THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1985 by The American Society of Biological Chemists, Inc.

Vol. 260, No. 18, Issue of August 25, pp. 10069–10074, 1985
Printed in U.S.A.

Response of *recA*-dependent Operons to Different DNA Damage Signals*

(Received for publication, January 14, 1985)

Cassandra L. Smith

From the Department of Human Genetics and Development, College of Physicians and Surgeons of Columbia University, New York, New York 10032

The responses of three *recA*-dependent operons (*recA*, λ, and φ80), with three different repressors, to five DNA damage treatments were compared. Each operon shows a unique induction onset time constant over a wide dose range. However, the extent of response is variable within the same dose range. Individual *Escherichia coli* cells show a graded SOS response to different levels of DNA damage. Apparently phage repressors have evolved to discriminate between lethal and sublethal DNA damage to a host by a wide variety of agents. Multiple induction signals could account for the complex behavior of this system. For example, the absence of the *recBC* enzyme from a cell leads to complex changes in SOS induction onset times and extents.

Escherichia coli repairs DNA damage by both constitutive and inducible pathways. The signal for inducing the SOS repair system may be generated when constitutive repair pathways attempt to deal with DNA damage. This SOS signals activates the constitutively made *recA* protein and results in the cleavage target repressors (for reviews see Refs. 1 and 2). For simplicity we will call *recA* protein a protease, even though a recent report suggests that the reaction is more complex (3). The only known *E. coli* repressor subject to cleavage by activated *recA* protein is the *lexA* gene product. This protein represses the *recA* operon, its own transcription (1, 4), and a variety of other SOS operons (5–8). These SOS gene products are involved in DNA repair, mutagenesis, recombination, and filamentation. Temperate lambdoid phage repressors are also cleaved by activated *recA* protein (9). This allows the prophage to come out of the chromosome and reproduce when DNA damage threatens the viability of the host.

The *recA-lexA* and temperate phage repressor control circuits are particularly interesting to study because they are each able to prevent unnecessary induction. With *recA*, inactivation of the *lexA* gene product first results in more *lexA* protein being synthesized (10, 11). If *recA* proteolysis cannot counter the increased *lexA* synthesis rate, the system simply returns to the constitutive level. If the proteolysis overcomes the enhanced *lexA* synthesis both the *recA* and *lexA* operons are induced.

The complex regulatory circuits of lambdoid phages also prevent accidental induction by small fluctuations in repressor levels (for reviews see Refs. 12 and 13). However, once the repressor level drops below a critical point, λ switches from the lysogenic to the lytic cycle, and further synthesis of repressor is inhibited. Thus, induction of λ becomes irreversible.

In vitro, activated *recA* protein cleaves the λ repressor (9). *In vitro* cleavage of the φ80 repressor mediated by *recA* protein has not been demonstrated. However, *in vivo* and in permeabilized cells, φ80 prophage induction is dependent on the *recA* gene product (14, 15), suggesting that activated *recA* protein can also cleave the φ80 repressor. Here, gene fusions will be used to follow the *in vivo* responses of *recA*, λ, and φ80 to different DNA damage signals. This allows a comparison of the cellular response to *recA* cleavage of three different substrates. Some of the results raise questions about basic characteristics of the *E. coli* SOS system.

MATERIALS AND METHODS

Bacteria, Phages, and Plasmids—All *E. coli* strains listed are isogenic to AB1157 and have the following genetic markers: *thi-1, thr-1, leu-6, his-4, proA2, argE3, lacY1, mtl-1, xyl-5, ara-14, galK2, str-3,* and *sup-37*. The *recB21 recC22* derivative used is JC5519. The *thyA*⁻ derivatives, CS2285 and CS2289, were selected as trimethoprim-resistant colonies of AB1157 and JC5519, respectively. For induction experiments *E. coli* AB1157, JC5519, CS2285, and CS2289 were (*a*) transformed with pCS16 to produce CS2421, CS2311, CS2478, and CS2309, (*b*) lysogenized with λpt60-3 to produce MO372, CS2083, CS2489, and CS2492, or (*c*) lysogenized with φ80pt190h to produce CS2082, MO5012, CS2493, and CS2494, respectively. Most of the data shown here were obtained using the *thy*⁺ strain. To simplify the reader's task, most of the data have been attributed to the *thy*⁻ derivative. The construction of pCS16 is described in Smith *et al.* (16). The *trp* phages λpt60-3 and φ80pt190h are described in Smith and Oishi (17).

Plasmid pCS17 was constructed by inserting a *Sac*II fragment of pLC1842 (18) containing the entire *recA* operon into the *Sac*II site of pKC16 (19). Plasmid pCS17 contains an intact *recA* gene which can be transcribed from the λ P_R promoter under the control of the temperature-sensitive *ind*⁻ (resistant to *recA* cleavage), cI_{857} repressor, or from the authentic *recA* promoter. *E. coli* strain CS2455 is derived from AB2463. It is a φ80pt190h lysogen, contains pCS17, and also contains *recA13* (from AB2463) and *lexA3* (from DM49) mutations. The *recA*⁻ mutation inactivates chromosomal *recA* operon; the *lexA3* mutation prevents induction via the authentic *recA* promoter on both the plasmid and the chromosome. Thus, the *recA* protein can be controlled by thermal induction of λ P_R.

Complex media are described in Smith *et al.* (16). SSA minimal media, T-Broth, and T1 Buffer are described in Smith and Oishi (17).

Induction Experiments—Induction experiments were carried out using the appropriate *E. coli* strain grown to early log phase (16). All experiments were at the same cell density, under conditions of vigorous log growth. Mitomycin C (Sigma) was made up in H_2O and stored at 4 °C protected from light. MNNG[1] (Sigma) was made up in 10 mM CH_3COONa (pH 4.8) stored at −20 °C protected from light. Bleomycin (a gift of Bristol Laboratories) was made up in H_2O and stored at 4 °C. UV irradiation and Thy starvation were as described

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] The abbreviations used are: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; UV, ultraviolet light irradiation.

by Smith and Oishi (14). Zero time is the time of addition of inducing agent or the time of resuspension in supplemented SSA medium lacking thymine.

Induction of *recA* was studied using a *recA-trp* gene fusion contained on a pBR322 derivative plasmid, pCS16 (Fig. 1*a*). The *recA-trp* fusion closely mimics the behavior of the authentic *recA* operon and is under the control of *lexA* (16, 20). The fusion lacks the authentic *trp* control region and the *trp* attenuator.

Phage induction was studied using *E. coli* lysogens containing λ and *ϕ80trp* phage (Fig. 1*b*). In these phages, transcription initiated at the phage P_L promoter can read through the *trp* control region even in the presence of the *trp* repressor (17). Most previous work used a *ϕ*80 superinfection system, where *ϕ80ptrp* was introduced into *E. coli* lysogenized with a *ϕ*80 wild type phage (15, 17). The induction behaviors of the superinfection and the *ϕ80trp* prophage systems are quite similar. The induction behavior of the *ϕptrp* phage is also consistent with the known behavior of λ prophages studied by other means. Induction of all three SOS operons was measured by following anthranilate synthetase activity (*trpED* gene product) in *E. coli* trp^+ cuts in the presence of tryptophan.

Analysis of Induction Data—The anthranilate synthase activity of uninduced controls increases linearly over short times, reflecting cell growth. This increase was fit to the line $b' + a't$ by least squares. The time dependence of anthranilate synthase activity for induced samples of pCS16 and the two phage lysogens always showed a significant linear region immediately after detectable induction. This region was fit to the line $a + bt$ by least squares. The onset time, t, is the intersection of the two straight lines, which yields $t = (b' - b)/(a - a')$. The extent of induction in the region of linear response was calculated as $(a - a')/b'$. This is the increased anthranilate synthase appearance rate due to induction normalized by the original anthranilate synthase activity. Since the original activity reflects the number of cells and operons, the extent measures the initial rate of anthranilate synthase synthesis per operon due to induction. Since all of the inducing conditions used stop DNA synthesis within 5 min, the number of operons in induced samples remains constant.

Infectious Center Assay—*E. coli* strain CS2082 was grown to 1×10^8 cells/ml in SSA medium, diluted with an equal volume of T1 Buffer containing tryptophan, and spun at room temperature for 5 min at 14,000 rpm. The cells were washed with an equal volume of T1 Buffer, spun, and resuspended in fresh prewarmed minimal medium and treated with mitomycin C. The number of viable cells present at the beginning of the mitomycin C treatment and after 30 min was determined by dilution plating. The number of infectious centers was determined at 15 min and 40 min by dilution plating using T-Broth agar overlays of AB1157 cells.

RESULTS

Detection and Analysis of Induction—λ and *ϕ*80 induction were studied in *λptrp* and *ϕ80ptrp* lysogens, respectively, each with phage integrated at the *trp* operon; *recA* induction was studied using a *recA-trp* fusion on a multicopy plasmid, pCS16 (Fig. 1). Each system was studied in separate but isogenic cells by monitoring the level of anthranilate synthase activity coded for by the *trpED* genes.

An inducing agent is added at time zero. After a lag period (onset time) a sudden increase in the rate of anthranilate synthase activity is observed (induction extent). The extent is the enhanced rate of anthranilate synthase synthesis per operon due to induction. Onset times and extents of induction were analyzed by the least squares fitting procedure described under "Materials and Methods." Although *trpED* gene fusions are used to follow expression of all three operons, the extent data are quite strain- and operon-dependent. Thus, relative induction extents were calculated by normalizing data for each operon, in each strain, to the maximum extent seen for that operon in that strain. This allows all operons and strains to be compared directly. For all three operons the maximum extent was seen with mitomycin C in $recB^+C^+$ strains and with UV irradiation in $recB^-C^-$ strains.

Responses of Three Operons in Wild Type E. coli—Five different inducing agents have been studied with three different *recA*-dependent operons, each having a different repressor. Over a wide range of induction doses and conditions, the onset times of the three operons are generally constant while the extents of response vary. The temporal order of induction of the three operons usually remains constant: *recA* is the fastest and *ϕ*80 is the slowest (Fig. 2 and Table I).

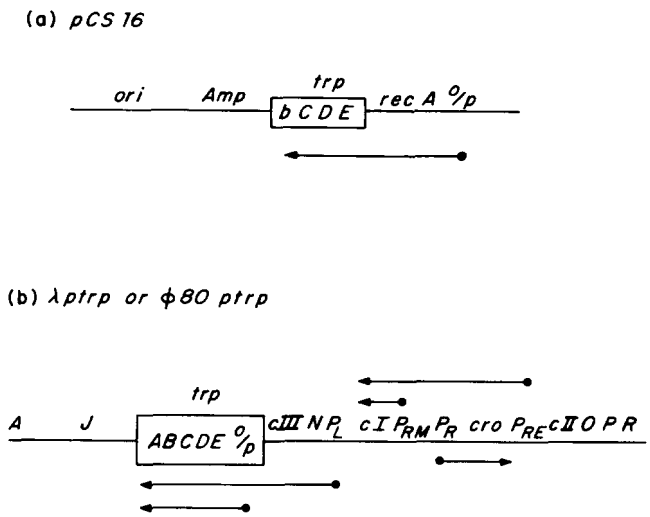

FIG. 1. **Transcriptional maps of gene fusions.** *a*, plasmid pCS16 containing a gene fusion between the *E. coli recA* operon and the *Salmonella typhimurium trpEDC* genes. *b*, bacteriophages λ and *ϕ*80 containing the *E. coli trp* operon. *Trp* genes can be transcribed from either the authentic *trp* promoter, P_T, or the phage promoter, P_L. The other major phage promoter used during lytic growth is P_R. The phage repressor gene, cI, can be transcribed from P_{RE}, its establishment promoter, or P_{RM}, its maintenance promoter.

The onset times observed for *recA* coincide with the timing of maximum proteolysis rates of *lexA* protein *in vivo* (10) and induction of the chromosomal *recA* operon (21). The λ onset times coincide with the kinetics of λ induction observed by other methods and with the disappearance of greater than 90% of λ repressor from the cell (22–24). Thus, the onset times themselves are not surprising. What is surprising is that the onset times are constant over most of the doses while the extent of induction varies widely (Fig. 2 and Table I). For example, with mitomycin C the extent of induction varied 10-fold for all three operons.

The induction response to bleomycin is complex; the dose dependence of onset times is biphasic. At high doses *ϕ*80 is actually induced before λ. The extent of bleomycin induction also has complex dose dependence. Bleomycin is a good inducer of *recA* and the extent shows peaks at both low and high doses. However, bleomycin is only a good inducer for λ at low doses, and it is not a good inducer for *ϕ*80. In contrast to mitomycin C and UV induction, it is striking that the phages are induced at lower bleomycin doses than *recA*. Early *ϕ*80 induction kinetics occur with agents that cause DNA strand breaks (14). Bleomycin cause both single and double strand breaks (25, 26), and the biphasic response behavior may be a consequence of these two types of DNA damage.

Both phages have surprisingly small dose windows for MNNG induction, but the onset times are similar to those obtained with other agents. Until recently (16), low doses of this agent were not thought to induce the *E. coli* SOS system, in part because the response of *recA* and bacteriophages to MNNG is very different. Although, MNNG induces *recA* at doses as low as 0.01 μg/ml, the phages are not induced until cells are treated with 5 μg/ml of MNNG (Fig. 3).

Effect of recBC Function on SOS Induction—The *recBC*

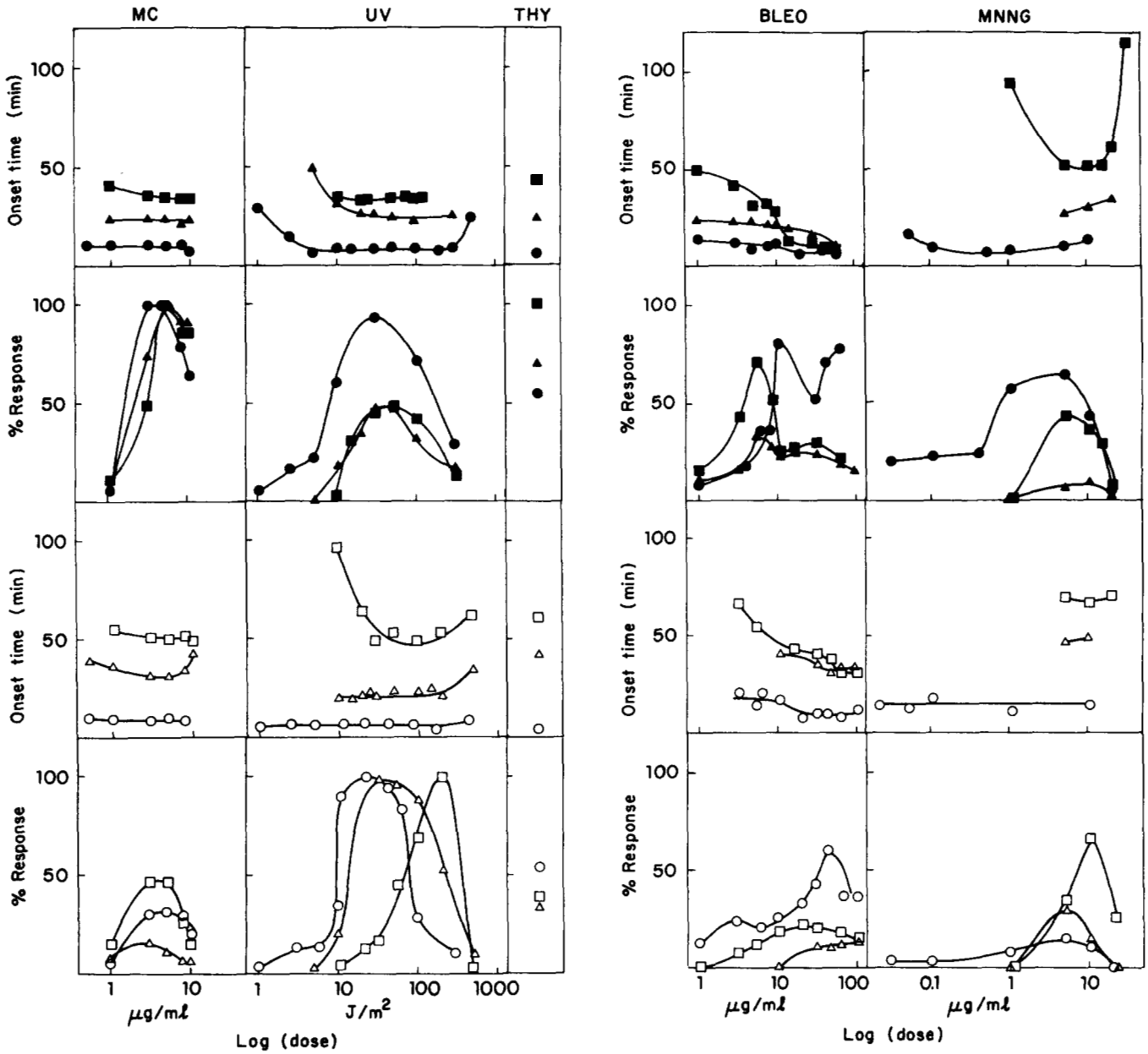

FIG. 2. **Dose dependence of induction onset time and relative extents for three different operons subjected to five different treatments.** In each panel ● and ○ indicate *recA*, ▲ and △ indicate λ, and ■ and □ indicate φ80. *Closed symbols* indicates measurements taken in wild type *E. coli* whereas open symbols indicates measurements taken in *recB*$^-$*C*$^-$ cells. *MC*, mitomycin C; *UV*, ultraviolet light irradiation; *THY*, thymine starvation; *BLEO*, bleomycin; *MNNG*, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

enzyme helps generate most induction signals and is absolutely required for those generated by DNA gyrase-inhibitor complexes (14, 20, 21). A simple model predicts that a decrease in signal in *recB*$^-$*C*$^-$ cells should lead to slower proteolysis, delayed induction onset times, and a decreased response extent, affecting all three operons in the same manner.

We examined the response of *recA*, λ, and φ80 to different DNA-damaging treatments in separate, isogenic *recB*$^-$*C*$^-$ cells. The results show that the *recBC* enzyme is much more important for phage response than for the *recA* response. The temporal order of induction seen in wild type cells is maintained in *recB*$^-$*C*$^-$ cells, but the onset times are usually longer (Fig. 2 and Table I). The onset times for mitomycin C and Thy starvation induction of the *recA* are unchanged while the onset time for λ by UV is actually shortened. A striking feature is that λ is no longer induced by low doses of bleomycin.

In general the dose that produces the maximum extent of induction is the same in *recB*$^+$*C*$^+$ cells and *recB*$^-$*C*$^-$ cells. However, there are several exceptions: φ80 induction by UV shifts to higher doses; low dose bleomycin induction peaks are reduced; and MNNG induction of λ shifts to lower doses, while induction of φ80 is shifts to higher doses. The absence of the *recBC* enzyme has no systematic effect on the extent of induction.

In view of the multifunctional nature of the *recBC* enzyme it may not be surprising that the absence of this enzyme leads to complex changes both in induction onset times and extents of response. The *recBC* enzyme is an exonuclease, endonuclease, and helicase (27, 28). *In vivo* it is inhibited during an

TABLE I

Induction onset times of recA, λ, and φ80

Agent	Onset time		Delay in
	recB⁺C⁺	*recB⁻C⁻*	*recB⁻C⁻*
	min		*min*
Mitomycin C			
recA	10	10	0
λ	22	35	13
φ80	35	51	16
UV			
recA	8	8	0
λ	25	20	−5
φ80	35	50	15
Thy starvation			
recA	5	5	0
λ	25	40	15
φ80	42	61	19
Bleomycin (low)			
recA	6	9	3
λ	12	None	∞
φ80	50	70	20
Bleomycin (high)			
recA	6	9	3
λ	12	34	22
φ80	10	32	22
MNNG			
recA	8	15	7
λ	30	45	15
φ80	50	70	20

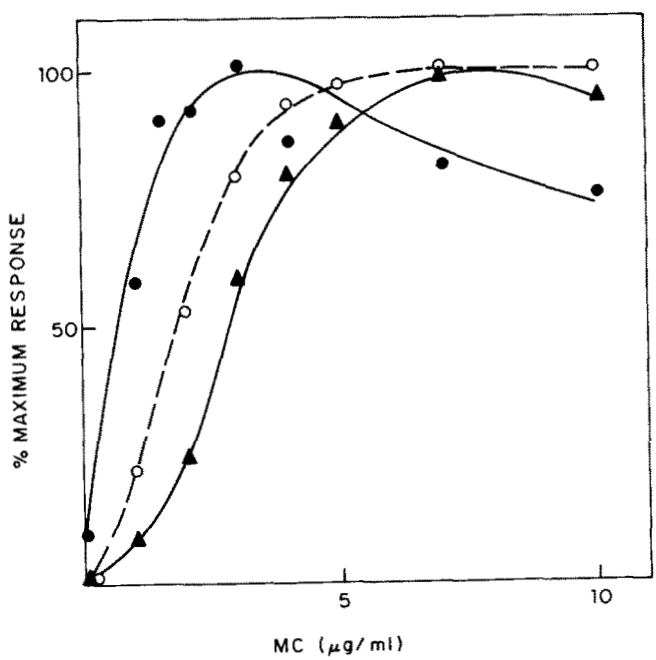

FIG. 3. **Relative cellular and operon responses of *E. coli* strain CS2082 to different mitomycin C (*MC*) doses.** The cellular response (●——●) is the fraction of *E. coli* φ80pt190h cells converted to infectious centers. The operon response (▲——▲) is the relative induction extent. Also shown is the fraction of cells killed (O- - -O). Data shown are the averages of two separate experiments.

SOS response (29). *In vitro*, inhibition of the *recBC* protein by *recA* protein and single strand binding (*ssb*) protein results in long single strand reaction products instead of short oligonucleotides (30, 31). Since both *recA* and *ssb* synthesis (32) are regulated by the SOS system, as time passes during an SOS response, the *recBC* reaction rates and products could be changing. Thus, the effect of this nuclease on induction of a particular operon would depend on the operon's onset time.

Induction Responses in Individual Cells—Over a wide dose range each agent described above produced a constant induction onset time but variable response for each operon. This would be explained simply if the extent was equal to the fraction of cells induced. In such an all or none model, in each individual cell the *recA*-dependent operons are either fully induced or fully repressed. An alternative, a graded model, allows all cells to behave the same. Here, for example, a 10% response means that all of the cells are induced to 10% the maximum level.

These two models can be distinguished by comparing the dose response of single cells, and the average response of all cells. Single cell responses are followed by their ability to act as infectious centers for phage production in a lawn of susceptible nonlysogenic cells. Average extents are followed by using a gene fusion. In an all or none model, single cell and average responses coincide at all doses. In a graded model the fraction of cells responding will reach a maximum at lower doses than the average extent.

The results of such an experiment with strain CS2082, treated with various doses of mitomycin C are shown in Fig. 3. The infectious center data plateaus at 3 μg/ml of mitomycin C, where 55% of the original viable cells can be induced to form infectious centers. The average extent data reaches a maximum at 7 μg/ml of mitomycin C under the conditions of the infectious center experiments. Thus, the single cell response occurs at lower mitomycin C doses than the average extent. This excludes the all or none model and implies that each cell shows a graded response to mitomycin C damage.

Graded Induction Responses—The dose of inducing agent required for a minimum onset time is always much lower than the dose required for an extensive response (Fig. 2). Higher doses create more signal and more activated *recA*, suggesting that response may be limited by the rate of proteolysis. This rate could depend on the concentration of *recA* protein. Thus, the effect of *recA* concentration on φ80 phage induction was examined directly by overproducing *recA* protein prior to DNA damage, using plasmid pCS17 in strain CS2455 as described under "Materials and Methods."

Phage φ80 repressor inactivation and *recA* protein levels were measured at 37 °C after mitomycin C and bleomycin treatment following a 20-min pulse at 43 °C (Fig. 4). This heat pulse changed the level of *recA* protein in strain CS2455 but not wild type cells (results not shown). The heat pulse has no direct effect on induction because the behavior of *φ80ptrp* in a wild type strain is unaffected (compare Fig. 4*a* and Table I). In strain CS2455 the bleomycin onset time was unaffected by the heat pulse, but the mitomycin C onset time was decreased to 20 min. The response generated by both agents increased 3-fold. These results indicate that overproduction of *recA* protein can change both onset times and the response. They suggest that the extent of induction is indeed limited by the overall proteolytic rate.

Multiple Induction Signals—Various treatments differentially induce some *recA*-dependent operons (5, 33–38). This is still consistent with the simplest model for *recA*-dependent induction, where a unique signal molecule has a single target, the *recA* protein. Different damage doses presumably lead to different signal concentrations and different amounts of activated *recA* protein. In a simple SOS model, the amount of activated *recA* protein required to induce a particular SOS operon should be independent of the type of damage. Thus, for each phage a particular *recA* level should correlate with the onset of induction, and a higher *recA* level should correlate with maximum induction. These *recA* levels should be the same for all damage agents. However, the data in Fig. 3 do not conform to such a simple model.

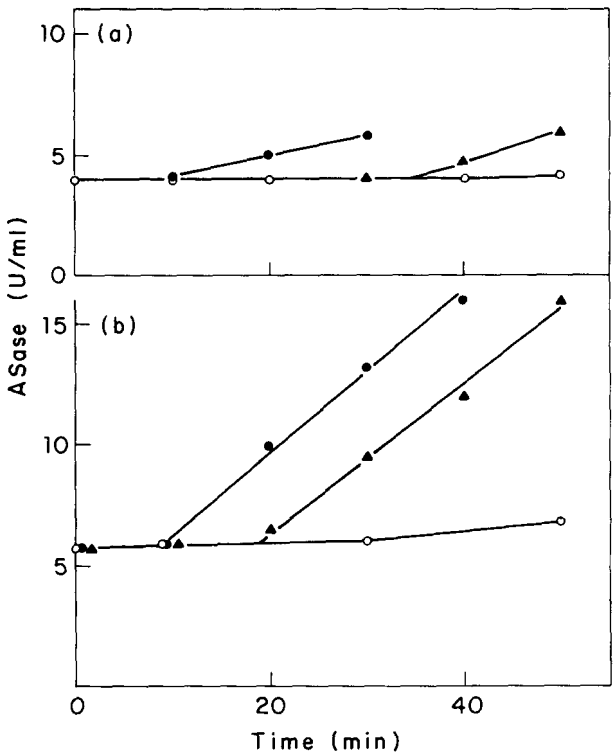

FIG. 4. **Effect of the constitutive *recA* level on ϕ80 induction.** *a*, induction by bleomycin and mitomycin C in wild type *E. coli* strain CS2082. *b*, induction by the same agents in *E. coli* strain CS2455, a *recA13 lexA3* mutant, in which the constitutive *recA* level was increased 10-fold by the use of λ P_L-*recA* fusion. This fusion was thermally triggered 30 min before addition of DNA-damaging agents at time zero. ●——●, bleomycin; ▲——▲, mitomycin C; ○——○, untreated controls. *ASase*, anthranilate synthase.

TABLE II

Response extent of λ and ϕ80 at doses giving low recA induction

	Mitomycin C[a]	Bleomycin[b]	UV[c]	MNNG[d]	Thy[e]
	% maximum response				
Wild type (pCS16)	20	20	20	20	55
Wild type (λpt60-3)	10	30	0	0	70
Wild type (ϕ80pt190h)	15	60	0	0	100
recB⁻C⁻ (pCS16)	20	20	20	15	54
recB⁻C⁻ (λpt60-3)	30	0	10	30	34
recB⁻C⁻ (ϕ80pt190h)	12	5	0	60	40

[a] Response of wild type cells (CS2478, CS2489, and CS2493) at 1.5 μg/ml and *recB⁻C⁻* cells (CS2309, CS2492, and CS2494) at 1.8 μg/ml.

[b] Response of wild type cells at 5 μg/ml and *recB⁻C⁻* cells at 2 μg/ml.

[c] Response of wild type cells at 5 J/m^2 and *recB⁻C⁻* at 7 J/m^2.

[d] Response of wild type cells at 0.05–0.5 μg/ml and *recB⁻C⁻* cells at 0.5 μg/ml. Fifteen per cent was the maximum response seen with pCS16 in *recB⁻C⁻* cells.

[e] Only one condition, complete thymine deprivation, was examined.

Table II compares the response of the phages to damage conditions that produce comparable low levels of *recA* response. The phages are not induced when the *recA* expression simply reaches some threshold value. UV and MNNG doses that induce 20% of the maximal response of the *recA* operon do not induce phages, while equivalent mitomycin C and bleomycin doses lead to efficient phage induction. A simple model predicts a constant temporal order of induction of the three operons. The temporal order observed with mitomycin C, UV, Thy starvation, MNNG, and low doses of bleomycin *recA* < λ < ϕ80. However, when wild type cells are treated with high doses of bleomycin, ϕ80 is induced before λ. Kenyon *et al.* (5) have noted a similar phenomenon with UV induction of the *E. coli lexA* controlled operon, *dinD*.

One explanation for all of these observations is that there are multiple induction signals which differentially activate the cleavage of different repressors. This could also explain the complex responses of the three operons. It would also account for the differences in the nucleotide sequences required for λ repressor cleavage *in vitro* and ϕ80 phage repressor inactivation in permeabilized cells (15, 39–41) and allow selective responses to DNA damage (see "Discussion").

DISCUSSION

Most of our results fit the current SOS model. The differences in kinetics and response of the three operons can reflect operon-specific repressor inactivation reaction parameters such as proteolytic rates, monomerization, and DNA binding. In general, the amount of DNA damage needed to produce a minimum onset time does not lead to extensive induction. Maximum induction of the operons can require up to a 100-fold increase in the amount of DNA damage. Therefore, onset time is not determined by the amount of DNA damage. However, the extent of induction is limited by the amount of DNA damage and the amount of *recA* protein. Thus, during an induction response the rate-limiting step apparent changes. Initially induction is prevented by whatever controls the onset time. However, once that barrier is overcome, another barrier, related to the proteolytic rate, can still limit induction.

In λ, the repressor monomerization rate may limit induction onset times since λ repressor monomers are cleaved more efficiently than repressor dimers (42, 43). Shea and Ackers (13) assessed λ repressor decay following UV irradiation, using the λ monomer repressor cleavage rate. Their modeling shows that the repressor concentration falls near to zero 20–30 min after UV treatment where we will see an onset of induction with all agents except bleomycin. At this point what could prevent λ and presumably ϕ80 from inducing fully?

When first faced with DNA damage the *E. coli* SOS system increases its *lexA* repressor synthesis (10, 11). An increasing proteolytic rate is required for the SOS system to progress towards *recA* induction. This control mechanism prevents unnecessary turn on. By analogy with the SOS system, phages may also resist induction by enhancing the rate of repressor synthesis. One possible mechanism is activation of the phage establishment promoter, P_{RE}. During induction some N protein is transiently made (44). This antitermination protein could allow proteins cII and cIII to be made and these, in turn, could allow P_{RE} expression and the synthesis of λ repressor. An increasing proteolytic rate would be needed to counter this synthesis.

Massive amounts of *recA* protein are made during an SOS response. Our results suggest that this synthesis is required to increase the proteolytic rate and overcome the ability of the *recA*-dependent systems to fight induction. The extent of response also depends on the proteolytic rate, which allows for a graded response to DNA damage signals.

The induction of *E. coli* SOS genes and prophages serves different functions. The *E. coli* genes facilitate repair whereas induction of a resident prophage would lead to destruction of its host. The destruction of a hospitable host at the cost of phage production would seem quite timely only under circumstances where the host is not likely to survive anyway. This is consistent with the fact that most agents require higher levels of damage to induce phage than to induce the *recA*

operon. However, the *recA* level is not the only factor which decides whether a prophage will be induced. If it were, one would predict that a particular prophage should be induced once a certain threshold level of *recA* proteolytic activity is reached. This is not what we observe (Table II). However, if multiple induction signals are produced, and phages can respond selectively to some of them, it would allow the phages to distinguish between lethal and nonlethal damage.

Acknowledgments—I would like to thank Charles Cantor, Gary Ackers and members of his research group, and Doug Ishii for helpful discussion and encouragement; John Cairns and Richard Kolodner for comments on this manuscript, and Junji Takeshita for excellent technical assistance.

REFERENCES

1. Little, J. W., and Mount, D. W. (1982) *Cell* **29,** 11–22
2. Witkin, E. M. (1976) *Bacteriol. Rev.* **40,** 867–904
3. Little, J. W. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 1375–1379
4. Brent, R., and Ptashne, M. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 1932–1936
5. Kenyon, C. J., Brent, R., Ptashne, M., and Walker, G. C. (1982) *J. Mol. Biol.* **160,** 445–457
6. Bagg, A., Kenyon, C. J., and Walker, G. C. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 5749–5753
7. Miller, H. I., Kirk, M., and Echols, H. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 6754–6758
8. Huisman, O., and D'Ari, R. (1981) *Nature (Lond.)* **289,** 196–198
9. Roberts, J. W., Roberts, C. W., and Craig, N. L. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 4714–4718
10. Little, J. W. (1983) *J. Mol. Biol.* **167,** 791–808
11. Brent, R., and Ptashne, M. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 4204–4208
12. Gussin, G., John, A., Pabo, C., and Sauer, R. (1983) in *Lambda II*, pp. 93–122, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY
13. Shea, M. A., and Ackers, G. (1984) *J. Mol. Biol.* **181,** 211–230
14. Smith, C. L., and Oishi, M. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 1657–1661
15. Oishi, M., Smith, C. L., and Friefeld, B. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 897–906
16. Smith, C. L., Siegfried, E., and Ruvolo, P. R. (1983) *DNA* **2,** 291–296
17. Smith, C. L., and Oishi, M. (1976) *Mol. Gen. Genet.* **148,** 131–138
18. Sancar, A., and Rupp, W. D. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 3144–3148
19. Rao, R. N., and Rogers, S. G. (1978) *Gene* **3,** 247–263
20. Smith, C. L. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 2510–2513
21. Gudas, L. J., and Pardee, A. R. (1976) *J. Mol. Biol.* **101,** 459–477
22. Monk, M., and Kinross, J. (1975) *Mol. Gen. Genet.* **137,** 263–268
23. Bailone, A., Levine, A., and Devoret, R. (1979) *J. Mol. Biol.* **131,** 553–572
24. Shinagawa, H., and Itoh, T. (1973) *Mol. Gen. Genet.* **126,** 103–110
25. Haidle, D. W. (1971) *Mol. Pharmacol.* **7,** 645–652
26. Müller, W. E. G., Yamazaki, Z., Breter, H.-J., and Zahn, R. K. (1972) *Eur. J. Biochem.* **31,** 518–525
27. Karu, A. E., MacKay, V., Goldmark, P. J., and Linn, S. (1973) *J. Biol. Chem.* **248,** 4874–4884
28. Rosamond, J., Telander, K. M., and Linn, S. (1979) *J. Biol. Chem.* **254,** 8646–8652
29. Marsden, H. S., Pollard, E. C., Ginoza, W., and Randall, E. P. (1974) *J. Bacteriol.* **118,** 465–470
30. MacKay, V., and Linn, S. (1976) *J. Biol. Chem.* **251,** 3716–3719
31. Prell, A., and Wackernagel, W. (1981) *J. Biol. Chem.* **256,** 10415–10419
32. Brandsma, J. A., Bosch, D., Bakendorf, C., and van de Putte, P. (1983) *Nature* **305,** 243–245
33. Tessman, E. S., and Peterson, P. K. (1980) *J. Bacteriol.* **143,** 1307–1317
34. Melechen, N. E., and Go, G. (1980) *Mol. Gen. Genet.* **180,** 147–155
35. Sedgwick, S. G., Levine, A., and Bailone, A. (1978) *Mol. Gen. Genet.* **160,** 267–276
36. Morand, P., Blanco, M., and Devoret, R. (1977) *J. Bacteriol.* **131,** 572–582
37. George, J., Devoret, R., and Radman, M. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71,** 144–147
38. Dharmalingam, K., and Goldberg, E. B. (1980) *Mol. Gen. Genet.* **178,** 51–58
39. Craig, N. L., and Roberts, J. W. (1980) *Nature (Lond.)* **283,** 26–30
40. Craig, N. L., and Roberts, J. W. (1981) *J. Biol. Chem.* **256,** 8039–8044
41. Irbe, R. M., Morin, L. M. E., and Oishi, M. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 138–142
42. Phizicky, E. M., and Roberts, J. W. (1980) *J. Mol. Biol.* **139,** 319–328
43. Cohen, S., Knoll, B. J., Little, J. W., and Mount, D. W. (1981) *Nature (Lond.)* **294,** 182–183
44. Johnson, A. D., Pabo, C. O., and Sauer, R. T. (1981) *Methods Enzymol.* **65,** part I, 839–856