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Loss of an Essential Function of *Escherichia coli* by Deletions in the *thyA* Region

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In an attempt to obtain deletions in the *thyA* gene, an abnormal lysogen of λ having the prophage inserted between the *thyA* and *lysA* genes was induced, and the surviving cured cells were examined for Thy⁻ and Lys⁻ mutants. In nearly 10,000 cured cells, 184 Lys⁻ but no Thy⁻ mutants were found. At the same time, the induced λ phage contained an approximately equivalent number of λ*thyA*⁺ and λ*lysA*⁺ transducing particles. By contrast, in a strain with the genotype F′ *thyA*⁻*lysA*⁺/*thyA*⁺*lysA*⁺, induction of the abnormal λ lysogen gave rise to many Thy⁻ mutants in the cells cured of the prophage. In these Thy⁻ mutants it was not possible to eliminate the episome with acridine orange, although the episome could be removed in control cultures with a *thyA*⁺ allele in the resident gene. Therefore, it was suggested that deletion of a gene in the region of the chromosome from the position of the insertion of the λ prophage through the *thyA* gene caused loss of an essential and diffusible function.

Shimada et al. (9), employing a host with the bacteriophage λ attachment site deleted, obtained abnormal lysogens in which λ prophage was inserted into the *Escherichia coli* chromosome at a number of other specific sites. In one of the abnormal lysogens, λ prophage was inserted between the *lysA* and *thyA* genes [λ(*lysA-thyA*)].

In the course of studies on thymidylate synthetase (3, 8; B. A. Dale, Ph.D. thesis, University of Michigan, Ann Arbor, 1968), it was considered desirable to use this lysogen to investigate the genetics and regulation of the enzyme. We found no evidence in the literature (1, 7) of a *thyA* deletion. However, since λ*thyA*⁺ transducing lysates were obtained by induction of the λ(*lysA-thyA*) lysogen (9), we expected to obtain *thyA* deletions in surviving cured cells, as has been shown for *gal* and *bio* genes on induction of λ prophage (4, 9) at the normal λ attachment site. Surprisingly, from some 10,000 cured clones resulting from induced cultures of this lysogen, we obtained 184 Lys⁻ mutants but no Thy⁻ mutants. Simultaneously, the induced λ lysates contained an approximately equal number of λ*thyA*⁺ and λ*lysA*⁺ transducing phage particles. In the presence of an F′ episome carrying *thyA*⁻ and *lysA*⁺ chromosomal genes, induction of the lysogen gave rise to many Thy⁻ mutants in the strains cured of the prophage. We therefore conclude that deletion of the *thyA* region, but not necessarily of the *thyA* gene, removes a diffusible function which is necessary for growth.

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MATERIALS AND METHODS

E. coli K-12 and phage strains. The bacterial strains employed are listed in Table 1. Strain KS 245 carries a deletion at the λ attachment site between the *gal* and *uvrB* genes [Δ(*gal*...*uvrB*)] and a single heat-inducible λ prophage between *lysA* and *thyA*. This strain was derived from strain 72 described in the paper by Shimada et al. (9). Bacteriophage λvir and λcI857 were provided by David Friedman of the Department of Microbiology, The University of Michigan.

Media. Tryptone broth (TB) with 1 μg of thiamine hydrochloride (Thi) per ml and 1 μg of biotin (Bio) per ml added was used to grow strain KS 245 and its derivatives. Strains GM 911 and GM 914 were grown in TB plus 0.2% maltose, 0.01 M $MgSO_4$, and 20 μg of thymine per ml. Agar plates prepared from the minimal medium (MM) of Vogel and Bonner (13) contained 0.2% glucose with appropriate requirements added at 20 μg/ml each. Diluting broth contained 0.08% nutrient broth and 0.5% NaCl; 0.9% NaCl was used as a nonnutritional dilutant.

thy mutants. Thymine-requiring mutants were isolated by the trimethoprim selection procedure (11), as modified in this laboratory (3). Thy⁻ mutants of strain KS 245, (e.g., strain GM 915, Table 1) isolated by this procedure require high levels of thymine or thymidine (dT) (100 μg/ml) for normal growth.

Isolation of λvir-resistant strains. Cultures grown at 32 C were spread on tryptone agar plates

TABLE 1. *Bacterial strains employed*

Strain	Lab no.	Relevant genotype	Source
JC 5467	GM 713	λ^r, λ^-, *mal lysA thyA argA thi his*	D. Mount
CP 154	GM 714	λ^r, λ^-, *mal lysA argA thi his*	D. Mount
	GM 773	F'_{15} *lysA*$^+$*thyA*$^+$*argA*$^+$/713	F'_{15}/*E. coli* B → 713[a]
	GM 776	F'_{15} *lysA*$^+$*thy argA*$^+$/713	From 773[a]
	GM 777	F'_{15} *lysA*$^+$*thy argA*$^+$/713	From 773[a]
CP 154 Thy$^-$	GM 791	λ^r, λ^-, *mal lysA thyA argA thi his*	[a]
C_{600}	GM 842	*thr leu thi*	D. Friedman
KLF43/KL253 (4291[b])	GM 845	F'_{143} *lysA*$^+$*thyA*$^+$*tyrA*$^+$/*thyA tyrA recA*	
KS 245	GM 902	Δ(*gal . . . uvrB*), λcI857 between *lysA*$^+$ and *thyA*$^+$, HfrH	K. Shimada (9)
	GM 905	845 *thy*	[a]
KS 245 λvir^r	GM 907	λvir-resistant strain of KS 245	[a]
713 Mal$^+$	GM 911	mal$^+$ revertant of 713, λ^s	[a]
791 Mal$^+$	GM 914	mal$^+$ revertant of 791, λ^s	[a]
KS 245 Thy$^-$	GM 915	*thy* derivative of KS 245	[a]
KS 245 Thy$^-$	GM 916	*thy* derivative of KS 245	[a]
	GM 949	902 *argA*	φ714 → 916[a]
	GM 950	902 *lysA argA*	φ714 → 916[a]
902 Att$_\lambda^+$	GM 954	(*gal . . . uvrB*)$^+$ derivative of 949	Hfr6 → 949[a]
	GM 956	(*gal . . . uvrB*)$^+$ derivative of 949	Hfr6 → 949[a]
954 λvir^r	GM 957	λvir-resistant strain of 954	[a]
956 λvir^r	GM 958	λvir-resistant strain of 956	[a]
	GM 973	F'_{15} *thy*/958	776 → 958[a]
	GM 974	F'_{15} *thy*/958	777 → 958[a]
	GM 975	F'_{143} *thy*/958	905 → 958[a]
	GM 982	F'_{15} *thy*/954	776 → 954[a]
	GM 983	F'_{15} *thy*/956	776 → 956[a]
	GM 984	F'_{143} *thy*/956	905 → 956[a]
	GM 995	F'_{15} *thy*/949	776 → 949[a]
	GM 1001	F'_{15} *thy*/950	777 → 950[a]
4236[b]	GM 1008	Hfr6	D. Oxender

[a] This study. The arrows indicate the direction of the genetic transfer, either by episomal transfer (F′) or by transduction (φ714 → 916). GM 776 and 777 are separate isolates as are GM 915 and 916, and GM 954 and 956.

[b] *E. coli* Genetic Stock Center; Department of Microbiology, Yale University.

previously seeded with λvir phage. Resistant colonies (λvir^r) developed after incubation at 32 C for 1 or 2 days. These strains were purified on tryptone agar at 32 C, and the properties of λvir resistance and λcI857 temperature sensitivity were verified again.

Transduction. Transductions were mediated with bacteriophage P1bt by using procedures described earlier (6). P1bt will transduce in both *E. coli* B and K-12 strains.

Mal$^+$ revertants. Since strains GM713 and GM791 are λ resistant because they are Mal$^-$, they can be converted to λ-sensitive strains by selecting for Mal$^+$ revertants. Overnight cultures of Mal$^-$, λ-resistant strains were spread on MM plates containing maltose as the energy source and with other required additions, and the plates were incubated at 37 C. Mal$^+$ revertants were purified on L agar plates and tested for phage λ sensitivity (GM 911 and GM 914).

Preparation of a λcI857 lysate from strain KS 245. The λcI857 lysates from strain KS 245 were obtained by heat induction, and the number of phage was measured by plaque formation on strain C_{600}.

Transduction by λlysA$^+$ or λthyA$^+$. The *lysA thyA* strains (GM 911 and 914) were grown overnight at 37 C in TB containing thymine, maltose, and $MgSO_4$, and then starved in 0.01 M $MgSO_4$ for 1 h. A 0.1-ml volume of a culture containing 2×10^9 cells per ml was mixed with the lysate at a phage multiplicity of 5. The mixture was incubated for 30 min at 32 C, and the cells were collected by centrifugation and suspended in saline. A 0.1-ml volume of an appropriate dilution was spread on minimal agar plates containing histidine, thiamine, arginine, and thymine for selection of Lys$^+$ transductants, or with histidine, thiamine, arginine, and lysine for Thy$^+$ selection. These plates were incubated at 32 C.

Episomal transfers. F′ episomal transfers were carried out essentially as described previously (6). To introduce an F′ episome into an Hfr strain, the Hfr strain was converted into a F$^-$ phenotype by aeration of a saturated culture (2) overnight at 32 C. The donor strain, grown to log phase, was added at a ratio of approximately 5 Hfr recipient cells (F$^-$ phenotype) to one F′ donor cell. The mixture was incubated at 32 C for 1 h, diluted 100-fold with diluting broth, and vigorously agitated 1 min with a Vortex mixer. A 0.1-ml volume of the diluted mixture was spread on MM plates containing 1 μg of Bio per ml and 1 μg of Thi per ml.

Genetic replacement of the gal. . .uvrB deletion.

To replace the *gal...uvrB* deletion of strain GM 949 (KS 245 *argA*), this Hfr strain was first converted to a F^- phenotype (2). A mating, Hfr6 (GM 1008)→949, was carried out by the method used for the F'→Hfr transfer. At the same time, Gal^+ and Bio^+ recombinants of strain GM 949 were selected on galactose minimal medium plus Thi. The resulting Gal^+ Bio^+ λcI857$^+$ strains (GM 954, GM 956) were purified on L agar plates.

Prophage curing procedure. A method of curing λ lysogens by a thermal treatment (9) was modified as follows. Cultures were grown overnight at 32 C in tryptone broth, or in MM plus Bio plus Thi medium, with those strains carrying an episome. Then, 0.1 ml of the culture at the proper dilution was spread on tryptone agar containing 100 μg of dT per ml, and the plates were incubated at 42 C for 1 or 2 days. It is necessary to prevent the plates from drying out. Sometimes such plates were shifted to 37 C after incubation overnight at 42 C. Most cured cells formed colonies within 24 h. To detect Lys^- or Thy^- mutants, colonies of cured cells were transferred by replica plating to the following types of agar plates, respectively: MM plus dT (100 μg/ml) and MM plus lysine.

Acridine orange treatment to eliminate F episomes. The acridine orange procedure was essentially that of Hirota (5). The culture, inoculated at 10^4 cells per ml, was grown overnight at 30 C in L broth (pH 7.7) containing 120 μg of acridine orange and 40 μg of dT per ml. An appropriate dilution was spread on tryptone agar plates containing 40 μg of dT per ml, and then the resulting colonies were replica-plated on minimal plates containing dT and the required nutrients. The cultures would not grow in the absence of arginine if the episome had been eliminated, since the resident gene was *argA*$^-$. To verify further the presence or absence of the F' episome, the colony was streaked on a minimal plate containing histidine and Thi and preseeded with strain GM714, which requires arginine, lysine, histidine, and thiamine. When induced Thy^- cultures are used as F' donors, only the recipient receiving the F' episome will grow on the plate.

RESULTS

Lys^-, but not Thy^-, mutants formed on induction of λ (lysA-thyA) lysogen. Strain KS 245 is a lysogen carrying a temperature-sensitive prophage, λcI857, inserted between the *lysA* and *thyA* genes. When such a strain is incubated at 42 C, λcI857 is released from the bacterial chromosome, replicates, and brings about lysis of the host cells. Some cells will survive, although at a low frequency. Among the survivors, one should expect to find deletion mutants, i.e., chromosomal deoxyribonucleic acid (DNA) will be deleted on either side of the λ insertion site as a λ hybrid is induced (4, 9). Table 2 shows the results of such experiments in which clones from cured cells, derived by induction of strain KS 245 and a λvir^r derivative of strain KS 245, were examined. The λvir^r strain was employed to increase the number of surviving cells. However, of 9,990 cured cells, 184 Lys^- mutants, but no Thy^- mutants, were found. Therefore, it appeared that deletions on the *thyA* side of the λ insertion site brought about loss of a gene essential for growth, and that the resulting mutant was not supported by the nutrients of tryptone agar with added dT.

TABLE 2. *Formation of Lys^-, but not Thy^-, in cured cultures after induction of a lysogen carrying λ prophage between lysA and thyA*

Strain	No. of cured cells	No. of mutants	
		Lys^-	Thy^-
KS 245 (GM 902)	1,977	19	0
KS 245, λvir^r (GM 907)	8,013	165	0

Both λthyA$^+$ and λlysA$^+$ transducing particles are formed by λ induction. The results in the previous section might arise if the induction of λ(*lysA-thyA*) lysogen were biased in favor of the production of transducing λ phage particles carrying *lysA*$^+$, but not *thyA*$^+$, genes. However, measurement of the number of λ*lysA*$^+$ and λ*thyA*$^+$ transducing particles in the lysates from strain KS 245 by transduction into *lysA thyA* recipients (GM 911 and GM 914) did not support such a bias. The number of Thy^+ transductants was, in fact, seven times greater than Lys^+ transductants with strain GM 911 as the recipient, and essentially equal to the Lys^+ transductants with strain GM 914 as the recipient (Table 3). Thus, although the transducing particles included both the *thyA*$^+$ and *lysA*$^+$ markers, only Lys^- mutants were found in the cured survivors.

Shizuya and co-workers (10) have found that a deletion in the *uvrB* region together with a *polA* mutant is lethal. To determine whether deletions of both the λ attachment site and the *thyA* region might also lead to a lethal condition, the *gal...uvrB* deletion was replaced by recombination after conjugation, and the result-

TABLE 3. *Production of both λthyA$^+$ and λlysA$^+$ transducing particles on induction of a λ (lysA-thyA) lysogen*[a]

Donor	Recipient	Selected marker	No. of transductants per ml
λ lysate from KS 245	*lysA thyA argA* (GM 911)	Lys^+	200
		Thy^+	1,460
λ lysate from KS 245	*lysA thyA argA* (GM 914)	Lys^+	720
		Thy^+	550

[a] The recipients, GM 911 and GM 914, were constructed from different strains (Table 1).

ing strains were made λvir resistant, as described above, to form strains GM 957 and GM 958. Many Lys⁻, but still no Thy⁻, mutants were obtained in cured cells after induction of λ phage from either GM 957 or GM 958 (Table 4). High concentrations of dT (1 mg/ml) were employed in the experiments in the event that dT could be limiting. These results demonstrate that the lethal condition is not related to the *gal...uvrB* deletion.

Formation of Thy⁻ mutants on induction of a λ(lysA-thyA) lysogen carrying the episome, F′ thyA⁻ lysA⁺. To test further whether an essential function was present in the *thy* region of the chromosome, partial diploids were constructed to complement this region. The F′ episome employed was made *thyA*⁻ so that deletions of the *thyA* gene of the chromosome could be detected and *lysA*⁺ so that Lys⁻ mutants would not be detected. With such λ(*lysA-thyA*) lysogens carrying the episome F'_{15} *thyA*⁻ *lysA*⁺, induction of λ phage gave a large number of Thy⁻ mutants in the cells cured of the prophage, but no Lys⁻ mutants, whether the *gal...uvrB* region was present (GM 973-975, GM 982-984) or deleted (GM 995, GM 1001) (Table 5). When these partially diploid, prophage-cured Thy⁻ cells were treated with acridine orange, 100% of the resulting cells tested still possessed the F′ episome. On the other hand, in the uninduced control cultures carrying these episomes (GM 982, GM 983, and GM 984), but having a *thyA*⁺ allele in the resident gene, 100% of the colonies examined showed loss of episome after identical treatment with acridine orange. These results then give further evidence that deletion of a function in the region of the chromosome from the position of the insertion of the λ phage through the *thyA* gene is either lethal or precludes growth.

TABLE 4. *Apparent lethality of deletions in the thy region produced by induction of a λ (lysA-thyA) lysogen not having the gal...uvrB deletion* (Att_λ^+)

Strain	No. of cured cells	No. of mutants	
		Lys⁻	Thy⁻
GM 957	270	48	0
	105[a]	50	0
GM 958	113	32	0
	195[a]	124	0

[a] The experiments were made independently, and these cured cells were obtained on tryptone plates plus 1,000 μg of dT/ml plus 1 μg of Thi/ml.

DISCUSSION

These studies provide strong evidence that a deletion in the region of the chromosome from the λ insertion site through the *thyA* gene, of the order of 0.1 to 0.2 min on the chromosome map (12), is lethal or prohibits growth. Thus, on induction of an unusual lysogen with λ prophage inserted between *thyA* and *lysA*, many Lys⁻, but no Thy⁻, mutants were found in the surviving cells cured of prophage. Nevertheless, both the *thyA* and *lysA* genes would be expected to have been deleted (4, 9) since approximately equal numbers of λ*thyA*⁺ and λ*lysA*⁺ hybrids were found in the induced lysates.

However, induction of λ(*lysA-thyA*) lysogens carrying in addition the episome F′ *thyA lysA*⁺ gave a large number of Thy⁻ mutants in the surviving cells cured of prophage, but no Lys⁻ mutants. It has not been possible to eliminate the episome from these cells by acridine orange. Thus, elimination either is lethal or prevents growth when the region from the λ insertion site through the *thyA* gene has been deleted.

Two possible reasons for the inability to obtain deletions in the *thyA* region may be

TABLE 5. *Formation of Thy⁻ cells by induction of λ (thyA-lysA) lysogen carrying a F′ thyA lysA⁺ episome*

Strain	Relevant genotype[a]	No. of cured cells	No. of mutants	
			Lys⁻	Thy⁻
GM 973	F'_{15} *thy lysA*⁺/958	520	0	328
GM 974	F'_{15} *thy lysA*⁺/958	928	0	68
GM 975	F'_{14} *thy lysA*⁺/958	256	0	16
GM 982	F'_{15} *thy lysA*⁺/954	145	0	110 (8/9)[b]
GM 983	F'_{15} *thy lysA*⁺/956	225	0	181 (41/41)
GM 984	F'_{14} *thy lysA*⁺/956	132	0	87 (9/10)
GM 995[c]	F'_{15} *thy lysA*⁺/949	303	0	192
GM 1001[c]	F'_{15} *thy lysA*⁺/950	250	0	182

[a] Strain GM 958 is λvir[r]; GM 954 and GM 956 are λvir[s].

[b] This ratio represents the fraction of Thy⁻ mutants of those tested which transferred the F′ episome to a F⁻ recipient.

[c] The region *gal...uvrB* was deleted in these strains.

suggested. First, a gene or region essential for growth exists between the λ insertion site and *thyA*. Second, the product of *thyA* has two functions: one as thymidylate synthetase, and the other at some unknown step in the growth process. If this were the case, all *thyA* mutants obtained by the usual trimethoprim-thymine selection would form a protein that is functional in the growth process, but it would not be possible to obtain a mutant which had lost both functions. However, a slow-growing mutant leaky in the unknown function, i.e., not fully by-passed by thymine, or a mutant with a temperature-sensitive second function would be conceivable. It is interesting to note that recent studies by Wovcha et al. (14) have provided evidence that deoxycytidine monophosphate hydroxymethylase has a second direct function in T4 DNA synthesis.

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