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Expression of the Arginine Regulon of *Escherichia coli* W: Evidence for a Second Regulatory Gene[1]

ELIZABETH C. THEIL, GEORGE W. FORSYTH, AND EVAN E. JONES

Nutritional Biochemistry Section, Department of Animal Science, and the Department of Biochemistry, North Carolina State University, Raleigh, North Carolina 27607

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Effect of the *M* (modifier) gene of *Escherichia coli* W on the expression of wild-type structural genes of four arginine biosynthetic enzymes was studied by examining enzyme activity in cell-free extracts of cultures grown in minimal medium and medium containing arginine. The mutant *M* gene was originally identified as causing arginine-induced synthesis of acetylornithine δ-transaminase in a strain deficient for the enzyme. The strains used in this study received the mutant *M* gene by recombination. Noncoordinate repression has been demonstrated for two more enzymes of the arginine regulon of *E. coli* W and the M^- gene increases the degree of noncoordinate repression for the regulon. Mutation of the *M* gene results in altered regulation of acetylornithine δ-transaminase, ornithine transcarbamylase, and acetylornithinase. In addition, a decreased growth rate is observed. It is proposed that the *M* gene is a regulatory gene. A model is presented to explain the data which involves changes in operator-repressor affinity for the structural genes and possibly for the gene controlling arginyl transfer ribonucleic acid synthetase.

During a study of the regulation of arginine biosynthesis, Vogel and Bacon (7) isolated a mutant of *Escherichia coli* W in which the arginine biosynthetic enzyme acetylornithine δ-transaminase was induced by arginine rather than being repressed by arginine; arginine still repressed the other enzymes of arginine synthesis that were examined. The transaminase inducible strain was isolated after mutagen treatment of a transaminase negative mutant (T^-) and after selection for growth in the absence of arginine. Strains with complete or partial restoration of transaminase activity which is repressed by arginine were also isolated. The mutation which led to inducibility has been shown to occur at a locus designated *M* (modifier; Fig. 1) and is not closely linked to the transaminase locus or to any of the other genes which specify synthesis of arginine biosynthetic enzymes (2). The *M* locus is also separate from the arginine regulatory gene *argR*. The presumed genotype of the inducible strain is M^-T^-.

The mutant *M* gene has been introduced into several other strains of *E. coli* W by recombination (2). It is the purpose of this study to investigate the function of the *M* gene by examining the level of activity of four of the arginine biosynthetic enzymes in the strains which carry combinations of normal and mutant *M* and *argR* genes and which have wild-type structural genes for the enzymes of arginine biosynthesis.

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MATERIALS AND METHODS

Strains and culture conditions. The four strains of *E. coli* that were used were obtained from Donald Bacon and Henry J. Vogel and have been described previously (2). They represent the following genetic constitutions: $argR^+$ M^+ (wild type), $argR^-$ M^+ pro^- (derepressed), $argR^+$ M^- pro^-, and $argR^-$ M^- pro^-. The proline marker was used to isolate recombinants. The $argR^-$ strain was isolated after mutagen treatment and selection for canavanine resistance; the same locus was present in all $argR^-$ mutants studied. The activity of the arginine biosynthetic enzymes in the $argR^+$ strains is high in minimal medium and is unchanged by added arginine. The structural genes for the arginine biosynthetic enzymes are all wild type.

Samples of overnight cultures grown in E medium (9), L-proline (25 μg/ml), and DL-methionine (100 μg/ml) with arginine (100 μg/ml) or without were added to fresh medium and harvested during the exponential phase of growth (ca. 50 Klett units, no. 66 K-S filter). The original M^- donor was met^-. Consequently, methionine and proline were routinely added to all cultures for purposes of comparison. Growth was at 37 C in Klett tubes containing 10 ml of medium and closed with rubber stoppers. Similar cultures were used to measure optical density (OD) changes during growth. Washed cells, suspended in

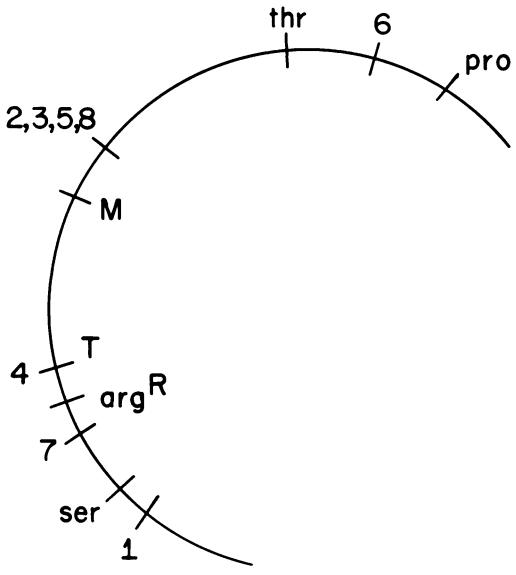

FIG. 1. *Genes of arginine biosynthesis of E. coli W are indicated in relation to other marker genes. Data were obtained by recombination (7). The enzymes studied in this work are enzyme 5, acetylornithinase; enzyme 8, argininosuccinate lyase; enzyme 6, ornithine transcarbamylase; and enzyme 4 is acetylornithine δ-transaminase which is also represented by the letter T. The regulatory gene for the arginine pathway is argR and M is the location of the mutation which leads to induction of the transaminase by arginine in an M^-T^- strain.*

0.1 M potassium phosphate buffer (*p*H 7.0) containing 1 mM reduced glutathione, were subjected to sonic disruption followed by centrifugation to remove unbroken cells and debris. The cell-free extracts were stored at −20 C prior to analysis of enzyme activity; enzyme activity remains unchanged by freezing and thawing.

Assay of enzyme activity. Acetylornithine δ-transaminase (enzyme 4; EC 2.6.1.11; α-*N*-acetyl-L-ornithine:2-oxoglutarate aminotransferase) was assayed by the procedure of Albrecht and Vogel (1).

Acetylornithinase (enzyme 5; EC 3.5.1.d; α-*N*-acetyl-L-ornithine aminohydrolase) was assayed by the procedure of Vogel and Bonner (9).

Ornithine transcarbamylase (enzyme 6; EC 2.1.3.3; carbamoylphosphate:L-ornithine carbamoyltransferase) was assayed by a modification of the procedure of Jones et al. (5) which was devised by Leon Unger. The cell extract (0.1 ml, ca. 0.7 mg of protein per ml) was added to a mixture containing 50 μmoles of tris (hydroxymethyl)aminomethane-hydrochloride (*p*H 7.8), 5 μmoles of L-ornithine, and 5 μmoles of carbamyl phosphate in 0.4 ml of water and incubated at 37 C. The reaction was terminated by the addition of 2.0 ml of a 5:3 mixture of 1% 2,3-butanedione-2 oxime in 5% acetic acid and 10% As_2O_3 in concentrated HCl. After mixing was completed, the tubes were covered, and the color was allowed to develop in the dark, at room temperature, overnight. The OD was read at 490 nm. One unit of activity is defined as that amount of enzyme necessary to produce 0.2 μmoles of citrulline in 10 min at 37 C. Corrections were made for enzyme blanks, and a citrulline standard was included in each assay. The amount of citrulline produced increases linearly during the incubation period.

Argininosuccinate lyase (enzyme 8; EC 4.3.2.1; L-argininosuccinate arginine-lyase) was assayed by the procedure of Jorge Ortigoza-Ferado as follows: 0.1 ml of the cell extract was added to a mixture of 1.5 μmoles of argininosuccinate (potassium salt) prepared from the barium salt (Calbiochem) by treatment with K_2SO_4, 0.2 mg of arginase (Worthington Biochemical Corp., Freehold, N.J.), and 10 μmoles of potassium phosphate (*p*H 7.5) in 0.4 ml of water and incubated at 37 C. One unit of activity is defined as that amount of enzyme which will produce 0.1 μmole of arginine, measured indirectly as the ornithine produced by arginase, in 15 min at 37 C. The ornithine was assayed by the procedure of Vogel and Bonner (9). Corrections were made for enzyme blanks, and an ornithine standard was included in each determination. Increasing the incubation up to 4 hr resulted in a linear increase in the amount of arginine produced.

Protein was assayed by the procedure of Lowry et al. (6) with appropriate buffer blanks.

RESULTS

Effect of the M^- gene on enzyme activity. The data in Table 1 indicate that the M^- mutation is pleiotropic, affecting the activity of three of the four enzymes studied (Table 1).

In minimal medium, the presence of the M^- gene results in a decrease in activity of enzymes 4, 5, and 6 as compared to the wild-type strain (M^+ $argR^+$). If the M^- gene had no effect on the regulation of enzyme synthesis, the ratio of enzyme activity obtained from M^- $argR^+/M^+$ $argR^+$ should be 1.0. Since the ratio of activities is 0.49 and 0.50 for enzymes 5 and 6, respectively, it is apparent that the M^- gene does have some effect and results in repression of synthesis of these enzymes.

Introduction of a mutation at the *argR* locus results in derepression of all the enzymes studied. A comparison of the double mutant M^- $argR^-$ to the M^+ $argR^-$ strain shows that only the synthesis of enzyme 6 is affected by the M^- gene and a ratio of 0.68 is obtained. Thus, the M^- mutation does not allow complete derepression of the enzyme 6 to occur in the *argR* mutant.

The M^- gene also affects regulation of enzyme synthesis when the mutants are grown in medium containing arginine. The ratio of specific activity of ornithine transcarbamylase is 0.32 for M^- $argR^+/M^+$ $argR^+$ and 0.70 for M^- $argR^-/M^+$ $argR^-$. The most striking effect of the M^- gene is on the activity of acetylornithine transaminase (enzyme 4). The M^- $argR^+/M^+$ $argR^+$ ratio for the transaminase is 5.3. The M^- gene

TABLE 1. *Activity of arginine biosynthetic enzymes in regulatory mutants of E. coli W*

Medium	Genetic constitution	Enzyme[a]			
		4	5	6	8
Minimal medium	M^+argR^+	7.0 ± 2.4	56.9 ± 14.6	34.9 ± 3.4	5.3 ± 3.2
	M^-argR^+	4.4 ± 1.1	27.8 ± 8.2	17.3 ± 5.8	5.4 ± 3.3
	M^+argR^-	11.6 ± 1.1	148.3 ± 25.6	1103 ± 196	12.0 ± 6.4
	M^-argR^-	10.6 ± 1.2	176.8 ± 18.3	737 ± 109	13.1 ± 7.2
Minimal medium plus arginine (100 μg/ml)	M^+argR^+	0.99 ± 0.94	11.8 ± 3.4	2.7 ± 0.3	0.32 ± 0.08
	M^-argR^+	5.2 ± 2.4	9.4 ± 5.9	0.86 ± 0.53	0.97 ± 1.6
	M^+argR^-	11.0 ± 2.1	137.6 ± 14.9	1112 ± 121	12.0 ± 5.6
	M^-argR^-	9.0 ± 1.3	165.2 ± 31.3	781 ± 255	12.9 ± 6.6

[a] Cell-free extracts were prepared from cells in the exponential phase of growth and assayed for enzyme activity. The data are expressed as units per milligram of protein. Analyses were performed on four to six independent preparations, and the error is presented as the standard deviation. Enzyme 4 is acetylornithine δ-transaminase, enzyme 5 is acetylornithinase, enzyme 6 is ornithine transcarbamylase, and enzyme 8 is argininosuccinate lyase.

actually prevents arginine repression of the transaminase but simultaneously results in greater arginine repression of ornithine transcarbamylase.

The differences between the numbers (Table 1) used to compute the above ratios are significant ($P < 0.05$).

Noncoordinate repression. The ratio of the specific activity of each enzyme in minimal medium to medium containing arginine is presented in Table 2. In an M^+ $argR^+$ strain, arginine represses all the enzymes studied. The extent of repression varies, and therefore regulation of synthesis of these enzymes is noncoordinate. Noncoordinate repression has been shown previously in *E. coli* K-12 for acetylornithinase, *N*-acetyl γ-glutamokinase, *N*-acetylglutamic γ-semialdehyde dehydrogenase, and argininosuccinate lyase (8) and in *E. coli* W for acetylornithinase and acetylornithine transaminase (2). The M^- gene results in greater noncoordinate repression by arginine than is present in the wild-type strain (Table 2).

TABLE 2. *Extent of derepression of arginine biosynthetic enzymes in regulatory mutants of E. coli W: ratio of enzyme activities in minimal medium to minimal medium containing arginine*

Genetic constitution	Enzyme 4	Enzyme 5	Enzyme 6	Enzyme 8[a]
M^+ $argR^+$	7.14	4.76	12.1	16.9
M^- $argR^+$	0.85	2.88	20.0	5.56
M^+ $argR^-$	1.05	1.08	0.99	1.00
M^- $argR^-$	1.18	1.08	0.93	1.02

[a] The apparent differences in the values for enzyme 8 are not significant due to the large error in the assay of this enzyme.

Effect of the M gene on growth. The *M* gene affects the regulation of several of the enzymes of arginine biosynthesis and is not enzyme-specific as was originally thought (2). Mutation of the *M* gene results not only in lowered levels of enzyme activity but also in a slower growth rate (Table 3; Fig. 2). The average doubling time is 2.0 hr for M^- strains and 1.3 hr for M^+ strains in minimal medium under the conditions employed in this study and is unchanged by added arginine.

DISCUSSION

The results suggest that the *M* gene is a regulatory gene affecting the activity of three of the four arginine biosynthetic enzymes that were examined. Strains carrying the M^- gene have reduced levels of acetylornithinase and ornithine transcarbamylase in minimal medium. In addition, the M^- mutation alters the control mechanisms so that acetylornithine transaminase synthesis is not repressible by arginine. The effect of the M^- mutation was initially observed as an increase in acetylornithine δ-transaminase activity when a transaminaseless strain (M^-T^- $argR^+$) was grown in the presence of arginine (2). Therefore, it was thought that the M^- mutation might result in the production of another transaminase. If the M^- gene is a structural gene which specifies the synthesis of a second transaminase, this enzyme would be involved in regulation of enzyme synthesis since the M^- mutation is pleiotropic and results in altered regulation of the arginine biosynthetic enzymes; thus, it seems unlikely that the M^- gene specifies a second transaminase. The nature of the acetylornithine transaminase produced by the M^-T^- $argR^+$ strain is currently

TABLE 3. *Doubling time for regulatory mutants of E. coli W*

Genetic constitution	Doubling time[a]									
	Minimal medium					Arginine (100 μg/ml)				
M+ *argR*+	1.4	1.4	1.1	1.3	(1.3)	1.2	1.1	1.4	1.0	(1.2)
M− *argR*+	1.9	2.6	1.6	1.8	(2.0)	2.8	2.0	1.9	1.5	(2.1)
M+ *argR*−	1.3	1.5	1.1	1.2	(1.3)	1.0	1.4	1.2	1.3	(1.2)
M− *argR*−	1.8	2.6	1.8	1.8	(2.0)	2.0	2.0	2.0	2.0	(2.0)

[a] Doubling time (expressed as hours) was determined from graphs made by plotting the log of the relative increas in OD of cultures in the exponential phase of growth versus time (*see* Fig. 2 for representative curves). Average doubling-time values for each mutant on minimal medium or medium with arginine are expressed parenthetically.

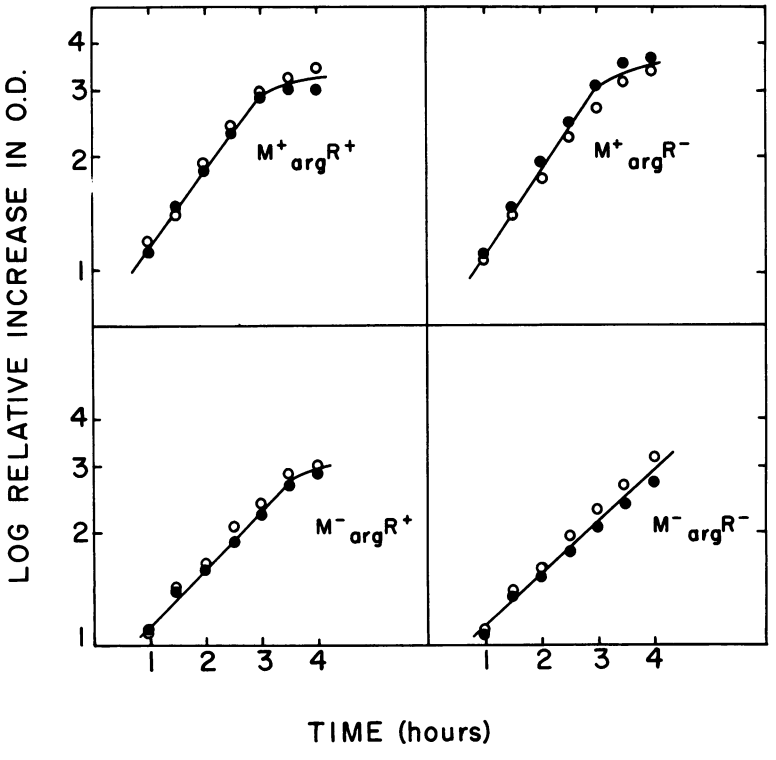

FIG. 2. *Effect of the M gene on growth. The OD of cultures grown under the conditions used for preparation of the cell-free extracts was measured during the exponential phase of growth. Symbols:* ○, *minimal medium;* ●, *minimal medium with arginine (100 μg/ml).*

under investigation in this laboratory. The transaminases obtained from the mutant and wild-type strains are being purified, characterized, and compared.

Since the genes which specify the synthesis of the arginine biosynthetic enzymes are not contiguous, there must be more than one repressor recognition site (operator) in the arginine regulon. If each repressor recognition site were identical, regulation should be coordinate. Regulation of the arginine biosynthetic pathway, however, is parallel but noncoordinate; therefore, the operators must be structurally similar but have different affinities for the respressor (*see* reference 4).

The fact that a single mutation affects the regulation of several enzymes in a regulon with nonidentical operators may be interpreted to mean that the *M*− mutation results in an alteration of interaction of the repressor with the operator sites. Such alterations could affect either the repressor or common regions of the operators. In minimal medium, the repressor formed in *M*−

strains is bound more tightly by the operators for acetylornithinase and ornithine transcarbamylase than the repressor in M^+ strains. The operator for ornithine transcarbamylase has a higher affinity for the M^- repressor than for the M^+ repressor in the presence of arginine. However, the operator for acetylornithine δ-transaminase has a low affinity for the M^- repressor; thus, the synthesis of this enzyme continues as a result of decreased binding of the repressor at the operator region in the presence of arginine.

The growth rate of the M^- strains is less than M^+ strains in minimal medium and is not increased by the addition of arginine. These observations may indicate poor arginine utilization which could result from a reduced concentration (absolute or effective) of arginyl transfer ribonucleic acid (tRNA) or a repressed level of arginyl tRNA synthetase. The possibility that the slow growth rate in the M^- strains is unrelated to arginine biosynthesis or metabolism has not been excluded. It has been shown previously that the arginyl tRNA synthetase of *E. coli* is regulated in a parallel, noncoordinate manner with the arginine biosynthetic enzymes (L. S. Williams and F. C. Neidhardt, Bacteriol. Proc., p. 131, 1968). The gene for the synthetase, therefore, may be in the same regulon as the biosynthetic enzymes and may be affected by the M^- repressor, resulting in reduced synthetase activity.

The effects of the M^- mutation indicate that the *M* gene is involved in regulation. Thus, regulation of the arginine biosynthetic enzymes in *E. coli* W is effectively the result of two genes, *argM* and *argR*. Multiple regulatory genes have been cited for other amino acid biosynthetic pathways, such as histidine and tryptophan (3). Multiple alleles of the *argR* locus have been reported (4), but, to our knowledge, no other reports have been made of more than one regulatory gene for arginine biosynthesis in *E. coli*.

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LITERATURE CITED

1. Albrecht, A. M., and H. J. Vogel. 1964. Acetylornithine δ-transaminase. Partial purification and repression behavior. J. Biol. Chem. 239:1872–1876.
2. Bacon, D. F., and H. J. Vogel. 1963. A regulatory gene simultaneously involved in repression and induction. Cold Spring Harbor Symp. Quant. Biol. 28:437–438.
3. Epstein, W., and J. B. Beckwith. 1968. Regulation of gene expression. Annu. Rev. Biochem. 37:411–431.
4. Jacoby, G. A., and L. Gorini. 1969. A unitary account of the repression mechanism of arginine biosynthesis in *Escherichia coli*. J. Mol. Biol. 39:73–77.
5. Jones, M. E., L. Spector, and F. Lipmann. 1955. Carbamyl phosphate, the carbamyl donor in enzymatic citrulline synthesis. J. Amer. Chem. Soc. 77:819–820.
6. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
7. Vogel, H. J., and D. F. Bacon. 1966. Gene aggregation: Evidence for a coming together of functionally related, not closely linked genes. Proc. Nat. Acad. Sci. U.S.A. 55:1456–1459.
8. Vogel, H. J., S. Baumberg, D. F. Bacon, E. E. Jones, L. Unger, and R. H. Vogel. 1967. Gene-ribosome-enzyme organization in the arginine system of *Escherichia coli*, p. 223–234. *In* H. J. Vogel, J. O. Lampen and V. Bryson (ed.), Organizational biosynthesis. Academic Press Inc., New York.
9. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: Partial purification and some properties. J. Biol. Chem. 218:97–106.