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Deletion Mapping of the *polA-metB* Region of the *Escherichia coli* Chromosome

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A λcI857 prophage inserted into one of the genes of the *rha* locus was used to select deletions unambiguously ordering the markers *polA-glnA-rha-pfkA-tpi-metBJF*. Transduction with phage P1 indicates at least 70% linkage between *glnA* and *polA*. The order of the *pfk* and *tpi* markers is reversed from that previously published. Despite the relatively large distance separating the *glnA* and *rha* loci, deletions removing this entire region have no obvious phenotype. The isolation of Tn*10* transposons integrated at different sites between *rha* and *glnA* greatly facilitated this work.

Since the recent focus of this laboratory has been the role of glutamine synthetase, the product of the *glnA* gene, which is located at about minute 85 on the *Escherichia coli* chromosome (3, 10), we were interested in genetically characterizing that region of the genome. A variety of approaches to this problem are possible. In *E. coli* strains missing the normal λ phage attachment site, this bacteriophage will integrate at a low frequency into other regions of the chromosome (12). Such lysogens will generate deletions of the region near the λ prophage, as well as specialized transducing phage, which can be used to deduce the order of adjacent chromosomal markers (12). Transposable drug resistance elements near the gene of choice can also be used to generate deletions. In addition, these drug resistance elements are useful as markers in multi-factor crosses and to move linked markers into other strain backgrounds (6). We report here data extending our knowledge of the region of the chromosome from *polA* (85 min) to *metB* (87 min) and establishing a new map order for certain genes in this region.

In *E. coli* the *glnA* gene is about 20% cotransducible by phage P1 with the genes of the *rha* (rhamnose utilization) locus (13). The growth of certain Rha$^-$ mutants (*rhaD*) can be inhibited by the presence of high levels (1%) of rhamnose (11). This rhamnose-sensitive phenotype (Rha^s), presumably due to the accumulation of ribulose-1-phosphate, depends on the activity of the other enzymes of the pathway. Therefore, inactivation of one of these enzymes, as by the insertion of λ, should make Rha^s strains resistant to this toxicity. Among five Rha$^-$ strains obtained from various strain collections we found one (LS517, from Larry Soll) that did not grow on glycerol minimal medium (3) in the presence of 1% rhamnose. The presumptive *rhaD* mutation in strain LS517 was moved into the LS519 (10) strain background by cotransduction with *metB* to construct strain ET2020 [F$^-$ Rha^s Δ(*gal-bio-uvrB*) (φ80h)], which is deleted for the λ attachment site. However, in this strain background the Rha^s phenotype was only evident with succinate as the source of carbon. This difference presumably reflects the effect of catabolite repression on expression of the *rha* genes, since the presence of glucose always prevented expression of the Rha^s phenotype and the optimal carbon source for observing this character varied in different strain backgrounds.

Strain ET2020 was infected with the phage (λcI857*b*515*b*519*xisamSam*7) as described by Shimada (12). Following their procedure, the frequency of λ-immune cells selected on succinate minimal medium was 10^{-5} per survivor, whereas the frequency of rhamnose-resistant (Rha^r), λ-immune cells was 3×10^{-7} per survivor. This strain reverted spontaneously to λ resistance at 8×10^{-7} per cell and to rhamnose resistance at 2×10^{-9} per cell. The Rha^r character of a number of (10/10) isolates selected after infection with phage λ was shown to result from insertion of this phage at the *rha* locus (*rhaD*::λ) by selecting Rha$^+$ transductants and demonstrating that these cells grew at high temperature, no longer carried λ immunity, and did not release λ phage.

One of these strains, ET2021 [*rha*::λ Δ(*gal-bio-uvrB*)], obtained after infection with λ, was used to select spontaneous heat-resistant (42°C) survivors on LB-gln agar (10). The frequency of survival was 5×10^{-7} at 42°C. The growth pattern of these survivors (Table 1) suggested that many of these strains contained deletions affecting the *glnA*, *rha*, *pfk*, and *tpi* loci. Enzyme

assays very kindly performed in the laboratory of D. Fraenkel confirmed the phenotypes of the *pfk* and *tpi* mutants (Table 1). Glutamine synthetase assays demonstrated that the Gln$^-$ phenotype is due to the absence of this enzyme. The frequency of double mutants (40% of the survivors for class III, 1% of the survivors for class IV) as well as data from reversion analysis (Table 1) suggested that the phenotypes of these mutants were due to the deletions of the relevant genes. No revertants were obtained except for the Pfk$^-$ character, which is known to revert at approximately this frequency via unlinked genetic and phenotypic suppression (4). The Pfk$^-$ phenotype was rescued via cotransduction with *metB* from four Pfk$^+$ revertants, which is consistent with the observations of Fraenkel and coworkers and supports the notion that these revertants are due to unlinked suppressors. Transductions performed with these pleiotropic mutants clearly showed that these strains contained deletions. All transductants selected for the wild-type character of a single gene lost in an apparent deletion (100 Rha$^+$ transductants, 60 Tpi$^+$ transductants) simultaneously acquired the donor phenotype for all other defective genes under that deletion. This is in contrast to the relatively low (5 to 50%) cotransduction frequency observed with combinations of point mutants in these genes (1, 10, 13). In addition, the Rha^r character of these 42°C survivors was easily cotransduced with *metB* (40%, 31/78), and such transductants simultaneously acquired all the growth characteristics of the donor strain, including, when appropriate, the Gln$^-$ phenotype, which is usually less than 4% linked to *metB*. From these results we have constructed the deletion map presented in Fig. 1. Our data show that, relative to *rha* and *metB*, the order of *pfkA* and *tpi* is reversed from that previously

TABLE 1. *Characterization of temperature-resistant derivatives of ET2021*

Deletion class[a]	Representative strain	Growth phenotype[b]				Revertants/10^{10} cells[c]				Enzyme activities[d]		
		Gln	Rha	Pfk	Tpi	Gln$^+$	Rha$^+$	Pfk$^+$	Tpi$^+$	GS	PFK	TPI
Wild type	LS519	+	−	+	+	NR	100	NR	NR	0.21	0.21	3.2
I	ET2034	+	−	+	+	NR	0	NR	NR	0.20	0.26	3.3
II	ET2022	−	−	+	+	0	0	NR	NR	<0.01	0.44	5.4
III	ET2029	+	−	−	+	NR	0	3,000	NR	0.21	0.02	3.0
IV	ET2025	−	−	−	+	0	0	3,000	NR	<0.01	0.02	3.6
V	ET2030	+	−	−	−	NR	0	0	0	<0.01	0.02	0.00
VI	ET2027	−	−	−	−	0	0	0	0	0.19	0.02	0.04

[a] The following number of deletions of each class were isolated. Class I, 63; class II, 11; class III, 69; class IV, 20; class V, 7; class VI, 3. The different number of isolates in each class reflects not only the relative frequency at which that class appeared, but also the ease with which such mutants could be identified. Thus, class III was easily identified on indicator plates, whereas class V must be identified by screening.

[b] Growth was determined at 30°C on minimal agar medium (3) containing 0.2% ammonium sulfate as the nitrogen source. Carbon sources were added to 0.4% final concentration and glutamine to 0.2%. The Rha$^-$ phenotype was scored as the inability to utilize rhamnose as sole carbon source. The Pfk$^-$ phenotype (cells missing phosphofructokinase) was scored as poor utilization of a variety of carbohydrates normally metabolized via glycolosis (5). Our putative *pfkA* deletion strains grew slowly on glucose and not at all on mannitol. They grew normally on fructose since it can be metabolized in the sequence fructose → fructose-1-phosphate → fructose-1,6-diphosphate and hence does not require phosphofructokinase. Pfk$^-$ strains could be identified directly on the 42°C selection plate as white colonies on MacConkey indicator plates containing 1% mannitol. Cells missing triose phosphate isomerase cannot grow on gluconeogenic carbon sources (such as lactate, acetate, and glycerol) and, in addition, fail to grow on many sugars, probably due to the accumulation of a toxic product, methylglyoxal (5). The Tpi$^-$ phenotype of our putative *tpi* deletion strains was scored as the inability to grow on glycerol, succinate, or fructose, in addition to those compounds which did not support the growth of *pfkA* mutants. Since gluconate is metabolized mainly by the Entner-Doudoroff pathway, *tpi* mutants can grow on this carbon source (5). The Gln$^-$ phenotype was therefore scored on gluconate ammonia minimal medium. Since L-broth medium (3) contains a limiting amount of glutamine, putative *glnA* deletion strains were initially identified as tiny colonies on L-broth agar.

[c] Reversion was tested by plating 10^{10} cells from a saturated culture grown on gluconate-ammonia-glutamine minimal medium. The cells were spread onto agar containing either rhamnose-ammonia-glutamine (Rha$^+$), mannitol-ammonia-glutamine (Pfk$^+$), fructose-ammonia-glutamine (Tpi$^+$), or gluconate-ammonia (Gln$^+$). Pfk$^-$ mutants revert via unlinked suppressors (4), and hence the fact that our Pfk$^-$ strains revert is not inconsistent with their being deletions. The Pfk$^-$ deletion was rescued via cotransduction with *metB* from four Pfk$^+$ revertants, confirming that they were due to an unlinked suppressor. NR, Not relevant.

[d] Enzyme data is expressed as micromoles of product formed per minute per milligram of protein. Glutamine synthetase (GS) assays were performed as previously described (10). Phosphokinase (PFK) and triose phosphate isomerase (TPI) activities were determined in the laboratory of D. Fraenkel (14).

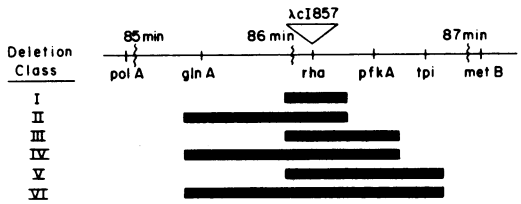

FIG. 1. *Deletion map of the glnA region of E. coli. The rha::λ insertion site is indicated at the top, and bars represent the extent of the various deletion classes based on the data in Table 1.*

reported (1). We place *metB* distal to *tpi* with respect to *rha* and *pfkA*, since none of the deletions requires methionine.

Since we also found that *polA* is at least 70% cotransducible with *glnA*, we wanted to order *polA* with respect to *glnA* and *rha*. It has been reported that the combination of the *polA* and *uvrB* mutations is lethal (8). We had obtained our Gln$^-$ deletions in strain ET2021, which lacks *uvrB*, so it would appear that *polA* is not between *glnA* and *rha*. However, we could not score for the methylmethane sulfonate (MMS)-sensitive phenotype of *polA* mutants since *uvrB* mutants are also sensitive to MMS. Therefore, to conform this gene order, we moved the *rha*::λ into a UvrB$^+$ strain background where deletions could be scored for MMS sensitivity. To facilitate construction of such a strain, we isolated two Tn*10* insertions near *glnA* using the procedure described by Kleckner et al. (6). One of these insertions, *zig2*::Tn*10*, is 85% linked (127/150) to *glnA* and 20% linked to *rha* (25/125). The other insertion, *zig1*::Tn*10*, is 30% linked (55/171) to *glnA* and 90% linked (210/229) to *rha*. (Notation such as *zig*::Tn*10* indicates the position of an insertion with no mutant phenotype by describing its position on the genetic map. Letters *i* and *g* indicate the approximate location in minutes [i.e., *aa* is 00; *ab* is 01; *bb* is 11; *ig* indicates location at minute 86]; 6.) The insertion *zig1*::Tn*10* was used to transduce the *rha*::λ via P1 transduction into strain ET1195 (*glnA202*). After the Tn*10* element had been removed by cotransduction with *glnA*, this strain (ET1250) was used to generate survivors at 42°C. Two of these UvrB$^+$ survivors were Gln$^-$, MMS resistant, and sensitive to chlorate. Additional UvrB$^+$ strains, constructed by transducing two other (*rha-glnA*) deletions into a *metB*$^-$ mutant, were also MMS resistant and chlorate sensitive. Therefore, assuming that a deletion of *polA* results in sensitivity to MMS, we have not been successful in generating *polA* deletions. This observation could be due to the fact that a *polA* deletion would be lethal (7).

However, an essential gene, designated Am, controlling the amounts of RNA polymerase core subunits may lie between *polA* and *rha* (9). It is conceivable, therefore, that *polA* deletions are not lethal and we were unable to isolate such mutants because the Am gene lies between *glnA* and *polA*. According to this idea, the conditionally lethal phenotype caused by some *polA* mutations would be attributable to an imbalance of the various functions of this complex protein (7).

The placement of the Am mutation between *polA* and *rha* depends on the location of an uncharacterized nutritional marker, X, between *polA* and *rha*, based on unpublished results (9). This auxotrophic mutation, X, is reported to be 25% linked to *rha*, with a three-point cross indicating that Am is between X and *rha* (9). It is possible that this uncharacterized auxotroph may in fact be *glnA*. Another possibility is that it is a purine requirement, since we find that in *E. coli* C λcI857 insertions in *rha* generate purine-requiring deletion strains, all of which are Gln$^-$. What is clear is that Am, X, and purine genes, as well as *polA*, must be distal to *glnA* with respect to *rha*, since none of these phenotypes is found in class II deletions. It is interesting that, despite the distance between *glnA* and *rha* (about 40 kilobases [14]), neither of two Tn*10* insertions in, nor deletions of, this region has any obvious phenotype.

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