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Structural Genes for Ornithine Transcarbamylase in *Salmonella typhimurium* and *Escherichia coli* K-12[1]

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Mutations of *Salmonella typhimurium* affecting the structural gene for ornithine transcarbamylase (*argI*) have been isolated and mapped. The two ornithine transcarbamylase loci in *Escherichia coli* K-12 have been demonstrated by F′ episome transfer.

The enzyme ornithine transcarbamylase (OTCase) catalyzes the conversion of ornithine and carbamyl phosphate to citrulline and acts in the biosynthetic pathway for arginine. Mutants defective at this step will grow on a minimal-glucose medium supplemented with arginine or citrulline but fail to grow on medium supplemented with ornithine. In the present work, mutant strains of *Salmonella typhimurium* lacking OTCase activity are described, and the gene for OTCase (*argI*) is mapped. The *argD* mutants which were previously described have the arginine or citrulline growth requirement (4). These were thought to affect the structural gene for OTCase. Below, we show that *argD* mutants affect some other function, probably carbamyl phosphate synthetase (*pyrA*).

In *Escherichia coli* K-12, it has been reported that two distinct structural genes for OTCase exist and that these loci are separated 14 min on the *E. coli* map (6). We confirm the existence of redundant OTCase genes in *E. coli* K-12 and show that only one gene codes for this activity in *S. typhimurium*.

MATERIALS AND METHODS

Bacterial strains. All strains are derived from *S. typhimurium* strain LT-2. The strains carrying *argD*, *pyrA*, and *pyrB* markers were obtained from K. E. Sanderson. The mutation *arg-513* which proved to be an *argI* mutation was obtained from B. N. Ames. The F′ episomes used are derived from *E. coli* K-12. The F′ episomes KLF-17 and -18 were obtained from B. Low. Strains carrying these episomes are listed in Table 1.

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Media. The E medium of Vogel and Bonner (14) with 2% dextrose added was used as minimal salts. Difco nutrient broth in 0.85% saline was used as maximally supplemented medium. Solid medium contained 1.5% agar. Minimal medium was supplemented with uracil (20 μg/ml) or arginine (100 μg/ml) when needed. The mutagen ICR-191 was a gift from Hugh Creech, Institute for Cancer Research, Fox Chase, Philadelphia, Pa.

P-22 mutagenesis. The *argI* strains were isolated according to the mutagenized P-22 transduction method of Hong and Ames (7). A nonlysogenizing mutant (*int-4*) of phage P-22 was mutagenized with hydroxylamine according to the method of Tessman (13). Strain *pyrB64* was exposed to mutagenized phage and Pyr$^+$ transductants were selected. Among these transductants, temperature-sensitive mutants or arginine auxotrophs were selected.

OTCase assays. The bacteria were grown in minimal-salts medium supplemented with arginine. Three hours after arginine exhaustion, the cells were harvested by centrifugation, resuspended in 0.1 volume of 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.1), and sonically disrupted. After centrifugation at 18,000 × *g* for 1 hr, the crude extracts were assayed for OTCase activity under the conditions described by Rogers (11). The production of citrulline was measured by the color assay for ureido groups described by Gerhart and Holoubek (5). A 0.1-μmole amount of citrulline in 3 ml of the standard assay solution gave an A_{550} of 0.70.

Transfer of episomes. F′episome transfer was performed by spot tests on solid medium. An Arg$^-$ recipient was mixed with the episome donor strain on minimal medium. The Arg$^+$ clones which arose were shown to be merodiploid by the appearance of segregants which again showed the Arg$^-$ phenotype.

RESULTS

Isolation of argI mutants. The first *argI* mutant was encountered in the course of isolating mutants linked to the *pyrB* locus by the

method of Hong and Ames (7). This *arg* mutation (*argI536*) is temperature-sensitive; the mutant grew at 40 C on citrulline or arginine but was not stimulated by ornithine. The mutation was 64% cotransducible with *pyrB*. Table 2 lists the cotransduction frequencies of several *arg* mutations with the *pyrB* locus. In addition to the mutants in Table 2 isolated by transducing phage mutagenesis, two *argI* mutants (*argI538* and *argI539*) were isolated after direct mutagenesis of cells with ICR-191 (2).

The argD locus. At first it was thought that the *argI* mutant isolated might be similar to the previously described *argD* mutations since they show the same nutritional phenotypes (4). Since the *argD* locus had already been shown to be significantly removed on the genetic map from the *pyrB* gene (4), a comparison of the *argD* and *argI* strains was made. The strains *argD22*, *25*, *36*, and *42* were tested for linkage to *pyrB*. As is shown in Table 2, no linkage could be demonstrated between *argD* and *pyrB*. Abd-el-al et al. (1) have described a mutation in *E. coli* B/r which shows a nutrient requirement similar to that of *argD* and *argI* but which maps at the *pyrA* locus, the structural gene for carbamyl phosphate synthetase. Therefore, linkage to the *pyrA* locus was tested and, as can be seen in Table 2, the *argD* gene was found to be very closely linked to the *pyrA* locus.

TABLE 1. *Bacterial strains with F' episomes used in this study*[a]

Strain	Genotype
TR116	*pro*⁻ (A or B) *purE66*/F'13 *lac*⁺
TR132	Str^R *purC7 proA46 gal-501* azaserine^R/F' *pro*⁺ *lac*⁺
TR1603	*trp294 thr115 pyrB92*/KLF17
TR1604	*trp294 thr115 pyrB92*/KLF18

[a] All strains are derived from *S. typhimurium* strain LT-2; KLF17 and KLF18 are F' episomes isolated from *E. coli* K-12 by Brooks Low.

TABLE 2. *Frequency (per cent) of cotransduction between various markers*

Recipient	Donor			
	pyrB64	*argD22*	*argD36*	LT-2 (wild type)
argI536	64			
pyrB643 argI537				75
pyrB647 argI537				68
argD22	<0.2			
argD36	<0.2			
pyrA129		83	84	

It was also noted that on minimal medium all of the *argD* mutants tested (*argD22*, *25*, *36*, and *42*) showed very slight growth. The addition of arginine did not fully restore wild-type growth rates. For example, in arginine-supplemented minimal medium at 37 C, the strains *argD22*, *25*, and *36* gave doubling times of 105 min and the strain *argD42* gave a doubling time of 300 min. The addition of uracil and arginine (the normal supplements for strains carrying the *pyrA* mutation) to minimal medium restored the wild-type doubling time of 50 min for all *argD* mutants tested. The addition of uracil alone inhibited completely even the very slight growth observed on minimal medium.

OTCase activity. The activity levels of OTCase was measured in the *argD* and *argI* strains. An *argF* (arginino succinase mutant in *S. typhimurium*) was included as a control. The cells for these assays were grown in minimal medium supplemented with enough arginine to allow the complete arginine auxotrophs to reach a density of 2×10^8 cells per ml. The *argD* strains continued to grow slowly beyond this density. At 3 hr after this density was reached, the cells were harvested. As can be seen in Table 3, the *argD* strains showed high levels of OTCase whereas the *argI* strains showed no detectable activity. The *argF88* mutant showed less derepression than the *argD* mutants, probably because it does not continue growth under the condition of arginine limitation.

Episomes. Various F' episomes derived from *E. coli* K-12 were tested to see whether they carried the OTCase gene. It has been reported that *E. coli* K-12 has two structural genes for OTCase, one which maps near the *pyrB* locus and another which maps between *proA* and *lac* (6). Therefore, episomes were tested which

TABLE 3. *Ornithine transcarbamylase (OTCase) activity in argD and argI strains*

Strain	OTCase activity[a]
argD22	17.0
argD25	4.8
argD36	20.0
argD42	21.1
argI513	<0.04
argI537	<0.04
argF88	0.8

[a] Micromoles of citrulline formed per hour per unit of optical density at 280 nm in crude extract. Enzyme levels reflect derepression on limiting arginine; variability of high levels results from variation in the amount of derepression achieved.

carry these regions of the *E. coli* K-12 chromosome; F′ episomes KLF-17 and KLF-18 carry the *pyrB* region and episomes F′ *pro-lac* and F′ 13 carry the *lac* region. Table 4 lists the results of these crosses. As is shown, all four episomes restore *argI* mutants to Arg$^+$. None of the *argD* mutations, on the other hand, are restored to Arg$^+$ by the presence of the episomes, which reaffirms the distinction between the *argI* and *argD* loci.

DISCUSSION

Mutants of *S. typhimurium* LT-2 affecting the structural gene for OTCase, the *argI* locus, have been isolated. The *argI* locus is shown to be 64 to 75% cotransducible with *pyrB* at 137 min on the genetic map (12). The *argD* locus, previously reported to be the gene for OTCase, appears actually to affect the structural gene for carbamyl phosphate synthetase at the *pyrA* locus, probably resulting in an enzyme with reduced activity. The usual phenotype for mutations in the *pyrA* gene is a requirement for both arginine and uracil, since carbamyl phosphate is required in the pathways for both arginine and pyrimidine biosynthesis. However, there have been two previous reports in which the *argD* phenotype, growth on arginine or citrulline but not ornithine, has been attributed to mutations in the *pyrA* gene. Yan and Demerec (15) reported that revertants of a certain class of *pyrA* mutations, containing unlinked suppressors plus the original mutation, had the *argD* phenotype; that is, only the uracil requirement had been suppressed. Abd-el-al et al. (1) isolated mutants in *E. coli* B/r which grew on arginine or citrulline but not ornithine. These turned out to have normal OTCase activities but reduced carbamyl phosphate synthetase activities.

TABLE 4. *Transfer of F′ episomes to argI and argD recipients*[a]

Recipient	F′ episomes carried by donors			
	F′ *pro-lac*	F′13	KLF-17	KLF-18
argI513	+	+	+	+
argI537	+	+	+	+
argD22	−[b]	−[b]	−	−
argD36	−[b]	−[b]	−	−

[a] Symbols: +, cross resulted in Arg$^+$ merodiploid; −, no Arg$^+$ clones resulted under standard mating conditions.

[b] Successful transfer of episomes was demonstrated by selection of Lac$^+$ clones on minimal-lactose medium supplemented with arginine. All merodiploid strains remained Arg$^-$.

The data presented here are entirely consistent with the *argD* mutations belonging to this class of *pyrA* mutations. Since all of the strains carrying *argD* mutations which we have tested have high levels of OTCase activity, it is certain that these mutations do not affect the structural gene for OTCase. The 85% frequency of cotransduction between *argD* and *pyrA* is consistent with both of these markers lying within the same gene. As was noted above, all of the *argD* strains tested are leaky. Arginine enhances this slow growth, but only the addition of both uracil and arginine restores the growth rate to the wild-type level. The fact that uracil alone inhibits the leaky growth of *argD* mutants indicates a similarity to the uracil-sensitive mutations known to map within the *pyrA* locus [see O'Donovan and Neuhard (10) for a review of the different classes of *pyrA* mutations].

The mutagen ICR-191 is known to produce frameshift mutations which in most cases will result in a protein with complete loss of activity (2, 3, 16). When *S. typhimurium* cells were treated directly with ICR-191, all mutations causing the arginine or citrulline growth requirement were located in the *argI* gene, not the *argD* gene. This is to be expected if *argD* mutations cause a partial loss of carbamyl phosphate synthetase (*pyrA*) activity.

The mutants now designated *argD* probably represent a mixed class. Most of them, including all of them tested here, are of the *pyrA* type. However, some *argD* mutations may prove to affect OTCase. In view of this possibility, we suggest that the OTCase gene be designated *argI* in agreement with the *E. coli* nomenclature. Further tests are required to know which of the *argD* mutations now in various collections actually affect *argI* and which affect *pyrA*. The relevant genes and enzymes involved in these pathways are presented in Fig. 1.

The *pyrB* gene codes for the catalytic subunit of aspartate transcarbamylase. This enzyme, like OTCase (*argI*), utilizes carbamyl phosphate as substrate and operates in a closely related pathway (see Fig. 1). The fact that such similar enzymes are encoded by genes that are so closely linked may have significance in terms of the regulation and evolution of aspartate transcarbamylase and OTCase. However, no coordination between the levels of aspartate transcarbamylase and OTCase in *S. typhimurium* or *E. coli* has been observed (6; Syvanen and Roth, *unpublished data*). Thus, the close linkage between *pyrB* and *argI* has no obvious relationship to the

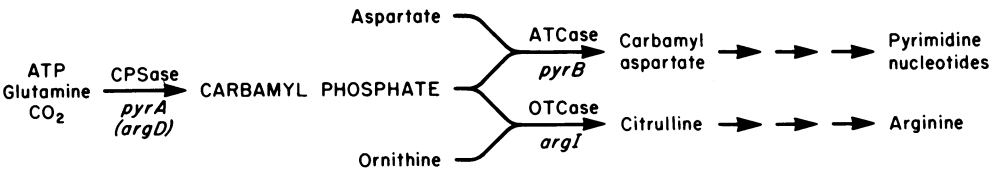

FIG. 1. *Pathway showing synthesis and utilization of carbamylphosphate. Enzymes designated above the arrows: CPSase, carbamyl phosphate synthetase; ATCase, aspartate transcarbamylase; OTCase, ornithine transcarbamylase. The standard designations for S. typhimurium genetic loci coding for the various enzymes are listed below the arrows.*

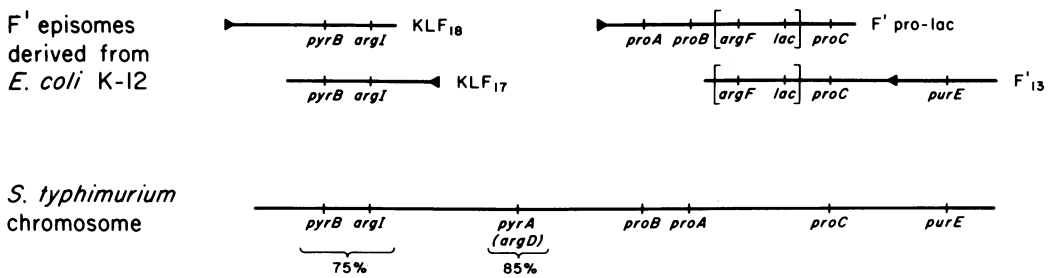

FIG. 2. *Map of Salmonella typhimurium chromosome and F' episomes derived from Escherichia coli K-12. Genes presented in brackets are found in E. coli K-12 chromosome but not S. typhimurium chromosome. Per cent cotransduction with Salmonella phage P-22 are given below the S. typhimurium chromosome. The genes presented on the F' episomes reflect the results of complementation tests and do not permit conclusions as to genetic map order in E. coli K-12. It is possible that the particular episomes used have undergone mutation or rearrangement since their original formation from the E. coli chromosome. Gene designations: argI designates the E. coli and S. typhimurium gene coding for OTCase which is closely linked to the pyrB locus; argF designates the second structural gene for OTCase in E. coli K-12; argD designates a subclass of pyrA mutations (requires arginine or citrulline only, see text). Map position of argF in E. coli K-12 is from Glansdorff et al. (6). The order of the pyrB and argI genes for the E. coli B chromosome is from Jacoby (8).*

regulation of their respective enzymes.

The isolation of the *argI* mutants in *S. typhimurium* indicates that this bacterium possesses a single gene for OTCase. This is contrasted to the case of *E. coli* K-12 where mutants that are OTCase minus are extremely difficult to isolate since there are two genes which must be damaged before the OTCase activity can be eliminated (6, 9). One of the *E. coli* genes is located near the *pyrB* locus and is designated *argI*; the other is located near *lac* and is designated *argF* in *E. coli*. The single OTCase gene of *S. typhimurium* (*argI*) maps in the same region as the *argI* locus of K-12. There is no gene in *S. typhimurium* analogous to the *argF* gene of *E. coli*.

In the present work, we have confirmed the existence of redundant structural genes for OTCase in *E. coli* K-12. It is shown that *E. coli* F' episomes covering the *pyrB* region, around 84 min, and F' episomes covering the *pro-lac* region, around 17 min, both have the capacity to restore the Arg^+ phenotype to *argI* (OTCase-deficient) mutants.

Figure 2 shows selected markers on the *S. typhimurium* and *E. coli* genetic maps which illustrate the differences of the various gene locations.

G. O'Donovan (*personal communication*) has also isolated OTCase-deficient mutations in *S. typhimurium* which are located near the *pyrB* locus. Recently, Jacoby (8) showed that *E. coli* B has a single OTCase gene (*argI*), which maps at a position analogous to the *argI* of *S. typhimurium* and *E. coli* K-12.

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