Mutagenic Response of Ames Strains Cured of Their Inducible Fels 1 and Fels 2 Prophages

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ABSTRACT

Ames strain TA100 was cured of its Fels 1 and Fels 2 prophages to yield the corresponding nonlysogenic derivative designated TAQ100. The two monolysogenic strains corresponding to TA100 lysogenic for Fels 1 (TAQ100F1) and for Fels 2 (TAQ100F2) were also isolated. In addition, the equivalent strains lacking pKM101 and designated TAQ1535, TAQ1535F1, and TAQ1535F2 were obtained. Ames strains TA98 and TA1538 are lysogenic for Fels 2 and were observed by colony hybridization to contain cryptic Fels 1 DNA sequences. Strains corresponding to TA98 and TA1538 cured of Fels 2 were isolated and designated TAQ98F1d and TAQ1538F1d, respectively.

Fels 1 grew poorly on Fels 1-cured strains, and Fels 2 grew not at all on Fels 2-cured strains. The cured strains had therefore to be identified as such by their failure to react in colony hybridization to contain cryptic Fels 1 DNA sequences. The specificity of the labeled probes was confirmed as did the standard Ames strains to a variety of well-known mutagens, including aflatoxin B1, 7,12-dimethylbenz[a]anthracene, daunorubicin, 2-amino-dipyrido[1,2-a:3',2'-d']imidazole, and β-naphthylamine. Also, mitomycin C, bleomycin, and diethylstilbestrol were nonmutagenic to TAQ100 and TAQ98F1d as they are to TA100 and TA98. Since the Fels prophages are inducible by aflatoxin B1, by daunorubicin, and by other agents, it seems that mutagenesis and Fels prophage induction occur in separate subpopulations of cells; this situation had previously been reported to occur for mutagenesis and prophage induction in Escherichia coli. In any case, the Fels prophages appear to have no major influence on the mutagenic response of the Ames strains.

INTRODUCTION

The Ames test (3) is widely used in research and industry to detect potential carcinogens as mutagens for Salmonella typhimurium (12). A battery of strains are used to identify different types of mutagenic chemicals. TA1535 and TA100 respond to agents causing base substitutions, whereas TA1538 and TA98 (as well as TA1537) respond to frame-shift mutagens (3). Strains TA1535 and TA100 are identical, except for the presence of plasmid pKM101 in the latter; similarly, TA1538 and TA98 differ only in that TA98 carries pKM101 (16). The plasmid enhances both frame-shift and base substitution mutagenesis (16), and muc, the gene (or genes) responsible, has been identified and localized to a small region of the plasmid DNA (23). In Escherichia coli, the recA+ lexA+ genotype is required for pKM101 to enhance mutagenesis (26). This indicates that the muc gene(s) of plasmid pKM101 increase(s) mutagenesis by participating in the recA+ lexA+-dependent, inducible cellular response to DNA damage (8, 28). In E. coli, induction of prophage λ is also a consequence of DNA damage (8, 28).

Wheeler et al. (27) and Yamamoto (27) have shown that the Ames strains are lysogenic for one or both of the bacteriophages Fels 1 and Fels 2. Strains TA1535 and TA100 release Fels 1 and Fels 2 into the culture medium, whereas strains TA1538 and TA98 release only Fels 2 (27). Based on present knowledge (8, 28) of bacterial mutagenesis and prophage induction, the presence of the Fels prophages in the Ames strains might interfere with the detection of certain mutagens. Many agents that are mutagenic in S. typhimurium (15) also cause induction of prophage λ in E. coli (4, 18, 19). Clearly, any cell in which Fels 1 or Fels 2 is induced should be incapable of giving a revertant colony. Indeed, induction of the Fels 1 and Fels 2 prophages has been observed after treatment of Ames strains with agents such as AB1 (27) and DNR.

To determine what effect, if any, the Fels prophages might have on the mutagenic response of the Ames strains, we isolated derivatives free of Fels 1 and/or Fels 2. We report here that the Ames strains and the cured derivatives respond similarly to a variety of chemical mutagens. We also show that all the Ames strains contain DNA sequences that will hybridize to [32P]DNA probes specific for Fels 1 or for Fels 2; this demonstrates that a cryptic Fels 1 prophage is present in TA1538 and TA98 despite the failure (27) of these strains to release Fels 1 into the culture medium.

MATERIALS AND METHODS

Materials. AB1, EB, DMBA, diethylstilbestrol, and mitomycin C were from Sigma Chemical Company, St. Louis, Mo. Bleomycin (Blenoxane) was from Bristol Laboratories of Canada, Belleville, Ontario, Canada. DNR was a gift from Rhône-Poulenc Industries, Vitry-sur-Seine, France. Glu-P-2 and β-NA were gifts of G. Saint-Ruf, Centre National de Recherche Scientifique, Orléans, France. [α-32P]dCTP was from New England Nuclear, Boston, Mass. Nick translation labeling kits were

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from BRL, Gaithersburg, Md. Agar was from Difco Laboratories, Detroit, Mich. Oxoid was from Oxoid Canada, Ltd., Nepean, Ontario, Canada.

**Bacterial and Viral Strains and Culture Conditions.** A list of the *S. typhimurium* strains used, along with their origins, is provided in Table 1. The characteristic properties of the Ames strains (spontaneous revertants per plate, mutagenic response to the diagnostic mutagen N-methyl-N-nitro-N-nitrosoguanidine, sensitivity to UV and to gentian violet) were routinely verified as described by Ames et al. (3). Ampicillin resistance of pKM101-carrying strains was verified with the aid of discs containing 10 μg ampicillin.

Rich liquid medium for bacterial growth was Oxoid. Solid medium for phage titration contained Oxoid and 1.2% agar; soft agar contained 0.6% agar, also in Oxoid. Unless otherwise indicated, the growth temperature for bacteria and viruses was 37°.

The Fels 1 and Fels 2 strains used for DNA isolation were mutants, isolated in this laboratory, forming clear plaques on Q1. They were grown on Q1 to titers of 10^9 to 10^10 plaque-forming units/ml. For tests of phage growth on the different bacterial hosts, the clear mutants, as well as wild-type Fels phages isolated from Q1 (Fels 1) and Q1 (Fels 2), were used. Lysates of Fels 1 were prepared in Vogel-Bonner (25) liquid medium. Plate lysates (17) of Fels 2 were obtained by growth on Oxoid plates.

**Mutagenesis Testing.** Mutagenesis assays were carried out as described by Ames et al. (3), except that overnight cultures were grown in Oxoid (14). Bleomycin, mitomycin C, and DNR were tested in the absence of S9; all other agents were tested in the presence of 25 μl of S9 per plate. S9 was prepared (3) from Aroclor 1254-induced rats. For a given agent, all comparisons of the mutagenic response of a given Ames strain and one or more of its corresponding cured derivatives were based on assays performed in parallel on the same day with the same reagents, S9, etc. Such a procedure is essential to avoid the problem of day-to-day variations (6, 20, 22) in the mutagenic response.

**Purification of Fels 1 and Fels 2 and Preparation of 32P-labeled Viral DNA Probes.** Each virus was purified by differential centrifugation (1) to remove bacterial DNA. Crude lysates (10 ml) were centrifuged at 70,000 x g for 1 hr, and the pellet was resuspended in 10 ml of 0.1 M ammonium acetate. Any insoluble material was removed by low-speed centrifugation (5,000 x g, 5 min), and the supernatants were again centrifuged at 70,000 x g for 1 hr. The pellets were resuspended in 1 ml of 0.1 M ammonium acetate. The resulting solutions were used for viral DNA isolation by the rapid SDS procedure described by Davis et al. (7), except that diethylpyrocarbonate was omitted. The purified DNA was subjected to phenol extraction, chloroform extraction, and ethanol precipitation in preparation for labeling by nick translation (21).

** Colony Hybridization.** Clones to be tested for the presence of Fels 1 or Fels 2 DNA were grown overnight on a master Oxoid plate. Replicas were made from the master plate and again grown overnight. Nitrocellulose filters were applied to the surface of the replicas to pick up the colonies. After treatment with alkali and neutralization, the filters were washed with 2 x SSC and heated at 80° for 2 hr. The rest of the procedure was modeled after that of Kloussis et al. (13). The filters were prehybridized for 4 hr at 68° in 4 x Denhardt’s/3 x SSC. Hybridization was for 48 hr at 68° in 8 ml of 4 x Denhardt’s/6 x SSC with 0.5% SDS, yeast tRNA (50 μg/ml), salmon sperm DNA (100 μg/ml), and 1 to 10 x 10^6 cpm of labeled viral DNA. The filters were washed at 50° in 3 changes of 0.1 x SSC containing 0.5% SDS over a 3-hr period. After drying at 37°, the filters were subjected to autoradiography. Exposure was for 1 to 7 days.

**Qualitative Test for Release of Fels Phage into the Culture Medium.** Fresh overnight cultures of the strains to be tested were treated with chloroform and further incubated at 37° for 10 min. Ten μl of the chloroform-treated cultures were then spotted on the surface of Oxoid plates overlaid with 0.3 ml of overnight cultures of Q1, Q1 (Fels 1) or Q1 (Fels 2) in soft agar. The plates were incubated overnight and scored. Plaques are formed by Fels 1 on Q1 and on Q1 (Fels 2) and by Fels 2 on Q1 and Q1 (Fels 1) (Table 1).

**Test for Growth of the Fels Phages on Bacterial Strains.** The strain to be tested (0.3 ml of an overnight culture) was spread in soft agar on an Oxoid plate. Lysates of Fels 1 and Fels 2 (or suitable dilutions thereof) were then spotted on the surface of the soft agar, and the plates were incubated. In these spot tests, Fels 1 formed plaques with low efficiency (≤0.01) on the Fels 1-cured derivatives of the Ames strains. The plaques were best seen after incubation of the test plates for 12 to 16 hr at 37° and then for 2 to 3 days at room temperature (25°). When Q1, Q1 (Fels 1), or Q1 (Fels 2) were the strains being verified, incubation of the plates for 12 to 16 hr at 37° was adequate.

<table>
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<th>Table 1 List of S. typhimurium strains used</th>
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<td><strong>Strain</strong></td>
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1 The TAQ strains are numbered according to the Ames strain to which they correspond. The presence of Fels 1, Fels 2, or cryptic Fels 1 DNA sequences was initially verified by placing F1, F2, or F1d after the strain number. Thus TAQ100F1 is TA100 cured of Fels 2 but still lysogenic for Fels 1.
2 The construction of the TAQ strains will be described in more detail elsewhere.
3 Fels 1 formed plaques with low efficiency on Fels 1-cured Ames strain derivatives; see "Materials and Methods."
We have never observed Fels 2 to form plaques on the Fels 2-cured derivatives of the Ames strains (Table 1).

RESULTS

Identification of Fels 1 and Fels 2 Lysogens by Colony Hybridization. As a first step in our procedure to obtain cured derivatives of the Ames tester strains, we isolated clones that failed to release Fels 1 and/or Fels 2 into the culture medium. In our first trial, approximately 25 such clones were obtained among survivors of TA100 cells treated with various agents known to cause prophage induction in other systems. No Fels 1- or Fels 2-sensitive strains were found in this group of potential cured clones. This led us to suspect that the Ames strains might be resistant to the Fels phages. [As it turned out, Fels 1 grows very poorly on Fels 1-cured Ames strain derivatives, and Fels 2 grows not at all on Fels 2-cured Ames strain derivatives (Table 1; see also “Materials and Methods”); these effects are probably due in part to the hisG46 mutation carried (2) by the Ames strains.]

We therefore decided to use colony hybridization (11) to identify those potential cured clones that had actually lost their Fels 1 and/or Fels 2 DNA sequences. Probes of Fels 1 and Fels 2 DNA, labeled with 32P by nick translation, were prepared. The specificity of the probes was verified using the nonlysogenic strain, S. typhimurium Q1, and its monolysogenic derivatives Q1 (Fels 1) and Q1 (Fels 2). Q1 gave little or no reaction with either probe (Fig. 1). Q1 (Fels 1) reacted with the Fels 1 probe but not appreciably with the Fels 2 probe, whereas Q1 (Fels 2) reacted with the Fels 2 probe but not appreciably with the Fels 1 probe (Fig. 1). Using colony hybridization, we found that one member of the first series of potential cured clones, isolated from a culture treated with DNR, was cured of Fels 2 (data not shown). This prompted us to search for other cured clones using the same agent (see below).

Presence of Cryptic Fels 1 DNA Sequences in Ames Strains TA98 and TA1538. Both the Fels 1 and the Fels 2 probes reacted in colony hybridization with all of the Ames tester strains, as well as with hisG46 and hisD3052 (Fig. 1). Since all of these strains release Fels 2 into the culture medium (Ref. 27 and data not shown), it was not surprising that they all reacted with the Fels 2 probe. Strains TA98 and TA1538 release no active Fels 1 phage into the culture medium (27), but they still contain cryptic Fels 1 DNA sequences as indicated by their reaction with the Fels 1 probe (Fig. 1). We refer to the cryptic Fels 1 DNA sequences in TA98 and TA1538 as F1d.

Cured Derivatives of the Ames Strains. Ames strain derivatives cured of Fels 1 and/or Fels 2 (Table 1) were identified among survivors of DNR-treated cells, as will be described in more detail elsewhere. Briefly, potential cured clones, identified by their failure to release active Fels 1 and/or Fels 2 into the culture medium, were screened by colony hybridization to identify those that had lost their Fels 1 or Fels 2 DNA sequences. Most (≥75%) of the potential Fels 1- or Fels 2-cured clones turned out to be similar to TA98 and TA1538 in that they still contained cryptic Fels DNA sequences. However, clones no longer containing DNA sequences reacting appreciably with the Fels 2 probe could be isolated routinely among survivors of pKM101-containing strains treated with DNR. Such Fels 2-cured derivatives of TA100 and TA98 were designated TAQ100F1 and TAQ98F1d, respectively (Fig. 1; Table 1). Only one primary Fels 1-cured clone was isolated. It was a survivor of DNR-treated TA100 which had also lost pKM101; it was therefore designated TAQ1535F2 (Table 1). The plasmid was reintroduced into this Fels 1-cured strain by conjugal transfer (17) to yield the monolysogen TAQ100F2 (Fig. 1; Table 1). A Fels 2-cured derivative of TAQ100F2 was isolated as before to yield TAQ100, a strain carrying neither Fels 1 nor Fels 2 (Fig. 1; Table 1). From TAQ100, the plasmidless strain designated TAQ1535 was derived (Table 1); Fels 2-cured clones (TAQ1535F1 and TAQ1538F1d) corresponding to the plasmidless strains TA1535 and TA1538 were also obtained (Table 1). All of the plasmidless cured strains were identified by colony hybridization (data not shown). We have not attempted to cure TA98 and TA1538 of their cryptic Fels 1 prophage.

Mutagenic Response of the Cured Strains. There were no...
major differences in the mutagenic response of the cured strains, as compared to that of their lysogenic parents. TA100 and its nonlysogenic analogue TAQ100 gave essentially identical dose-response curves for AB1, for DMBA, for Glu-P-2, and for β-NA (Chart 1, a to c) as well as for the weakly mutagenic (3, 15) DNR (data not shown). In analogous experiments, the 2 monolysogenic strains TAQ100F1 and TAQ100F2 each gave dose-response curves not detectably different from that of TA100 for AB1 (data not shown). Similarly, TA98 and its Fels 2-cured derivative TAQ98F1d gave virtually the same dose-response curves for DNR, for EB, and for DMBA (Chart 1d) as well as for Glu-P-2 (Chart 3c) and AB1 (data not shown). Furthermore, diethylstilbestrol, bleomycin, and mitomycin C, 3 agents with carcinogenic activity that are nonmutagenic to the standard Ames strains (5, 10, 15), were also nonmutagenic to TAQ100 and to TAQ98F1d (Chart 2).

Strain TA1535 does not carry the plasmid pKM101 but is otherwise identical to TA100 (16). TA1535 is insensitive to many mutagens to which TA100 responds, including AB1 (16); the 2 monolysogens TAQ1535F1 and TAQ1535F2, as well as the nonlysogen TAQ1535, were also insensitive to AB1 (data not shown). TA1535 does respond to β-NA (15), and the dose-response curves for TAQ1535F1, TAQ1535F2, and TA1535 (Chart 3a) and for TAQ1535 (data not shown) were very similar.

The only consistent difference that we observed between an Ames strain and one of its cured counterparts was a lowering of the sensitivity to EB mutagenesis in TAQ1538F1d as compared to TA1538 (Chart 3b). We isolated 2 clones of TAQ1538F1d, and both gave substantially fewer mutants with EB than does TA1538 (Chart 3b). TAQ1538F1d and TA1538 were, however, equally sensitive to the mutagenic effects of Glu-P-2 (Chart 3c). Note also that TAQ98F1d, the parent strain

![Chart 1. Dose-response curves for Ames strains TA100 and TA98 and their cured analogues TAQ100 and TAQ98F1d with known mutagens.](image-url)
Chart 2. Absence of mutagenic effect of diethylstilbestrol, bleomycin, and mitomycin C on Ames strains TA100 and TA98 and on their cured analogues TAQ100 and TAQ98F1d.

from which the 2 clones of TAQ1538F1d were derived by spontaneous loss of pKM101 (Table 1), responded to EB in the same way as did TA98 (Chart 1d).

**DISCUSSION**

When we undertook this work, we suspected that the presence of the Fels 1 and Fels 2 prophages in the Ames strains might reduce their mutagenic response to certain chemicals. Fels 1 and Fels 2 are both inducible by AB1 (27), by DNR, and by other agents. As we pointed out in the Introduction, a cell in which Fels 1 or Fels 2 is induced should not give a revertant colony. Therefore, the effect of Fels 1 and/or Fels 2 in the Ames strains might have been to reduce the observed number of mutants for a given dose of mutagen.

As it turned out, the response of our cured strains to the agents used was, with the exception of the response of TAQ1538F1d to EB, remarkably similar to that of the Ames strains to which they corresponded (Charts 1 to 3). For the mutagens tested, this was so both for the quasilinear portions of the dose-response curves (Charts 1 and 3) and at higher dose ranges where toxic effects may reduce the number of mutants (data not shown). Note in particular the similar mutagenic response of the Ames strains and their cured derivatives to DNR (Charts 1 and data not shown), which was the agent used to obtain the cured strains. Concerning the 3 false negatives, bleomycin, mitomycin C, and diethylstilbestrol, it was conceivable that they fail to cause detectable mutagenesis because of toxic effects that they exert on the tester cells as a consequence of Fels prophage induction. This possibility was rendered highly unlikely by the failure of these agents to mutate TAQ100 and TAQ98F1d (Chart 2).

The only consistent effect that we observed of curing on mutagenesis was the decreased mutagenic response of
TAQ1538F1d to EB (Chart 3b). A trivial explanation for this result would be that the TAQ1538F1d that we isolated is poorly responsive to mutagens in general. This possibility can be excluded for 2 reasons: (a) TAQ1538F1d was obtained by spontaneous loss of pKM101 from TAQ98F1d (Table 1), which gives the same mutagenic response as TAQ98 to EB (Chart 1d); (b) TAQ1538F1d and TAQ1538 respond identically to Glu-P-2 (Chart 3c). It seems, therefore, that in the absence of pKM101 the Fels 2 prophage potentiates the mutagenic response to EB.

That our Fels 2-cured derivatives of the Ames strains appear to be resistant to Fels 2 (Table 1) is probably a consequence of the ips mutation carried (2) by the Ames strains. This mutation affects the cell surface, and indeed the ips strains were selected by their resistance to another phage, C21 (2). We have recently eliminated the unlikely possibility that the cured strains retain the Fels 2 DNA sequences necessary to maintain immunity; by Southern blot analysis (24), no Fels 1 phage-specific Pst I fragments are retained by TAQ1535F2, and no Fels 2-specific Pst I fragments are present in TAQ100F1.6

Fels 1 grows on our Fels 1-cured strains, albeit with low efficiency (Table 1). We are presently investigating this phenomenon in order to determine which of several possible explanations may account for the low efficiency of plaque formation. In any case, however, the strains that we have identified by colony hybridization as being cured of Fels 1 (Fig. 1 and other data not shown) are no longer immune to Fels 1.

TA1538 and TA98 both carry cryptic Fels 1 DNA sequences (Fig. 1; Table 1). Their similarity in this respect is not surprising, since TA98 was obtained by conjugal transfer of pKM101 into TA1538 (16). Furthermore, strains cryptic for Fels 1 and/or Fels 2 are by no means difficult to obtain; they made up the majority of our potential cured strains. Presumably, a mutation...
rendering Fels 1 cryptic was introduced during the construction (2) of strain TA1538.

In conclusion, we have found the Fels prophages to have no negative influence on the use of the Ames strains for mutagen detection. The only difference that we have observed between the mutagenic response of the Ames strains and their counterparts cured of Fels 1 and/or Fels 2 suggests that the Fels 2 prophage may actually increase the mutagenic response for at least one agent. However, the Fels 1 and Fels 2 prophages are both inducible by mutagenic agents like DNR and AB1. It thus appears that, in mutagen-treated Fels 1 and/or Fels 2 lysogens of S. typhimurium, as in UV-irradiated lysogens of E. coli (9, 29), prophage induction and mutagenesis occur in different subpopulations of cells. This situation presumably arises because mutation and lysogenic induction occur in different individuals, chosen at random from a large cellular population.

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