

The Proton Channel Is the Minimal Structure of ATP Synthase Necessary and Sufficient for Microcin H47 Antibiotic Action

Eliana Rodríguez and Magela Laviña*

Sección de Fisiología y Genética Bacterianas, Facultad de Ciencias,
Montevideo, Uruguay

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It had been previously determined that the presence of F_0F_1 ATP synthase was required for microcin H47 antibiotic action. In this work, microcin-resistant *atp* mutants were genetically analyzed. Their mutations, originated by Tn5 insertion, in all cases were found to affect determinants for the F_0 portion of ATP synthase. To discern if microcin action required the presence of the entire complex or if the F_0 proton channel would suffice, recombinant plasmids carrying different segments of the *atp* operon were constructed and introduced into an *atp* deletion strain. The phenotypic analysis of the strains thus obtained clearly indicated that the presence of the F_0 proton channel was absolutely required for microcin H47 action, while the F_1 catalytic portion was found to be dispensable. Furthermore, when any of the three components of the proton channel was missing, total resistance to the antibiotic ensued. Complementation analysis between *atp::Tn5* chromosomal mutations and recombinant *atp* plasmid constructions further supported the idea that the proton channel would be the minimal structure of the ATP synthase complex needed for microcin H47 antibiotic action.

The production of peptides with antibiotic action is a resource frequently employed in nature and, as judged by its widespread occurrence among bacteria, can be considered a successful strategy in mediating bacterial antagonistic relationships. Microcins are a group of antibiotic peptides produced by strains of Gram-negative bacteria. At present, only a few microcins have been thoroughly studied. Nevertheless, it is already clear that they exhibit a noteworthy diversity in their mechanism of action; e.g., colicin V and microcin E492 act as ionophores, microcin C7 inhibits protein synthesis, microcin B17 inhibits DNA replication due to its interaction with DNA gyrase, and microcin J25 acts upon RNA polymerase (6, 8, 11, 21, 24). Therefore, the study of microcins' mode of action presents a particularly interesting aspect: the identification of new antagonistic activities against basic cellular functions.

In the present work, we deal with microcin H47 (MccH47), a gene-encoded peptide antibiotic produced by a naturally occurring strain of *Escherichia coli* isolated in Uruguay. The MccH47 genetic system is clustered in a 10-kb DNA segment located in the chromosome (13). The information encoded by this system determines the three basic functions related to antibiosis: synthesis of the antibiotic, its secretion to the extracellular medium, and immunity. In brief, the MccH47 genetic system comprises four genes involved in microcin synthesis (the smallest being the structural gene), two further genes devoted to microcin secretion, and an immunity gene whose product protects the cell against its own antibiotic production (2, 7, 16, 17).

MccH47 is ribosomally synthesized as a peptide precursor which already possesses antibiotic activity of the same specificity as that of mature microcin. This toxic effect was detected

in cells carrying the MccH47 structural gene and lacking the remaining genes from the microcin system. Although cells with such a genetic construction were nonviable, some mutant clones grew which exhibited Atp^- and MccH47-resistant (MccH47^r) phenotypes. This fact, together with the isolation and characterization of MccH47^r mutants affected in the *atp* locus, led us to consider ATP synthase as the possible target of MccH47 antibiotic action (17, 20).

ATP synthase of *E. coli* consists of a membrane-bound F_0 sector to which a cytoplasmic F_1 sector is bound. This complex is made up of eight different polypeptides: three subunits form the F_0 proton channel in a ratio of a_1, b_2, c_{9-12} ; and five subunits compose the catalytic F_1 portion in a stoichiometry of $\alpha_3, \beta_3, \gamma_1, \delta_1, \epsilon_1$. The *atp* operon, which encodes these subunits, is arranged as follows: *atpIBEFHAGDC*, corresponding, respectively, to *i* (a protein of unknown function that does not form part of ATP synthase) and subunits *a, c, b, $\delta, \alpha, \gamma, \beta,$ and ϵ* (23).

To enhance our understanding of the mechanism of action of MccH47, we concentrated on two aspects: (i) the genetic characterization of previously isolated MccH47^r *atp* mutants and (ii) the identification of the minimal structure of the ATP synthase complex capable of conferring sensitivity to MccH47.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. The strains, plasmids and bacteriophages used in this study are listed in Table 1.

Media and culture conditions. Luria-Bertani (LB) rich medium, R medium, and M63 minimal medium supplemented either with glucose or succinate were used (15). The following antibiotics were added to media at the indicated final concentrations: ampicillin, 50 $\mu\text{g/ml}$; chloramphenicol, 30 $\mu\text{g/ml}$; kanamycin, 30 $\mu\text{g/ml}$; tetracycline, 12 $\mu\text{g/ml}$. When necessary, L-amino acids were added at a final concentration of 40 $\mu\text{g/ml}$. All strains were grown at 37°C, except for those carrying *Mu*cts, which were grown at 30°C.

Phenotype assays. Microcin sensitivity was assayed by patch test as described previously (20), the level of sensitivity being estimated by the diameter of the zone of inhibition. The *Atp* phenotype was analyzed through the ability of clones to grow on minimal medium supplemented with succinate. Mutants affected in

* Corresponding author. Mailing address: Sección de Fisiología y Genética Bacterianas, Facultad de Ciencias, Iguá 4225, Malvín Norte, Montevideo 11.400, Uruguay. Phone: (5982) 525 86 18, ext. 143. Fax: (5982) 525 86 29. E-mail: magela@fcien.edu.uy.

TABLE 1. Bacterial strains, bacteriophages, and plasmids used in this study

Strain, phage, or plasmid	Genotype and/or phenotype	Source or reference
<i>E. coli</i> K-12 strains		
BZB1011	<i>gyrA</i>	Laboratory collection
FGB64	BZB1011 <i>atpB</i> ::Tn5 64	20
MC4100	<i>araD139 lacU169 relA rpsL thiA</i>	Laboratory collection
RYC816	<i>gyrA</i> (λ +) <i>recA56 srl</i> ::Tn10	Laboratory collection
MC1000 Δ (<i>uncI-uncC</i>)	<i>araD139</i> Δ (<i>araABC-leu</i>)7679 Δ (<i>atpI-atpC</i>) <i>galK galU</i> Δ (<i>lac</i>)X74 <i>recA rpsL thi</i>	19
FGB084	MC4100 <i>atpF</i> ::Tn5 84	This work
FGB089	MC4100 <i>atpF</i> ::Tn5 89	This work
FGB091	MC4100 <i>atpE</i> ::Tn5 91	This work
FGB099	MC4100 <i>atpB</i> ::Tn5 99	This work
Bacteriophages		
Mu _{cts} Ap ^f	Mu _{cts} carrying the <i>bla</i> gene of Tn3	14
MudII4042	Mu _{cts} A ⁺ B ⁺ <i>repP15A lac</i> (<i>'ZYA</i>)931 (Cm ^r)	9
Plasmids		
pEG109	MudII4042:: <i>phoA proC</i> (Cm ^r)	9
pBJC917	pBR322 carrying <i>atpBEFHAGDC</i> (Ap ^r)	22
pER900	<i>Bam</i> HI deletion of pBJC917	This work
pER20	pBR322 with an <i>Eco</i> RI- <i>Sph</i> I fragment carrying <i>atpBEFH</i> (Ap ^r)	This work
pER90	<i>Bam</i> HI deletion of pER20	This work
pER1	pBR322 with an <i>Hind</i> III- <i>Ava</i> I fragment carrying <i>atpB</i> (Ap ^r)	This work
pER12	<i>Hpa</i> I deletion of pBJC917	This work
pER2	<i>Bam</i> HI deletion of pER12	This work
pER13	<i>Nnu</i> I deletion of pER20	This work
pER3	<i>Hinc</i> II partial deletion of pER13	This work
pMVD31	pACYC184 with an <i>Eco</i> NI- <i>Eco</i> NI fragment carrying <i>atpHAGDC</i> (Cm ^r)	This work

the *atp* locus are impaired for oxidative phosphorylation and, consequently, are unable to grow on minimal medium supplemented with a nonfermentable carbon source, such as succinate (4). Strains to be assayed were streaked on minimal medium supplemented with succinate and, in parallel, on the same medium supplemented with glucose instead. The plates were incubated 24 h at 37°C. Strains able to grow on either carbon source were considered Atp⁺, while those only capable of growing on glucose were scored as Atp⁻.

Genetic techniques. P1 transduction was performed as described (15). MccH47^r *atp* strains were made deficient for homologous recombination by introducing the *recA56* allele from RYC816 by cotransduction with the neighboring *srl*::Tn10 marker, selecting transductants on LB plates supplemented with tetracycline. The RecA phenotype of the transductants was detected by UV sensitivity. Tn5 insertions of MccH47^r *atp* mutants were cloned in vivo from the chromosome by means of phage MudII4042 lytic propagation as described previously (9, 20). The transducing phage lysates were used to infect MC4100 Mu_{cts} Ap^f, and transductant clones were selected on LB plates supplemented with chloramphenicol and kanamycin.

Manipulation and sequencing of DNA. Routine DNA manipulations were carried out as described previously (18). DNA sequencing was performed by the Centro Técnico de Análisis Genéticos (Facultad de Ciencias, Montevideo, Uruguay). A specific Tn5 sequencing primer was used: 5' CGA TGA AGA GCA GAA GTT AT 3'.

RESULTS

Localization of the Tn5 insertion sites in MccH47-resistant *atp* mutants. As was previously described, a group of chromosomal Tn5 insertion mutants was preliminarily characterized as presenting a high level of resistance to MccH47; phenotypic and complementation analyses revealed they were affected in the *atp* locus. The transposon insertion in one of these, FGB64, was located after nucleotide 1038 of the reported *atp* sequences (accession number J01594), interrupting the expression of the *atpB* gene, which encodes subunit a of the proton channel (20). P1 transducing phage was grown on four additional MccH47^r *atp* mutants, and the lysates were used to infect

MC4100, selecting clones on LB plates supplemented with kanamycin. All the transductants were MccH47^r, thus confirming that the Tn5 insertion was responsible for the MccH47^r phenotype in each case. The insertions were now called Tn5 84, 89, 91, and 99. One transductant from each group was chosen (FGB084, FGB089, FGB091, and FGB099) and used in further studies.

To identify the gene affected by each of the Tn5 insertions mentioned above, as well as to precisely identify, at the nucleotide level, the sites of insertion, these mutations were first cloned in vivo using the chimerical phage MudII4042, as described in Materials and Methods. In each of the four cloning experiments, transductants of strain MC4100 Mu_{cts} Ap^f should carry recombinant plasmids containing MudII4042 DNA, part or all of the Tn5 sequence, and, presumably, chromosomal DNA adjacent to the transposon. These plasmid constructions were used as start points for cloning DNA segments containing the chromosomal DNA-Tn5 junction sites into multicopy vectors. Nucleotide sequencing was performed upon these latter constructions. In this way, the mutations of strains FGB084, FGB089, FGB091, and FGB099 were cloned; this attempt was extended to the analysis of insertion Tn5 64, whose left end had already been precisely located (20). The following junction sites were cloned and sequenced: the right end of Tn5 64, 84, and 91; the left end of Tn5 89; and both ends of Tn5 99 (Fig. 1). It should be noted that in those cases where information was available from both junction sites (Tn5 64 and 99) a distance of 9 nucleotides was found between them, corresponding to the duplication of the Tn5 target (3). The Tn5 insertions were all found to interrupt the *atp* operon: Tn5 64 and 99 were located to *atpB*, Tn5 91 was located to *atpE*, and Tn5 84 and 89 were located to *atpF*. These three genes are

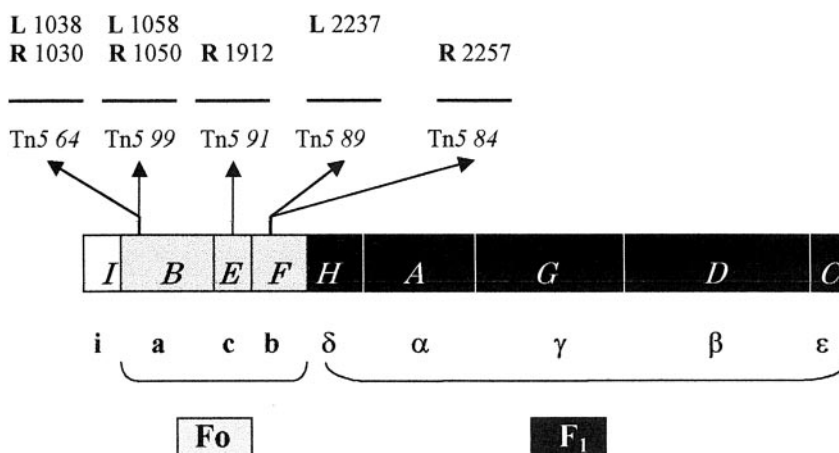


FIG. 1. Tn5 insertions in the *atp* operon conferring resistance to MccH47. The *atp* operon is represented as follows: white box, *atpI*; grey boxes, *atp* genes encoding F₀ polypeptides; black boxes, *atp* genes encoding F₁ polypeptides. The corresponding Atp products are designated below. The sites of Tn5 insertions are shown by arrows, and the left (L) and right (R) nucleotides immediately adjacent to the transposon sequences are indicated, numbered according to the sequence of Walker et al. (23).

precisely those that encode the three subunits that constitute the F₀ proton channel. To discern if the presence of the entire ATP synthase complex was required for antibiotic action or if the proton channel by itself would suffice, the following experimental series was performed.

Identification of the minimal *atp* genetic information capable of conferring sensitivity to MccH47. In order to obtain strains carrying different genetic information for ATP synthase, plasmid pBJC917 was used as a start point to construct a series of derivative plasmids with different DNA segments of the *atp* operon (Table 1 and Fig. 2). These plasmids were used in transformation experiments, and in all cases, a completely deficient *atp* chromosomal mutant, MC1000 $\Delta(\textit{uncI-uncC})$, was used as the receptor strain. In the resulting transformant clones, two phenotypes were systematically analyzed: (i) the Atp phenotype, related to the capacity to carry out oxidative phosphorylation, as evidenced by growth on succinate as the sole carbon source, and (ii) the MccH47 sensitivity phenotype, detected by the appearance of growth inhibition halos in patch tests performed with a MccH47-producing strain, as described in Materials and Methods (Fig. 2). It should be mentioned that, as expected, MC1000 $\Delta(\textit{uncI-uncC})$ exhibited Atp⁻ and MccH47^r phenotypes.

With respect to the Atp phenotype of the transformant clones, all of them were defective for oxidative phosphorylation with the exception of transformants with pBJC917. As to MccH47 sensitivity, whenever a recombinant plasmid carried the genes encoding the three subunits of the F₀ proton channel, the presence of the plasmid conferred antibiotic sensitivity. This was the case regardless of the remaining *atp* context, thus indicating that the minimal structure necessary for MccH47 action to take place could be the proton channel. Accordingly, total resistance to MccH47 was found in those constructions lacking one or more genes for components of this channel. Among the MccH47-sensitive clones, the degree of sensitivity differed depending on the recombinant plasmid. The strain carrying pBJC917 exhibited the highest level of sensitivity: inhibition halos about 12 mm in diameter were observed.

When the same assay was carried out with MC1000 $\Delta(\textit{uncI-uncC})$ with pER20 (encoding subunits a, c, b, and δ) or with pER13 (encoding subunits a, c, and b), the inhibition halos had a diameter of ca. 9 mm (see below).

Complementation assays. We knew from previous results that when MccH47^r *atp* mutants were transformed with plasmid pBJC917 the resulting strains were MccH47^s and Atp⁺; i.e., the *atp* operon complemented for both phenotypes (20). The recombinant plasmids mentioned above, encoding different portions of ATP synthase, were now used in complementation assays with the five chromosomal MccH47^r *atp* mutants under study. Prior to these assays, all five strains were made deficient for homologous recombination by means of P1 transduction, as described in Materials and Methods. Once these derivative strains were obtained, they were transformed with all of the recombinant plasmids carrying F₀ determinants shown in Fig. 2. The same two phenotypes, oxidative phosphorylation and MccH47 sensitivity, were assayed for in each transformant clone (Table 2).

As to the capacity to carry out oxidative phosphorylation, only the entire operon (pBJC917) restored the Atp⁺ phenotype in all five mutants. However, the complementation pattern differed upon transformation with plasmid pER900: those mutants defective for subunit a remained Atp⁻, whereas mutants defective for subunits c or b turned Atp⁺. In no other context was the Atp⁺ phenotype restored (Table 2).

In all five mutants, sensitivity to MccH47 was restored by introduction of plasmids carrying the wild-type *atp* gene corresponding to the chromosomal gene affected by Tn5 insertion. In the strains where complementation took place, different degrees of microcin sensitivity were observed: in general terms, the more *atp* information was present in *trans*, the greater the sensitivity to MccH47 was (Table 2 and Fig. 3). It is noteworthy that the single presence in *trans* of the wild-type *atp* gene affected in each of the five mutants gave rise to complementation for the MccH47 sensitivity phenotype.

Although the results so far obtained clearly demonstrated that subunits a, c, and b were all three absolutely required for

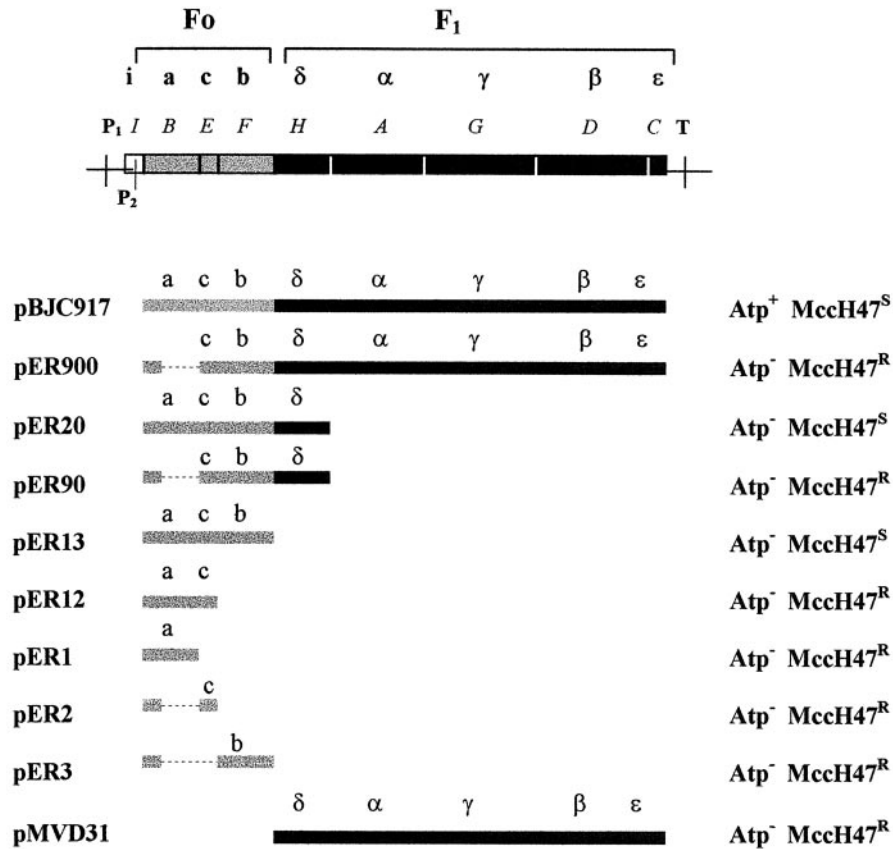


FIG. 2. Recombinant *atp* constructions and conferred phenotypes. The *atp* operon is represented at top: white box, *atpI*; grey boxes, *atp* genes encoding F_0 polypeptides; black boxes, *atp* genes encoding F_1 polypeptides. The corresponding Atp products are indicated. P₁, main promoter; P₂, secondary promoter; T, terminator. Below, pBJC917 and a series of derivative plasmids are depicted, following the same color code as described above; only *atp* DNA is represented, with the encoded polypeptides indicated in each case. Deletions are shown as dashed lines. pBJC917 and all the plasmids containing F_0 genes keep the P₂ promoter. A more detailed description of the construction of each plasmid is given in Table 1. To the right, the Atp and MccH47 sensitivity phenotypes conferred by plasmids to strain MC1000 $\Delta(uncI-uncC)$ are indicated.

MccH47 action, they did not prove that these subunits were indeed arranged into a proton channel in our constructions. To elucidate this point, the following complementation assay was performed: strain MC1000 $\Delta(uncI-uncC)$ carrying pMVD31

(coding for the five F_1 subunits) was transformed with plasmid pER13 (coding for the *a*, *c*, and *b* subunits). The resultant strain recovered the wild-type Atp⁺ and MccH47 sensitivity phenotypes, indicating that a functional ATP synthase complex

TABLE 2. Complementation analysis for oxidative phosphorylation and for MccH47 sensitivity^a between *atp*::Tn5 chromosomal mutations^c and recombinant plasmids carrying *atp* genes

Plasmid ^b	FGB64 and FGB099 (a)		FGB091 (c)		FGB084 and FGB089 (b)	
	Phenotype	Sensitivity	Phenotype	Sensitivity	Phenotype	Sensitivity
Vector alone	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^r
pBJC917 (acb δ α γ β ϵ)	Atp ⁺	MccH47 ^s ***	Atp ⁺	MccH47 ^s ***	Atp ⁺	MccH47 ^s ***
pER900 (cb δ α γ β ϵ)	Atp ⁻	MccH47 ^r	Atp ⁺	MccH47 ^s ***	Atp ⁺	MccH47 ^s ***
pER20 (acb δ)	Atp ⁻	MccH47 ^s **	Atp ⁻	MccH47 ^s **	Atp ⁻	MccH47 ^s **
pER90 (cb δ)	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^s **	Atp ⁻	MccH47 ^s **
pER13 (acb)	Atp ⁻	MccH47 ^s **	Atp ⁻	MccH47 ^s **	Atp ⁻	MccH47 ^s **
pER12 (ac)	Atp ⁻	MccH47 ^s *	Atp ⁻	MccH47 ^s *	Atp ⁻	MccH47 ^r
pER1 (a)	Atp ⁻	MccH47 ^s *	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^r
pER2 (c)	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^s *	Atp ⁻	MccH47 ^r
pER3 (b)	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^r	Atp ⁻	MccH47 ^s *

^a The level of sensitivity to MccH47 was assigned according to the diameter of the antibiosis halos detected by patch test (***, 12 to 15 mm; **, 6 to 8 mm; *, 3 to 4 mm).

^b Plasmid-encoded Atp polypeptides are shown.

^c The Atp subunit affected is indicated in parentheses after each mutant or group of mutants.

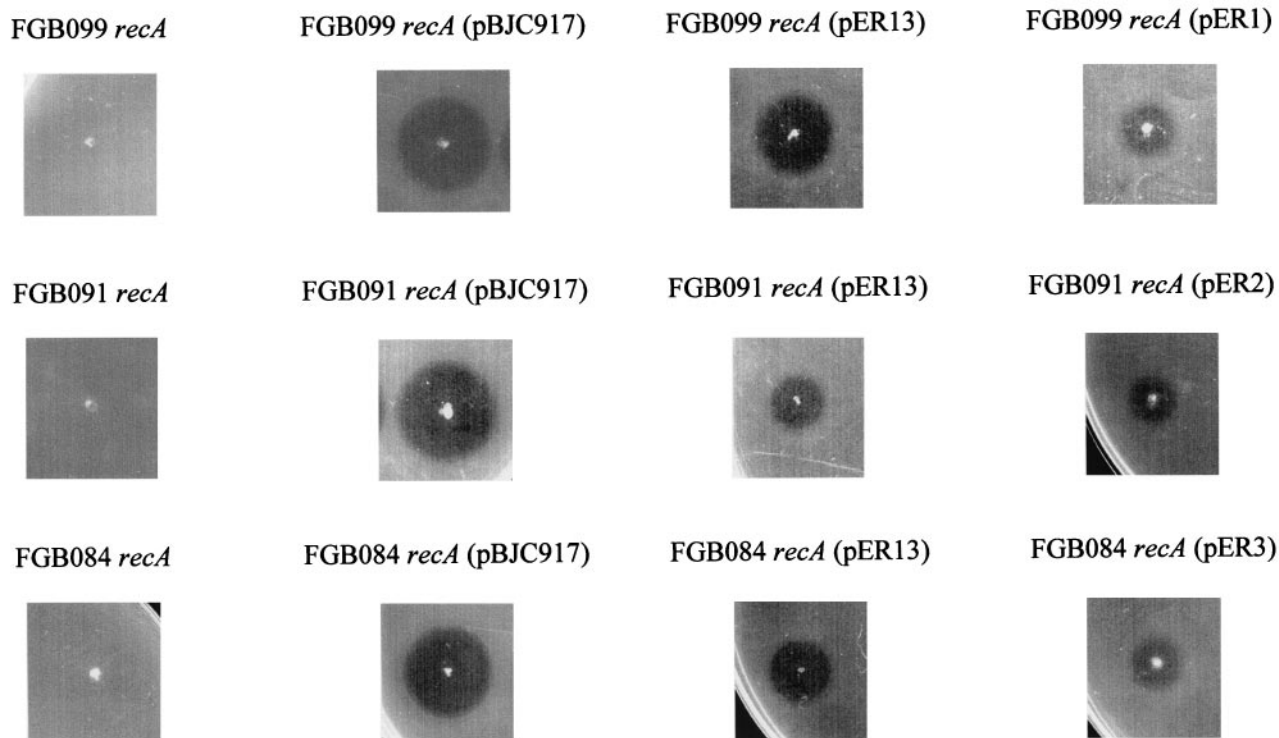


FIG. 3. Complementation for MccH47 sensitivity between *atp*::Tn5 chromosomal mutations and recombinant plasmids carrying *atp* genes. A patch test was performed on the strains indicated, as described in Materials and Methods.

had indeed been assembled. In a parallel assay, the same result was obtained when pER20 (coding for a, c, b, and δ subunits) was employed instead of pER13; as expected, no such complementation was observed with pER12 (coding for a and c subunits) (Table 3).

DISCUSSION

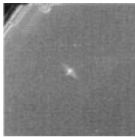
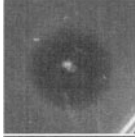
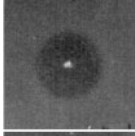
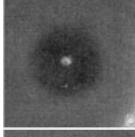
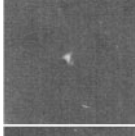
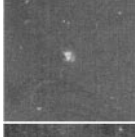
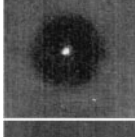


MccH47^r *atp* Tn5 insertion mutants were extensively analyzed, and their mutations were located at the nucleotide level. It was important to discern if their MccH47^r phenotype was due to the disruption of one or more *atp* genes. Complementation assays provided the certainty that only one *atp* gene had been affected in each mutant analyzed: in every case, when the single wild-type *atp* gene corresponding to the chromosomal gene affected by Tn5 insertion was introduced, mutants regained sensitivity to the antibiotic. These results showed that there was only one *atp* gene affected in each case and that its mutation was the sole responsible for the MccH47^r phenotype, ruling out the possibility that rearrangements or secondary mutations had occurred. This fact enabled us to analyze our set of results on the basis of a strict correlation between genotype and phenotype.

The Tn5 insertions of the *atp* mutants analyzed clustered in the genes encoding the three subunits that compose the F_o proton channel; conversely, no Tn5 insertion was found to affect determinants for the F₁ catalytic portion of ATP synthase. These results indicated that each of the components of the proton channel would be directly involved in the mode of action of MccH47, but they did not enable us to discard the

participation of the catalytic portion. The construction of strains with genetic information for different portions of ATP synthase as well as the results from complementation experiments clearly indicated that the presence of subunits a, c, and b was at the same time necessary and sufficient for conferring MccH47 sensitivity. Whenever the three components of the proton channel were present, the strain was sensitive to the antibiotic. This proved to be the case regardless of whether the F_o polypeptides were encoded by the chromosome, by a recombinant plasmid, or by a combination of both. These results discard the catalytic F₁ portion as being directly involved in MccH47 mode of action. On the other hand, the Atp⁺ phenotype was not always restored, even in the presence of all the information necessary for the ATP synthase complex. The importance of a *cis* arrangement of the *atp* genes to produce a correctly assembled ATP synthase has already been reported (5).

It was still necessary to discern if the subunits a, c, and b encoded by our constructions were correctly assembled into a proton channel. The complementation assays carried out in the MC1000 $\Delta(\textit{uncI-uncC})$ context provided information on this matter. When this strain was transformed with two plasmids, pMVD31 (encoding only F₁ polypeptides) and pER13 (encoding only F_o polypeptides), the resulting strain regained full sensitivity to MccH47 as well as the capacity to carry out oxidative phosphorylation. This would imply that each set of *atp* genes separately determined the adequate formation of F_o and F₁, and that both portions would meet to conform the entire ATP synthase complex. Other authors arrived at the

TABLE 3. Complementation assays between plasmids carrying F_o and F₁ determinants

Strain	Encoded Atp polypeptides	Atp phenotype	MccH47 phenotype ^a
MC1000 $\Delta(uncI-uncC)$		–	
MC1000 $\Delta(uncI-uncC)$ (pBJC917)	a, c, b, δ , α , γ , β , ϵ	+	
MC1000 $\Delta(uncI-uncC)$ (pER20)	a, c, b, δ	–	
MC1000 $\Delta(uncI-uncC)$ (pER13)	a, c, b	–	
MC1000 $\Delta(uncI-uncC)$ (pER12)	a, c	–	
MC1000 $\Delta(uncI-uncC)$ (pMVD31)	δ , α , γ , β , ϵ	–	
MC1000 $\Delta(uncI-uncC)$ (pMVD31, pER20)	δ , α , γ , β , ϵ , a, c, b, δ	+	
MC1000 $\Delta(uncI-uncC)$ (pMVD31, pER13)	δ , α , γ , β , ϵ , a, c, b	+	
MC1000 $\Delta(uncI-uncC)$ (pMVD31, pER12)	δ , α , γ , β , ϵ , a, c	–	

^a MccH47 phenotype refers to sensitivity of the strains to the antibiotic, detected by patch test as described in Materials and Methods.

same conclusion through *in vitro* reconstitution experiments in membranes, making use of similar F_o- and F₁-encoding constructions. Although the assembly of the ATP synthase complex might involve interactions between the proton channel and the catalytic portion, they found that each moiety would be able to assemble separately *in vivo* (1, 10). Thus, it could be deduced that cells only bearing the F_o determinants succeed to assemble the proton channel and, therefore, that F_o itself would be the minimal structure necessary for MccH47 action.

We had previously considered that ATP synthase could be the target of MccH47 antibiotic action (17, 20); now we focus this presumption on the F_o proton channel. In light of this

hypothesis, and knowing that MccH47 exerts a bactericidal effect on sensitive *E. coli* K-12 cells carrying the entire ATP synthase or only the proton channel (12; our unpublished results), it could be reasoned that the interaction of MccH47 with the latter structure would be responsible for cell death. If this were the case, antibiotic action would be related to F_o function, i.e., proton translocation. The passage of protons through F_o can conceivably be altered in three ways: blockage of translocation, proton pumping against the gradient, and proton entry downstream of the gradient. The first possibility cannot account for cell death, since cells would survive by means of substrate-level phosphorylation under most of the culture con-

ditions employed. The second possibility has an energetic cost; with the entire ATP synthase complex, the energy required to extrude protons could be provided by the coupled hydrolysis of ATP by F_1 , but in cells with only F_o , no such energy would be supplied. Therefore, only the third possibility remains clearly feasible, since it is the only one that can account for the bactericidal action of MccH47 in both contexts, with the entire ATP synthase and with only F_o : the membrane potential would be dissipated by the action of MccH47, which would provoke an unregulated entry of protons. It should also be taken into account that the presence of the entire ATP synthase confers higher levels of sensitivity to MccH47 than does the presence of the single proton channel, indicating that the F_1 portion, although not being strictly required for antibiotic action, enhances microcin's effect in a way that remains to be elucidated.

In summary, results presented in this work support our previous findings on the need of F_oF_1 ATP synthase for MccH47 antibiotic action (20). Here we demonstrate that the entire enzymatic complex is not required: the F_o proton channel is both necessary and sufficient for conferring sensitivity to MccH47. So far, all the *in vivo* analyses performed on MccH47 mode of action indicate that the F_o portion of ATP synthase would be the target of this antibiotic. MccH47 thus appears to be a natural product with an unprecedented mode of action on prokaryotes. This hypothesis will need to be tested in *in vitro* studies.

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