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# *tif*-Dependent Induction of Colicin E1, Prophage Lambda, and Filamentation in *Escherichia coli* K-12

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To help understand how the *tif-1* mutation of the *recA* gene of *Escherichia coli* confers adenine activability on the *recA* protein, we used the fact that cytidine plus guanosine inhibits induction of prophage lambda and cell filamentation in a *tif-1* mutant, and that adenine reverses this inhibition. We varied the amount of adenine in agar plates containing a fixed amount of cytidine and scored for survivors of three different *tif*-dependent lethal induction processes. Much more adenine was required for cell killing when cytidine was present than when it was absent. Therefore adenine does not override cytidine inhibition, but instead appears to compete with it for a site of action which may be on the *recA* protein. The competition is not at the cell transport level. Our results lead to a model in which the *tif* form of the *recA* protein is an allosteric enzyme that binds both negative and positive modulators. By varying the adenine-cytidine ratio of the medium it is possible to control the degree of induction in a *tif-1* cell. For the three different *tif*-dependent inductions studied here, least adenine was required for lambda induction and most for lethal filamentation, presumably reflecting requirements for different amounts of activated *recA* protein in each process. Varying the adenine-cytidine ratio revealed two stable intermediate stages in lambda induction, as well as a stage of colicin E1 induction in which the cells produced colicin without cell death. The rate of filament formation could be similarly controlled. Experiments with *tif*(ColE1, λ) gave evidence of a competition between colicin repressor and lambda repressor for activated *recA* protein.

The *tif-1* mutation (10) of the *recA* gene (8, 12, 24, 25) permits the induction by heat or adenine of processes that in a normal (*tif*$^+$) strain are induced by DNA damage. These processes are dependent on an intact *recA* gene and have been collectively termed the SOS repair response (35, 49). *tif*-mediated SOS responses include prophage induction (10), induction of colicins (43), cellular mutagenesis (9, 47–49), reactivation of UV-irradiated phages (3, 9, 27), cell filamentation (18), and induction of *recA* synthesis (13). In this paper the designations *tif-1* and *tif* will be used interchangeably, although new *tif* alleles have recently been isolated (28). (*tif* denotes thermally induced filamentation.)

Our current knowledge of how the *recA* protein induces an SOS response comes from the studies of Roberts and co-workers (5, 36–38), who found that the *recA* protein cleaves phage lambda repressor into two fragments. The proteolytic activity of the *recA* protein in vitro is absolutely dependent on both ATP and polynucleotide, preferably single-stranded (5). A ribopolymer has also been shown to be effective. The polynucleotide required in vitro may play the same role as the damaged DNA that is required in vivo. The data of Craig and Roberts (5) indicate that the polynucleotide interacts with *recA* protein rather than with repressor protein. The requirement for added polynucleotide in vitro is the same for both the Tif$^-$ and Tif$^+$ forms of the *recA* protein. The mechanism by which ATP and polynucleotide convert *recA* protein to its activated state, i.e., its proteolytically active state, is not yet understood.

Activated *recA* protein may act as a protease in induction of all *recA*-dependent functions. One of the major *recA*-dependent functions is induction of synthesis of *recA* protein itself above the constitutive level; i.e., *recA* protein synthesis is self-regulated (25). Evidence from experiments using strains mutated in the *recA* and *lexA* genes led to a model (13, 30) which states that the *lexA* gene product is a repressor of the *recA* gene and is destroyed by activated *recA* protein. Further support for this model has been provided by the demonstration that mutational inactivation of *lexA* function by mutations called *spr* results in extreme derepression of *recA* synthesis (8, 23, 25, 33).

Evidence that induction in the *tif-1* mutant involves a conformational change of the *recA* protein is provided by the finding that protein synthesis is not required for *tif*-mediated cleav-

age of lambda repressor (41, 45). Goldthwait and Jacob (10) and Kirby et al. (18) found that the inducing activity of the *tif* mutant is inhibited by cytidine plus guanosine, and that both adenine and UV reverse this inhibition. These authors assayed induction of prophage lambda and induction of cell filamentation to measure *tif* activity. We have extended their experiments in an attempt to understand the mechanism of activation of the *tif* and wild-type forms of *recA* protein. By varying the ratio of adenine to cytidine in the culture medium of *tif-1* we have found evidence for a competition between these agents in induction of three different *recA*-dependent processes. This competition suggests that the *tif* form of *recA* is an allosteric enzyme. From the results of these competition experiments we propose a model for activation of the *tif* form of *recA* as well as for radiation-induced activation of the wild-type form.

Varying the adenine-cytidine ratio of the medium has revealed unexpected stable intermediate stages in induction of lambda and colicin E1 and has also provided evidence that different repressor species can compete for activated *recA* protein.

## MATERIALS AND METHODS

**Media and reagents.** Tryptone broth contains 13 g of Difco tryptone and 7 g of NaCl per liter. Broth plates contained 10 g of tryptone, 5 g of yeast extract, and 12 g of agar (all Difco) per liter. After autoclaving, 12.5 g of glucose and 2.5 mM $CaCl_2$ were added per liter. Top agar was tryptone broth to which were added 1 mM $CaCl_2$ and 7 g of agar per liter. Lambda plates were as described by Gottesman and Yarmolinsky (11). For plating phage lambda, the top agar described above was supplemented with 50 mM NaCl and 10 mM $MgSO_4$ per liter. M9-CAA medium was prepared as described by Miller (26) for M9 and supplemented with 5 g of vitamin-free Casamino Acids and 2 g of glucose per liter, with 10 $\mu$M $FeCl_3$, 1 mM $MgSO_4$, and 2 mg of thiamine per liter. For M9-CAA plates, Difco agar was added to 15 g/liter. For addition to liquid cultures, guanosine and adenine were dissolved in 0.05 N NaOH at 5 mg/ml. Adenine (synthetic), cytidine, and guanosine were obtained from Calbiochem. Bleomycin was a gift from William Bradner of Bristol Laboratories. Streptomycin was used at 200 $\mu$g/ml and ampicillin was used at 50 $\mu$g/ml in lambda plates for selecting drug-resistant colonies.

**Bacterial and phage strains.** Wild-type lambda was used. The bacterial strains used are listed in Table 1. Strain JM1 is *thr-1 leuB6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44* (2). The *tif-1* allele of the *recA* gene in strains JM12 and GC3217 is the *tif* mutation of strain C600 T44 (10). The mutation *sfiA11* (9) suppresses the lethal filamentation that occurs in *tif-1* cells at high temperature. The plasmid pDMS630 was kindly provided by Joseph Inselburg and was previously called ColE1 6-30 (14). It bears the transposon Tn*3*, previously called Tn*A*, inserted into a nonessential region of the plasmid, and confers ampicillin resistance upon the cell in which it resides. The plasmid pDMS630 was introduced into JM12 *tif* or GC3217 *tif sfi* by brief mating at 30°C with HfrH5(pDMS630). Two classes of *tif*(ColE1) exconjugant strains were obtained in each mating; one was sensitive and one was resistant to the colicin E1 they produce after induction on adenine plates. This difference depended on whether, in the course of conjugal transfer of the ColE1 plasmid from HfrH5(pDMS630), the recipients had also received an uncharacterized mutation for colicin resistance. It is unlikely that this mutation lies in *btuB* since it is transferred proximally to HfrH. The sensitive class of *tif sfi*(ColE1) exconjugants, typified by EST203, was unable to form colonies on adenine plates at 41°C because of cell death due to colicin formation. The resistant *tif sfi*(ColE1) class, typified by EST204, formed colonies with 100% efficiency of plating at 41°C even on very high adenine plates. All *tif*(ColE1) strains gave approximately the same colicin yields in liquid culture.

**Phenotype tests.** The spot test for colicinogeny used exposure to chloroform vapor followed by overlay with any colicin E1-sensitive bacteria (21). The *tif-1* phenotype is absence of growth at 41°C on M9-CAA plates containing 200 $\mu$g of adenine per ml. In *tif sfi* strains, the presence of the *tif* mutation is detected by induction of phage lambda or colicin E1, since lethal filamentation is suppressed. In the present work the *tif* phenotype of *tif sfi* was checked by the spot test for *tif*-dependent colicin production (43) after conjugal transfer of pDMS630 into a single-colony isolate of *tif sfi* and by induction of lysogens.

**Colicin assay.** Two milliliters of a culture was shaken briskly by hand with about 5 drops of chloroform. Use of the Vortex mixer must be avoided, since colicin E1 will be inactivated. In an experiment testing the efficacy of different lysing procedures, chloroform treatment using shaking by hand gave the same colicin yield as sonication, lysozyme lysis, or lysis with 1% sodium deoxycholate, whereas chloroform treatment with the Vortex mixer gave very low yields. Colicin E1 can tolerate moderate chloroform shaking only when it is protected by the presence of a turbid bacterial culture. Supernatant or high-salt wash fractions containing colicin E1 are inactivated by even gentle chloroform shaking. The high-salt wash fraction was obtained as described previously (43). The colicin content of each sample of culture was determined by spotting serial dilutions onto plates seeded with indicator bacteria and determining the end point dilution, i.e., the highest dilution factor at which some clearing of the bacterial lawn was still detectable. We found that the presence of adenine, cytidine, or guanosine at any dose used and bleomycin up to 5 $\mu$g/ml had no effect on the spot test.

## RESULTS

**Modulation of induction of *tif*(λ), *tif*(ColE1), and *tif* on plates.** The effect of varying the adenine-cytidine ratio of the medium was measured for three lethal *recA*-inducible processes to distinguish whether adenine

TABLE 1. *Bacterial strains*

| Strain | Description[a] | Reference or source |
|---|---|---|
| JM1 | *tif*$^+$ *sfi*$^+$ | (2) |
| JM1(pDMS630) | *tif*$^+$ *sfi*$^+$ (ColE1::Tn*3*) | Transformation of JM1 with pDMS630 |
| JM12 | *tif-1 sfi*$^+$ | (2) |
| JM12(λ) | *tif-1 sfi*$^+$(λ) | Lysogenization of JM12 with λ |
| EST200(pDMS630) | *tif-1 sfi*$^+$(ColE1::Tn*3*) | Mating HfrH5(pDMS630) with JM12 |
| EST201(pDMS630,λ) | *tif-1 sfi*$^+$(ColE1::Tn*s*)(λ) | Lysogenization of EST200 with λ |
| GC3217 | *tif-1 sfiA11* | (9) |
| EST203(pDMS630) | *tif-1 sfiA11*(ColE1::Tn*3*) | Mating HfrH5(pDMS630) with GC3217 |
| EST204(pDMS630) | *tif-1 sfiA11*(ColE1::Tn*3*), Col$^r$ | Mating HfrH5(pDMS630) with GC3217 |
| GC579 | *tif*$^+$ *sfiA11* | (9) |
| GC579(pDMS630) | *tif*$^+$ *sfiA11*(ColE1::Tn*3*) | Transformation of GC579 with pDMS630 |
| DM1187 | *spr-51 tif-1 sfiA11* | (27) |
| DM1187(ColE1) | *spr-51 tif-1 sfiA11*(ColE1) | (43) |
| HfrH5 | Hfr (ColE1::Tn*3*) | (15) |
| Plasmid | | |
| pDMS630 | ColE1::Tn*3* | (14) |

[a] Only relevant mutations are listed; auxotrophic requirements are given in the text.

competes with cytidine or instead overrides cytidine in *tif*-dependent induction. The three lethal processes were lambda induction, colicin induction, and cell filamentation. Survival curves were obtained by spreading cultures of JM12 *tif*, EST200 *tif*(ColE1), and JM12 *tif*(λ) on plates containing variable amounts of adenine plus a constant amount of cytidine at 41°C. The same cultures were also spread on plates containing adenine alone (Fig. 1). It was seen that at any adenine level, cell survival was many-fold greater when cytidine was present in the plates than when it was absent.

It is known from *spr tif sfi* strains (27, 33) that the presence of a large amount of activated *recA* protein in a cell does not affect cell survival as long as lethal filamentation is suppressed by an *sfiA* mutation (9). This is confirmed by the survival of *tif sfiA* colonies on plates even at 400 μg of adenine per ml (Fig. 1) and at 600 μg/ml (not shown).

The survival curves on adenine plates for all three strains were biphasic, suggesting that the populations might be genetically heterogeneous. Cultures of single-colony isolates of *tif*(λ), *tif*(ColE1), and *tif* all gave the same biphasic type of curve as each original mass culture. For tif(λ) and *tif*, many of the survivors required more adenine for killing than the parental *tif* strain. Beginning with JM12 *tif*, 24 surviving colonies were picked from plates containing 50 μg of adenine per ml. Twelve of these were genetically resistant to killing by 50 μg of adenine per ml, but of these, eight were still killed on plates with 600 μg of adenine per ml. These mutants were not characterized further.

The plating efficiency for *tif*(λ) at zero adenine concentration was very variable, but was always

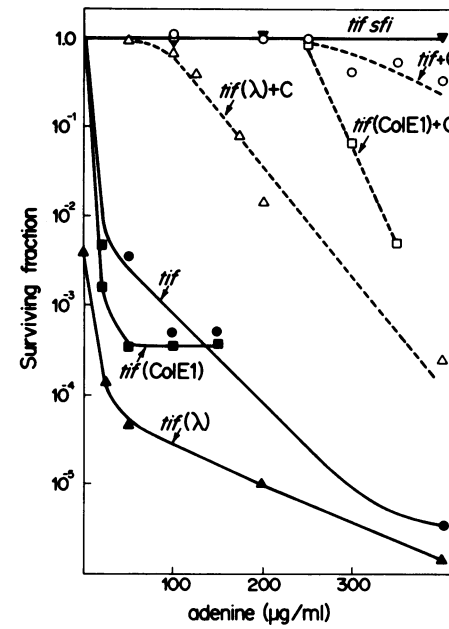


FIG. 1. *Colony survival at 41°C as a function of adenine concentration in agar plates for strains GC3217 tif sfi (▼); JM12 tif (●, ○); EST200 tif(ColE1) (■, □); and JM12 tif(λ) (▲, △). Cells were grown in M9-CAA medium at 30°C to log phase, then diluted through the same medium and spread on M9-CAA plates having different adenine concentrations. The plates were incubated at 41°C for about 20 h. Colony survival on plates containing adenine alone is shown by the solid curves and closed symbols. Values for 100% survival were obtained from broth plates at 30°C. The dashed curves and open symbols show the surviving fractions when 300 μg of cytidine (C) per ml was present in the plates. The data for the dashed curves are presented in more detail in Fig. 2.*

less than $10^{-2}$. For *tif*(ColE1) and *tif*, colonies were formed with 100% efficiency on plates lacking adenine, but the colonies were small and thin. Formation of normal-sized colonies at 41°C for all three strains required addition of 150 μg of cytidine per ml to the plates.

The survival curves of Fig. 1 for adenine and cytidine in combination are shown in greater detail in Fig. 2. A different survival curve was obtained for each of the three induction processes, presumably reflecting a need for different amounts of activated *recA* protein in each process. It is seen that lethal filamentation contributes negligibly to killing of *tif*(λ) or *tif*(ColE1) (Fig. 2).

Examination of the colicin halos around *tif*(ColE1) colonies on adenine plus cytidine plates revealed that induction of colicin synthesis can occur without loss of colony-forming ability. For cytidine-protected *tif*(ColE1), survival of colony-forming ability was 100% until the adenine concentration exceeded 150 μg/ml (Fig. 2). However, at adenine doses as low as 25 μg/ml, each colony was producing colicin in large amount, as seen by overlaying the *tif*(ColE1) colonies with colicin-sensitive indicator bacteria without exposure to chloroform (Fig. 3). We assume that cells that give 100% efficiency of plating on adenine-cytidine plates form colonies whose individual cells also are all alive on these plates. This assumption is supported by the observation that colony size decreased sharply when plating efficiency decreased due to increased adenine. On the basis of this observation we infer that *tif*(ColE1) cells can produce colicin E1 abundantly in the absence of cell death.

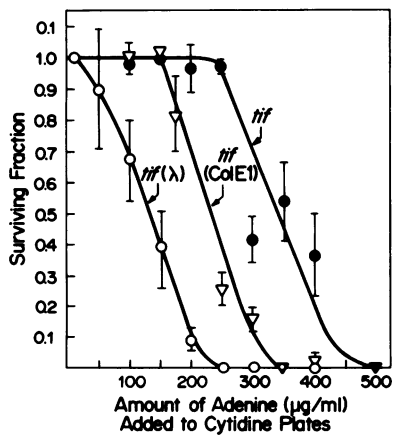


FIG. 2. *Colony survival at 41°C as a function of adenine concentration in cytidine plates at 41°C. Cultures of JM12 tif (●); EST200 tif(ColE1) (▽); and JM12 tif(λ)(○) were spread on M9-CAA plates containing 300 μg of cytidine per ml plus adenine in the amount given on the abscissa. Procedures were as described for Fig. 1. Values for 100% survival were obtained from cytidine plates at 41°C and broth plates at 30°C, both of which gave the same counts. Each survival point is the average of at least four experiments, except for two 150-μg/ml points. The error bars show the standard deviation of the mean.*

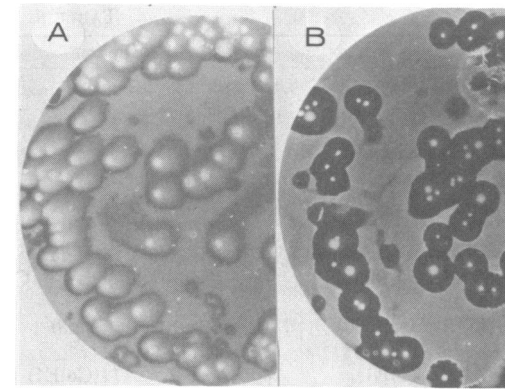


FIG. 3. *Colicin halos formed by colonies of EST200 tif(ColE1). (A) Colonies on M9-CAA plates containing 300 μg of cytidine per ml; (B) colonies on M9-CAA plates containing 300 μg of cytidine plus 100 μg of adenine per ml. Survival at this adenine-cytidine ratio was 100%. Cells were grown to log phase at 30°C in M9-CAA medium, diluted in the same medium, spread on plates, and incubated at 41°C for about 20 h. There was no exposure to chloroform vapor. After overlay with colicin-sensitive bacteria, the plates were again incubated at 41°C overnight. The colonies seen on cytidine plates were very large due to dispersion of cells during the overlay procedure, since JM12 cells cohere very poorly to each other.*

In the experiments to test competition between adenine and cytidine, lethal filamentation at 41°C by adenine did not vary significantly with increase in cytidine concentration above 150 μg/ml (Table 2). We hypothesize that the cytidine transport system is saturated at 150 μg/ml and that cytidine transport is independent of adenine transport. Measurements of transport by standard techniques (22, 39) confirm the independence of these transport systems (22, 39).

**Modulation of *tif*(λ) induction in liquid culture: intermediate induction states.** The original experiments on *tif*-mediated lambda induction (10) measured turbidity of *tif*(λ) cultures as a function of time after shift-up to 40°C. Those data showed that cytidine plus guanosine permitted growth of the induced culture, i.e., protected against induction, whereas adenine

TABLE 2. *Effect of varying the cytidine concentration in adenine plates at 41°C on survival of JM12 tif at 41°C*[a]

| Addition (μg/ml) | | Surviving cells (%) |
|---|---|---|
| Cytidine | Adenine | |
| 150 | 500 | 3.2 |
| 300 | 500 | 4.1 |
| 600 | 500 | 7.2 |

[a] A culture of JM12 was grown to log phase in M9-CAA medium at 30°C, then spread on plates containing cytidine plus adenine and incubated at 41°C overnight.

reversed this protection, resulting in prophage induction and cell lysis. It might be expected that for liquid cultures of *tif*(λ) containing different adenine-to-cytidine ratios, a set of growth curves should be obtained whose shape depends on the fraction of cells that survive prophage induction at each different adenine-cytidine ratio.

Such a set of growth curves was obtained (Fig. 4), but in addition an unexpected phenomenon was found. At certain adenine-cytidine ratios, a large fraction of the *tif*(λ) culture was killed but failed to lyse, even after almost 4 h of incubation. This result is seen in Fig. 4 with 300 μg of cytidine plus 20 μg of adenine per ml. In this medium 60% of the cells lysed and 40% of the cells stopped growth but did not lyse, with the final optical density remaining constant for at least 160 min. Three-fourths of these nonlysing cells were killed, i.e., incapable of colony formation. If more adenine was added at a late time, many more cells lysed (Fig. 4). This modulation of prophage induction to a stage between killing and lysis was observed repeatedly. During the period in which there was plateau of optical density, the cells failed to show the characteristic morphological change which precedes lysis, namely, a conversion of cells to opaque spheres. In adenine alone, without cytidine, this conversion of rods to spheres occurred about 15 min before lysis.

In cultures that showed this plateau of optical density, about one-fourth of the cells that were blocked in lysis were not killed but entered a state of reversible cessation of growth that persisted until at least 4 h after the initiation of induction. The colonies formed by these survivors consisted of cells that were still lysogenic for lambda.

In liquid culture, much less adenine was needed to overcome the cytidine protection of *tif*(λ) induction than on plates. Moreover, in liquid culture the amount of adenine needed to overcome such protection varied considerably from one experiment to another, presumably due to variations in the concentration of intracellular adenine derivatives. In experiments such as that of Fig. 4, complete lysis was usually observed at a ratio of 40 μg of adenine per ml to 300 μg of cytidine per ml, whereas killing without lysis was observed at 5 to 25 μg of adenine per ml in the presence of 300 μg of cytidine per ml.

Kirby et al. (18, 19) reported that cytidine plus guanosine gives better inhibition of *tif*-mediated induction than either nucleoside alone. In most of our experiments on *tif*(λ) induction in liquid culture, cytidine alone (300 μg/ml) gave good protection against induction (Fig. 4). However, in several experiments cytidine alone had little protective effect. In these experiments a mixture of cytidine plus guanosine gave excellent protection against *tif*(λ) induction. The duration of this inhibition depended on the concentrations used (Fig. 5). The protective effect of the cytidine-guanosine combination was due not to the increase in total concentration of inhibitory nucleosides but to a requirement for both inhibitors (Fig. 5). Optimum inhibition of lambda induction requires 300 μg of cytidine plus 200 μg of guanosine per ml. Despite the effective inhibition by the combination of cytidine plus guanosine, addition of adenine at 40 μg/ml resulted in almost complete lysis of *tif*(λ) at 41.5°C.

**Filamentation.** In the presence of cytidine plus guanosine in liquid culture, JM12 *tif* was very resistant to filament formation by adenine at 41.5°C. Only a small fraction of the cell pop-

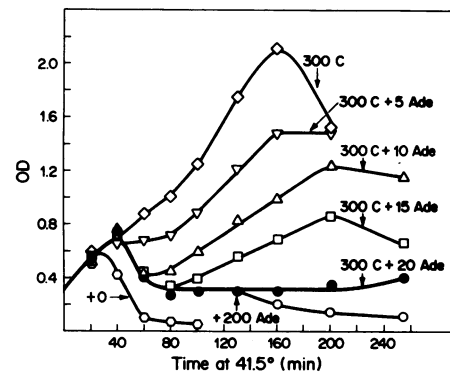


FIG. 4. *Effect of varying the adenine-cytidine ratio on tif-mediated lysis of strain JM12 tif(λ) at 41.5°C. Concentrations of cytidine (C) and adenine (Ade) are in micrograms per milliliter. Cells of JM12 tif(λ) were grown at 30°C in M9-CAA medium to about 2 × 10⁸/ml, and then cytidine and adenine were added to 10-ml samples as indicated, the cultures were shaken at 41.5°C, and optical density was measured at 550 nm. The sample with 300 μg of cytidine plus 20 μg of adenine per ml was further subdivided at time zero. At 130 min, 200 μg of additional adenine per ml was added to one of the two flasks.*

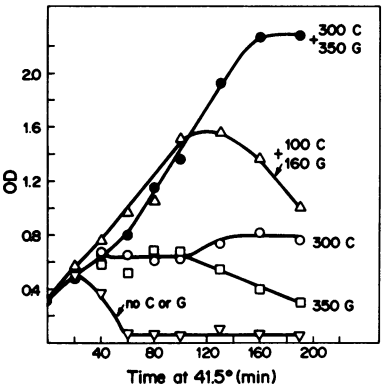


FIG. 5. *Inhibition of prophage induction at 41.5°C of JM12 tif(λ) by the addition of a combination of cytidine plus guanosine. Cells of JM12 tif(λ) were grown as described for Fig. 4, and then to each sample of culture were added either 300 μg of cytidine per ml (○); 350 μg of guanosine per ml (□); a mixture of 100 μg of cytidine plus 160 μg of guanosine per ml (△); a mixture of 300 μg of cytidine plus 350 μg of guanosine per ml (●); or no additions (▽). The cultures were then shifted to 41.5°C with aeration, and optical density at 550 nm was measured.*

ulation formed filaments within 4 h even at very high adenine (500 μg/ml). In contrast, adenine alone at 5 μg/ml resulted in rapid filamentation of the whole culture. The ratio of adenine to cytidine plus guanosine determined the average length of those cells that did filament, as well as the rate of filament formation. It should be noted that high adenine alone (1,000 μg/ml) in liquid culture produces *recA*-independent filamentation in both *sfiA*$^+$ and *sfiA* strains (JM1 and GC579).

**Induction of lysis in *tif*(ColE1,λ).** Strain *tif*(ColE1) was lysogenized with wild-type lambda, yielding EST201 *tif*(ColE1,λ). Induction of this strain in liquid culture gave the results expected if colicin repressor strongly competes with lambda repressor for activated *recA* protein. When protected by cytidine or by cytidine plus guanosine, strain EST201 *tif*(ColE1,λ) required much more adenine for induction than strain JM12 *tif*(λ) (Fig. 6). For the *tif*(λ) strain, adenine at 40 μg/ml completely overcame the protective effect of cytidine plus guanosine, i.e., complete lysis was observed. In contrast, for the *tif*(ColE1,λ) strain, adenine at 300 μg/ml produced lysis in only 60% of the cell population (data not shown). Lysis of the *tif*(ColE1,λ) strain required considerably more adenine, and presumably more activated *recA* protein, than did lysis of *tif*(λ). *tif*(λ) cells always lysed in liquid culture at 41.5°C without added adenine; in most experiments (though not in Fig. 6), lysis of *tif*(ColE1,λ) required exogenous adenine (5 μg/ml). Thus activated *recA* protein may be in short supply for lambda induction in strain *tif*(ColE1,λ).

***tif*-dependent colicin E1 synthesis.** In previous work on *tif*-dependent colicin synthesis (43), we compared the colicin E1 yield from a heat-induced *tif sfi*(ColE1) strain with the colicin yield from a constitutive *tif* strain (27), DM1187 *spr tif sfi*(ColE1), and were surprised to find a low yield from the *tif sfi* strain. In the present work we found that this was an artifact of the earlier assay method. Most of the colicin E1 in the constitutive *tif* strain was found in the supernatant fraction, in apparent support of the traditional notion that colicins are secreted proteins. Colicin production in the *tif sfi* and the *spr tif sfi* strains was examined. The results from the supernatant fluid and high-salt wash of both cultures showed that thermal shift-up of *tif sfi*(ColE1) resulted in induction of colicin synthesis, but the yields were very low and variable compared to the same fractions from *spr tif sfi*(ColE1) cultures. The reason for the low yields was apparent when we found that most of the colicin E1 produced by *tif* or *tif sfi* strains actually remained in the cell. In a *tif sfi*(ColE1) culture grown for 4 h at 41.5°C, approximately 90% of the colicin was in the cell pellet, 5% was in the supernatant fluid, and 5% was in the high-salt wash.

It was recently shown by Jakes and Model

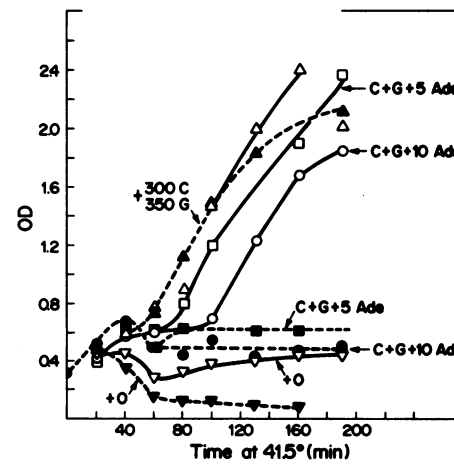


FIG. 6. *Induction at 41.5°C of prophage lambda in EST201 tif(ColE1,λ) compared with induction in strain JM12 tif(λ). Cells of each strain were grown to an optical density of 0.3 as described for Fig. 4. To samples of each culture were added: 300 μg of cytidine plus 350 μg of guanosine per ml (▲, △); cytidine plus guanosine plus 5 μg of adenine per ml (■, □); cytidine plus guanosine plus 10 μg of adenine per ml (●, ○); or no additions (▼, ▽). Closed symbols indicate tif(ColE1,λ), and open symbols indicate tif(λ).*

(16) that colicin E1 is not a normally secreted protein. It is not known how colicin eventually is released from the cell to form a halo on an indicator lawn. The extracellular location of most of the colicin E1 produced by DM1187 *spr tif sfi* in liquid culture is characteristic of derivatives of *tif*(ColE1) which are constitutive for colicin E1 synthesis due to either plasmid or cell mutations. In such strains partial lysis of the cultures was observed (unpublished data).

The kinetics of colicin formation after shift-up to 41.5°C are shown for EST200 *tif*(ColE1) (Fig. 7). There was an induction lag of about 60 min after shift to high temperature. The induction lag time was the same for the *tif*(ColE1) and the *tif sfi*(ColE1) strains. The maximum colicin concentration was reached by 5 h after temperature shift. Maximum yields at 41.5°C for both EST200 *tif*(ColE1) and EST204 *tif sfi*(ColE1) were in the range of 500 to 2,000 U and were not strictly correlated with maximum optical density. For cultures of strain DM1187 *spr tif sfi*(ColE1) grown overnight at 30°C, colicin yields were in the range of 1,000 to 2,000 U. Experiments involving shift-up of the *spr tif sfi*(ColE1) strain to 41.5°C showed no increase above the colicin that had been produced at 30°C. For *tif*(ColE1) strains grown at 30°C, the maximum colicin yield was about 20 U. For *tif*$^+$ (ColE1) strains (JM1 or GC579), the maximum

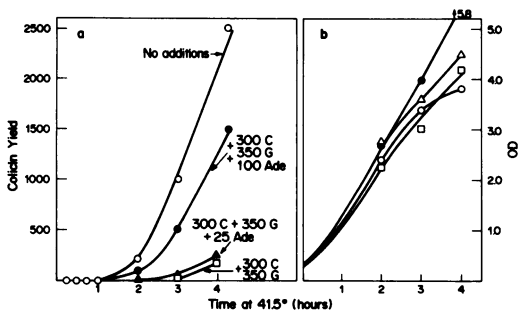


FIG. 7. *Kinetics of colicin E1 synthesis after thermal shift-up of strain EST200 tif(ColE1). (a) Colicin yield as measured by endpoint dilution, i.e., the highest dilution of the culture that will give detectable clearing of a lawn of colicin-sensitive indicator bacteria on plates. A culture of EST200 tif(ColE1) was grown with aeration at 30°C in M9-CAA medium to about 2 × 10$^8$ cells per ml, and then to 10-ml samples of the culture were added: no additions (○); 300 μg of cytidine plus 350 μg of guanosine per ml (□); cytidine plus guanosine plus 25 μg of adenine per ml (▲), cytidine plus guanosine plus 100 μg of adenine per ml (●). The flasks were aerated at 41.5°C. (b) Optical density at 550 nm of the culture samples whose colicin titer is shown in (a). The same symbols are used in both panels. The difference in optical density at 4 h was not found in repetitions of this experiment.*

spontaneous colicin yields at 30 or 41.5°C were also about 20 U. Thus *tif*-dependent colicin production is 25-fold to 100-fold higher than spontaneous colicin production.

*tif*-dependent colicin E1 synthesis, like lambda induction, was severely inhibited by cytidine plus guanosine (Fig. 7). Inhibition by cytidine alone or guanosine alone was much less effective than the combination of the two nucleosides. The cytidine-guanosine inhibition of colicin synthesis was reversed by adenine, with the rate of colicin synthesis increasing with the adenine concentration (Fig. 7). Strains *tif*(ColE1) and *tif sfi*(ColE1) showed the same response to these modulators.

In the absence of cytidine-guanosine inhibition, adenine (100 to 200 μg/ml) had no effect on *tif*-mediated colicin synthesis in liquid culture. Surprisingly, addition of 400 μg of adenine per ml caused marked inhibition of colicin synthesis by both *tif*(ColE1) and *tif sfi*(ColE1) (EST200 and EST204), although the increase in turbidity was not affected. Adenine at 400 μg/ml did not inhibit prophage induction of lambda lysogens of these colicinogenic *tif* strains. The inhibition of colicin synthesis by high adenine did not involve replication of the ColE1 plasmid (data not shown) and is not understood. However, adenine did enhance colicin production on plates, and this could be seen by the increase in size of the halos surrounding the colonies. The *tif sfi*(ColE1) strain, EST204, which is not killed at 41°C because of its colicin resistance and *sfi* mutation, was used (Fig. 8). Cytidine inhibited colicin production, whereas adenine enhanced it.

**Effect of bleomycin on *tif*-mediated colicin induction.** To observe the effect of DNA-damaging agents in overcoming cytidine-guanosine inhibition of *tif*-dependent induction, the potent DNA-damaging agent bleomycin (42) was used. Bleomycin was very effective in inducing colicin E1 synthesis in wild-type strains (unpublished data). Table 3 shows the effect of bleomycin on colicin yield by EST200 *tif*(ColE1) in the presence of cytidine plus guanosine at 41°C. It was seen that bleomycin reversed the inhibition of colicin induction caused by cytidine plus guanosine.

## DISCUSSION

**Intermediate induction stages.** Our results show that the degree of induction of three *recA*-dependent processes in a *tif-1* strain depends on the adenine-to-cytidine ratio of the medium. Since the degree of induction is probably a function of the concentration of activated *recA* protein, it appears likely that the amount of activated *recA* protein in a *tif-1* cell can be con-

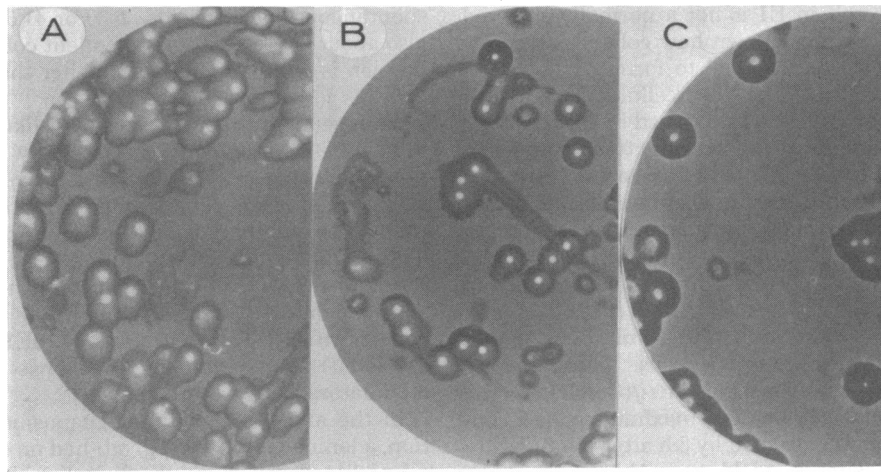


FIG. 8. *Effect of adenine and cytidine in plates on the size of colicin E1 halos produced at 41°C by strain EST204 tif sfi(ColE1), which is colicin resistant. Cells were grown to log phase at 30°C in M9-CAA medium, then spread on M9-CAA plates containing either (A) 200 μg of cytidine per ml; (B) no additions; or (C) 400 μg of adenine per ml. Procedure was the same as for Fig. 3.*

TABLE 3. *Bleomycin reversal of inhibition of colicin E1 synthesis in EST200 tif(ColE1)*[a]

| Addition | | Colicin yield (U/ml) | Turbidity ($OD_{550}$[b]) | Ratio: colicin/OD |
|---|---|---|---|---|
| Cytidine plus guanosine | Bleomycin (μg/ml) | | | |
| 0 | 0 | 350 | 4.0 | 88 |
| +[c] | 0 | 10 | 4.5 | 2 |
| + | 1 | 75 | 3.3 | 22 |
| + | 10 | 350 | 1.9 | 180 |
| 0 | 10 | 75 | 1.3 | 58 |

[a] A culture of EST200 *tif*(colE1) was grown in M9-CAA medium at 30°C to log phase, then divided into five aliquots as indicated and aerated at 41.5°C for 4 h. Colicin assay was as described in the text.

[b] $OD_{550}$, Optical density units at 550 nm.

[c] +, 100 μg of each per ml.

trolled by varying the adenine-cytidine ratio. Adenine alone leads to uncontrolled induction, at least for prophage lambda. The presence of large amounts of nonactivated *recA* protein in a cell does not result in induction of lambda (27) or of colicin E1 (unpublished data), even in the presence of high adenine.

Stable intermediate stages in the induction of lambda and colicin E1 have been revealed by varying the adenine-cytidine ratio of the medium. By adjustment of this ratio for lambda induction, *tif*(λ) cells could be brought into a state in which they were killed but did not lyse even after several hours of aeration at 41°C. Also, a fraction of the culture entered a state of reversible cessation of growth. Both of these states probably are the result of steady-state synthesis of autogenously regulated (17) lambda repressor, elicited by discrete amounts of activated *recA* protein. We suggest that the following events are occurring: some *recA* molecules in each cell are activated by an adenine derivative, the number activated depending on the adenine-cytidine ratio. These activated *recA* molecules cleave lambda repressor catalytically (5), eliciting continued synthesis of lambda repressor. The *kil* gene is expressed transiently, but complete expression of the lambda genome is prevented by rebinding of repressor to the phage operator regions. Any newly synthesized *recA* protein is not activated because of insufficient adenine derivative. Addition of extra adenine at a late time pushes the cells into lysis by activating more *recA* protein, resulting in cleavage of lambda repressor at a rate that cannot be matched by repressor synthesis. This finding of intermediate induction stages is complementary to the finding of Bailone et al. (1) of "subinduction," a state in which appreciable cleavage of lambda repressor occurs in the absence of phage synthesis.

For colicin E1 induction we found that *tif*(ColE1) cells, at certain adenine-cytidine ratios of the agar plates, could enter a state in which they synthesized colicin abundantly but were not killed. Increasing the adenine-cytidine ratio of the plates resulted in loss of colony-forming ability due to colicin production. In liquid culture we found that the rate of colicin synthesis was dependent on the adenine-to-cytidine ratio. Possibly, when the rate of colicin synthesis exceeds a certain value the cells are killed. What may be involved in cell death is how fast the colicin is synthesized compared to

how fast it is exported. The mechanism of colicin export is not known except that colicin E1 is not secreted (16).

Each of the three inducible processes studied here is negatively controlled by a different repressor. For prophage λ, the repressor is the product of the cI gene and has been studied in great detail (17, 34). For colicin E1, the existence of a plasmid-coded product which acts in *trans* to repress colicin synthesis has recently been deduced from the study of derepressed ColE1 mutants (32). For the inducible inhibitor of septum division (46), i.e., the filamentation protein, the repressor appears to be the *lexA* protein (29), which is also the repressor of *recA* synthesis. Evidence for this conclusion comes from the fact that the *tsl-1* mutation, which maps in *lexA*, produces lethal filamentation at high temperature (29). In a *tsl-1* strain, lethal filamentation occurs in the absence of functional *recA* protein (31). The *tsl-1* mutation also produces a large increase in synthesis of *recA* protein (40), as expected for a mutation that inactivates *lexA* protein. The gene which encodes the filamentation protein has not yet been identified but is probably not the *sfiA* gene (9) since in strains bearing the mutation *sfiA11*, normal filamentation still occurs after UV (unpublished data). We have not examined *sfiB* mutants.

Evidence is presented here of apparent competition between lambda repressor and colicin repressor for activated *recA* protein, since much more adenine is required to induce lysis of *tif*(ColE1,λ) than of *tif*(λ) when both strains are grown in cytidine plus guanosine. This case of competition between repressor species is in contrast to the case of preferential affinity observed by Bailone et al. (1). These workers showed that the presence of large amounts of lambda repressor in a cell reduced the frequency of induction of lambda lysogens but did not prevent induction of phages 434 and 80. Thus lambda repressor does not compete with 434 and 80 repressors for activated *recA* protein.

**Evidence for a modulator-binding site on the *recA* protein.** It was originally shown (18, 19) for the *tif-1* mutant that cytidine plus guanosine inhibits induction of phage lambda and that adenine and UV reverse this inhibition. Our results (Fig. 1 and 2) show that the role of adenine is to compete with cytidine rather than to override it. It is not yet known whether UV-damaged DNA overcomes guanosine-cytidine inhibition of the *tif* (*recA*) protein competitively or by an override mechanism. The apparent competition between adenine and cytidine observed here for *tif* activation suggests that the *tif* form of *recA* is an allosteric protein that can bind both negative and positive modulators. It is known that cytidine and adenine have different transport mechanisms (22, 39), so the competition we observe is not a competition for a transport protein. The fact that effective production of the inhibited state of a *tif-1* mutant requires two such different molecules supports the idea that the *tif* protein is an allosteric enzyme, since inhibition of many allosteric enzymes requires binding of two different negative modulators.

Any model of *tif* activation must be consistent with the results of Roberts and co-workers (5, 37, 38) on the mechanism of *recA* activation in vitro. These workers have shown that purified *recA* protein, either *tif* or *tif*$^+$, requires the addition of ATP and polynucleotide for cleavage of lambda repressor (5). It is possible that the adenine derivative required in vivo for *tif* activation is the ATP required in vitro for activation of both *tif* and *tif*$^+$ proteins and that the ATP-binding site on *recA* is also an inhibitor-binding site.

We propose that in the *tif-1* mutant an inhibitor-binding site normally present on *recA* protein is altered so that it has a much reduced affinity for binding negative modulators or a much enhanced affinity for binding a positive modulator, i.e., the site is now an allosteric site. Spontaneous activation of wild-type *recA* protein would occur by spontaneous dissociation of the normal inhibitor from its binding site and its replacement by ATP. To explain how DNA breakdown products or gapped DNA cause activation of wild-type *recA* protein, we postulate that there is a binding site for DNA that may be separate from the inhibitor site. When DNA binds to the DNA-binding site, we assume that this binding causes a conformational change at the inhibitor site, so that it now assumes a conformation like the inhibitor site of an extreme *tif* mutant, i.e., a *tif* mutant whose modulator site binds ATP very strongly.

Evidence that there may be a DNA-binding site distinct from the inhibitor site comes from the isolation by Clark of the mutant *recA142* (4). This strain, when lysogenic, cannot be induced by mitomycin C but shows a wild-type level of spontaneous lambda induction. It retains proteolytic activity (36) but apparently does not respond to DNA. More definite proof on whether there are separate DNA-binding and inhibitor-binding sites awaits the construction of a *tif* derivative that is activable by heat plus adenine but which cannot be activated by DNA-damaging agents.

We predict that the "hyper-*tif*" mutants recently isolated by Mount (28) will have *recA* protein with a higher affinity for binding of ATP than does the *tif-1* mutant. Curves for lethal

induction of hyper-*tif* mutant cells should be shifted very much to the left of the curves in Fig. 2. Still to be explained is the fact that, in vitro, *tif* protein, like wild-type *recA*, is completely dependent upon added polynucleotide for its proteolytic activity (5). D'Ari et al. (6) have recently shown that *tif*-dependent induction can be suppressed at the level of *recA* activation by a certain *dnaB* mutation and also by a *dnaA* mutation. These authors suggest that activation of the *tif* protein may require specific protein-protein interactions with certain other enzymes of a replication complex. In the framework of our model, these protein-protein interactions would cause a conformational change in the *tif* protein, producing an allosteric site.

At present there is no direct proof that there is a site on the *recA* protein which can bind both the negative modulators and ATP. The possibility exists that some protein other than *recA* is the target of the cytidine-adenine competition. Our model of *recA* as an allosteric enzyme suggests in vitro studies of binding of presumptive modulators to purified *recA* protein, as a test.

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