MOI of 5 with the indicated phage, and labeled at 32°C from 0 to 10 min after infection with either $10 \mu\text{Ci}$ of ^{14}C -amino acids per ml (first two tracks) or $50 \mu\text{Ci}$ of ^{35}S -methionine per ml (last four tracks). Labeled polypeptides were separated by SDS-PAGE through 15% polyacrylamide gels. Although there was always some variability in the expression of host proteins after UV irradiation, the background of proteins synthesized by the uninfected host cells was unusually high in this particular experiment. (B) *E. coli* 159 was UV irradiated ($1,400 \text{ J/m}^2$), infected at an MOI of 10 with the indicated phage, and labeled at 37°C from 0 to 10 min after infection with $50 \mu\text{Ci}$ of ^{35}S -methionine per ml of culture. Labeled proteins were separated by SDS-PAGE through a 15% polyacrylamide gel. (C) *E. coli* 159 was UV irradiated ($1,000 \text{ J/m}^2$), infected at an MOI of 5 with the indicated phage, and labeled at 37°C for 1 min at 20 min after infection with $10 \mu\text{Ci}$ of ^{14}C -amino acids per ml. Labeled proteins were separated by SDS-PAGE through a 14 to 20% polyacrylamide gradient gel. (D) *E. coli* 159 was UV irradiated (450 J/m^2), infected at an MOI of 10 with the indicated phage, and labeled at 37°C from 5 to 10 min after infection with $75 \mu\text{Ci}$ of ^{35}S -hydrolysate per ml of culture. Labeled proteins were separated by SDS-PAGE through a 15% polyacrylamide gel. (E) *E. coli* 159 was UV irradiated (450 J/m^2), infected at an MOI of 10 with the indicated phage, and labeled at 37°C from 30 to 40 min after infection with $75 \mu\text{Ci}$ of ^{35}S -hydrolysate per ml. Labeled proteins were separated by SDS-PAGE through a 15% polyacrylamide gel.

infected cells could have resulted, as previously suggested (14), from N -dependent transcription, initiated at p_R , extending past t_{R2} into the late region. As shown (Fig. 2 and 3), synthesis of λ late proteins after high UV doses ($>2,000 \text{ J/m}^2$) was severely inhibited; under these conditions Q -dependent transcription from p_R' probably did not occur.

(iv) **Chemical stability of λ early proteins.** Proteolytic degradation of λ regulatory proteins is known to be a necessary event in the control of λ gene expression (24; C. Epp, Ph.D thesis, University of Toronto, Toronto, Ontario, Canada), and therefore, the chemical stability of λ proteins in UV-irradiated cells was examined. Most early λ proteins, including the early b region proteins and the proteins Ssb, Bet, Ea22, and Ea9 encoded by the p_L operon, were found to have chemical half-lives of approximately 20 min at 37°C . This is illustrated for bEa59 in Fig. 5d. Proteins O, CII, and N were considerably less stable, with chemical half-lives of 2 to 5 min (24, 85). Increasing the preinfection UV dose caused only minor changes in the rates of degradation of most early proteins; however, a slight increase in the rate of degradation of O protein was measured (Fig. 5d).

Minicells. (i) Kinetics of λ protein synthesis in unirradiated minicells. The patterns of λ proteins synthesized at different times after infection of unirradiated minicells are compared in Fig. 6 with the patterns obtained early and late after infection of UV-irradiated cells. Quantitative measurements of the rates of synthesis in infected minicells of proteins encoded in the early p_R and p_L operons, in the λb region, and by the late morphogenetic genes are shown in Fig. 7A and B. Synthesis of most λ early proteins reached maximal rates approximately 6 min after infection of UV-irradiated cells (Fig. 3A), but maximal rates of early protein synthesis were not reached until approximately 40 min after infection of minicells (Fig. 7A). Although the rates of synthesis of the early λ gene products did eventually decrease in minicells, some synthesis of early proteins continued even at very late times after infection (Fig. 7A). The kinetics of synthesis of the early b region proteins bEa59 and bEa47 differed markedly from that of other early λ proteins. These b region proteins were synthesized rapidly after infection of minicells, and their syntheses continued unabated for very extended periods at high levels (Fig. 7B). The identity of these

FIG. 5. Chemical stability of early λ proteins in UV-irradiated cells. Aliquots of a culture of *E. coli* 159 were UV irradiated with (a) 600, (b) 1,000, and (c) 1,400 J/m², infected with λ cI857Sam7 at an MOI of 5, and pulse-labeled at 37°C with [³⁵S]methionine (50 μ Ci/ml) for 1 min, starting 5 min after infection. The radioactive label was chased with unlabeled methionine (final concentration of 1 mg of methionine per ml). At the indicated times after the start of the chase, samples were taken, the cells were lysed, and labeled proteins were separated by SDS-PAGE through 15% polyacrylamide gels. (d) Quantitation by scanning densitometry of bands in the autoradiograms shown in panels a and c.

b region proteins was confirmed by infection of minicells with phage deleted for various regions of the *b* region (69; data not shown).

Synthesis of the cII- and cIII-dependent delayed-early proteins CI, RexA, or Int could not be detected after infection with wild-type phage of minicells prepared from the nonlysogenic strain *E. coli* DS410.

Synthesis of late λ proteins (e.g., E protein) was initiated 10 to 20 min after infection of minicells (Fig. 7B), approximately the same time as observed for the initiation of late protein synthesis in infected cells exposed to low UV doses. The rate of synthesis of late proteins in minicells was,

however, approximately 5 times lower than in lightly UV-irradiated cells (200 J/m²), although the different late proteins appeared to be synthesized in the same relative amounts in both irradiated cells and minicells.

(ii) **Genetic control of λ protein synthesis.** Minicells from the lysogenic strain *E. coli* DS410(λ cI⁺) were infected with λ cI857Sam7 at MOIs from 0.2 to 5 to determine whether the CI repressor was active in minicells. Results obtained with an MOI of 1 are shown in Fig. 8A. Most λ proteins were synthesized in very reduced amounts, indicating repression of transcription from the *p_R* and *p_L* promoters. As the MOI was increased, the amounts of synthesis of λ proteins increased, consistent with there being only a limited amount of CI protein present in minicells. Although cII- and cIII-dependent synthesis of CI and RexA proteins from the establishment promoter, *p_{RE}*, could not be detected in minicells from DS410 (Fig. 6), CI and RexA proteins were synthesized in substantial amounts after infection of minicells from DS410(λ cI⁺) (Fig. 8A). Synthesis of CI and RexA by transcription from the maintenance promoter, *p_{RM}*, must therefore have been stimulated, as would be expected (15, 60), by having CI protein present in the minicells. Unexpectedly, proteins encoded by the λ *b* region, such as bEa59 and bEa47, were found to be made in large amounts in minicells

FIG. 6. Kinetics of λ protein synthesis in minicells. Minicells from the *E. coli* DS410 were infected with λ cI857Sam7 at an MOI of 5 and labeled at 37°C with 25 μ Ci of [³⁵S]methionine per ml for 10 min at the indicated times after infection. Labeled proteins were separated by SDS-PAGE through a 14 to 20% polyacrylamide gradient gel. A comparison with λ proteins synthesized early and late after infection of UV-irradiated cells (UV'd cells) is shown. *E. coli* 159 was UV irradiated (1,000 J/m² for the sample at time 0 and 450 J/m² for sample at 30 min), infected with λ cI857Sam7 at an MOI of 5, and labeled at 37°C with ³⁵S-hydrolysate (75 μ Ci/ml) for 10 min, starting at the time indicated after infection. uninf., Uninfected.

FIG. 7. Kinetics of synthesis of representative λ proteins in minicells. Autoradiograms from experiments as described in the legend to Fig. 6 were quantitated by scanning densitometry. (A) Rates of synthesis of Ea9 protein, a representative early λ protein encoded in the p_L operon, and O and CII, which are early proteins encoded in the p_R operon. (B) Relative rates of synthesis of the b region protein bEa47 and the late major head protein E, plotted on an arbitrary scale.

prepared from either DS410(λ CI⁺) (Fig. 8A) or from DS410 containing a plasmid to overproduce cI repressor (64) (data not shown). The promoter(s) from which their message(s) was transcribed could not have been repressed by the CI protein; transcription of these b region proteins must not therefore have been initiated at p_L in minicells.

Synthesis of λ proteins in minicells was found to be largely dependent on the function of N protein (Fig. 8B). Minicells infected with λ CI857Nam7am53 synthesized only limited

amounts of the products of the promoter-proximal genes O , P , and ssb as a result of some readthrough of the initial transcription termination signals t_{R1} , t_{L1} , and t_{L2} . None of the products of promoter-distal genes transcribed from p_R , such as Q and late protein genes located beyond t_{R2} , or those transcribed from p_L , such as $Ea22$, $Ea9$, and $Ea8.5$ located beyond t_{L3} , were synthesized (Fig. 8B). Synthesis of proteins encoded in the b region of lambda was, however, found to be independent of N function in infected minicells. In fact,

FIG. 8. Proteins synthesized in minicells infected with mutant λ phages. (A) Minicells from *E. coli* DS410(λ CI⁺) were infected at an MOI of 1 with λ CI857Sam7 and labeled at 37°C with 25 μ Ci of [³⁵S]methionine per ml from 30 to 60 min after infection. The proteins synthesized in uninfected (uninf.) minicells are the products of translation of stable *E. coli* mRNAs trapped within minicells at the time of their formation (45). (B) Minicells from *E. coli* DS410 were infected at an MOI of 5 with the indicated phage and labeled at 37°C with 25 μ Ci of [³⁵S]methionine per ml from 30 to 60 min after infection. (C) Minicells isolated from *E. coli* DS410 were infected with either λ CI857Sam7 or λ CI857cro27 at an MOI of 5 and labeled at 37°C for 10 min with 25 μ Ci of [³⁵S]methionine per ml beginning at the indicated time after infection. (D) Minicells from *E. coli* DS410 were infected with either λ CI857Sam7 or λ CI857Qam73am501 at an MOI of 5 and labeled at 37°C with 25 μ Ci of [³⁵S]methionine per ml from 120 to 130 min after infection. Labeled proteins were separated by SDS-PAGE through 14 to 20% gradient polyacrylamide gels.

a major proportion of the proteins synthesized after infection with λN mutant phage (Fig. 8B) were absent after infection with $\lambda b2$ (data not shown). Infection by $\lambda bio10Nam7$ or by $\lambda bio256Nam53$ (77) also resulted in the synthesis of *b* region proteins in minicells (Fig. 8B) but not in UV-irradiated cells. Transcription of the *b* region in minicells would appear therefore to be initiated at a promoter located within the *b* region and not between p_L and *att*. This would be consistent with the observed synthesis of *b* region proteins in the presence of CI protein in minicells from DS410(λcI^+) (Fig. 8A).

Functioning of Cro protein in minicells was detected as a decline in the rate of synthesis of λ early proteins at later times after infection with $\lambda cI857Sam7$, (Fig. 8C). In the absence of Cro protein, at late times after infection with $\lambda cI857cro27$, synthesis of the early proteins O, Ssb, and CII continued at high levels and synthesis of late proteins, such as E, Lom, Nu3, and F₁, was much reduced. Although there was no detectable *cII*- and *cIII*-dependent synthesis of CI, RexA, or Int proteins in minicells infected with $\lambda cI857Sam7$, infection with $\lambda cI857cro27$ did result in a small amount of Int protein synthesis, a result consistent with overproduction of CII and CIII proteins.

The synthesis of late proteins in minicells was partially dependent upon Q protein, as shown by the reduced synthesis of late proteins in minicells infected with $\lambda cI857Qam73am501$ compared with their synthesis in minicells infected with $\lambda cI857Sam7$ (λQ^+) (Fig. 8D). Transcription of late genes from p_R' must therefore have been activated by Q protein in minicells. The limited expression of late genes observed in minicells infected by λQ mutants probably resulted from *N*-dependent transcription from p_R (14).

(iii) **Chemical stability of λ proteins.** Pulse-chase experiments were performed to examine the chemical stability of λ proteins in minicells (Fig. 9). Most λ proteins were found to be very stable in minicells; bEa59 polypeptide, for example, had a chemical half-life of approximately 2 h at 37°C. The early proteins O (85), N (24, 25, 41), and CII (24, 70, 84), known to be subject to proteolysis in infected nucleated cells, were found however to be degraded in minicells also. Rates of protein degradation were much lower in minicells than in UV-irradiated cells. The half-life of O protein was approximately 2 min in UV-irradiated cells compared with approximately 10 min in minicells (Fig. 5 and 9).

(iv) **Cleavage of λ morphogenetic proteins.** Cleavage and fusion of λ proteins occurs during the assembly of the morphogenetic proteins to form phage head and tail structures (30, 31, 62). The proteolytic cleavage of B protein to B* protein and the proteolytic degradation of C protein and Nu3 protein (not resolved in Fig. 10) were detected, albeit at slow rates, in infected minicells (Fig. 10). Two polypeptides, with electrophoretic mobilities corresponding to those of the fusion peptides X₁ and X₂, known to be produced by fusion of peptides derived from E and C proteins (29), were synthesized in minicells (Fig. 10). As expected for fusion peptides, the amounts of these polypeptides increased as the length of the chase was increased.

Cleavage of H protein to H* protein, a reaction which occurs during tail morphogenesis (30), was not detected in minicells.

(v) **Effects of UV irradiation on λ protein synthesis.** Stable mRNAs, which encode *E. coli* outer membrane proteins, are occluded within minicells at the time of minicell formation (45, 69). Translation of these mRNAs was inhibited by very low UV doses (<500 J/m²), presumably because these mRNAs are highly sensitive to UV irradiation (57) (Fig. 11A). With increasing UV doses, the ability of minicells to

FIG. 9. Chemical stability of λ proteins in minicells. (A) Minicells from *E. coli* DS410 were infected with $\lambda cI857Sam7$ at an MOI of 5 and labeled at 37°C with 25 μ Ci of [³⁵S]methionine per ml from 45 to 65 min after infection. The radioactivity was then chased by addition of unlabeled methionine (final concentration of 1 mg of methionine per ml). Aliquots were removed after the indicated times of chase, and proteins were separated by SDS-PAGE through a 14 to 20% gradient polyacrylamide gel. (B) The autoradiogram in panel A was scanned, and the percent radioactivity remaining in proteins O and bEa59 was determined.

synthesize λ proteins upon subsequent infection decreased, presumably reflecting increasing damage to the transcription and translation machinery within the minicells (Fig. 11B). However, even after very high UV doses (3,500 J/m²) which completely inhibited late λ protein synthesis in nucleated cells (data not shown), late λ protein synthesis could be detected in infected minicells (Fig. 11B).

DISCUSSION

Synthesis of λ proteins in infected, nucleated cells exposed to only low UV doses (<1,000 J/m²) follows expected

FIG. 10. Cleavage of λ morphogenetic proteins in minicells. (A) Minicells from *E. coli* DS410 were infected at an MOI of 5 with λ b2bio10 and labeled at 37°C with 25 μ Ci of [35 S]methionine from 115 to 120 min after infection. The label was chased by the addition of unlabeled methionine (final concentration of 1 mg of methionine per ml). Aliquots were taken at the indicated times after the beginning of the chase, and proteins were separated by SDS-PAGE through a 12.5% polyacrylamide gel. uninf., Uninfected. (B) The autoradiogram in panel A was quantitated by scanning densitometry, and at the indicated times, the label in proteins B, C, and B* was plotted as a percentage of the maximum label measured in that protein.

kinetics and appears to be regulated appropriately by the action of CI, N, CII, CIII, Cro, and Q proteins. In more heavily irradiated cells ($>1,000$ J/m²), the patterns of protein synthesis are aberrant in that CII and CIII activation of transcription of the *cl*, *rexA*, and *int* genes fails to occur,

synthesis of early proteins is not terminated, and the synthesis of late proteins is dramatically reduced. In minicells, as in lightly UV irradiated cells, all proteins previously observed in a lytic lambda infection are synthesized. Protein expression occurs with the previously documented relative kinetics and is controlled by the regulatory proteins CI, N, Cro, and Q; however, progress through the temporal sequence of regulatory events is slower, inhibition of early protein synthesis is always incomplete, synthesis of *b* region proteins is not regulated by the actions of CI, N, or Cro proteins, and proteolytic cleavage of λ proteins is much reduced.

Failure of a regulatory mechanism to operate in UV-irradiated cells or in minicells presumably reflects an inadequate level of synthesis of a phage-regulatory protein(s) or an inappropriate concentration of a host factor(s) known to be required for the λ regulation. The products of the host genes *hflA*, *hflB* (7, 84), *himA*, *himD* (35), *lon* (24), *rho* (17), *groEL*, *groES* (21, 81), *nusA*, *nusB*, *nusC*, *nusD*, *nusE* (19) and DNA replication enzymes (20) are examples of host proteins needed for λ regulation which could be inactivated by UV irradiation or differentially partitioned during minicell formation. It is, in fact, unnecessary to limit this argument to host proteins specifically involved in λ development. For instance, if the overall effective concentration of RNA polymerase were reduced, it could significantly alter promoter selection and thereby the patterns of gene expression. Similarly, reduced ribosome function could affect choices between different available ribosome-binding sites or lowered amounts of DNA-binding proteins could change the conformation of infecting λ DNA molecules, resulting in aberrant patterns of transcription. A decrease in a parameter as general as the level of ATP could cause the changes observed in protein processing.

In UV-irradiated cells, synthesis of early proteins encoded in the *b* region is repressed by the CI protein, requires the action of N protein, and is at least partially inhibited by Cro protein (Fig. 4) (28, 54, 55). None of these regulatory proteins controls the synthesis of early *b* region proteins in minicells (Fig. 8). Transcription of the *b* region in vivo in unirradiated nucleated cells is thought to be initiated at *p_L* and extended leftward by the antitermination action of N protein into the early *b* region (10, 53). This also seems to be the case for early *b* region transcription in UV-irradiated cells but is apparently not the mechanism of *b* region transcription in infected minicells. In minicells, transcription of the genes encoding the early *b* region proteins is independent of *p_L* and N function. It seems most likely that this transcription is initiated at a promoter(s) within the *b* region. In the absence of N protein action, transcription initiated to the right of *att* from a lambda promoter or a *bio* promoter (Fig. 8B) would be expected to terminate at *t₁* approximately 200 bases to the left of *att* before reaching the *b* region genes (56). This strongly suggests that a promoter(s) used to transcribe the early *b* region in minicells is located to the left of *t₁* and to the right of *bEa59*. In addition, infection of *cl* repressor-containing minicells with *b1018* (a deletion with its left endpoint within *bEa59* which extends rightward through *att* to a right endpoint within *exo*) results in the synthesis of high levels of *bEa47* protein (data not shown). This result suggests that an additional promoter(s) lies between the left endpoint of the *b1018* deletion and the right of *bEa47*. The *b* region does contain RNA polymerase-binding sites and promoters which direct leftward and rightward transcription in vitro (3, 9, 37, 43, 73, 82). The in vivo significance of most of these promoters is unknown, although there is some

FIG. 11. Protein synthesis in UV-irradiated minicells. (A) Minicells from *E. coli* DS410 were UV irradiated with doses of 0, 150, 500, 900, 1,200, 1,700, 2,500, and 3,500 J/m² (tracks 1 through 8, respectively) and labeled by addition of 25 μ Ci of [³⁵S]methionine per ml from 30 to 60 min after being placed at 37°C. Labeled polypeptides were separated by SDS-PAGE through a 14 to 20% gradient polyacrylamide gel. (B) Minicells from panel A, UV irradiated with doses from 150 to 3,500 J/m² (tracks 1 through 7, respectively), were subsequently infected with λ cI857Sam7 at an MOI of 5 and labeled at 37°C with 25 μ Ci of [³⁵S]methionine per ml from 30 to 60 min after infection. The *E. coli* envelope proteins Ia, Ib, II*, Tsx, and lipoprotein (Lip) synthesized by translation of mRNAs trapped in minicells (45) are indicated to the left of the figure. Lambda gene products are indicated to the right of the figure. Infection by λ was found to reduce the amount of protein synthesized by translation of resident *E. coli* mRNAs.

published evidence for weak in vivo rightward (33) and leftward promoters (48) within the *b* region. If one or more of these *b* region promoters were in fact used in minicells, it would seem that these promoters should also be considered as sites for transcription initiation in infected nucleated cells under some conditions.

As λ protein synthesis in minicells and in lightly UV-irradiated cells is qualitatively very similar, it raises the question of which experimental system to use. Both systems appear to have advantages and disadvantages. After low UV doses of nucleated cells (<600 J/m²), lambda control mechanisms function normally and all λ -encoded polypeptides appear to be synthesized. At these low UV doses, detecting the synthesis of phage proteins, especially those with molecular weight below 15,000, may however be very difficult. Continued synthesis of host polypeptides produces a high background. High UV doses reduce this background but also cause significant changes in λ gene expression. High UV doses would probably also cause abnormalities in the regulation of expression of nonlambda genes (36) after infection of heavily irradiated cells with λ -transducing phages. Minicells offer the advantage of a very low background of host protein synthesis. This background, if caused by translation of entrapped host mRNAs (45), can be completely eliminated by incubating the minicells at 37°C for 1 to 2 h before infection or by preinfection exposure to a low dose of UV irradiation (400 J/m²). Infection of minicells is therefore

particularly useful for detecting the synthesis of low-molecular-weight polypeptides or polypeptides synthesized in very low amounts or very unstable polypeptides. On the other hand, the regulation of phage gene expression in minicells is clearly aberrant so that patterns of gene expression in minicells should be interpreted with care. This caution in interpretation ought also to be extended to analyses of regulation of non- λ genes expressed in minicells from λ -transducing phages (36) or from DNA sequences cloned in vitro into λ vectors (64). As with the expression of the early *b* region genes, transcription of cloned genes might be directed from unanticipated promoters in minicells.

ACKNOWLEDGMENTS

We appreciate the excellent technical assistance of Lynn Cutler. This work was supported in part by grant MT-3323 from the Medical Research Council of Canada and Public Health Service grant RO1-GM24361 from the National Institute of General Medical Sciences to M.L.P. and by Public Health Service grant 1RO1 AGO1456 from the National Institute on Aging to J.N.R.

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