Energy Coupling Factor as Target of Colicin K: Characterization of a Colicin K-Insensitive *ecf* Mutant of *Escherichia coli*¹

JEN-SHIANG HONG,* D. LYNNE HAGGERTY, AND MICHAEL A. LIEBERMAN

Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

Received for publication 23 February 1977

We isolated a colicin K-insensitive energy uncoupled mutant of *Escherichia coli*. This mutant was presumed to be an *ecf* mutant as evidenced by its similarity to a known *ecf* mutant (M. A. Lieberman and J.-S. Hong, 1974) with respect to the mutational site, reversion pattern, and defects in transport and growth. The mutation conferring the colicin K-insensitivity resided in the *ecf* gene as the majority of the secondary mutations overcoming the *ecf* phenotype reverted the colicin K-insensitive phenotype to colicin K-sensitive. The insensitivity of the mutant to colicin K was not due to either a defect in adsorption or to a lack of the energized membrane state. The defect was most probably due to the inability of colicin K molecules to interact with their target. Our previous studies concerning the role of the *ecf* gene product in energy coupling to active transport and oxidative phosphorylation support the contention that the ECF protein is itself the direct target of colicin K.

The primary action of colicins K and E1 appears to be a de-energization of the cytoplasmic membrane such that active transport and oxidative phosphorylation are abolished (3, 11). This de-energization also contributes to numerous cellular alterations, including the lowering of intracellular adenosine 5'-triphosphate (ATP) levels (2) and the cessation of deoxyribonucleic acid, ribonucleic acid, and protein synthesis (5, 14). Plate et al. (17) showed that the cessation of macromolecular synthesis is largely attributable to the lowering of intracellular ATP levels. They also suggested that the ATP levels decrease because ATP is hydrolyzed by the adenosine 5'-triphosphatase in an effort to re-energize the membrane.

Other cellular alterations brought about by colicin K or E1 include the excretion of glycolytic metabolites (2), the efflux of accumulated potassium ion (20), and an alteration of membrane permeability to Mg^{2+} and Co^{2+} (12; for review see Luria [11]). The biochemical basis for these alterations is not known. Based largely on the observed inhibition of active transport and oxidative phosphorylation, it has been suggested that the primary target of these colicins may be the membrane-bound energy transducing machinery (11).

During the investigation of a novel energy-uncoupled mutant [*metC** *ecf*(Ts)] (8) we found

that although the mutant was isolated as being defective in active transport, it also exhibited many of the characteristics of cells that have been treated with colicins E1 or K (10). Therefore, we suggested that the ECF protein may be the biochemical target of these colicins (10). In this paper we report the isolation of an *ecf* mutant that is insensitive to the action of colicin K. Genetic evidence is presented showing that the mutation conferring the colicin K-insensitive phenotype resides in the *ecf* gene. We also demonstrate that the insensitivity of the mutant to colicin K is due to a drastically slowed initiation process (the interaction of colicin K with the target molecule; also known as stage 1 [11] of colicin action).

MATERIALS AND METHODS

Bacterial strains and media. Strains used were JSH202 (*F*⁻ *thi* *strA*) and MAL303 (*F*⁻ *thi* *strA* *metC* *ilv*). Both are derivatives of *Escherichia coli* K-12. Media used were nutrient broth and minimal salts medium E (19) supplemented with 0.5% glucose and 40 μ M vitamin B1. When required, amino acids were added to 0.4 mM. Solid media contained 1.5% agar.

Viability assay. Cultures were aerobically grown in minimal glucose with shaking at 25°C and harvested during mid-log phase. Cells were washed twice with 70 mM potassium phosphate buffer (pH 7.0) containing 1 mM $MgCl_2$ and suspended in the same buffer; sterile conditions were maintained throughout. These cells were used for both transport assays and colicin experiments.

¹ Publication no. 1134 from the Department of Biochemistry, Brandeis University, Waltham, MA 02154.

Standard colicin experiments were carried out as follows. A 1-ml portion of washed cells at an optical density at 660 nm = 3.0 (equivalent to 1.3×10^9 to 1.6×10^9 cells per ml) in a 25-ml flask was preincubated for 3 min with shaking at either 25 or 43°C. At zero time a portion (100 μ l) of the cell suspension was withdrawn and added to a tube (13 by 100 mm) containing 20 μ l of colicin. Each tube was incubated for a desired length of time after which trypsin (10 μ l; 6.6 mg/ml) was added, and the incubation continued for an additional 5 min. Viable cells were determined by diluting in nutrient broth, plating on nutrient broth plates and incubating overnight at 36°C.

Genetic methods. Transductions were performed as described (8).

Colicins. Colicin E2 was a generous gift from M. Nomura, and colicin E3 was from L. Hager. Preparation of colicins E1 and K have been described (3). The number of killing units in the colicin preparation was calculated from the equation $S/S_0 = e^{-m}$, where m is the multiplicity of killing units, and S/S_0 is the survival ratio.

Isolation of colicin K-resistant energy uncoupled mutants. JSH202 cells were mutagenized with diethylsulfate, grown in minimal glucose medium at 25°C, harvested at mid-log phase, washed twice with 70 mM potassium phosphate (pH 7.0), and resuspended at a final density of 2.1×10^9 cells per ml in 70 mM potassium phosphate (pH 7.0) containing 1 mM $MgCl_2$ and 0.5% Casamino Acids. Cells (1 ml) in a test tube (100 by 13 mm) were incubated at 42°C for 30 min after which glucose was added to a final concentration of 0.4 mM. After 5 min, colicin K ($m = 10$) was added. Ten minutes later trypsin (Sigma) was added to a final concentration of 0.8 mg/ml, and the incubation continued for 5 min. Minimal glucose medium (100 ml) was then inoculated with the colicin K-treated cells (0.2 ml), and the cycle was repeated. After each colicin treatment, survival was determined by plating on nutrient broth plates at 25°C. Nutrient broth plates with 0.2% deoxycholate were used to determine the number of tolerant mutants present (15).

After the second cycle, the cells were diluted, plated on minimal succinate plates, and incubated at 25°C. When the colonies were still small (24 to 48 h), the plates were shifted to 42°C. After 12 to 24 h, small colonies were isolated, purified, and tested for transport ability at 25 or 43°C.

Isolation of Suc^+ revertants. These were isolated as described for JSH4 (8).

RESULTS

Isolation of the mutant MAL321. Since colicins K and E1 appear to require the energized membrane state (6; Hong and Haggerty, manuscript submitted for publication) to initiate their lethal activity (initiation process, stage 1), mutants unable to form the energized membrane state should be resistant to colicins K and E1 and therefore could be selected for as uncoupled mutants.

The method we devised is based on an observation made with a previously isolated temperature-sensitive energy uncoupled mutant, MAL300 (10). Basically, when the susceptibility of MAL300 to colicin K was checked after glucose (0.4 mM) addition at the nonpermissive temperature, a loss of trypsin-rescuable viability was observed if colicin K (5 min incubation) was added 2 min after the glucose addition; however, no decrease in cell viability was observed if colicin K was added 5 min after the glucose addition. This result has been related to the finding that MAL300, at 42°C, is capable of establishing and maintaining a membrane potential 2 min after glucose addition, but this potential has been dissipated within 5 min after glucose addition (Lieberman, Simon, and Hong, J. Biol. Chem., in press). The strain is thus colicin-insensitive due to a lack of membrane energy, which is necessary for stage 1 of the colicin attack process (6, 11). Cells were therefore exposed to colicin K and treated with trypsin under the conditions described above. To determine if the procedure is applicable to our cells, a mixture of JSH210 (wild-type) and JSH205 (an energy-uncoupled mutant) (9) at a ratio of 10⁶:1 was subjected to two cycles of the selection procedure. The resulting ratio was 67.5:1, representing an enrichment of 1.5×10^4 . The selection was then performed on mutagenized JSH202. One mutant that displayed a temperature-sensitive phenotype for transport ability was found. P1 phage were grown on the mutant and MAL303 was transduced to $MetC^+$. Three of the eight resulting transductants exhibited an energy-uncoupled phenotype, and one, MAL321, was used for the studies described here.

The mutant is an *ecf* mutant. The mutant MAL321 is capable of utilizing succinate, fumarate, malate or D-lactate as a carbon source at 25 but not at 43°C.

We found by means of P1 phage transduction using JSH210 (*metC*) as a recipient that the mutation conferring the Suc^- and transport phenotype is approximately 85% (40/47) linked with *metC*. This linkage is similar to that reported with MAL300 (Lieberman et al., J. Biol. Chem., in press).

The data in Fig. 1 demonstrate that in the absence of an exogenous energy source, the mutant was unable to transport proline and serine at 43°C (compare with wild-type MAL303). The cells could only accumulate the substrate sixfold, whereas the wild-type could do so 300-fold. It was also clear that low levels of glucose (0.4 mM) stimulated the transport of these amino acids, although at 43°C this stimulation was transitory. These results are very

FIG. 1. Uptake of proline and serine by MAL303 (wild-type) and MAL321 (mutant). Cells were grown in minimal glucose medium at 25°C and transport assayed as described previously (8). Radioactive proline used was L-[U-¹⁴C]proline (134.5 Ci/mol) at 7.7 μ M. Radioactive serine used was L-[U-¹⁴C]serine (159 Ci/mol) at 12.9 μ M. Both radioactive compounds were obtained from New England Nuclear Corp. When glucose was used, it was added to the cells immediately after substrate (radioactive amino acid) addition to a final concentration of 0.4 mM. Transport was then assayed as a function of time after the glucose addition. Symbols: ▲, MAL303 at 25°C; △, MAL303 at 43°C; ●, MAL321 at 25°C; ○, MAL321 at 43°C.

similar to the transport results obtained with the *ecf*(Ts) *metC** mutants JSH4 (8) and MAL300 (Lieberman et al., J. Biol. Chem., in press), except that MAL321 exhibited a more severe defect in transport than did the other mutants.

Revertants of MAL321 were selected for their ability to grow on succinate at 43°C. They were found to be of two classes: class I, which is prototrophic, and class II, which is auxotrophic for methionine. The methionine requirement in class II revertants was due to the absence of cystathionase (*metC* gene product) activity (8). This reversion pattern is identical to that observed previously with the *ecf* mutant JSH4. Both classes of revertants grow on succinate

and transport normally at 43°C (data not shown).

Since this mutant, MAL321, is similar to MAL300 (or JSH4) with respect to mutational site, reversion pattern, and defects in transport and growth, we tentatively concluded that MAL321 is also an *ecf* mutant. This would give MAL321 the genotype F⁻: *thi ilv strA metC** *ecf*(Ts). The *metC** notation denotes that the *metC* gene in MAL321, while retaining wild-type levels of cystathionase activity, is different from the wild-type *metC* gene (8; Lieberman et al., J. Biol. Chem., in press).

Insensitivity to colicin K and its relation to *ecf*. We found that, when incubated with colicin K in the presence of 0.4 mM glucose, MAL321 cells were essentially fully rescuable by trypsin against the lethal action of colicin K for at least 10 min (Fig. 2).

To determine if the mutation conferring the colicin K insensitivity in MAL321 is associated with the *ecf* mutation, we isolated 19 independent class I and 7 independent class II revertants. It is important to realize that the reversion events leading to class I and class II revertants occur within different genes: those leading to class II revertants apparently undergo an *metC** to *metC*⁻ transition (8; Lieberman et al., J. Biol. Chem., in press), whereas those leading to class I revertants can occur in either the *ecf*(Ts) gene [*ecf*(Ts) to *ecf*⁺] or in the *metC** gene.

FIG. 2. Insensitivity of MAL321 to colicin K. Trypsin-rescuable cell viability was determined as described in the text. Glucose was added to 0.4 mM 1 min prior to the addition of colicin K ($m = 9.8$).

gene (*metC** to *metC*⁺). Both classes of revertants occurred at approximately the same frequency (one in 10⁵ to one in 10⁶), and this frequency suggests that each reversion event was in one gene. Work with both JSH4 (8) and MAL300 (Lieberman et al., J. Biol. Chem., in press) has demonstrated that both the *metC** and *ecf*(Ts) mutations are required for the energy-uncoupled phenotype to be expressed. Thus, if a reversion event occurs in either gene the energy-uncoupled phenotype is lost.

The 26 revertants of MAL321 were examined for their response to colicin K using the same conditions as were used for MAL321 (Fig. 2) except that the incubation with colicin K was for 2 min at 43°C. We found that 17 (17/19) of the class I revertants were sensitive to colicin K; at least 60% of the cells were killed for each revertant under these conditions. The most sensitive of these revertants, 321-I-7 and 321-I-14, were as sensitive to colicin K as was the wild-type MAL303 (Fig. 3). Two class I revertants (321-I-1 and 321-I-13), however, remained insensitive to colicin K (Fig. 3; data for 321-I-13 not shown). All of the class II revertants (7/7) remained as insensitive to colicin K as MAL321 (data not shown).

These results are interpreted as follows: if the reversion event did not occur within the *ecf*(Ts) gene (as in class II revertants) all revertants retained colicin K insensitivity. This strongly suggests that the *metC** gene is not involved in conferring colicin K insensitivity. The data with class I revertants indicate that a single reversion event in the *ecf*(Ts) gene can simultaneously restore the energy-coupling processes as well as the sensitivity to colicin K. The

varying sensitivity to colicin K among these revertants indicates that the reversion event can occur at a second site in the *ecf*(Ts) gene rendering a protein capable of wild-type energy coupling activity, while it still confers a slight insensitivity to colicin K. The two class I revertants that remain fully resistant to colicin K (321-I-1 and 321-I-13) could be either *metC** to *metC*⁺ reversions [which could leave the *ecf*(Ts) gene product unaltered] or could be a second site reversion in *ecf*(Ts) which confers energy coupling activity but retains complete insensitivity to colicin K.

The most consistent explanation for the reversion studies is that the mutation rendering the colicin K-insensitive phenotype is located in the *ecf*(Ts) gene.

Colicin K-insensitive mutation maps at 64 min. By means of P1 transduction using MAL303 as a recipient, we found that the colicin K-insensitive mutation in strain 321-I-1 was greater than 90% linked with *metC*, placing the mutation at 64 min on the revised *E. coli* genetic map (1). This result indicates that the colicin K-insensitive marker has the same linkage with *metC* as does the *ecf*(Ts) marker, and this specific location rules out the possibility that the mutation maps in the gene coding for the colicin K receptor, which is known to map at 12 min (1).

Normal adsorption. To rule out the possibility that the rescuability of cell viability by trypsin seen with the colicin K-insensitive mutant is not due to an adsorption defect, viability tests were carried out in the absence of trypsin. We utilized revertants for this study (321-I-1 and 321-I-7) such that we could be sure that an energized membrane would be established by the cells, since the initiation process appeared to be dependent on the generation of such a state. The extent of killing observed with colicin K-insensitive strains was the same as with the sensitive strains (Fig. 4). This suggests that adsorption is normal. However, the killing could result from readsorption after diluting and plating rather than from adsorption before dilution. To rule this out, an experiment was carried out under the same conditions used in Fig. 4 except that cells and colicin were diluted separately (to 2×10^{-4}), mixed, incubated for 10 min, and then plated. A dilution to 10^{-4} was done in the experiment shown in Fig. 4. Since no killing was observed, we conclude that the killing seen in the absence of trypsin was not due to readsorption. This makes unlikely the possibility that a defect in adsorption is responsible for the trypsin reusability seen with the colicin K-insensitive mutants.

FIG. 3. Response of class I revertants to colicin K. Trypsin-rescuable cell viability was determined under the same conditions as described in Fig. 2.

Slowed initiation. The results in Fig. 5 demonstrate that the rate of killing was much slower in the colicin K-insensitive strains. A 1-min incubation of colicin K with sensitive cells was sufficient to kill more than 90% of these cells (Fig. 3). To obtain the same amount of killing with insensitive cells, approximately 70 min of incubation at 43°C or 100 min of incubation at 26°C was required.

Rescuability against colicin K is specific. Under conditions in which they are insensitive to colicin K, 321, 321-I-1, 321-I-7, and two class II revertants were all sensitive to colicins E1, E2, and E3 (Table 1). The colicin K-insensitive mutation is therefore distinct from *tolA* and *tolB*, which map at about 16 min on the *E. coli* genetic map. Both *tolA* and *tolB* mutants are pleiotropically resistant to colicins E2, E3, and K (13, 15). The fact that the colicin K-insensitive strains are sensitive to colicin E1 indicates that the protection observed against colicin K is not due to a lack of energy supplies since colicin

FIG. 4. Killing by colicin K in the absence of trypsin. The conditions used were exactly the same as those described in the text except that the incubation time with colicin K was 1 min. Glucose was added to 0.4 mM 1 min prior to the addition of colicin K. Temperature was 26°C. Symbols: ○, MAL321-I-1; ●, MAL321-I-7.

FIG. 5. Slowed initiation process in colicin K-insensitive strains. Trypsin-rescuable cell viability was determined under the same conditions as those used in Fig. 2 with the exception that glucose was added to 10 mM.

TABLE 1. Sensitivity to colicins E1, E2, and E3^a

Strain	Trypsin-rescuable survival (%)				
	Control	E1	E2	E3	K
MAL303	100	6.0	2.0	4.7	4.0
MAL321	100	2.4	1.0	2.2	99
MAL321-I-1	100	2.4	6.7	1.6	90
MAL321-I-7	100	8.6	7.4	7.0	4.5
MAL321-II-1	100	8.5	5.1	5.0	100
MAL321-II-2	100	6.0	3.8	3.1	100

^a The assays were carried out at 43°C under the conditions described in the text. Glucose (0.4 mM) was added to the cells 1 min before the addition of colicin. Incubation time with colicin was 5 min. The colicins were added at the following killing concentrations: E1, $m = 7.4$; E2, $m = 9.2$; E3, $m = 7.4$; K, $m = 8.1$. MAL321-I-1 and 321-I-7 are class I revertants; MAL321-II-1 and 321-II-2 are class II revertants.

E1 also requires an energized membrane state for initiation (6).

DISCUSSION

The results presented in this paper indicate that the colicin K-tolerant mutation probably resides in the *ecf* gene. This conclusion is based on the finding that the majority of the class I revertants [selected to overcome the *ecf*(Ts) phenotype] are capable of reverting the colicin K-insensitive phenotype to colicin K-sensitive. The fact that these revertants occur frequently (10^{-6} to 10^{-5}) indicates that the colicin K-insen-

sitive phenotype is due to a lesion in a single gene.

It is not surprising to find that the class II (*metC*) revertants remained insensitive to colicin K. We previously showed that the mutations giving rise to class II reversion are located in the *metC* gene and concluded that these mutations are intergenic suppressors of the *ecf*(Ts) gene (8). Recent experiments using antiserum prepared against cystathionase (*metC* gene product) indicated that these mutations are structural mutations in the *metC* gene since cross-reacting materials were detected in these *metC* revertants (S. Kagan and J-S. Hong, unpublished data). The mechanism of the *metC* suppression of the *ecf*(Ts) phenotype is not yet understood. The finding that all of the class II revertants examined were insensitive to colicin K suggests that reversion to *metC* cannot suppress the colicin K-insensitive phenotype, whereas it can suppress the *ecf*(Ts) phenotype.

It is apparent that the process leading to cell death is drastically slowed in the colicin K-resistant cells as a result of the colicin K-insensitive mutation. This enables us to observe a full rescuability of cell viability by trypsin during the early stage in the initiation process. It is possible that the colicin K resistance observed may be due to a defect in the adsorption process or a lack of an energized membrane state, which has been shown to be required for the initiation process (6; Hong and Haggerty, manuscript submitted for publication). The first possibility is unlikely because: (i) the colicin K-tolerant mutation does not reside in the colicin K receptor gene; and (ii) in the absence of trypsin no difference is observed in the extent of killing between sensitive and tolerant strains. The second possibility is ruled out since all of the class II revertants (as well as some of those of class I) isolated from MAL321 remain insensitive to colicin K. These *Suc*⁺ revertants transport amino acids and grow on succinate, fumarate, or D-lactate, as do the wild type, at 42°C, which is an indication of the presence of the energized membrane state.

The colicin K insensitivity observed with our mutants is most probably due to a defect in some other aspect of the initiation process, perhaps an inability of colicin K to interact with its target protein. The demonstration that the *ecf* gene product is essential for colicin K-induced cellular damage suggests that the *ecf* gene product is either the target of colicin K or is required for colicin K to reach the target. The following findings strongly support the contention that the *ecf* gene product is itself the direct target of colicin K: (i) the *ecf* gene product is

essential for energy coupling in active transport (8) and oxidative phosphorylation (Lieberman et al., J. Biol. Chem., in press), two functions which are altered by colicin K, and (ii) *ecf* mutants exhibit many cellular alterations characteristic of cells that have been treated with either colicin E1 or K (10). It is apparent that the colicin K-insensitive mutation we have studied affects the susceptibility of the ECF to colicin K molecules but not to colicin E1 molecules (Table 1). These two colicins (7, 18) differ considerably in amino acid composition and in isoelectric point and conceivably could have different target proteins or different sites on the same target protein. Therefore, we suggest that the observed insensitivity of the colicin K-insensitive mutants towards colicin K is due to a reduced affinity of the colicin K target protein for colicin K itself.

While this work was in progress, Plate (16) reported the isolation of an energy-uncoupled mutant of *E. coli* also unable to respond to colicin K. It was suggested that the gene product may be the target of colicin K. No mapping of the mutation was reported. If this gene is different from the *ecf* gene, it will raise the question of what is the functional and structural relationship between these two targets.

ACKNOWLEDGMENTS

This work was supported by grant GB40237 from the National Science Foundation and by Public Health Service grant GM22576, training grant GM212, and research career development award KO4-GM-0092, all from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Bachmann, B. J., K. B. Low, and A. L. Taylor. 1976. Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriol. Rev.* 40:116-167.
2. Fields, K. L. and S. E. Luria. 1969. Effects of colicins E1 and K on cellular metabolism. *J. Bacteriol.* 97:64-77.
3. Fields, K. L. and S. E. Luria. 1969. Effects of colicins E1 and K on transport systems. *J. Bacteriol.* 97:57-63.
4. Fredericq, P. 1965. *Zentralbl. Bakteriell. Parasitenkunde. Hyg. Abt. 1: Orig.* 196:142.
5. Jacob, F., L. Siminovitch, and E. L. Wollman. 1952. Sur la biosynthèse d'une colicine et sur son mode d'action. *Ann. Inst. Pasteur Paris* 83:295-315.
6. Jetten, A. M., and M. E. R. Jetten. 1975. Energy requirement for the initiation of colicin action in *Escherichia coli*. *Biochim. Biophys. Acta* 387:12-22.
7. Kunugita, K., and Matsushashi, M. 1971. Purification and properties of colicin K. *J. Bacteriol.* 104:1017-1019.
8. Lieberman, M. A., and J-S. Hong. 1974. A mutant of *Escherichia coli* defective in the coupling of metabolic energy to active transport. *Proc. Natl. Acad. Sci. U.S.A.* 71:4395-4399.
9. Lieberman, M. A., and J-S. Hong. 1976. Energization of osmotic shock-sensitive transport systems in *Esche-*

- richia coli* requires more than ATP. Arch. Biochem. Biophys. 172:312-315.
10. Lieberman, M. A., and J-S. Hong. 1976. Changes in active transport, intracellular adenosine-triphosphate levels, macromolecular synthesis, and glycolysis in an energy-uncoupled mutant of *Escherichia coli*. J. Bacteriol. 125:1024-1031.
 11. Lurai, S. E. 1973. Colicins, p. 293-320. In L. Leive (ed.), Bacterial membranes and walls. Marcel Dekker, Inc., New York.
 12. Lusk, J. E., and D. L. Nelson. 1972. Effects of colicins E1 and K on permeability to magnesium and cobaltous ions. J. Bacteriol. 112:148-160.
 13. Nagel de Zwaig, R., and S. E. Luria. 1967. Genetics and physiology of colicin-tolerant mutants of *Escherichia coli*. J. Bacteriol. 94:1112-1123.
 14. Nomura, M. 1963. Mode of action of colicins. Cold Spring Harbor Symp. Quant. Biol. 28:315-324.
 15. Nomura, M., and C. Witten. 1967. Interaction of colicins with bacterial cells. III. Colicin-tolerant mutations in *Escherichia coli*. J. Bacteriol. 94:1093-1111.
 16. Plate, C. A. 1976. Mutant of *Escherichia coli* defective in response to colicin K and in active transport. J. Bacteriol. 125:467-474.
 17. Plate, C. A., J. L. Suit, A. M. Jetten, and S. E. Luria. 1974. Effects of colicin K on a mutant of *Escherichia coli* deficient in Ca, Mg-activated adenosine triphosphatase. J. Biol. Chem. 249:6138-6143.
 18. Schwartz, S. A., and D. R. Helinski. 1971. Purification and characterization of colicin E. J. Biol. Chem. 246:6318-6327.
 19. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. 218:97-106.
 20. Wendt, L. 1970. Mechanism of colicin action: early events. J. Bacteriol. 104:1236-1241.