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A Mutation Defining Ultrainduction of the *Escherichia coli gal* Operon

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Tn*10* insertion in the *galS* (ultrainduction factor) gene of *Escherichia coli* allows the *gal* operon to be constitutively expressed at a very high level, equal to that seen in a Δ*galR* strain in the presence of an inducer. The insertion has been mapped by criss-cross Hfr matings and by marker rescue into Kohara phages at 46 min on the *E. coli* chromosome.

The *gal* operon of *Escherichia coli* is regulated both positively and negatively at the level of transcription initiation. There are two *gal* promoters, P1 and P2; P1 is activated by the cyclic AMP-cyclic AMP receptor protein complex bound in the −40 region, whereas P2 is inhibited by this binding. Both promoters are negatively regulated by a Gal repressor (GalR), the product of the unlinked *galR* gene, by binding to the two *gal* operators, O_E and O_I (1). Addition of an inducer such as D-galactose or D-fucose causes derepression of *gal*. Heretofore, this induction had been ascribed to the sugar's binding to the Gal repressor and causing the latter to release its binding to the DNA. Recently, we reported another level of regulation of *gal*. Strains that have the entire *galR* gene deleted and are thus constitutive for *gal* expression still showed further induction when an inducer was added. We called this phenomenon ultrainduction (10). We proposed the existence of a second repressor capable of binding to sites which are the same as or which overlap with *gal* operators and inactivable by D-galactose and D-fucose. In this study, we have isolated a mutation in the gene encoding the second *gal* repressor and have mapped it to 46 min on the *E. coli* chromosome.

To isolate a mutation in the gene encoding the second repressor, we started with a strain that was deleted for the *galR* gene and had a *galE-lacZ* protein fusion (JT247). (The strains used in this study are shown in Table 1.) In such a strain, ultrainduction was indicated by a threefold increase in β-galactosidase activity when the inducer was added (10). Colonies of such a strain appeared red on MacConkey lactose plates, even in the absence of an inducer, because of the high level of expression (approximately 500 Miller units; see Table 2). When the β-galactosidase competitive inhibitor thio-ethyl-phenyl-β-D-galactoside (TEPG) (5) was added to a concentration of 1 mM, JT247 produced white colonies on MacConkey lactose plates. The addition of D-fucose or D-galactose to MacConkey lactose plates containing 1 mM TEPG resulted in red colonies. We reasoned that a mutation in the gene encoding the proposed repressor would result in red colonies in the presence of TEPG, even without an inducer. Bacteriophage λ NK1098 containing a defective Tn*10* transposon (termed ΔTn*10*) was used to make random insertions into strain MC4100 (9, 11). Approximately 10,000 of these MC4100 Tc^r colonies were pooled, and phage P1 was grown on the bacterial pool. The resulting P1 lysate was used to infect JT247, selecting for tetracycline resistance on Luria broth plates containing 15 μg of tetracycline per ml. Tetracycline-resistant colonies were replicaplated to MacConkey lactose plates containing 15 μg of tetracycline per ml and 1 mM TEPG. Red colonies were found at a frequency of approximately 1.0%.

To ensure that the red phenotype on MacConkey lactose-tetracycline-TEPG plates was caused by the ΔTn*10* insertion, phage P1 was used to transfer the ΔTn*10* from six of the mutants into a fresh JT247 strain. In one of the six, there was no linkage (0 of 226 tetracycline-resistant colonies) between tetracycline resistance and the MacConkey lactose plate phenotype. In each of the other five, 100% of at least 2,000 tetracycline-resistant colonies were red on the MacConkey lactose-tetracycline-TEPG plates. Only these five were studied further.

β-Galactosidase in the mutants was measured and compared with that in the parent strain. Results are shown in Table 2. Two classes of mutants were found: class I, four mutants that in the absence of an inducer had a high level of *gal-lacZ* expression which increased further upon addition of an inducer (represented by strain AG708); and class II, one mutant that in absence of an inducer had a high level of expression of *gal-lacZ* which did not increase upon addition of an inducer (strain AG701). The phenotype of the second class was what we expected from a mutation in a gene encoding the proposed second *gal* repressor. Expression was at a high level regardless of whether an inducer was present. We call the gene into which the ΔTn*10* was inserted *galS*. The mutation in AG708 is probably not allelic to that in AG701, because the AG708 ΔTn*10* insertion causes a decrease in growth rate, whereas *galS*::ΔTn*10* does not. Furthermore, it does not appear to map to the same location (see below).

When this *galS*::ΔTn*10* mutation was introduced into a $galR^+$ strain, there was no effect on the basal level of expression. As shown in Table 2, however, the induced level was higher than that in a $galS^+$ $galR^+$ strain, showing that *gal* can be induced to a higher level of expression in a strain lacking the second repressor.

The *galS*::ΔTn*10* was introduced by phage P1 transduction into two Hfr strains for mapping. Hayes Hfr has its origin of transfer at 97.5 min, and it transfers in a clockwise direction. KL16's origin of transfer is at 60 min, and it transfers in a counterclockwise direction. Interrupted mating

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TABLE 1. *E. coli* strains used

Strain	Origin or genotype	Source or reference
AG701	JT247 *galS*::ΔTn*10*	This study
AG708	JT247 *zzz*::ΔTn*10*	This study
G1053	Hfr KL16	Laboratory collection
JT234	MC4100 *galE*::*lacZ*/F′*gal*$^+$	10
JT247	JT234 Δ*galR*	Laboratory collection
MJW103	Hfr KL16 *galS*::ΔTn*10*	G1053 × P1 · AG701[a]
MJW104	Hfr Hayes *galS*::ΔTn*10*	SA814 × P1 · AG701[a]
MJW105	Hfr Hayes *clpA182*::ΔTn*10*	SA814 × P1 · SG12049[a]
MJW106	Hfr KL16 *clpA182*::ΔTn*10*	G1053 × P1 · SG12049[a]
SA313	F$^-$ Str^r *his*[λcI857Δ(λ*attL int*)]	Laboratory collection
SA814	Hayes Hfr	Laboratory collection
SG12049	C600 *clpA182*::ΔTn*10*	Susan Gottesman
VS20102	MC4100 *manA108*::ΔTn*10*(Kan)	Valerie Stout

[a] The ΔTn*10* insertion in AG708 is in an unmapped locus.

between these two strains and VS20102 (Str^r) was performed by the method of Miller (7), but with interruption by vortexing for a full minute. After interruption, cultures were plated on Luria broth plates containing tetracycline and streptomycin. As a control, the same experiment was performed simultaneously with Hayes Hfr and with KL16 into which a *clpA*::ΔTn*10* mutation had been introduced by P1 transduction. The *clpA*::ΔTn*10* had been previously mapped to 19.5 min on the chromosome (4). Results are shown in Fig. 1. This plot of the transfer of a marker in two directions gives the approximate map position on the *E. coli* chromosome. The *galS*::ΔTn*10* marker mapped to approximately 45 to 50 min. We noticed that some of the strains with the *galS*::ΔTn*10* allele were unable to grow with fructose as a carbon source. Investigating further, we found that the fructose phenotype was independent of and unrelated to the *galS* phenotype, but the inability to grow on fructose was about 60% cotransducible by phage P1 with *galS*::ΔTn*10*. The operon for fructose utilization is at 47 min on the *E. coli* chromosome. Location of the *galS*::ΔTn*10* near the *fru* operon was consistent with the Hfr mapping results. The ΔTn*10* insertion of AG708 is unlinked to *fru*.

To map more precisely the location of the ΔTn*10* insertion, we used the Kohara et al. (6) *E. coli* phage bank to marker rescue the ΔTn*10* in *galS* from the mutant. The Kohara phages containing *E. coli* chromosomal fragments from the 45- to 48-min region were grown on strain MJW104, which contained the *galS*::ΔTn*10* mutation. Lysates were used to infect SA313, expression was allowed, and the phage-infected cells were plated on Luria broth plates containing tetracycline. Phages 7F1, 7H12, and 7H11 gave rise to tetracycline-resistant colonies, and the other phages did not. The *E. coli* region that the three phages have in common is at precisely 46 min, near the previously mapped *mgl* operon, encoding proteins necessary for galactose and methylgalactoside transport (2, 3, 8). Whether *galS* is involved in *mgl* expression remains to be studied.

In summary, we have defined by mutation and mapped a new gene, *galS*, involved in the regulation of the *gal* operon.

TABLE 2. Expression from the *galE-lacZ* fusion

Strain	Relevant genotype	β-Galactosidase activity[a]	
		−Fucose	+Fucose
JT234	Wild type	68	388
JT247	Δ*galR*	516	1,209
AG708[b]	Δ*galR zzz*::ΔTn*10*	1,115	1,760
AG701	Δ*galR galS*::ΔTn*10*	2,266	2,300
JT234/P1701	*galS*::ΔTn*10*	69	1,115

[a] In Miller units (7).
[b] Mini-Tn*10* in an unknown location.

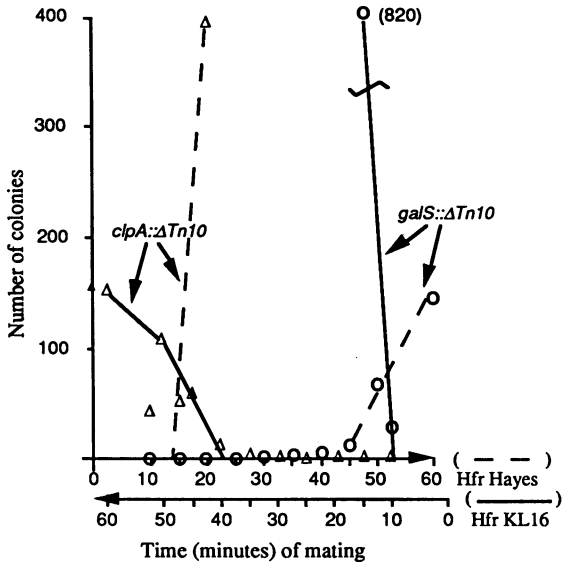

FIG. 1. Criss-cross Hfr mapping of the *galS*::ΔTn*10*. Simultaneous matings from two overlapping directions were plotted so that the plots cross over the region containing the locus selected for in the mating. Time points of *galS*::ΔTn*10* (○) and *clpA*::ΔTn*10* (△) matings are shown. Arrows on the time axis indicate the direction of markers transferred. The origins of transfer of Hfr Hayes and KL16 are 62.5 min apart, and they mobilize transfer in opposite directions. The chromosomal location is equal to the Hayes Hfr time minus 2.5 min. The *clpA*::ΔTn*10* locus is known to map at 19.5 min on the chromosome (4) and was within the region predicted by the criss-cross matings. The *galS*::ΔTn*10* was similarly localized between 43 and 50 min on the chromosome.

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