JOURNAL OF BACTERIOLOGY, Apr. 1981, p. 415–417
0021-9193/81/040415-03$02.00/0

Vol. 146, No. 1

Insertion of IS3 Can "Turn-On" a Silent Gene in *Escherichia coli*

M. ZAFARULLAH, D. CHARLIER, AND N. GLANSDORFF*

Erfelijkheidsleer en Microbiologie, Vrije Universiteit Brussel and Onderzoekingsinstituut van het COOVI, B-1070 Brussels, Belgium

Received 22 October 1980/Accepted 12 January 1981

A silent *argE* gene became reactivated by the integration of IS3 in orientation II. IS3 itself is responsible for this effect, at least in part.

Insertion of an IS2 element in orientation II adjacent to the proximal end of a silent *Escherichia coli* gene can turn it on (8, 9). We show that IS3 behaves similarly to IS2 in that respect.

We used strain P4XSUP102 (3, 4), harboring a silent *argE* gene, to select for mutations able to turn on gene activity. *argE* (coding for acetylornithine deacetylase, EC 3.5.1.16) is transcribed from a promoter (PE) which faces the promoter for *argCBH* (PCBH) over the operator region of the divergent *argECBH* operon (4; Fig. 1). In P4XSUP102, a DNA segment extending from *argB* to PE is deleted (2, 4; Fig. 1); consequently, *argE* transcription is almost abolished, although *argH* (the gene for arginosuccinase, EC 4.3.2.1) undergoes no decrease in activity.

N^2-Acetyl-L-arginine is a low-affinity substrate for acetylornithine deacetylase; it is therefore possible to select for reactivation of *argE* without bringing *argH* into play by plating a strain carrying the SUP102 deletion on minimal medium supplemented with acetyl-L-arginine (4). We performed such a selection with strain MN42(λ⁻)*metB* Δ(*ppc-argECBH*)*42* (4) lysogenized with both the thermoinducible bacteriophage λ199 and the transducing derivative λ13SUP102 (2). Using the SUP102 deletion on a phage enabled us to screen directly for acetyl-L-arginine utilizers carrying chromosome rearrangements, by isopycnic centrifugation of lysates on CsCl gradients.

Growth of cells, preparation of phage lysates, transductions, enzyme assays, and electron microscope heteroduplex analysis were performed as described previously (3, 4; see also legend to Fig. 1 and Table 1, footnote *a*).

Cells of MN42 (λ199)⁺(λ13SUP102)⁺ grown at 32°C were plated on minimal medium (4) supplemented with 0.5% glucose (wt/vol; Difco Laboratories, Detroit, Mich.), 50 μg L-methionine per ml, and 200 μg of acetyl-L-arginine per ml, both from Sigma Chemical Co., St. Louis, Mo. About 10^{-7} mutants per cell plated appeared after 2 to 3 days at 32°C. In independent selections, 30 to 50% of the mutants were found to produce phage transducing the acetyl-L-arginine-utilizing phenotype. Roughly a third of these displayed an increase in buoyant density. By heteroduplex analysis a particular batch was found to consist mainly of mutants harbouring IS2 (seven cases) or IS3 (four cases) in the *arg* region (Fig. 1).

Phage lysates from six such mutants (AA4, AA10, AA41, AA44, AA59, and AA203) were used to transduce at a low multiplicity of infection (less than 0.01 phage per cell) an *argR*⁺ and an *argR* (genetically derepressed) derivative of strain MN42(λ⁻)*metB* Δ(*ppc-argECBH*)*42* *recA* lysogenized with λ199 (2). Selection was for transductants having acquired the *ppc* gene, which is located immediately to the left of *argE*. They were obtained on glucose minimal plates supplemented with L-arginine (100 μg/ml) and L-methionine (50 μg/ml); they were purified, checked for the ability to transduce further the capacity to utilize acetyl-L-arginine, and grown for enzyme assays (Table 1) in glucose minimal medium supplemented with methionine and arginine, as above.

Mutants AA41, 44, and 203 harbor IS2 elements in orientation II with respect to *argE*. Heteroduplex mapping (Fig. 1) showed that the insertions are located to the right of PCBH, very close to the endpoint of the material deleted in P4XSUP102. Since they are in orientation I with respect to *argH*, they exert the expected polar effect on the activity of this gene (Table 1). With respect to *argE*, the IS3 elements found in mutants AA4, AA10, and AA59 are also in orientation II if we consider the polar IS3 element of *lacZ* mutant MS505 (6) to be in orientation I relative to *lacY*. Indeed, we conclude that (i) with respect to phage genes (Fig. 1) *argE* and *lacZ* are both transcribed leftwards on their respective vectors, λ13 and λ *placZMS*505; (ii)

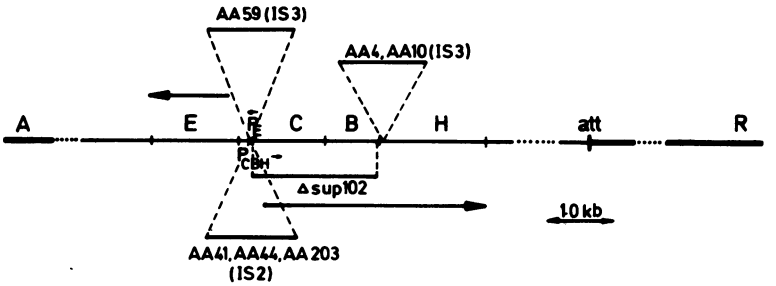

FIG. 1. *The argECBH divergent operon in transducing phage λ 13 (3). Arrows indicate direction of DNA transcription. The gene-enzyme correspondence in arginine biosynthesis is as follows: glutamate* $\xrightarrow{A}$ *N^2-acetyl-L-glutamate* $\xrightarrow{B}$ *N^2-acetyl-L-glutamyl phosphate* $\xrightarrow{C}$ *N^2-acetyl-glutamate-L-semialdehyde* $\xrightarrow{D}$ *N^2-acetyl-L-ornithine* $\xrightarrow{E}$ *ornithine* $\xrightarrow{F,I}$ *citrulline* $\xrightarrow{G}$ *argininosuccinate* $\xrightarrow{H}$ *arginine. The location and nature of the IS elements reactivating argE on λ13 carrying the SUP102 deletion were ascertained by electron microscope heteroduplex mapping (3) with reference phage λ13 dppc-argECBH, λ13 A010::IS2, λ13 A07::IS2 (3), and λ placZMS505::IS3 (6).*

TABLE 1. *Effect of IS2 and IS3 on the expression of argE and argH in P4XSUP102*

Strain	Enzyme sp act[a]	
	Acetylornithine deacetylase (*argE*)	Arginosuccinase (*argH*)
P4X (wild type)[b]	8	0.18
P4X *argR*[c]	102	10.9
P4XSUP102	<0.2	1.0
P4XSUP102 *argR*	<0.2	5.7
AA41 (IS2)[d]	10.1	0.18
AA41 *argR*	18.0	0.29
AA44 (IS2)	11.4	0.22
AA44 *argR*	18.5	0.44
AA203 (IS2)	9.1	<0.02
AA203 *argR*	10.9	<0.02
AA4 (IS3)	22.7	<0.02
AA4 *argR*	9.5	<0.02
AA10 (IS3)	23.5	<0.02
AA10 *argR*	9.7	<0.02
AA59 (IS3)	8.9	0.07
AA59 *argR*	11.5	0.07

[a] Enzyme specific activities expressed as units (micromoles of product formed per hour) per milligram of protein.

[b] First four strains are described in reference 4.

[c] *argR*: Genetically derepressed.

[d] This and all following strains are transductants (see text) of MN42 (λ⁻) Δ(*ppc-argECBH*) (λ199)⁺ *recA*, *argR*⁺ or *argR*, by phage lysates from the above-mentioned AA mutants.

only DNA strands of the same chemical polarity were seen to make molecular hybrids of 1.3 ± 0.1 kilobases at the site of insertion.

In mutant AA59, IS3 is integrated very close to *argE*. The low *argH* activity can be ascribed to the polar effect which IS3 exerts in orientation I (6). In AA4 and AA10, IS3 was found about 0.2 kilobase further to the right, and *argH* expression was completely abolished, suggesting that IS3 is integrated within *argH*. Curiously, acetylornithine deacetylase exhibited lower activities in *argR* than in *argR*⁺ derivatives. This may be due to interference between convergent RNA polymerases when transcription at PCBH becomes derepressed. In the normal situation, or in AA59, AA41, and AA44, where the IS element falls within the control region, initiation of leftwards and rightwards transcription may be mutually exclusive events (7).

The complete *argH* deficiency of mutants AA4 and AA10 enabled us to assess the role played by IS3 in the reactivation of *argE*, by selecting for *argH*⁺ derivatives without having to bring expression of *argE* into play; such derivatives were obtained by plating AA4 and AA10 on minimal medium supplemented with L-ornithine (100 μg/ml). Out of 30 derivatives obtained from either mutant, all had reverted to the phenotype of P4XSUP102. A few of them were picked at random and found to be identical with P4XSUP102 as far as enzyme assays, CsCl gradient analysis, and heteroduplex mapping could tell (data not shown). Since loss of the insertion and return to low *argE* activity are concomitant, the IS3 element must, at least in part, be responsible for turning on *argE*.

In conclusion, the present analysis shows that IS3 is able to turn on gene expression when integrated in a particular orientation, a property previously reported for IS2 (8, 9). The data would, however, not warrant the conclusion that IS3 constitutes a mobile promoter per se. As discussed elsewhere in detail (Glansdorff et al., Cold Spring Harbor Symp. Quant. Biol., in press; D. Charlier, and J. Besemer, submitted for pub-

lication), all reported cases of gene activation promoted by IS elements could be explained by the formation of promoters at the novel joint created by the insertion events. The same could be true for recently reported activations of *lac* genes by Tn*5* (1) and of yeast genes by other transposons (Errede et al., Cell, in press). Identifying RNA transcription startpoints will settle this question.

The stimulation of gene transcription by integration of transposable elements is not very rare; other rearrangements displaying promoter activity have been reported (2, 5). Such phenomena may have played a prominent role in evolution.

This work was supported by the Belgium FKFO. M.Z. acknowledges a fellowship from the Belgian-Pakistanese Cultural Agreements.

LITERATURE CITED

1. **Berg, D., A. Weiss, and L. Crossland.** 1980. Polarity of Tn*5* insertion mutations in *Escherichia coli*. J. Bacteriol **142**:439–446.
2. **Charlier, D., M. Crabeel, R. Cunin, and N. Glansdorff.** 1979. Tandem and inverted repeats of arginine genes in *Escherichia coli*. Structural and evolutionary considerations. Mol. Gen. Genet. **174**:75–88.
3. **Charlier, D., M. Crabeel, S. Palchaudhuri, R. Cunin, A. Boyen, and N. Glandsdorff.** 1978. Heteroduplex analysis of regulatory mutations and of insertions (IS1, IS2, IS5) in the bipolar *argECBH* operon of *Escherichia coli*. Mol. Gen. Genet. **161**:175–184.
4. **Elseviers, D., R. Cunin, N. Glansdorff, S. Baumberg, and E. Ashcroft.** 1972. Control regions within the *argECBH* gene cluster of *Escherichia coli* K12. Mol. Gen. Genet. **117**:349–366.
5. **Ghosal, D., and H. Saedler.** 1978. DNA sequence of the mini-insertion IS2-6 and its relation to the sequence of IS2. Nature (London) **275**:611–617.
6. **Malamy, H. H., H. Fiandt, and W. Szybalski.** 1972. Electron microscopy of polar insertions in the *lac* operon of *Escherichia coli*. Mol. Gen. Genet. **119**:207–220.
7. **Piette, J., R. Cunin, M. Crabeel, A. Boyen, N. Glansdorff, C. Squires, and C. Squires.** 1980. Nucleotide sequence of the control region of the *argECBH* bipolar operon in *Escherichia coli*. Arch. Int. Physiol. Biochim. **89**:B243.
8. **Pilacinski, W., E. Mosharrafa, R. Edmundson, J. Zissler, H. Fiandt, and W. Szybalski.** 1977. Insertion sequence IS2 associated with *int*-constitutive mutants of bacteriophage lambda. Gene **2**:61–74.
9. **Saedler, H., H. J. Reif, S. Hu, and N. Davidson.** 1974. IS2, a genetic element for turn-off and turn-on of gene activity in *E. coli*. Mol. Gen. Genet. **132**:265–272.