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COLICINS AND OTHER BACTERIOCINS WITH ESTABLISHED MODES OF ACTION

Jordan Konisky

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

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INTRODUCTION

The literature abounds with examples of antagonism between particular bacterial strains. In many instances the mediating agent is a bacteriocin. In the most general sense, these substances are defined by two criteria—they are proteins or complexes of proteins, and they are not active against the

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producer bacterium. Such a definition is based entirely on function and avoids more restrictive considerations, such as whether (*a*) the toxin is encoded by plasmid or chromosomal genes, (*b*) the host range is narrow or broad, or (*c*) the bacteriocin is a simple protein (such as a colicin) or a complex structure composed of several distinct subunit species (such as a phage tail-like pyocin).

The bacteriocins have been the subject of several reviews (53, 92, 128, 141). These have considered many aspects of these antibiotic-like toxins, including structure, mode of action, genetics, evolution, and ecology. In contrast to these comprehensive treatments, this review focuses on a discussion of the mode of action of those few bacteriocins whose primary target has been identified. There is no treatment of the less well understood bacteriocins.

COLICIN E2 AND E3 AND CLOACIN DF13

Mode of Action

The toxins colicin E2 and E3 and cloacin DF13 are the most thoroughly understood bacteriocins (for reviews see 27, 66, 73). Their modes of action and immunity systems have been determined to the molecular level, and a great deal of information is available concerning their functional architecture. Because of their many similarities, they are discussed together. These proteins are enzymes—E3 and DF13 have RNase activity and E2 has endonuclease activity on DNA. They are toxins because their amino acid sequences endow them with some structural feature that allows them to traverse the bacterial envelope.

Early experiments demonstrating that treatment of *Escherichia coli* cells with colicin E3 led to specific inhibition of protein synthesis suggested that the cellular target was a component of the machinery of protein synthesis (108). This was verified when it was shown that 70*S* ribosomes isolated from E3-treated cells are defective in their capacity to support in vitro protein synthesis (95). A similar situation was found in the case of cloacin DF13-treated *Enterobacter cloacae,* with the exception that the cloacin caused, in addition, the leakage of cellular potassium (30). The basis for such K^+ efflux has not been subsequently investigated. In both cases ribosome damage is localized to the 16*S* RNA of the 30*S* ribosomal subunit, which sustains a single nucleolytic cleavage at the same position near the 3'-terminus of the molecule, thereby leading to the generation of a fragment of 49 nucleotides (10, 29, 137). Since an identical cleavage occurs when isolated ribosomes are treated with highly purified E3 or DF13 (8, 11, 29), the in vivo action of these bacteriocins most certainly involves penetration of all or part of these molecules through the bacterial surface layers, thereby allowing direct interaction of toxin with ribosomes.

Although early in vitro studies allowed the possibility that these bacteriocins acted indirectly (for example, by activating a latent RNase of the ribosome), there is now compelling evidence that colicin E3 (and, thus, presumably cloacin DF13) has endogenous RNase activity (113).

Treatment of *E. coli* with colicin E2 leads to the specific inhibition of DNA synthesis and induces DNA degradation (108). It seems likely that the additional effect of colicin E2 on active transport of cells lysogenic for phage lambda (6) is secondary to the primary action of this colicin. In vitro, highly purified E2 exhibits DNase, but not RNase, activity (133, 135). There is little doubt that the ability of this colicin to act as a DNA endonuclease is the basis for its action against cells. Here again, the colicin must somehow penetrate the cell envelope, at least to the extent that it is able to interact with the bacterial chromosome.

General Structure

Each of these bacteriocins is released from producing cells as a 1 : 1 complex of two polypeptides with a composite molecular weight of 60,000 for colicin E2 and E3, and 67,000 for the cloacin (28, 74, 75, 135). Upon dissociation, E2 and E3 yield polypeptides of 50,000 and 10,000 daltons, whereas DF13 is composed of proteins of 58,000 and 9,000 daltons. In each case the larger polypeptide, termed E2*, E3*, or DF13*, is the active moiety of the complex (28, 74, 135). Since E2*, E3*, and DF13* are also active against whole cells and display the same host specificity as the complex, each of these subunits must also contain the structural information required for receptor selection (see below) and penetration through the outer and inner bacterial membranes.

Each of the smaller subunits has no bacteriocin-like activity but instead inhibits the nuclease activity of the larger polypeptide (28, 78, 135). The native complexes are only weakly active in vitro and addition of purified small subunit to E2*, E3*, and DF13* completely prevents their action on isolated ribosomes. Such inhibition is absolutely specific. For example, the smaller polypeptide derived from native colicin E2 neutralizes the in vitro action of E2* but not E3*, and vice versa (75, 135). The same specific interaction with only the homologous catalytic subunit is displayed by the smaller subunits of the E3 or DF13 complex (120). Clearly, any model for explaining the in vivo mode of action sequence must provide for removal of the inhibitor subunit to activate the bacteriocin.

Immunity System

The highly specific interaction between homologous catalytic and inhibitor subunits is the basis for the immunity system of these bacteriocins. The structural genes for the inhibitory subunits have been shown to reside on

the bacteriocin-determining plasmids (90, 139), and expression is such that even under uninduced conditions, in which only a small fraction of plasmid-carrying cells are producing bacteriocin, all the cells in the population contain some free immunity protein.

In considering a general model for immunity to these three bacteriocins, workers in the field have settled on the following scheme (27, 56, 73, 93). Upon synthesis, E2*, E3*, and DF13* interact with their respective immunity proteins to form inactive complexes. Thus, the producing organism is protected from the action of endogenous bacteriocin and maintains synthetic capacity for continuous toxin synthesis until eventual release of the complex into the medium. The mechanism whereby immunity protein is removed from the complex to generate active nuclease is unknown. Although there have been several suggestions that such activation might be mediated by interaction of complex with the cellular outer membrane receptor, in vitro attempts to verify this mechanism have not been successful (121). Nevertheless, it seems likely that removal of the immunity protein occurs during penetration through the cell surface layer and that only active molecules enter the cell interior. Immunity from exogenous bacteriocin would result from neutralization of such activated bacteriocin by free intracellular immunity protein produced under the direction of the relevant immunity-determining plasmid.

Functional and Structural Domains

The analysis of fragments derived from protease digestion of these bacteriocins has proven to be a very useful approach in delineating their functional and structural domains. The general pattern obtained is supported by studies on mutant colicin E3 and DF13 (1, 44, 104). The nuclease activity of these proteins resides in the C-terminal portion of E2*, E3*, and DF13*, comprising about 25% of the total polypeptide (31, 105, 114, 116, 162). Although this region of each molecule is very basic and quite sensitive to trypsin digestion, its interaction with each corresponding acidic immunity protein not only neutralizes the activity of each polypeptide, but renders the region resistant to protease attack (31, 140, 162). This may serve to protect these bacteriocins against inactivation by proteases in the cell envelope (9, 15, 18, 151) and, thus, explains several reports that the bacteriocin complex is more active in vivo than the corresponding E3* and DF13* subunits (28, 56, 74).

The N-terminal region (about 25% of the polypeptide) of each E2*, E3*, and DF13* is thought to be involved in translocation across the cell membranes (17, 116). Its rather hydrophobic character may reflect this function (31, 67, 115). The structural domain that interacts with each cognate outer membrane receptor occupies the central portion of each catalytic subunit (27, 116).

Digestion of colicins E2* and E3* with trypsin leads to the generation of a N-terminal fragment that represents approximately 70% of the intact polypeptide and a C-terminal fragment that comprises the rest of the molecule. Analysis of these fragments shows that the similarity of amino acid composition and antigenic specificity of native colicin E2 and E3 (54) is entirely a result of the almost identical amino acid composition of the N-terminal fragment (115). These results are expected since these two colicins bind to the identical outer membrane receptor protein (43, 102) and probably have similar modes of entry into cells (82). In contrast, the C-terminal tryptic fragments of E2* and E3* are significantly different in composition (115) reflecting both the difference in the catalytic function of the two colicins and the fact that this is the region that binds homologous, but not heterologous, immunity protein. Although E2- and E3-immunity proteins are of similar size, are very acidic in character, and bind to common functional domains of their respective homologous E2* and E3* polypeptides, they display no obvious structural relatedness (115).

Studies on the protein chemistry of DF13* have not revealed sequence similarities to colicins E2* and E3*. This is not surprising since the cloacin absorbs to a different cell surface receptor. In spite of the fact that the immunity proteins of colicin E3 and cloacin interact only with their respective homologous E3* and DF13* subunit, these inhibitors do have extensive regions of primary sequence homology (149). It is possible that a direct comparison of amino acid sequence in the catalytic domain of these bacteriocins will reveal similarities.

COLICINS A, E1, Ia, Ib, AND K

Mode of Action

The proteins colicin A, E1, Ia, Ib, and K form ion-permeable channels in the bacterial cytoplasmic membrane. It seems likely that the many reported structural and metabolic changes seen in treated cells are a secondary result of the colicin-induced collapse of the membrane proton motive force. This discussion focuses on the primary action of these colicins. Readers desiring a more comprehensive treatment of the various biochemical changes observed in affected cells should consult recent reviews (58, 92).

Early mode of action studies implicated the cytoplasmic membrane as a primary target of colicins A, E1, K, Ia, and Ib. These bacteriocins inhibit protein and nucleic acid biosynthesis and uncouple electron transport from active transport of thiomethyl-β-D-galactoside and potassium (37, 38, 45, 106). Treated cells leak potassium (22, 34, 45, 55, 106, 109, 161), and in the case of colicin E1 and K, it has been shown that affected cells become more permeable to magnesium and cobalt (101). This loss of cellular potassium and magnesium has been implicated as the primary cause of cell death (97).

In common with several antimicrobial agents that act by dissipating the transmembrane potential, colicin-treated cells display enhanced transport of glucose via the phosphoenolpyruvate-phosphotransferase system (45, 76). Colicin E1 or K-treated cells[1] show reduced activity of membrane-associated ATP-linked reverse transhydrogenase (131). Although these colicins cause a lowering of intracellular ATP levels, this decrease is not responsible per se for the effects of these molecules on membrane function (34). The inhibition of macromolecular synthesis by these toxins probably derives from several factors, including (*a*) low levels of ATP, (*b*) inability to accumulate substrates by active transport, and (*c*) inability to maintain sufficient levels of cofactors, such as cations, etc.

Although the pattern of changes seen in inhibited cells led several groups to conclude that these colicins act by collapsing the energized membrane state, it was not until Gould et al (46, 47) observed that colicin E1 or K treatment leads to a more rapid rate of proton extrusion and a higher amplitude of the H^+/O ratio that more direct evidence was obtained. These results suggested that upon colicin treatment the cell membrane becomes freely permeable to counterions, leading to a collapse in the membrane electrical potential. This was verified when it was shown that colicins Ia, Ib, and A_k inhibit uptake of triphenylmethyl phosphonium cation, whose accumulation is a measure of $\Delta\psi$ (145, 154, 160). Since the proton motive force is composed solely of the $\Delta\psi$ term at physiological pH ($\Delta pH = 0$ at pH 7.5), the colicin-induced depolarization of the cytoplasmic membrane leads to dissipation of the sole driving force for many active transport systems, as well as ATP production by oxidative phosphorylation.

The finding that addition of colicins Ia, Ib, E1, A_1, and K to planar bilayer membranes prepared from soybean phospholipids increases the electrical conductance across such artifical membranes by forming voltage-dependent channels marked a critical advance (136). Significantly, colicins E2 and E3 are without effect in this system. Thus, colicins that collapse $\Delta\psi$ in whole cells are also able to induce transmembrane ion flow in a protein-free artificial membrane. It was proposed that in vivo membrane depolarization results from insertion of colicin molecules into the cytoplasmic membrane where they form aqueous channels. Although this scheme awaits in vivo verification, several other in vitro studies are supportive. When added to liposomes of heterogeneous composition (soybean or *E. coli* phospholipids), colicins E1, Ia, and A_k (but not E2 or E3) induce the rapid transmembrane flux of several ions, as well as the slower leakage of small

[1]It has recently become known that several papers that purport to study colicin K action actually dealt with colicin A (100a). This resulted from a mixup in colicin-producing strains. Where it was relevant, I used the notation A_k to designate such instances.

molecules such as choline, sucrose, leucine, and glucose-6-phosphate (88, 146). This effect is not a result of general disruption of the membrane, since neither intravesicular inulin nor dextran is released. Although colicin E1 treatment increases the permeability of liposomes of homogeneous composition (dimyristoyl-phosphatidyl-choline), treated vesicles do not leak nonelectrolytes of the size of glycerol or larger (147). The fact that colicin-treated liposomes leak a variety of molecules suggests that these colicins do not function as mobile carriers in this system, but do form nonspecific channels of finite size. Direct support for such a channel function was obtained when it was determined that colicin E1 depolarizes dimyristoyl-phosphatidyl-choline vesicles both above and below the membrane-phase transition temperature (147). In contrast to the voltage dependence for channel formation in planar artificial membranes (136), the action of these colicins on liposomes is not dependent on a transmembrane electrical potential (88, 146). The basis for this discrepancy has not been clarified.

The colicin Ia-induced proton permeability seen in liposomes (146) is different from the situation seen in whole cells or membrane vesicles (outside pH adjusted to 5.5 to allow the generation of ΔpH), which upon colicin Ia treatment display a transient enhancement in ΔpH (144, 145). Indeed, it seems likely that the colicin does promote proton permeability in whole cells or vesicles, but that the flux of protons is simply very small compared to the rate of flux of other ions in the system, which are present in much higher concentration. It is also possible that the colicin-induced proton flux across the membrane is too small to collapse ΔpH because of the low proton concentration compared to the large internal buffering capacity (136). The observed slight enhancement of ΔpH is in keeping with the many observations that a collapse in $\Delta\psi$ is accompanied by an increase in ΔpH (127).

The formation of single channel in the cytoplasmic membrane having the same selectivity and conductance properties of those channels formed in the artificial planer membrane system would account for the observation that these colicins display single-hit killing. Such a channel would elicit sufficient ion flow to depolarize the membrane within a few minutes (136).

Structural Features

In aqueous solution colicins exhibit physical properties indicative of overall structural asymmetry (91, 92). However, colicins E1 (56,000 daltons), Ia (79,000), Ib (80,000), and K (45,000) are by far the most elongated, a feature that may be related to their mechanism of action. With prolate-shaped molecules, estimated axial ratios are 15, 11.8, 10.8, and 9.6 for E1, Ia, Ib, and K, respectively. Assuming oblates, the corresponding calculated ratios are 20, 14.9, 13.4, and 11.8. Although such estimates are mere approximations, the high proportion of polar amino acids found in these colicins

dictates that they assume elongated forms in solution to maximize their interaction with the aqueous environment. Obviously, these colicins may take on a very different overall structure when integrated into the hydrophobic environment of the cell envelope membranes.

Protease digestion of colicin E1 yields a C-terminal fragment (18,000 daltons), which is enriched in nonpolar amino acids (24). This fragment depolarizes both whole cell-derived membrane vesicles and liposomes composed of dimyristoylphosphatidyl-choline (24; W. Cramer, personal communication). In contrast, a 40,000-dalton N-terminal fragment is thought to contain the domain associated with receptor recognition. There has been a suggestion that the channel-forming colicins share a common functional domain of similar primary structure (150). However, this work is difficult to evaluate, since some of the reported relevant colicin E1 amino acid sequence data conflicts with results obtained in three separate laboratories (personal communication from S. Luria, W. Cramer, and J. Lebowitz).

Immunity System

As in the case of colicins E2 and E3 and cloacin DF13, immunity to the channel-forming colicins does not involve an alteration in receptors (94, 102). However, in contrast to these enzymes, there is no evidence that colicins E1, K, Ia, Ib, or A are released from cells in complex with another polypeptide.

Although the immunity system for any one of these colicins has not been clarified, the molecular interaction involved must be highly specific. For example, strains harboring the Col Ia plasmid are immune to colicin Ia, yet sensitive to colicin Ib, and vice versa, even though these colicins share many physical and chemical properties, exhibit extensive homology in primary structure, and adsorb to a common receptor (91). Furthermore, no bacterial mutants described are insensitive to one but not the other (17, 26). Thus, it seems most reasonable that the immunity system involves some specific interaction between a plasmid-coded gene product and the colicin, rather than a plasmid-determined alteration in their mode of action sequence. It has recently been shown that immunity to colicin Ia is mediated by a plasmid-determined inner membrane protein of 14,500 daltons. Immunity operates at the level of the cytoplasmic membrane since membrane vesicles prepared from Ia-immune cells can be depolarized by colicins E1 and Ib, but not Ia (155). These results raise the possibility that immunity derives from neutralization of colicin by this immunity protein and that the association takes place at or within the cytoplasmic membrane. The formation of such stoichiometric complexes might well provide the explanation for the observation of immunity breakdown that occurs at high Ia or Ib concentra-

tion (100). Breakdown would occur when the cytoplasmic membrane is challenged with an amount of colicin in excess of the amount of immunity protein. Although several studies suggest the Col E1 plasmid gene that determines immunity to this colicin encodes a polypeptide of approximately 13,000–14,500 daltons (33, 68, 118, 123), its role in mediating immunity has not been explored.

COLICIN L

Colicin L is a bacteriocin produced by *Serratia marcescens* strain JF246 and is active against certain *E. coli* strains, but not those *Serratia* strains tested (42). This toxin (64,000 daltons) (40) inhibits synthesis of protein, DNA, and RNA and induces the efflux of accumulated leucine (39). General membrane damage does not occur, since treated cells can transport α-methyl-D-glucoside. Inhibited cells suffer a reduction in ATP levels. Although no outer membrane receptor protein has been identified for this colicin, mutants lacking a major outer membrane polypeptide, the *ompA* protein, are insensitive to the colicin. Colicin tolerance in these strains can be overcome by treatments that affect the outer membrane or peptidoglycan layers of the cell envelope (41). Furthermore, the colicin inhibits active transport in vesicles prepared from both *ompA*$^+$ and *ompA* cells. These results indicate that the *ompA* protein may play a role in mediating access of the colicin L molecule to the cytoplasmic membrane, which may be the primary cell target (42). Although the action of colicin L is reminiscent of the effects of colicins Ia, Ib, A, E1, and K, it remains to be established whether or not this reflects a similar action on the cytoplasmic membrane.

COLICIN M

Colicin M (27,000 daltons) (134) causes cell lysis, and under conditions of osmotic protection induces the formation of spheroplasts (14). Although the colicin exhibits no murein hydrolase activity in vitro, treatment of cells does lead to inhibition of murein synthesis and promotion of murein hydrolysis (cited in 134). This suggests that the cellular target may be the enzymes involved in peptidoglycan formation. Since this enzyme system is probably located at the outer surface of the inner membrane, action of this colicin may require only partial penetration of the cell envelope (134). There is experimental support in favor of this notion (134). Further delineation of the mode of action of this colicin will undoubtedly require the development of an in vitro system that responds to its action.

TO THE TARGET

Role of Receptors

Colicin action is initiated by adsorption of each toxin to a specific outer membrane receptor. The presence or absence of such receptors is a critical factor in defining the activity spectrum of a particular colicin against members of the *Enterobacteriacae* (48, 129). However, since the presence of receptors is not sufficient to ensure strain sensitivity (see below), other strain-specific properties undoubtedly contribute.

Many of the colicin receptors have been shown to be involved in outer membrane-mediated nutrient uptake (see 13, 82, 93 for reviews). Thus, the polypeptide that serves as the receptor for colicins E1, E2, and E3 functions in uptake of vitamin B12, whereas the colicin K receptor serves as a specific diffusion pathway for nucleosides (3, 32, 50, 98). Several colicin receptors are involved in iron uptake, serving as siderophore-binding proteins. Thus, the *E. coli tonA* protein is receptor for colicin M and ferrichrome (51, 152, 153), whereas enterochelin and colicins B and D (primary cell targets unknown) utilize a common polypeptide for adsorption (52, 60, 125, 126, 153). There is indirect, but suggestive, evidence that the colicin Ia, Ib receptor may also be involved in iron accumulation (93). These important physiological functions undoubtedly exert selective pressure for the maintenance of active receptors on the surface of sensitive organisms.

The molecular events between the initial adsorption to receptors and final interaction with a particular cellular target is not known for any colicin. However, there are indications that such translocation may be energy dependent (77, 107, 119, 124, 130). Fortunately, the lack of information in this area has stymied neither discussion nor speculations, and several reviews have dealt with this unsettled aspect of colicin action (13, 59, 82, 93).

In general, receptors are thought to provide a means whereby colicins are able to overcome the outer membrane barrier, which excludes access of exogenously added proteins to the periplasm and cytoplasmic membrane. This view derives from several studies in which it has been possible to demonstrate colicin-mediated de-energization of membrane vesicles prepared from resistant cells that lack receptors (7, 80, 142, 144, 154). Similarly, osmotic shock alleviates the need for receptors in cells challenged with colicins E3 or M (12, 148).

Possible Role of Proteases

Over the last several years, almost every discussion of colicin action has entertained a possible scheme involving toxin cleavage with subsequent translocation of an active fragment to the cell target. Receptor-mediated fragmentation seemed particularly intriguing. Although support for such an

hypothesis was claimed in a study that purported to demonstrate receptor-dependent cleavage of colicins E1, E2, E3, and K with the generation of an active polypeptide fragment (151), this work has been retracted (D. Sherratt, personal communication). Similary, the determination that cleavage of colicin E4 by whole cells is receptor dependent (18) is complicated by the later realization that the colicin studied was actually colicin A. The original conclusion of receptor dependence has proven to be incorrect (C. Lazdunski, personal communication).

Although there is no little doubt that under certain conditions addition of colicins to whole cells or isolated outer membranes leads to fragmentation, it has been convincingly established that receptors have no obligatory role in the proteolysis of colicin Ia, Ib, A, or E1 (9, 15). Furthermore, since colicin A activity is actually enhanced under conditions that prevent cleavage, fragmentation of the kind observed is clearly not required for action of this colicin (15). There is much need for further work in this area.

Colicin Uptake

Although one might expect that dissection of the steps intervening between adsorption and interaction with target might be amenable to genetic analysis, this general approach has proven disappointing. Although many such tolerant mutants have been described, and even in some cases characterized enough to identify the altered gene product as an envelope component, in no case has it been possible to define in molecular terms how that component facilitates colicin action.

According to its activity spectrum against a variety of mutants, a particular colicin can be unambiguously assigned to one of two groups (25, 26). Although type B colicins (B, Ia, Ia, V, D, and M) are inactive on strains that have a lesion in the *tonB* gene, but active against strains mutant in *tolA* or *tolB* genes, the type A colicins (E1, E2, E3, K, A, L) show the opposite specificity. This distinction is independent of mode of action, but it is thought to reflect two different modes of colicin uptake. There has been discussion (82) of the possibility that some A-type colicins gain entry or access by utilizing those sites of adhesion between inner and outer membranes described by Bayer (4) in a process that depends on the *tolA* and *tolB* gene function. In the case of colicins E2 and E3, supporting evidence has been presented (2, 3, 82). Although the exact function of the *tonB* gene product has not been determined, available information has led to the consensus that it plays a role in mediating transfer of outer membrane-bound nutrients (such as vitamin B12 and siderophores) or type B colicins from their respective surface receptors to the cytoplasmic membrane (13, 82, 93). Such transfer might occur with or without direct interaction of the two membranes.

A model has been considered that proposes that channel-forming colicins are able to interact with the cytoplasmic membrane while remaining adsorbed to their respective outer membrane receptors. For the type B colicins Ia and Ib, such a transenvelope orientation has been proposed to occur at hypothetical sites of apposition between inner and outer membrane (93, 96). In was further hypothesized that the formation of such regions of apposition is *tonB* dependent. According to this model, the A-type colicins E1, A, and K would span the envelope at Bayer adhesion sites. Based on calculated dimensions, these colicins are all of sufficient size to span the cell envelope. The earlier described assignment of receptor and action domains of colicin E1 to respective N-terminal and C-terminal regions (24) would predict the simultaneous attachment of these regions to outer and inner membrane, respectively.

Support for this model derives from the observation that addition of trypsin to inhibited cells reverses the effect of colicins Ia and K on macromolecular synthesis and the inhibition of active transport by colicin E1 (23, 100, 110). Presumably, digestion of colicin exposed at the cell surface suffices to disrupt or destroy that structural domain of the molecule forming channels in the inner membrane. However, an alternative mechanism involving a requirement for continuous translocation of surface colicins (trypsin sensitive) from receptor to cytoplasmic membrane (trypsin insensitive) cannot be ruled out. Such a mechanism involving transient channel formation for any particular colicin molecule would manifest trypsin reversibility. Although the finding that immobilized colicin E1 but not E2 or E3 kills sensitive cells (99) supports the idea that this colicin can span the cell envelope, cleavage of the Sephadex-bound molecule to yield an active penetrating fragment was not ruled out.

In the case of colicin E3, it is possible to bypass the need for receptors by subjecting cells to osmotic shock (143). Thus, receptors are not absolutely essential for translocation of this molecule across the inner membrane. However, efficient translocation through adhesion sites in whole cells may require that the colicin be presented to the inner membrane in an optimal orientation. This alignment might be assured by a fixed spatial relationship between the outer membrane receptor and the cytoplasmic membrane. Although the hydrophobic domains of colicins E2 and E3 and cloacin DF13 most likely play a role in their translocation across the inner membrane, there is absolutely no hint of how this is brought about.

PESTICIN A1122

Pesticin A1122 is a 65,000-molecular-weight polypeptide produced by *Yersinia pestis* (63). The activity spectrum of the toxin is defined by the presence of a specific outer membrane receptor elaborated by sensitive

organisms, which include serotype I strains of *Yersinia pseudotuberculosis,* some isolates of *Yersinia enterocolitica,* nonpesticinogenic *Y. pestis,* and *E. coli* ϕ, but not *E. coli* K12 (36).

Studies on the mode of pesticin action have been limited to its effects on *E. coli.* Since *ton B* derivatives of *E. coli* ø are insensitive to pesticin (36), its mode of uptake by cells probably has some feature in common with the B-type colicins. Although the pesticin receptor is distinct from those used by colicins I, B, D, or M, evidence suggests that it may operate as a component in some as yet undefined iron uptake system (16, 36, 64).

Pesticin A1122 is an enzyme whose toxic action results from its ability to degrade cellular murein (35). Addition of this toxin to either *E. coli* ϕ or sensitive Yersinieae induces the formation of osmotically stable spheroplast-like forms, which is paralleled by a normal increase in cell mass. There is no inhibition of DNA, RNA, or protein synthesis (35, 49). Its mode of action was established by the finding that in vitro the purified pesticin catalyzes the hydrolysis of the β-1,4 bond between N-acetyl glucosamine and N-acetylmuramic acid in the glycan backbone of the bacterial cell wall (35). Murein preparations from a wide variety of naturally insensitive gram-negative strains, as well as from the immune-producing organism and resistant mutants of sensitive strains, are degraded by the pesticin in vitro. These results demonstrate that the activity spectrum of this bacteriocin is not dictated by the cell target, but more likely reflects the ability of the toxin to gain access to the murein of the challenged organism.

STAPHYLOCOCCIN 1580

The bacteriocin staphylococcin 1580, which is produced by *Staphylococcus epidermidis* 1580, is active against many gram-positive, but not gram-negative, bacteria (79). Although early mode of action studies (78, 80) were carried out with pure material, which was shown to be a 150,000–400,000 dalton complex of subunits that contain protein, carbohydrate, and lipid (81), later studies utilize a less characterized but apparently pure preparation (157–159). The effect of staphylococcin 1580 on the energy metabolism of sensitive *Staphylococcus aureus* or *Bacillus subtilis* cells is similar to what is seen in *E. coli* cells treated with the channel-forming colicins. There is a rapid inhibition of macromolecular synthesis and active transport, depletion of cellular ATP, and efflux of preaccumulated rubidium ion and glutamic acid. In contrast, electron transport is not significantly inhibited and is even stimulated in pyrurate grown *S. aureus* cells.

Further study of staphylococcin action has made use of membrane vesicles prepared from both *S. aureus* and *B. subtilis* (80, 159). With fluorescent dyes to monitor the membrane potential in such membrane preparations, it has been shown that the toxin is able to collapse the $\Delta\psi$, whether gener-

ated from respiration or by a potassium diffusion potential. This finding strongly implicates the cytoplasmic membrane as the primary cell target and suggests that the many changes seen in staphylococcin 1580-treated cells result from its ability to depolarize the energy-transducing cytoplasmic membrane. This work is at a stage that would greatly benefit from the use of artificial membrane systems.

There is an energy requirement for the initiation of staphylococcin 1580 action that is thought to reflect some energy-dependent step in the killing mechanism (156). These steps have not been defined, and delineation will probably require a genetic approach. Two potentially useful mutant classes have already been reported (80). Although active transport in membrane vesicles prepared from resistant *S. aureus* mutants is inhibited by the staphylococcin, similar membrane preparations isolated from tolerant mutants of stable *S. aureus* L-forms are not affected by the toxin. Thus, resistant and tolerant mutants may define alterations in surface receptor and other components in the uptake process, respectively.

BUTYRICIN 7423

Clostridium butyricum 7423 produces the rather hydrophobic bacteriocin butyricin 7423 (mol wt 32,500), which is active against *Clostridium pasteurianum* (21). Treated cells are inhibited in DNA, RNA, and protein synthesis. In addition there is a lowering of ATP levels and an induced efflux of cellular K^+ (20, 21). Although the butyricin inhibits the F_1F_0-ATPase of vegetatively growing cells in vitro (19), the primary action of the toxin is thought not to be due to stoichiometric inhibition of this enzyme. The observed inhibition of $\Delta\psi$ is thought not to derive from the formation of ion-permeable channels in the cytoplasmic membrane, but to derive from some, as yet uncharacterized, interaction with this membrane (J. G. Morris personal communication).

PYOCIN R1

The particulate bacteriocins produced by various *Pseudomonas aeruginosa* strains are grouped in five classes according to differences in receptor specificity (84). Each producer strain is immune to the pyocin it produces. These toxins resemble contractile bacteriophage tails in structure, each being composed of a contractile sheath, a core, and fibers (69, 71). Their structures are very similar as viewed in the electron microscope, and they display antigenic relatedness. Of each of the pyocin's over 20 distinct subunit proteins only that polypeptide that comprises the main component of the fiber differs from pyocin to pyocin (117). Since these fibers function in the

adsorption of each bacteriocin to its cognate lipopolysaccharide receptor on the surface of sensitive organisms (71, 81, 103), such differences may well form the basis for the host range of each R-type pyocin.

All the evidence suggests that the R-type pyocins originated from a common temperate bacteriophage, which by mutation became defective in assembly of normal particles. Subsequent divergence of the fiber structure would have generated the various R-type pyocins. Such an origin is supported by the finding that several *P. aeruginosa* strains produce phages that display immunologic cross-reaction with the R-type pyocins (61, 62, 70, 87). Furthermore, the isolated tail of one of these phages has pyocin-like bacteriocidal action (138). There is genetic evidence that these bacteriocins are coded by allelic chromosomal genes (83, 86).

With regard to mode of action against sensitive *P. aeruginosa* strains, pyocin R1 is the most thoroughly studied R-type pyocin. Treatment leads to an immediate cessation of DNA, RNA, and protein synthesis with no cell lysis or DNA degradation (89). Ribosomes isolated from R1-treated cells are physically degraded (89). It has been proposed that the arrest in macromolecular synthesis may derive from the observed inability of treated cells to take up or/and maintain amino acids, nucleosides, and ions (65). The finding that pyocin R1 causes an uncoupling of respiration to solute transport implies that the pyocin causes damage to the energy-transducing membrane, rather than acting to inhibit those reactions that lead to oxygen reduction (85). The enhanced binding of hydrophobic fluorescent probes to R1-treated cell, with accompanying changes in certain parameters of fluorescence (149), is similar to the studies on the channel-forming colicins and, here too, probably reflects structural changes in the cell envelope as a result of membrane de-energization. The sum of these observations strongly suggests that the cytoplasmic membrane is the primary target of this bacteriocin. Whether or not the killing mechanism involves a direct interaction of the pyocin, or some component of its composite structure with this membrane, is unknown. It seems likely that the other R-type pyocins have a similar action mechanism (117).

PYOCIN AP41

The bacteriocin pyocin AP41 has been isolated as a complex of two polypeptides (90,000 and 6000–7000 daltons). Killing activity against sensitive *P. aeruginosa* strains resides in the larger component (132). In vivo the pyocin inhibits DNA synthesis preferentially and induces production of resident pyocins or phages. These results suggest a colicin E2-like mode of action and that the small subunit of the pyocin complex might correspond to an immunity protein. This scheme is supported by a report (M.

Kageyama, personal communication) that trypsinolysis of native AP41 leads to the generation of a 16,000-dalton fragment that displays in vitro DNA endonuclease activity. This activity is inhibited by addition of the small subunit.

MEGACIN A-216

Megacin A-216 (51,000 daltons) is a phospholipase (57, 72, 122). Bacteriocin-treated *Bacillus megaterium* leak intracellular material but do not lyse, and treated protoplasts are converted to cell ghosts. In vitro, the purified megacin catalyzes the formation of lysolecithin from lecithin and, thus, can be classified as an A-2 type phospholipase.

Immunity of the meg^+-producing *B. megaterium* strain is mediated by a proteinaceous inhibitor found in the culture medium (111, 112). This substance not only prevents the action of the megacin on protoplasts, but it inhibits megacin-dependent conversion of lecithin to lysolecithin. Furthermore, megacinogenic mutants displaying increased immunity produce higher levels of inhibitor when compared to the parental meg^+ strain, whereas mutants showing increased sensitivity produce less. These results, together with the finding that the megacin inhibitor is not a general inhibitor of phospholipase A activity and is not produced by nonmegacinogenic strains, establish its role in the megacin immunity system.

CONCLUDING REMARKS

Although it has been possible to discuss the mode of action of several bacteriocins in terms of interaction with primary cell targets, a complete understanding of their killing mechanism at the molecular level requires a more thorough delineation of how each toxin reaches its target. This particular aspect of bacteriocin action has proven quite refractory to experimental attack. A major technical problem in following the fate of bound bacteriocin is that although killing is single hit, not all bacteriocin-receptor interactions are functionally effective. Although the basis for such heterogeneity is not known, it might well derive from differences in molecular environment. For example, only a minority of receptors may be able to interact with envelope components that can function to mediate toxin uptake. A further complication is that any manipulation that involves cell breakage, fractionation of envelopes, etc, may well cause a redistribution of bacteriocin molecules or fragments. The possibility of nonspecific modification of absorbed colicin by envelope proteases must also be taken into account. Clearly, the sorting out of physiologically functioning toxin from bacteriocins not taking part in the mode of action scheme is very difficult. Ideally, one would like some means

to distinguish "killers" from "non-killers" and an approach that will allow the freezing of "killer molecules" at various steps in the uptake process.

The voltage dependence of the channels formed by certain of the colicins in the planar membrane system provides the membraneologist with a potentially very useful system for probing the nature of gated channel formation. Furthermore, the ability to assay a functional interaction (channel formation) between a membrane and an exogenously added protein soluble in aqueous solutions may allow one to discern certain features of a process that may be of general relevance to membrane biogenesis and protein secretion.

Although a start has been made in elucidating the functional domains of several colicins, progress has relied, for the most part, on the good fortune that protease cleavage of intact molecules has yielded useful fragments. What are now needed are more defined studies in which the investigator is able to dictate specific changes in protein structure. Such an approach is now feasible through DNA technology. Indeed, the cloning and sequence analysis of several colicin structural genes have been undertaken by several groups, and it should not be long before primary sequences are known. This kind of information should make possible the use of directed in vitro mutagenesis to generate interesting molecules for both in vitro and in vivo analysis. Undoubtedly this approach can be applied to all the bacteriocins.

Literature Cited

1. Andreoli, P. M., Overbeeke, N., Veltkamp, E., van Embden, J. D. A., Nijkamp, H. J. J. 1978. *Mol. Gen. Genet.* 160:1–11
2. Bassford, P. J., Kadner, R. J., Schnaitman, C. A. 1977. *J. Bacteriol.* 129:265–75
3. Bassford, P. J., Schnaitman, C. A., Kadner, R. J. 1977. *J. Bacteriol.* 130:750–58
4. Bayer, M. 1979. In *Bacterial Outer Membranes*, ed. M. Inouye, pp. 167–202. New York: Wiley
5. Beppu, T., Arima, K. 1976. *J. Bacteriol.* 93:80–85
6. Beppu, T., Yamamoto, H., Arima, K. 1975. *Antimicrob. Agents Chemother.* 8:617–26
7. Bhattacharyya, P., Wendt, L., Whitney, E., Silver, S. 1970. *Science* 168:998–1000
8. Boon, T. 1971. *Proc. Natl. Acad. Sci. USA* 68:2421–25
9. Bowles, L. K., Konisky, J. 1981. *J. Bacteriol.* 145:668–71.
10. Bowman, C. M., Dahlberg, J. E., Ikemura, T., Konisky, J., Nomura, M. 1971. *Proc. Natl. Acad. Sci. USA* 68: 964–68
11. Bowman, C. M., Sidikaro, J., Nomura, M. 1971. *Nature London New Biol.* 234:133–37
12. Braun, J., Frenz, J., Hantke, K., Schaller, K. 1980. *J. Bacteriol.* 142: 162–68
13. Braun, V., Hantke, K. 1977. In *Microbial Interactions*, ed. J. L. Reissig, pp. 101–37. London: Chapman and Hall
14. Braun, V., Schaller, K., Wabl, M. R. 1974. *Antimicrob. Agents Chemother.* 5:520–33
15. Brey, R. N. 1981. *J. Bacteriol.* 149:306–15
16. Brubaker, R. R., Surgalla, M. J. 1961. *J. Bacteriol.* 82:940–49
17. Cardelli, J., Konisky, J. 1974. *J. Bacteriol.* 119:379–85
18. Cavard, D., Lazdunski, C. 1979. *Eur. J. Biochem.* 96:525–33
19. Clarke, D. J., Fuller, F. M., Morris, J. G. 1979. *Eur. J. Biochem.* 98:597–612
20. Clarke, D. J., Morris, J. G. 1976. *J. Gen. Microbiol.* 95:67–77
21. Clarke, D. J., Robson, R. M., Morris, J. G. 1975. *Antimicrob. Agents Chemother.* 7:256–64

22. Dandeu, J. P., Billault, A., Barbu, E. 1969. *C. R. Acad. Sci.* 269:2044–47
23. Dankert, J., Hammond, S. M., Cramer, W. A. 1980. *J. Bacteriol.* 143:594–602
24. Dankert, J. R., Uratani, Y., Grabau, C., Cramer, W. A., Hermodson, M. 1981. *Fed. Proc.* 1844 (Abstr.)
25. Davies, J. K., Reeves, P. 1975. *J. Bacteriol.* 123:96–101
26. Davies, J. K., Reeves, P. 1975. *J. Bacteriol.* 123:102–17
27. DeGraaf, F. K. 1979. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reibe A* 244:121–34
28. DeGraaf, F. K., Klaasen-Boor, P. 1977. *Eur. J. Biochem.* 73:107–14
29. DeGraaf, F. K., Niekus, H. G. D., Klootwijk, J. 1973. *FEBS Lett.* 35: 161–65
30. DeGraaf, F. K., Planta, R. J., Stouthamer, A. H. 1971. *Biochim Biophys. Acta* 240:122–36
31. DeGraaf, F. K., Stukart, M. J., Boogerd, F. D., Metselaar, K. 1978. *Biochemistry* 17:1137–42
32. DiMasi, D. R., White, J. C., Schnaitman, C. A., Bradbeer, C. 1973. *J. Bacteriol.* 115:506–13
33. Ebina, Y., Kishi, F., Nakazawa, T. 1979. *Nucleic Acids Res.* 7:639–49
34. Feingold, D. S. 1970. *J. Membr. Biol.* 3:372–86
35. Ferber, D. M., Brubaker, R. R. 1978. *J. Bacteriol.* 136:495–501
36. Ferber, D. M., Fowler, J. M., Brubaker, R. R. 1981. *J. Bacteriol.* 146:506–11
37. Fields, K. L., Luria, S. E. 1969. *J. Bacteriol.* 97:57–63
38. Fields, K. L., Luria, S. E. 1969. *J. Bacteriol.* 97:64–77
39. Foulds, J. 1971. *J. Bacteriol.* 107: 833–39
40. Foulds, J. 1972. *J. Bacteriol.* 110: 1001–9
41. Foulds, J., Chai, T-J. 1978. *J. Bacteriol.* 133:158–64
42. Foulds, J., Shemin, D. 1969. *J. Bacteriol.* 99:655–60
43. Fredericq, P. 1958. *Symp. Soc. Exp. Biol.* 12:104–22
44. Gaastra, W., Oudega, B., DeGraaf, F. K. 1978. *Biochim. Biophys. Acta* 540: 301–12
45. Gilchrist, M. J. R., Konisky, J. 1975. *J. Biol. Chem.* 250:2457–62
46. Gould, J. M., Cramer, W. A. 1977. *J. Biol. Chem.* 252:5491–97
47. Gould, J. M., Cramer, W. A., van Thienen, G. 1976. *Biochem. Biophys. Res. Commun.* 72:1519–25
48. Graham, A. C., Stocker, B. A. D. 1977. *J. Bacteriol.* 130:1214–23
49. Hall, P. J., Brubaker, R. R. 1978. *J. Bacteriol.* 136:786–89
50. Hantke, K. 1976. *FEBS Lett.* 70:109–12
51. Hantke, K., Braun, V. 1975. *FEBS Lett.* 49:301–5
52. Hantke, K., Braun, V. 1975. *FEBS Lett.* 59:277–81
53. Hardy, K. G. 1975. *Bacteriol. Rev.* 39:464–515
54. Herschman, H. R., Helinski, D. R. 1967. *J. Biol. Chem.* 242:5360–68
55. Hirata, H. S., Fukui, S., Ishikawa, S. 1969. *J. Biochem.* 65:843–47
56. Hirose, A., Kumagai, J., Imahori, K. 1976. *J. Biochem.* 79:305–11
57. Holland, I. B. 1962. *J. Gen. Microbiol.* 29:603–14
58. Holland, I. B. 1975. *Adv. Microb. Physiol.* 12:56–139
59. Holland, I. B. 1976. In *Specficity and Action of Animal, Bacterial and Plant Toxins,* ed. P. Cuatrecasas, pp. 99–127. London: Chapman and Hall.
60. Hollifield, W. C., Neilands, J. B. 1978. *Biochemistry* 17:1922–28
61. Homma, J. Y., Shionoya, H. 1967. *Jpn. J. Exp. Med.* 37:395–421
62. Homma, J. Y., Watabe, H., Tanabe, Y. 1968. *Jpn. J. Exp. Med.* 38:213–24
63. Hu, P. C., Brubaker, R. R. 1974. *J. Biol. Chem.* 249:4749–53
64. Hu, P. C., Yang, G. C. H., Brubaker, R. R. 1972. *J. Bacteriol.* 112:212–19
65. Iijima, M. 1978. *J. Biochem.* 83:395–402
66. Imahori, K. 1979. *TIBS* 4:212–13
67. Imahori, K., Ohno, S., Ohno-Iwashita, Y., Suzuki, K. 1978. In *Versatility of Proteins,* ed. C. H. Li, pp. 167–82. New York: Academic
68. Inselburg, J., Applebaum, B. 1978. *J. Bacteriol.* 133:1444–51
69. Ishii, S., Nishi, Y., Egami, F. 1965. *J. Mol. Biol.* 13:428–31
70. Ito, S., Kageyama, M. 1970. *J. Gen. Appl. Microbiol.* 16:231–40
71. Ito, S., Kageyama, M., Egami, F. 1970. *J. Gen. Appl. Microbiol.* 16:205–14
72. Ivanovics, G. E., Afoldi, L., Nagy, E. 1959. *J. Gen. Microbiol.* 21:51–60
73. Jakes, K. 1982. In *The Molecular Actions of Toxins and Viruses,* ed. S. van Heyninger, P. Cohen. North Holland: Elsevier. In press
74. Jakes, K. S., Zinder, N. D. 1974. *Proc. Natl. Acad. Sci. USA* 71:3380–84
75. Jakes, K., Zinder, N. D., Boon, T. 1974. *J. Biol. Chem.* 249:438–44
76. Jetten, A. M. 1976. *Biochim. Biophys. Acta* 440:403–11

77. Jetten, A. M., Jetten, M. E. R. 1975. *Biochim. Biophys. Acta* 387:12–22
78. Jetten, A. M., Vogels, G. D. 1972. *Antimicrob. Agents Chemother.* 2:456–63
79. Jetten, A. M., Vogels, G. D. 1972. *J. Bacteriol.* 112:243–50
80. Jetten, A. M., Vogel, G. D. 1973. *Biochim. Biophys. Acta* 311:483–95
81. Jetten, A. M., Vogels, G. D., DeWindt, F. 1972. *J. Bacteriol.* 112:235–42
82. Kadner, R. J., Bassford, P. J., Pugsley, A. P. 1979. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig. Reihe A 244:90–104
83. Kageyama, M. 1974. *J. Gen. Appl. Microbiol.* 20:269–75
84. Kageyama, M. 1975. In *Microbial Drug Resistance,* ed. S. Mitsuhashi, H. Hashimoto, pp. 291–305. Tokyo: Univ. Tokyo
85. Kageyama, M. 1978. *J. Biochem.* 84:1373–79
86. Kageyama, M., Inagaki, A. 1974. *J. Gen. Appl. Microbiol.* 20:257–67
87. Kageyama, M., Shinomiya, T., Aihara, Y., Kobayaski, M. 1979. *J. Virol.* 32:951–57
88. Kayalar, C., Luria, S. E. 1979. In *Membrane Bioenergetics,* ed. C. P. Lee, G. Schatz, L. Ernster, pp. 297–306. New York: Addison-Wesley
89. Kaziro, Y., Tanaka, M. 1965. *J. Biochem.* 57:689–95
90. Konings, R. N. H., Andreoli, P. M., Veltkamp, E., Nijkamp, H. J. J. 1976. *J. Bacteriol.* 126:861–68
91. Konisky, J. 1973. In *Chemistry and Functions of Colicins,* ed. L. Hager, pp. 41–58. New York: Academic
92. Konisky, J. 1978. In *The Bacteria,* ed. L. N. Ornston, J. R. Sokatch, 6:319–59. New York: Academic
93. Konisky, J. 1979. In *Bacterial Outer Membranes,* ed. M. Inouye, pp. 319–59. New York: Wiley
94. Konisky, J., Cowell, B. S. 1972. *J. Biol. Chem.* 247:6524–29
95. Konisky, J., Nomura, M. 1967. *J. Mol. Biol.* 26:181–95
96. Konisky, J., Tokuda, H. 1979. *Zentralbl. Bakteriol. Parasitenkol. Infektionsk. Hyg. Abt. 1 Orig. Reihe A* 244:105–20
97. Kopecky, A. L., Copela d, D. P., Lusk, J. E. 1975. *Proc. Natl. Acad. Sci. USA* 72:4631–34
98. Krieger-Brauer, H. J., Braun, V. 1980. *Arch. Microbiol.* 124:233–42
99. Lau, C., Richards, F. M. 1976. *Biochemistry* 15:666–71
100. Levisohn, R., Konisky, J., Nomura, M. 1968. *J. Bacteriol.* 96:811–21
100a. Luria, S. E. 1982. *J. Bacteriol.* 149:386
101. Lusk, J. E., Nelson, D. L. 1972. *J. Bacteriol.* 112:148–60
102. Maeda, A., Nomura, M. 1966. *J. Bacteriol.* 91:685–94
103. Meadow, P. M., Wells, P. L. 1978. *J. Gen. Microbiol.* 108:339–43
104. Mock, M., Schwartz, M. 1980. *J. Bacteriol.* 142:384–90
105. Mooi, F. R., DeGraaf, F. K. 1976. *FEBS Lett.* 62:304–8
106. Nagel de Zwaig, R. 1969. *J. Bacteriol.* 99:913–14
107. Nieva-Gomez, D., Konisky, J., Gennis, R. B. 1976. *Biochemistry* 15:2747–53
108. Nomura, M. 1963. *Cold Spring Harbor Symp. Quant. Biol.* 28:315–24
109. Nomura, M., Maeda, A. 1965. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.* 196:216–39
110. Nomura, M., Nakamura, M. 1962. *Biochem. Biophys. Res. Commun.* 7:306–9
111. Ochi, T., Higashi, Y., Inoue, K., Amano, T. 1970. *Biken J.* 13:63–76
112. Ochi, T., Yano, K., Amano, T. 1971. *Biken J.* 14:423–24
113. Ohno, S., Imahori, K. 1978. *J. Biochem.* 84:1637–40
114 Ohno, S., Ohno-Iwashita, Y., Suzuki, K., Imahori, K. 1977. *J. Biochem.* 82:1045–53
115. Ohno-Iwashita, Y., Imahori, K. 1979. *FEBS Lett.* 100:249–52
116. Ohno-Iwashita, Y., Imahori, K. 1980. *Biochemistry* 19:652–59
117. Ohsumi, M., Shinomiya, T., Kageyama, M. 1980. *J. Biochem.* 87:1119–26
118. Oka, A., Nomura, N., Morita, M., Sugisaki, H., Sugimoto, K., Takanami, M. 1979. *Mol. Gen. Genet.* 172:151–59
119. Okamoto, K. 1975. *Biochim. Biophys. Acta* 389:370–79
120. Oudega, B., Klaasen-Boor, P., DeGraaf, F. K. 1975. *Biochim. Biophys. Acta* 392:184–95
121. Oudega, B., van der Molen, J., DeGraaf, F. K. 1979. *J. Bacteriol.* 140:964–70
122. Ozaki, M. Y., Higaski, Y., Saito, H., An, I., Amano, T. 1966. *Biken J.* 9:201–13
123. Patient, R. K. 1979. *Nucleic Acids Res.* 6:2647–65
124. Plate, C. A. 1973. *Antimicrob. Agents Chemother.* 4:16–24
125. Pugsley, A., Reeves, P. 1977. *Antimicrob. Agents Chemother.* 11:345–58
126. Pugsley, A. P., Reeves, P. 1977. *Biochem. Biophys. Res. Commun.* 74: 903–11
127. Ramos, S., Schuldiner, S., Kaback, H.

R. 1976. *Proc. Natl. Acad. Sci. USA* 73:1892–96
128. Reeves, P. R. 1972. *The Bacteriocins.* New York: Springer. 142 pp.
129. Reeves, P. 1979. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A* 244:7889
130. Reynolds, B. L., Reeves, P. R. 1969. *J. Bacteriol.* 100:301–9
131. Sabet, S. F. 1976. *J. Bacteriol.* 126:601–8
132. Sano, Y., Kageyama, M. 1981. *J. Bacteriol.* 146:733–39
133. Saxe, L. S. 1975. *Biochemistry* 14: 2058–63
134. Schaller, K., Dreher, R., Braun, V. 1981. *J. Bacteriol.* 146:54–63
135. Schaller, K., Nomura, M. 1976. *Proc. Natl. Acad. Sci. USA* 73:3989–93
136. Schein, S. J., Kagan, B. L., Finkelstein, A. 1978. *Nature* 276:159–63
137. Senior, B. W., Holland, I. B. 1971. *Proc. Natl. Acad. Sci. USA* 68:959–63
138. Shinomiya, T., Shiga, S. 1979. *J. Virol.* 32:958–67
139. Sidikaro, J., Nomura, M. 1975. *J. Biol. Chem.* 250:1123–31
140. Suzuki, K., Imahori, K. 1978. *J. Biochem.* 84:1021–29
141. Tagg, J. R., Dajani, A. S., Wannamaker, L. W. 1976. *Bacteriol. Rev.* 40:722–56
142. Takagaki, Y., Kunugita, K., Mitsuhashi, M. 1973. *J. Bacteriol.* 113:42–50
143. Tilby, M., Hindennach, I., Henning, U. 1978. *J. Bacteriol.* 136:1189–91
144. Tokuda, H., Konisky, J. 1978. *J. Biol. Chem.* 253:7731–37
145. Tokuda, H., Konisky, J. 1978. *Proc. Natl. Acad. Sci. USA* 75:2579–83
146. Tokuda, H., Konisky, J. 1979. *Proc. Natl. Acad. Sci. USA* 76:6167–71
147. Uratani, Y., Cramer, W. A. 1981. *J. Biol. Chem.* 256:4017–23
148. Uratani, Y., Kageyama, M. 1977. *J. Biochem.* 81:333–41
149. Van den Elzen, P. J. M., Gaastra, W., Spelt, C. E., DeGraaf, F. K., Veltkamp, E., Nijkamp, H. J. J. 1980. *Nucleic Acid Res.* 8:4349–63
150. Watson, D. H. 1980. *Biochim. Biophys. Acta* 622:287–96
151. Watson, D. H., Sherratt, D. 1979. *Nature* 278:362–64
152. Wayne, R., Frick, K., Neilands, J. B. 1976. *J. Bacteriol.* 126:7–12
153. Wayne, R., Neilands, J. B. 1975. *J. Bacteriol.* 121:497–503
154. Weaver, C. A., Kagan, B. L., Finkelstein, A., Konisky, J. 1981. *Biochim Biophys. Acta* 645:137–42
155. Weaver, C. A., Redborg, A., Konisky, J. 1981. *J. Bacteriol.* 148:817–28
156. Weerkamp, A., Geerts, W., Vogels, G. D. 1978. *Biochim. Biophys. Acta* 539: 372–85
157. Weerkamp, A., Heinen-von Borries, U. J., Vogels, G. D. 1978. *Antonie van Leeuwenhoek J. Microbiol. Ser.* 44: 35–48
158. Weerkamp, A., Vogels, G. D. 1978. *Antimicrob. Agents Chemother.* 13:146–53
159. Weerkamp, A., Vogels, G. D. 1978. *Biochim. Biophys. Acta* 539:386–97
160. Weiss, M. J., Luria, S. E. 1978. *Proc. Natl. Acad. Sci. USA* 75:2483–87
161. Wendt, L. 1970. *J. Bacteriol.* 104: 1236–41
162. Yamamoto, H., Nishida, K., Beppu, T., Arima, K. 1978. *J. Biochem.* 83:827–34