PERSPECTIVES IN TOXINOLOGY

Edited by

ALAN W. BERNHEIMER, Ph.D.

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New York University Medical Center

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SERIES PREFACE

Despite the dramatic contribution that antibiotic therapy has made to the control of infectious diseases, microbial pathogens persist and continue to present us with problems as they mutate, become more virulent, or acquire resistance to a wide variety of antibiotics and chemotherapeutic agents. Just as the invading microorganism develops new strategies in overcoming the host's defense mechanisms or our antibiotic "barrages," so must we be constantly thinking of new countermeasures as well as developing and broadening our understanding of the mechanisms of pathogenicity. The ultimate description of the basis of a microorganism's pathogenicity has to be in molecular, biochemical terms, and our success in controlling the "invader" is highly dependent on our understanding of the biochemical differences between the host and infectious agents. For these reasons, as well as many others, medical microbiology is far from being a static field of scientific and medical endeavor. This series has been launched in an effort to bring together developments in a rapidly changing and advancing area of microbiology. Our knowledge of the problems of infectious diseases can only be enhanced by understanding both the basic and practical, clinical aspects of medical microbiology.

The molecular basis for the interaction between a biological toxin—be it of microbial, plant, or other origin—and mammalian cells has been a preoccupation and fascination for all those who wish to understand mechanisms of pathogenicity and toxicity. It is not surprising in the light of the history of infectious diseases that bacterial toxins have occupied a prominent place in toxinology. It is both fitting and fortunate, therefore, that Dr. Alan W. Bernheimer and authoritative authors have devoted their efforts to the development of this contribution, *Perspectives in Toxinology,* to accompany *Mechanisms in Bacterial Toxinology,* the first volume of this series, Developments in Medical Microbiology and Infectious Diseases.

For many reasons, bacterial systems offer tremendous advantages over other biological systems for toxinology studies. The genetics of toxin production covered in this volume by Dr. Werner K. Maas provide just such an example where the wealth of knowledge of molecular genetics in bacteria can be put to good effect in studies of toxinology. To neglect other biological toxins at the expense of bacterial toxins would be inappropriate, and the inclusion of toxic plant lectins and cnidarian venoms adds valuable breadth to the topic and must inevitably stimulate and cross-fertilize fields of

interest not only in medical microbiology but also in basic responses to endotoxins such as tumor necrosis, dealt with by Dr. H. Francis Havas. Both lectins and endotoxins have profound and varied biological effects, and an understanding of their interactions with various types of cells has greatly broadened our biological horizons.

Milton R. J. Salton

PREFACE

The word toxinology first received wide use when it was incorporated into the name of an international society about 15 years ago. More restrictive than toxicology it has the advantage of being concerned only with poisonous agents produced by living entities rather than with all poisons. Current advances in the study of toxins synthesized by bacteria, animals, and plants suggest that this is an appropriate time for publication of a new collection of reviews. In this volume major emphasis is placed on toxins of bacterial origin, but the scope is broadened by inclusion of a chapter on cnidarian (coelenterate) toxins and by one on the plant toxins, ricin and abrin.

Timely and of general relevance to toxinology are a chapter concerned with modern principles of genetics as they pertain to bacterial toxinogenesis and a chapter on the biochemistry of gangliosides and their role as membrane receptors of toxins. The capacity of endotoxin to induce tumor necrosis is reviewed, and a detailed treatment is presented of the limulus lysate assay of endotoxin with special reference to its clinical usefulness. The exotoxins of certain gram-negative bacteria and the chemistry and pharmacology of botulinum and tetanus neurotoxins form the topics of separate reviews.

I am greatly indebted to the contributors whose participation and cooperation made this volume possible. I acknowledge with thanks the invaluable help of Lois S. Avigad who prepared the index. To the publishers I am grateful for expediting production of the finished work.

Alan W. Bernheimer

CONTRIBUTORS

Richard S. Blanquet, Ph.D. Associate Professor of Biology, Georgetown University, Washington, D.C.

Bibhuti R. DasGupta, Ph.D. Project Associate, Food Research Institute, University of Wisconsin, Madison, Wisconsin

H. Francis Havas, Ph.D. Professor of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania

Robert W. Ledeen, Ph.D. Associate Professor of Biochemistry in Neurology, Albert Einstein College of Medicine, New York, New York

Pinghui V. Liu, M.D., Ph.D. Professor of Microbiology and Immunology, University of Louisville School of Medicine, Louisville, Kentucky

Werner K. Maas, Ph.D. Professor of Microbiology, New York University Medical Center, New York, New York

Jane Mellanby, D. Phil. Fellow and Tutor in Physiology, St. Hilda's College, Oxford, England

Ronald Nachum, Ph.D. Assistant Professor of Pathology, University of Southern California School of Medicine, Los Angeles, California

Sjur Olsnes, M.D., Ph.D. Senior Biochemist, Norsk Hydro's Institute for Cancer Research, Oslo, Norway

Stuart E. Siegel, M.D. Associate Professor of Pediatrics, University of Southern California School of Medicine, Los Angeles, California

H. Sugiyama, Ph.D. Professor of Bacteriology, University of Wisconsin, Madison, Wisconsin

CONTENTS

CHAPTER 1

GENETICS OF TOXIN PRODUCTION BY BACTERIA

WERNER K. MAAS

1. INTRODUCTION

The genetics of toxin production by bacteria can be considered conveniently with respect to two areas: (*a*) the use of mutants and genetic methods to study the biosynthesis of toxins; and (*b*) the transmission of genes involved in toxin production in bacterial populations. The first area deals with the use of mutants to study an essentially biochemical problem, the nature and control of the expression of genes involved in toxin production. Genetic methodology is one of several tools that can be used to elucidate the steps of gene expression, transcription, translation, and further transformations leading to the final gene product. It has proved to be very powerful in the analysis of these steps for many bacterial proteins, especially enzymes of bacterial metabolism. The second area is concerned with the genetics proper of toxin production, the location of these genes within the bacterial genome, and the ways in which they are transmitted to other bacteria. Its contributions to toxinology are chiefly to the epidemiology of infections, the role of gene arrangements in the control of toxin production, and the understanding of the evolution of toxin-producing organisms.

In the following section we shall review the uses that have been made of genetic techniques to study toxin biosynthesis. We shall discuss mainly three examples, diphtheria toxin, cholera toxin, and *E. coli* enterotoxin, all of which have been studied extensively. One of the aims is to demonstrate the potentialities of the genetic approach by comparing studies on these toxins with other well-studied systems, such as the lactose operon and the enzymes of biosynthetic pathways. This is done, because it now seems an opportune time to apply the complete methodology of molecular genetics to toxin biosynthesis. In the following sections (3.1, 3.2) we shall consider the location and transmission of genes involved in toxin synthesis, with emphasis on recent studies with plasmid-located genes.

The three toxins that have been chosen as model systems have been reviewed elsewhere (1), and they therefore will not be considered in detail. Rather their genetics will be used to illustrate the general features of the genetic approach and the information that can be gained from the variety of genetic techniques available at the present time. For references to some of the older literature, the reader will be referred to the chapters in the volume cited and to other recent review articles (2,3).

2. BIOSYNTHESIS OF TOXINS

2.1. General

Historically, studies on the genetics of a given toxin have usually been preceded by groundwork laid by chemical characterization of the toxin, by studies on its mode of action, and, by investigation of the physiology of toxin production. Genetic studies, once initiated, have usually proceeded through the following stages: (*a*) Localization of the genes for toxin production, i.e., on the chromosome, on a temperate phage, or on a plasmid. (*b*) Isolation and characterization of mutants affecting toxin production. (*c*) Complementation tests with mutants to find out how many cistrons are involved in toxin synthesis. (*d*) Fine-structure mapping of mutations in *tox* genes. (*e*) Search for regulatory mutants affecting operator or repressor genes. During recent years genetic studies at the molecular level have been added, such as DNA-dependent toxin synthesis in cell-

free extracts and cloning of toxin genes for physical mapping studies by electron microscope (E.M.) heteroduplex (HD) analysis and for amplification of toxin production. The advances made with these genetic approaches vary a great deal from one toxin to another, the greatest having been made with the three toxins chosen as model systems.

The biosynthesis of toxin is in many respects like that of enzymes and other cellular proteins, and therefore the genetics of toxin production is like the genetics of the synthesis of other proteins. However, there are two features that distinguish toxins from many other proteins: they are usually exotoxins and therefore secreted into the culture medium; at least some of the toxin molecules have two portions associated with different functions, one being the binding to a surface receptor on the target cells, the other being inhibition of a reaction inside the cell which is vital for cellular functions; it is the latter that constitutes the actual toxic effect. The genetic basis for these two features gives a unique aspect to the genetics of toxin synthesis and adds a special relevance to this field for studies of the transport of proteins across membranes and for the formation of protein complexes.

2.2. Diphtheria Toxin

It has been known since 1951 that the genetic information for the production of diphtheria toxin is carried on a temperate phage. Since then studies on the genetics of this toxin have been guided by developments in phage genetics. Because of their relatively small size and extracellular existence, phages can be manipulated easily for genetic work, and this has been one of the contributing factors toward the success of genetic investigations on phages, including those for diphtheria toxin. Genetic information for a number of other toxins is also carried on temperate phages. These include the alpha toxin of *Staphylococcus aureus,* erythrogenic toxin of *Streptococcus pyogenes,* and type C and type D toxins of *Clostridium botulinum* (3). With these, genetic studies have not progressed so far as they have with diphtheria toxin.

Another contributing factor to the success of genetic studies on diphtheria toxin is the elucidation of the chemical structure of the toxin during the past 20 years. These studies make it possible to locate mutational sites within defined regions of the toxin molecule and thus to establish direct correlations between the structure of the gene and the structure of its protein product.

The chemical structure of the toxin is shown in Figure 1. It consists of a single polypeptide chain or molecular weight 63,000. Treatment with certain proteases, such as trypsin, liberates two fragments: A, containing the N-terminal portion and B, containing the C-terminal portion. Fragment A carries an enzymatic activity responsible for toxicity, the ADP-ribosylation of elongation factor EF-2. Fragment B has no known enzymatic activity, but is required for the binding of the toxin to receptors on the cell surface. Although these relationships are well known, they are restated here briefly, because they have contributed much to the understanding of mutants affecting toxin production.

It is no easy matter to isolate mutants affecting toxin production, since there are no selection or enrichment procedures available, as there are for other mutants, such as drug-resistant mutants and auxotrophic mutants. Even with highly efficient mutagenic procedures, such as treatment with N-methyl-N′-nitro-N-nitrosoguanidine (NG) the frequency of nontoxigenic mutants is at best 1%. It is thus necessary to have a simple assay procedure available that permits the testing of hundreds of colonies. For

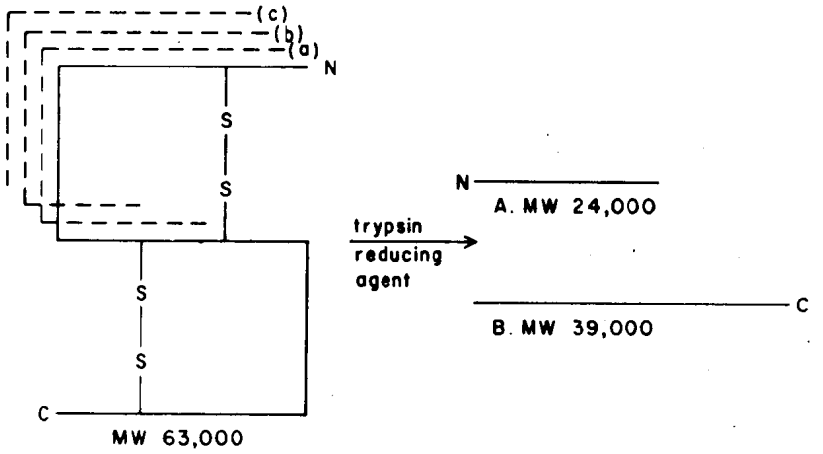

Figure 1. Diagram of the diphtheria toxin molecule and the two fragments generated by digestion with trypsin. Fragment A has ADP-ribosylation activity, fragment B does not. (a), (b), and (c) represent CRM fragments obtained from nonsense mutants a, b, and c (see Figure 2). (a) and (b) have ADP-ribosylation activity, (c) does not.

diphtheria toxin mutants have recently been isolated with relative ease by using as a preliminary screen either an immunological method, the Elek test (4), or a tissue culture assay (5).

Three types of phage mutants defective in toxin production (*tox* mutants) have been isolated: (*a*) those that produce an immunologically fully active protein (CRM) of molecular weight 63,000 and are presumably missense mutants; (*b*) those that produce weak CRMs with lower molecular weights and are known to produce fragments of the toxin molecule starting from the N-terminal end (Fig. 1); and (*c*) those that have no detectable activity by any of the available methods and produce no detectable protein. They may be mutations in the *tox* structural gene which preclude any protein synthesis (deletions, nonsense mutations), or they may be mutations in a regulatory gene which turns off the activity of the *tox* gene.

Of special interest are mutants of the second type, since they tell us the direction in which the *tox* gene is transcribed on the phage. Chain termination in these mutants can occur at the level of transcription as a result of a deletion or at the level of translation as a result of a nonsense mutation. Holmes (4) has demonstrated that the latter possibility is the correct one. In phage crosses involving *tox* mutants in which a common region of the toxin molecule was missing, he was able to obtain tox^+ recombinants that produce a normal toxin molecule. Such recombinants cannot be formed if the phages carry overlapping deletions but can be formed if they carry chain-terminating nonsense mutations. In these matings Holmes was able to order the mutations on the vegetative map of phage β in relation to other genes on either side of the *tox* gene. Since translation is known to start at the N-terminal end and translation and transcription proceed in the same direction, the direction of transcription with regard to other phage genes can be deduced (see Fig. 2).

The existence of mutants without detectable toxin or CRM raises the question of regulation of toxin synthesis, since, as pointed out above, such mutations can occur in a

regulatory gene. Complementation tests have shown that these mutants do not produce a repressor which acts in *cis* configuration or that they lack a diffusible activator substance which is produced by wild-type *tox*$^+$ phages. Mapping experiments are consistent with the notion that the mutations may be in a regulatory DNA sequence adjacent to the structural gene. This possibility is indicated in Figure 2, adapted from a recent paper by Holmes (4). Mutants in promoter or operator sites are known to show effects only in the *cis* configuration. However, as pointed out by Holmes and by Laird and Groman (6), such mutants can also be either nonsense mutants at the beginning of the structural gene or deletions for part of the structural gene.

The main physiological evidence for regulation of toxin synthesis comes from studies on the effect of iron concentration on the rate of toxin synthesis. It has been known for a long time that the iron concentration of the medium is critical for optimal yields of toxin. The mechanism by which iron controls toxin formation has not, however, been elucidated, and it is not known whether iron acts directly as a corepressor of the *tox* gene or indirectly, for example, by controlling toxin secretion. Yet a phage mutant that renders cells carrying this phage relatively insensitive to iron regulation has already been isolated (7). Further studies along these lines may eventually be helpful in elucidating possible mechanisms for the regulation of toxin synthesis.

Another approach toward the problem of regulation is the study of DNA-dependent cell-free synthesis of the toxin. This is achieved with extracts of *E. coli,* but not with extracts of the C7(-) corynebacterial strain (8). Yet the latter extracts are capable of promoting synthesis of other proteins. They appear to contain an inhibitor for toxin synthesis, effective in *E. coli* extracts, which acts either at the level of transcription or of translation of the *tox* gene. Identification of this inhibitor and its mode of action should also shed light on the way in which toxin synthesis is regulated.

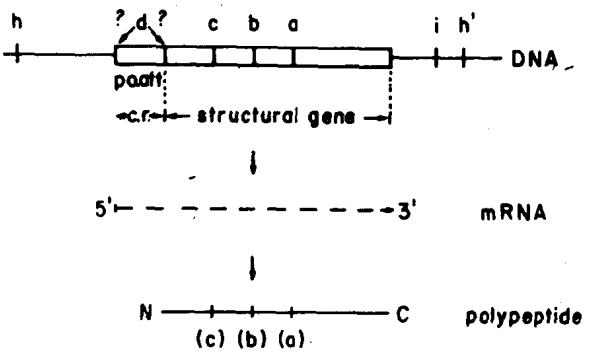

Figure 2. Hypothetical scheme for diphtheria *tox* gene transcription and translation [modified from Holmes (4)]. The control region contains a promoter site (p), an operator site (o), and an attenuator site (att). The sites of th. nonsense mutations on the *tox* genes and the corresponding sites of chain termination on the toxin molecule are indicated. Genetic markers on either side of the *tox* gene are h, h′, affecting host range and i, controlling immunity. c.r. = control region; d = CRM-negative mutation, affecting either a site in the control region or a site at the beginning of the structural gene. For further explanations, see text.

Finally, it should be noted that complementation studies with mutants have failed to reveal the presence of more than one phage gene for toxin production. This is in accord with the finding that the toxin consists of a single polypeptide chain.

2.3. Cholera Toxin

Cholera toxin, like diphtheria toxin has been characterized chemically and functionally. It is a protein of molecular weight 84,000. There are two unequal subunits: fragment A, molecular weight 29,000; and fragment B, molecular weight 10,600. The complete molecule consists of one copy of A and five of B, held together noncovalently. Fragment A consists of two peptides A1, molecular weight 24,000 and A2, molecular weight 5,000, linked by a single disulfide bond. The toxin exerts its effect by stimulating adenyl cyclase activity in intestinal cells, which leads to an increase in the level of cyclic AMP. This in turn stimulates the "sodium pump" and leads to a secretion of water into the lumen of the small intestine, causing diarrhea.

Genetic studies have not progressed so far as they have with diphtheria toxin. Mutants of *Vibrio cholerae* with reduced toxin production have been isolated following treatment with potent mutagenic agents such as NG and ethyl methanesulfonate (EMS) (9). For the screening of mutants, an immunological test is used that involves a precipitin reaction in agar plates containing antiserum and seeded with mutagenized bacteria. Nontoxigenic mutants can be recognized by the absence of a halo around the colonies after incubation. The amount of toxin produced in the mutants varies from one strain to another, but they all produce some toxin. One strain, M13, which was originally thought to be completely nontoxigenic, can be shown to produce a slight amount of toxin (0.1% of the wild type) in a very sensitive and specific-reversed passive hemagglutination assay (10). One mutant is found to produce large quantities of cell-associated toxin but little extracellular toxin. This strain may be blocked in the secretion of toxin (11).

There is a mating system in *V. cholerae* involving a sex factor P similar to the F factor of *E. coli*. Conjugal transfer mediated by P permits the mapping of genes. Two tox^- mutations including the one in M13 have been mapped by this procedure and shown to be near a gene for histidine biosynthesis on the chromosome (12). Among recombinants obtained in crosses with a multiauxotrophic recipient, strain linkage is found to a *his* locus but not to a number of other loci. These results show that at least one gene controlling the production of cholera toxin is located on the chromosome.

The toxin produced by the mutants used for mapping, though greatly reduced in amount, behaves in all testable respects like normal toxin. This finding suggests that the mutations mapped in these strains are in a regulatory gene controlling toxinogenesis rather than in a structural gene for the toxin. Thus the question of where the structural gene is located is open. It should also be noted that because of the presence of two dissimilar polypeptides in the toxin molecule, there may be two corresponding structural genes, although it has been shown that in rare cases one gene may code for two polypeptide chains (13).

2.4. Enterotoxins of *E. coli*

Enterotoxigenic strains of *E. coli* produce two kinds of toxin, a heat-labile one (LT) and a heat-stable one (ST). Some strains produce only LT, others only ST, and others

produce both. The genes for both kinds of toxin are carried on plasmids, called Ent plasmids. LT is a protein and is antigenic. Its mode of action is like that of cholera toxin, stimulation of adenyl cyclase activity. It also cross-reacts immunologically with cholera toxin. ST is not antigenic and has a molecular weight of less than 10,000. Its chemical nature and mode of action are not known.

LT has been purified in several laboratories and from a number of strains (14,15,16). The molecular weight estimations vary considerably, from 20,000 to 10^6. This may be due partly to strain differences and partly to the methods of purification, which may result in association of other molecules with LT. In our own work we have obtained a molecular weight of about 180,000 (17). We also have indications of the presence of two dissimilar subunits, one of molecular weight 40,000, the other of molecular weight 14,000. These results are preliminary and await further confirmation. So far there is no general agreement on the chemical nature of LT; indeed, more than one kind of LT may be produced in nature.

Recently we have observed synthesis of LT in cell-free extracts, which is dependent on the presence of Ent plasmid DNA. The toxin produced in the in vitro system is similar to the toxin obtained from culture filtrates in regard to behavior during purification and neutralization by antiserum against LT. The S 30 extract used was obtained from a nontoxigenic strain. Our results thus provide strong evidence for the notion that the structural gene for LT synthesis is actually located on the Ent plasmid.

So far no nontoxigenic mutants have been reported, and genetic studies have focused on the characterization of Ent plasmids, including physical studies on plasmid DNA (18,19). One observation of significance for the genetics and regulation of toxin synthesis is the enhancement of LT production when the cells are grown in the presence of mitomycin C (20).. This antibiotic also induces the production of colicins and phage lambda in a lambda lysogen. In phage lambda, induction is due to inactivation of lambda repressor. Mitomycin C fails to induce colicin production and prophage lambda in certain recombination-deficient (*rec A*) strains (21,22). We have introduced the *rec A* gene into enterotoxigenic strains and found that here also induction of toxin synthesis is abolished. These results suggest that LT synthesis is normally controlled in the cell and that the presence of mitomycin C interferes with this control mechanism, as it does in the case of phage lambda.

2.5. Problems for the Future

One of the main obstacles in genetic studies on toxin synthesis has been the lack of efficient methods for the isolation of mutants affecting toxin synthesis. One way in which recognition of mutants would be facilitated is the association of toxinogenicity with a phenotypic characteristic that can be scored in single colonies on agar plates. This is possible in the immunological test for cholera toxin production described above (9), but for such a method to work the cells must excrete large amounts of toxin. It would be most useful to have other easily recognizable characteristics, such as color changes of indicator dyes, associated with toxinogenicity, but so far such conditions have not been found. The introduction of specific mutations affecting easily recognizable characteristics into toxinogenic strains may be useful in this regard. For example, if a given toxin has an effect on adenylcyclase activity, introduction of a "leaky" adenylcyclase mutation into a toxigenic and a nontoxigenic strain may permit distinction between the two strains on the basis of characteristics based on the intracellular cyclic AMP

AMP concentration, such as the cAMP dependent fermentation of certain sugars (maltose, arabinose).

In the absence of a visually recognizable colony characteristics for toxinogenicity, one has to test individual clones directly for toxin production by one of the available assay methods. It thus becomes important to increase the frequency of mutants among clones to be tested in order to make mutant isolation a less laborious process. One way of doing this is to use "localized mutagenesis" (23). For example, it is known that NG-induced mutation occur mostly in the region of the DNA replication fork, which comprises in any one cell a short segment of the total genome (24). Because of the high efficiency of NG as a mutagen, one usually finds several mutations in a treated cell, clustered within a short segment. Thus if one can select for mutants in a gene near the *tox* gene, one has a good chance of finding mutants in the *tox* gene. This method is used to isolate mutants in a plasmid ColVB *trp*, which in addition to the tryptophan operon carries genes for colicin production and conjugal transfer. The plasmid used carries a mutation in the *trp* operon, which gives a high frequency of trp^+ revertants in response to NG treatment. Among such revertants, 37% are found to have a mutation for plasmid-mediated conjugal transfer (25). In experiments in which NG-treated cells are scored directly for transfer deficient mutants, this frequency is only 0.16%. Thus, by using the localized mutagenesis approach, the chance of finding a mutant among treated cells is increased by a factor of about 200.

Another problem for the future is the mechanism of transcription of *tox* genes into messenger RNA and the regulation of toxin synthesis at the transcriptional level. For several operons (tryptophan, lactose) it has been shown that the first structural gene is preceded by 50–200 base pair long control region of DNA, which contains the promoter site for initiation of transcription and the operator site for binding of repressor (26,27). For the *trp* and *his* operons, a second control site, the "attenuator," was defined (28,29) as the tryptophan, and lactose control regions were sequenced (26,27). It should be possible to carry out similar studies with *tox* genes. For example, by using the methodology of cloning restriction enzyme fragments, one should be able to obtain plasmids containing relatively short DNA sequences carrying *tox* genes. Cloning of a DNA sequence that carries the gene for ST enterotoxin has been described by So et al. (30). One could isolate promoter mutants for toxin synthesis in such plasmids and study the binding of RNA polymerase to DNA sequences carrying a wild-type or mutant promoter. In this way the promoter site for a *tox* gene could be defined. The distance between the promoter site and the beginning of the structural *tox* gene could then be determined by sequencing DNA segments surrounding the promoter sequence and determining the DNA sequence at the beginning of the structural gene by sequencing amino acids at the N-terminal end of the toxin molecule. Although this approach seems complicated, it may, in the absence of other biochemical or physiological methods, give useful information about regulation of toxin synthesis at the transcriptional level.

At the level of assembly of toxin molecules, it will be of interest to study the genetic relationship between the two portions of bifunctional toxin molecules (such as cholera toxin), the one involved in binding to the cell surface, the other in acting inside the cell. A somewhat analogous situation is seen in certain bacterial enzymes that have two activities. For example, carbamoylphosphate synthetase consists of two subunits coded by two adjacent genes (31). The *carA* subunit binds glutamine, the amino donor for carbamoylphosphate synthesis and displays glutaminase activity. The *carB* subunit performs the other steps of the reaction. The *carB* unit alone can catalyze carbamoylphosphate synthesis from NH_3, but not from glutamine. The *carA* gene coupled to *carB*

enables the enzyme to utilize glutamine. This dual arrangement is found in a number of other enzymes that utilize glutamine instead of NH_3. In the case of cholera toxin and possibly LT, we may have a similar arrangement. Two genes may be required to produce a complex molecule, one coding for a molecule having a specific function inside the cell, the other coding for a molecule having a more general function, namely, binding to a cell surface receptor. It will be of interest to see if the two postulated genes for cholera toxin or LT are adjacent to each other. This possibility is indicated by the fact that in the case of diphtheria toxin, there is a single gene for the bifunctional toxin molecule, which may have resulted from the fusion of two adjacent genes.

Finally, there is the question of how a toxin molecule is transported across the cell membrane during secretion. This may involve synthesis of a toxin molecule inside the cell containing a hydrophobic side chain that facilitates its passage through the membrane. This side chain may subsequently be cut off presumably by proteolytic enzyme activity. A mechanism of this kind appears to be involved in the secretion of a number of extracellular proteins, such as penicillinase in *B. licheniformis* (32) and secretory proteins in the dog pancreas (33). Evidence for such a mechanism can be provided by comparing intracellular or in vitro synthesized toxin with extracellular toxin. In preliminary experiments we have observed such a difference with LT of *E. coli*. We find that the larger of the subunits of LT synthesized in vitro has the same mobility in SDS gel electrophoresis as the one obtained from the exotoxin, but the smaller subunit obtained from the in vitro system moves somewhat more slowly, indicating that it is larger than the one obtained from the secreted toxin.

3. TRANSMISSION OF GENES INVOLVED IN TOXIN PRODUCTION

The genetic apparatus of many bacteria includes besides the chromosome, temperate phages, and plasmids. Genes for toxin production have been found in all three elements. In fact, in our examples, the *tox* gene for diphtheria toxin is located on a temperate phage, the *tox* genes for *E. coli* enterotoxins are located on plasmids, and the gene controlling cholera toxin synthesis is located on the chromosome. DNA of all three genetic elements can be transferred from one cell to another by any of the three known mechanisms of gene transfer: transformation, transduction, and conjugation. In addition, phage-located genes can be transferred by phage infection. In the first part of this section, we shall consider the transmission of *tox* genes from one cell to another.

During recent years it has become apparent that, within a cell, segments of DNA containing one or more genes can be transposed from one genetic element to another. As a result of these findings, the procaryotic genome is seen to be much more fluid than it was thought to be during the earlier years of bacterial genetics. The transposition of genes, which will be considered in the second part of this section, has become an important part of the general problem of gene transmission.

3.1. Gene Transmission Between Cells

The extent to which *tox* genes can be transmitted to different types of cells depends on the genetic element on which the *tox* genes are located. For chromosomally located genes, completed transmission to another strain requires incorporation of the transferred DNA into the chromosome of the recipient, and this in turn requires homology between donor and recipient DNA. Because of this requirement, the host range for chromosomal