JOURNAL OF BACTERIOLOGY, Oct. 1976, p. 35-38

Vol. 128, No. 1
Printed in U.S.A.

Escherichia coli Ornithine Carbamoyltransferase Isoenzymes: Evolutionary Significance and the Isolation of λ*argF* and λ*argI* Transducing Bacteriophages

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Received for publication 18 May 1976

Among the *Enterobacteriaceae*, *Escherichia coli* K-12 is the only strain known to have two structural genes (*argF* and *argI*) for ornithine carbamoyltransferase. The two gene products interact to form a family of four functional isoenzymes, respectively designated FFF, FFI, FII, and III. The FFF and III isoenzymes exhibit nearly identical kinetic parameters in the conditions applied. FFF is more thermolabile than III; this allows the straightforward characterization of new transducing phages carrying either *argF* or *argI*. The bearing of the available information regarding ornithine carbamoyltransferase isoenzymes on the evolution of the ancestral *E. coli* chromosome is reconsidered.

We have previously established that *Escherichia coli* K-12 contains two structural genes (*argF* and *argI*) encoding the information required for the synthesis of ornithine carbamoyltransferase, which catalyzes the sixth step of arginine biosynthesis (4). The active enzyme molecule is a trimer, and the products of the two genes become associated in a family of four isoenzymes (8); these are consequently designated FFF, FFI, FII, and III. This finding strongly suggests that the two genes are similar and originate from the duplication of an ancestral ornithine carbamoyltransferase gene. A recent heteroduplex analysis of λ*argI* and λ*argF* transducing phages (7) has provided a direct approach of the degree of likeness exhibited by the two enzymes; complementary deoxyribonucleic acid strands of the *argF* and *argI* genes pair along each other without visible interruption; given the sensitivity of the analysis, this means that the two sequences must be 85% or more homologous.

We have judged it necessary to characterize the FFF and III isoenzymes further, since an extensive comparison of the two enzymes, ultimately at the level of primary sequences, may provide some clue regarding the antiquity of the ancestral duplication and the possible significance of the existence of these two genes in the same cell. This topic has already encouraged some speculation (16; Nature, News and Views 256:676, 1975), which will be considered later. Furthermore, it was hoped that the study would provide criteria for the characterization of ornithine carbamoyltransferases of the F or I type. Such criteria would be useful not only to distinguish *E. coli* strains from one another (see accompanying paper, reference 9), as was done previously by genetic tests and diethylaminoethyl-Sephadex chromatography (8), but would also permit the isolation of new transducing phages carrying either the *argF* or *argI* region by a more direct approach than the one used previously (6).

MATERIALS AND METHODS

Strains. See Table 1 for list of strains used.

Genetic techniques. Preparation of λ phage stocks and transductions were performed as described previously (12).

Enzyme assays and ornithine carbamoyltransferase isoenzyme characterization. The detailed procedure of Legrain et al. (8) has been followed. Determination of kinetic parameters has been performed as described previously (C. Legrain and V. Stalon, Eur. J. Biochem., in press) except that imidazole-hydrochloride buffer, pH 6.8, was used instead of tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 8.0.

The thermal inactivation of ornithine carbamoyltransferase in extracts (8) of *E. coli* K-12 JEF8C23 (*argF* constitutive, with a negligible *argI* activity; 9) and *E. coli* K-12 NCI8 (*argF*$^-$ *argI* constitutive; 9) was performed as follows. Tubes containing 1.9 ml of 150 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 8.0, were heated at 60°C; at time zero, 0.1-ml extract samples (about 0.2 mg of protein) were added to the test tubes and rapidly mixed. At various time intervals, 50-μl samples of the solution were removed for assay of the remaining activity

TABLE 1. *List of strains*

Strain	Relevant genotype	Source or reference
Bacteria		
RW592	F⁻ *thi* Δ(*bio att* λ)[a]	14
3000X111	Hfr *thi* Δ(*pro lac argF*)	F. Jacob
30SOU4	Hfr *thi pyrB*	1
JEF8C23	Hfr *metB thr carB8*, constitutive for *argF* expression	9
NCI-8	Hfr *thr carB8* Δ(*pro lac argF*), constitutive for *argI* expression	9
P4XB2	Hfr *metB argR*	This laboratory
Phage		
λ 199	*CI857 susS7 susxis6 Δb515 Δ519*	

[a] Δ denotes deletion.

and chilled rapidly in test tubes containing the ornithine carbamoyltransferase reaction mixture lacking carbamoylphosphate (8). The reaction was initiated by adding carbamoylphosphate; subsequent steps were as described previously (8).

RESULTS AND DISCUSSION

Both FFF and III isoenzymes catalyze citrulline synthesis (4, 8). It was, however, not known whether they were equally efficient in this process or whether they differed in their capacity to promote the reverse reaction (phosphoro- or arsenolysis of citrulline). We have determined the apparent Michaelis constants of the FFF and III isoenzymes for their substrates and products in 150 mM imidazole-hydrochloride buffer at pH 6.8. The results do not reveal any conspicuous difference between the two enzymes. For both *argF* and *argI* ornithine carbamoyltransferases, 10^{-2} mM was obtained as the apparent Michaelis constant for carbamoylphosphate and 5 mM as that for ornithine. The kinetic parameters for the reverse reaction, the arsenolytic cleavage of citrulline, show that the apparent Michaelis constants for citrulline and arsenate are, respectively, 60 and 5 mM for both enzymes. The turnover number, expressed in terms of micromoles of citrulline formed per hour per milligram of protein, is about 140,000 for both FFF and III. The accompanying paper (9), moreover, shows that in vivo citrulline phosphorolysis can be efficiently achieved by both F and I types of ornithine carbamoyltransferases.

We have found that it is, nevertheless, possible to distinguish the FFF and III isoenzymes by their relative thermolability (see Fig. 1). *E. coli* K-12 *argI*⁺ *argF*⁻ and *E. coli* B and W (which make only *argI*; 8; Legrain and Stalon, in press) produce an enzyme resisting a 20-min exposure at 60°C (in extracts containing about 2 mg of protein per ml). Under the same conditions, the ornithine carbamoyltransferase activity of the FFF isoenzyme vanishes almost completely. *E. coli* K-12 *argF*⁺ *argI*⁺, in which the two hybrid forms are predominant (8), exhibits an intermediary situation.

We have used this property to characterize strains lysogenized by a λ*argF* or λ*argI* transducing phage. Applying Shrenk and Weisberg's method (14) as described in detail by Mazaïtis et al. (12), we were able to obtain phages transducing to Arg⁺, a stable ornithine carbamoyltransferase-less strain (recovered by us as a spontaneous mutant of the *argF* deletion strain 3000X111 after penicillin counterselection of wild-type cells). These phages were recovered from a "mixed lysate" of strain RW592, which was shown by diethylaminoethyl-Sephadex chromatography (8) to produce all four ornithine carbamoyltransferase isoenzymes. Table 2 shows which types of phages were recovered from this experiment. They are presently being used in in vitro enzyme synthesis (P. Halleux, N. Glansdorff, M. Crabeel, and A. Piérard, Abstr. 849, 10th Fed. Eur. Biochem. Soc. Meet., Paris, 1975). Independent proof that the transductants producing a heat-labile ornithine car-

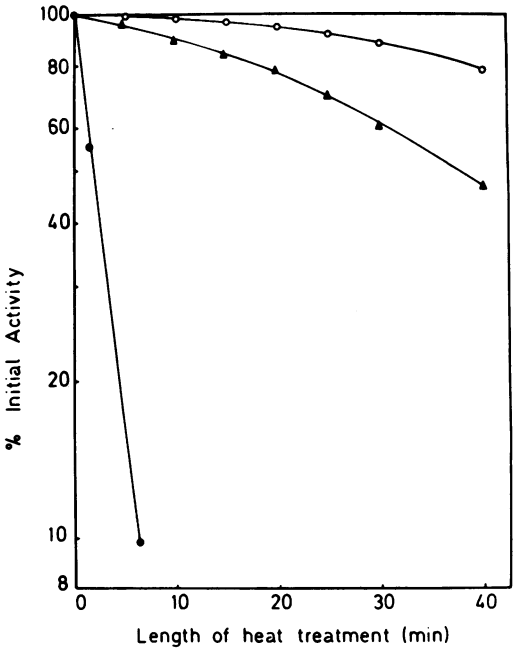

FIG. 1. *Thermic inactivation of ornithine carbamoyltransferase in E. coli K-12 JEF8C23 (●, argF constitutive, with a negligible argI activity; 9), E. coli K-12 NCI-8 (○, argF⁻ argI constitutive; 9), and P4XB2 (▲, argF⁺argI⁺).*

TABLE 2. *Types of transducing phages recovered from lysogens prepared with a "mixed lysate" of strain RW592*

Lysogen[a]	Genes transduced by the phage[b]	Thermostability of ornithine carbamoyltransferase in the lysogen
N-1	*argI pyrB valS*	High
N-3	*argI pyrB*	High
N-4	*argF*	Low
N-5	*argF*	Low
N-12	*argI pyrB valS*	High

[a] The strain is the ornithine carbamoyltransferase-less mutant of 3000X111 mentioned in the text.

[b] The presence of the *valS* gene was examined by the rapid assay described by Kikuchi et al. (6). The test does not indicate if only a part, rather than the whole, of *valS* is present on the phage.

bamoyltransferase harbor a λ*argF* phage was obtained as follows. Strains N4 and N5 (presumably λ*argF* lysogens) were superinfected with λ*pyrB argI* from lysogen N3 and 12; colonies having acquired the second phage were recognized by their capacity to produce phages transducing strain 3OSOU4 to Pyr$^+$. Cultures of such strains were examined for ornithine carbamoyltransferase isoenzymes and found to exhibit a mixed pattern, attesting the presence of both the *argF* and *argI* genes in a functional state.

The present data thus add to our previous appreciation of the degree of similarity exhibited by the two gene products and at the same time provide a simple criterion for their straightforward characterization. The reported differences in thermolability and the elution pattern on diethylaminoethyl-Sephadex columns are the only physicochemical differences presently allowing us to distinguish FFF from III. The primary sequences of the *argF* and *argI* gene products are presently being determined. It is already known (D. Gigot, personal communication) that their respective amino acid compositions do not differ by more than 5%. It will be interesting to compare their sequences, not only between themselves, but also with the primary sequence of the catalytic subunit of aspartate carbamoyltransferase, which bears striking similarities to the *argF* and *argI* products (8).

A final comment can be made regarding the evolutionary significance of the similarity encountered between *argF* and *argI*. To explain certain regularities observed in the distribution of biochemically related genes in *E. coli*, Zipkas and Riley (16) have suggested that the chromosome of this organism was formed by two successive duplications of a primitive genome one-quarter of the size of the present-day chromosome. If *argF* and *argI* had been created by such an event, one would have expected them to be 90° or 180° apart; this is not the case. In addition, in the light of all the evidence presently available, it cannot be considered likely that the two genes evolved independently, as Zipkas and Riley suggested. We would wonder, rather, whether the duplication responsible for the existence of *argF* is an ancient one and has any bearing on the presumably distant origins of the *E. coli* chromosome. The two genes involved exhibit much greater similarities than their counterparts of other isoenzyme systems, lactate dehydrogenase and aldolase in mammals, for instance (5, 10); this is particularly striking with respect to kinetic parameters. Moreover, it should be stressed that no *argF* gene has been detected in *E. coli* W or *E. coli* B (see reference 8); in the latter case the analysis has been carried far enough to conclude that the gene is absent and not merely nonfunctional (Epstein, personal communication). Furthermore, no *argF* equivalent been found in *Klebsiella pneumoniae* (11), *Enterobacter aerogenes* (M. Matsumoto, personal communication), *Salmonella typhimurium* (15), *Proteus mirabilis* (13), and *Serratia marcescens* (Matsumoto, personal communication), the genetic linkage maps of which show important similarities to that of *E. coli*. One should therefore consider that *argF* might be the product of a relatively recent event (duplication or transposition) peculiar to *E. coli* K-12; the latter types of chromosomal rearrangements are known to occur in bacteria by a variety of mechanisms (2, 3).

ACKNOWLEDGMENTS

This work has been supported by the Belgian Funds for Fundamental and Collective Research (FRFC-FKFO) and by the Belgian National Funds for Scientific Research (FNRS).

We thank A. Piérard for critical reading of the manuscript.

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