Intracellular Bacterial Toxins: Origins and Effects

Bacterial genetics and host pathology have traditionally been two separate disciplines in microbiology, but recently they have been merging in studies of intracellular bacterial toxins. The ways that four such toxins affect host cells have been determined, as have some of the genetic mechanisms that allow bacteria to produce these toxins. These results are leading to new ways to provide active immunity against certain diseases and, because the toxins inactivate vital components of host cells, the results are of importance to those who study the molecular biology of mammalian cells.

The four intracellular bacterial toxins thus far studied in this connection are diphtheria toxin, *Pseudomonas aeruginosa* toxin (exotoxin A), cholera toxin, and *Escherichia coli* enterotoxin. Results of studies on the production of the three toxins other than diphtheria toxin are still scant. But recently substantial progress has been made toward understanding the synthesis of diphtheria toxin.

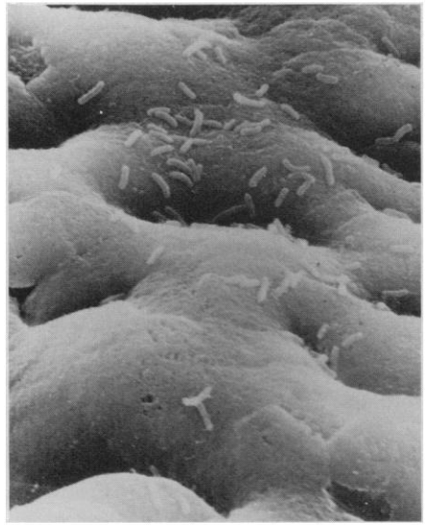
Diphtheria toxin is produced by strains of *Corynebacterium diphtheriae* that are infected with specific bacterial viruses, called β phage, that carry the toxin gene. John Murphy and his associates at Harvard University Medical School report that the viral gene coding for diphtheria toxin is under a different sort of control from that of the other genes of the β phage —a control that involves a factor synthesized by the bacterial host.

It has been known for about 40 years that substantial amounts of diphtheria toxin are produced only by infected *C. diphtheriae* when little inorganic iron is present. However, Murphy and his colleagues find that other proteins coded by genes of the β phage are synthesized regardless of the amount of iron in the culture medium. Murphy suggests that *C. diphtheriae* cells produce a protein that binds iron and then binds to a control region of the DNA of β phage to prevent the expression of the diphtheria toxin gene when iron is present.

In support of his model, Murphy points out that bacteria, other than *C. diphtheriae*, apparently do not produce such an iron-binding protein. For example, when the β phage DNA is translated in vitro by extracts from the bacterium *E. coli*, toxin is synthesized whether or not iron is present. The addition of extracts from *C. diphtheriae* cells that have no β phage DNA integrated into their DNA, however, inhibits toxin production in this in vitro system if iron is present.

If Murphy's model is correct, it should be possible to isolate β phage with mutations in the control region of the phage DNA that is recognized by the bacterial iron-binding protein. Murphy and his associates now believe they have isolated such a phage mutant. When bacteria of the species *C. diphtheriae* are infected with this mutant, they synthesize diphtheria toxin even in the presence of high concentrations of iron. The mutation is apparently in a control region of DNA to which the iron-binding protein adheres since the synthesis of diphtheria toxin cannot be prevented when DNA containing the toxin gene and its control region is introduced into cells infected by this mutant phage. This is evidence that the iron-binding protein can no longer recognize the mutant DNA and prevent synthesis of the toxin.

Like the control of toxin production, the molecular mechanism whereby diphtheria toxin kills mammalian cells is fairly well known. About 15 years ago, investigators at the laboratory of Alwin M. Pappenheimer, who is now at Harvard University, showed that diphtheria toxin kills cells by preventing protein synthesis. More recently, D. Michael Gill and his associates at Harvard University found that the toxin acts enzymatically inside the host cell. R. John Collier and his associates at the University of California at Los Angeles showed that the toxin requires nicotinamide adenine dinucleotide (NAD), which functions as a coenzyme in many cellular reactions, for its actions. Diphtheria toxin catalyzes the transfer of the adenosine diphosphate (ADP)-ribosyl fraction of NAD to elongation factor 2 (EF-2), which is involved in peptide chain elongation in mammalian cells. This transfer inactivates EF-2, and so the toxin prevents protein synthesis and kills the host cells.



Vibrio cholerae in the small intestine of the rabbit. ×4000 [Source: Edward T. Nelson, John D. Clements, and Richard Finkelstein]

Recently, Barbara Iglewski and David Kabat of the University of Oregon Medical School and their associates discovered that exotoxin A kills mammalian cells in the same way as diphtheria toxin. The mechanisms of action of the two toxins are so alike that they catalyze the transfer of the ADP-riboysl portion of NAD onto the same amino acid of EF-2 in a stereochemically similar way.

This identity between the modes of action of diphtheria toxin and exotoxin A is unexpected because the toxins are produced by unrelated bacteria, they are immunologically and molecularly different, they cause quite different diseases, and they have different species specificities. (The role of exotoxin A in human diseases has not yet been established, but this toxin does cause hypotensive shock in dogs and monkeys.) Iglewski and Kabat thus advance the hypothesis that the two toxins may have a common evolutionary origin. This implies that other bacterial toxins may be found that also act by this mechanism, Iglewski and Kabat believe. Nevertheless, they and others point out that, at present, it remains possible that the two toxins may have had different evolutionary origins and may have, by chance, evolved so as to have the same mechanism of action.

Apparently, bacteria can acquire genes for toxin production in several ways. The genes for diphtheria toxin are carried by a virus, but the genes for cholera toxin and *E. coli* enterotoxin are transmitted differently. Michael Vasil, Randall Holmes, and Richard Finkelstein of the University of Texas Southwestern Medical School found that the gene regulating the production of cholera toxin is located on the chromosome of the bacterium *Vibrio cholerae*, which produces this toxin. Eight years ago, genes coding for *E. coli* enterotoxin were shown by H. William Smith, now at Haughton Poultry Research Station in Huntingdon, England, to be carried by plasmids, which are small pieces of DNA that are separate from bacterial chromosomes and that can be transmitted from bacterium to bacterium.

Coincidentally, the modes of action of cholera toxin and *E. coli* enterotoxin, although different from that of diphtheria toxin and exotoxin A, are very similar to each other, according to Gill and his associates. Several groups of investigators

showed that both toxins cause host cells to produce large amounts of adenosine 3′, 5′-monophosphate (cyclic AMP) by activating the enzyme adenylate cyclase, which catalyzes the synthesis of cyclic AMP. Moreover, although both toxins normally act on cells of the small intestine, causing diarrhea, the toxins can also activate the adenylate cyclases of other kinds of mammalian cells.

Cholera toxin consists of two parts designated A and B, that are noncovalently associated. Gill and others have shown that the B subunits bind to specific receptors on the surfaces of cells, but do not themselves enter the cells. The A subunits enter the cells, whereupon a piece of each A subunit, called A_1, acts enzymatically to activate the cell's adenylate cyclase. (Each A subunit of cholera toxin consists of two peptides, A_1 and A_2, that are joined by a disulfide bond.)

By working with lysates (extracts of burst cells) from pigeon erythrocytes, Gill determined that components of the host cell are involved in the activation of adenylate cyclase catalyzed by cholera toxin. Intact pigeon erythrocytes respond only to the complete cholera toxin molecule, consisting of both portions A and B. Lysed erythrocytes, on the other hand, respond to fragment A_1 of subunit A but not to fragment A_2 or to the B portion of the toxin. However, erythrocyte ghosts (the membranes of lysed erythrocytes), on which adenylate cyclase is located, do not respond to A_1 unless the erythrocyte cytoplasm is present. This indicates that some components of the host cell cytoplasm are necessary for A_1 to exert its effects. Gill discovered that these components consist of NAD, adenosine triphosphate (ATP), and a soluble protein.

Gill, together with Doyle J. Evans, Jr., and Dolores Evans, both at the University of Texas Medical School, now find that a fragment of *E. coli* enterotoxin behaves the same way as fragment A_1 of cholera toxin. It too requires NAD, ATP, and another component of the host cell cytoplasm to activate the host cell's adenylate cyclase. Moreover, the active fragment of *E. coli* enterotoxin is about the same molecular weight (about 24,000) as fragment A_1 of cholera toxin and is inactivated by antibody against cholera toxin.

In addition to resembling cholera toxin in its enzymatically active portion, *E. coli* enterotoxin may also resemble cholera toxin in its segment that binds to host cell surfaces. Various investigators have found that antibody prepared against the B portion of cholera toxin inactivates *E. coli* enterotoxin. However, Gill reports that the two toxins bind to different surface receptors on host cells, so the B portions of the two toxins cannot be identical.

Finkelstein and his associates expect to use the immunological cross-reaction between cholera toxin and *E. coli* enterotoxin together with their ability to isolate mutants of *V. cholerae* with altered toxigenicity in order to develop new ways to immunize against cholera and similar diseases. According to Finkelstein, various nonspecific diarrheal diseases caused by bacterial toxins that immunologically cross-react with cholera toxin are responsible for greater morbidity and mortality than cholera. For example, a significant portion of cases of traveler's diarrhea are, apparently, caused by *E. coli* enterotoxin.

Since cholera and related diseases are localized infections caused by bacteria that inhabit the surface and the lumen of the small intestine, it has proved difficult to develop effective ways to immunize people against these diseases. Field studies conducted during the past decade have demonstrated that, although some degree of immunity to cholera can be produced with vaccines consisting of killed *V. cholerae* cells, the immunity is generally short-lived and the vaccines do not protect highly susceptible people, including infants and children, from developing cholera. Finkelstein and his associates are of the opinion that the most effective way to provide immunity to these diseases would be to develop stable mutant strains of *V. cholerae* that produce an altered toxin that does not harm mammalian cells. People could ingest cells of this mutant strain, whereupon the bacteria would colonize their intestines and the people would continually produce antibodies against the altered toxin. These antibodies, Finkelstein expects, would also inactivate cholera toxin, *E. coli* enterotoxin, and other related bacterial toxins. Moreover, the mutant bacteria would be passed from person to person in a population, as are other intestinal bacteria, so the immunity to the toxins would spread.

Finkelstein and his colleagues have screened large numbers of mutagenized *V. cholerae* in order to select suitable mutant strains to provide immunity. They have already isolated one avirulent strain and have given it to Richard Hornik and his associates at the University of Maryland Medical School who administered it to volunteers. Although the volunteers developed immunity to cholera without developing symptoms of cholera, some virulent *V. cholerae* were recovered from one volunteer. It is possible, but not yet established, that these virulent bacteria arose by a reversion of the mutant strain of *V. cholerae* to the wild type. Thus this first attempt at providing localized immunity to cholera and related diseases was not a complete success.

Since the four intraceulular bacterial toxins studied thus far affect essential components of host cells, molecular biologists are interested in understanding exactly why and how they affect those components and what other cellular processes the toxins may affect. Cholera toxin, in particular, has been used with success by biologists to study the effects of cyclic AMP in mammalian cells.

Cholera toxin is so selective in activating adenylate cyclase that investigators often assume that, if cholera toxin does not mediate a particular effect, the effect is not brought about by cyclic AMP. Cholera toxin inhibits delayed hypersensitivity, stimulates glycogenolysis causes release of growth hormone from the pituitary, causes insulin release by the pancreas, and affects numerous other events in which cyclic AMP plays a major role.

Various investigators are studying the ways bacterial toxins enter cells as a model of the way other substances, such as viruses, enter cells. According to Pappenheimer there are apparent similarities between the way diphtheria toxin enters cells and the way certain hormones, including chorionic gonadotrophin, luteinizing hormone, and thyroid stimulating hormones, may enter cells. For example, each of these hormones, like diphtheria toxin, consists of two subunits. One subunit binds to specific receptors on the surface of the target cell, whereupon the other subunit enters the cell. Thomas Moehring and Joan Moehring of the University of Vermont believe that studies of how diphtheria toxin enters cells may yield information on how certain viruses enter cells. They find that mutant cell lines that are impermeable to diphtheria toxin are also impermeable to a number of viruses.

New prospects for immunization against cholera and related diseases and for understanding the biochemistry of mammalian cells are emerging from the coupling of genetic and pathological studies of intracellular toxins. From analyses of how toxin production is genetically controlled, investigators can devise ways to select for bacteria that produce altered toxins. These altered toxins can be used in immunizations and in studies of how toxins affect host cells. And research on how these toxins react with EF-2 or adenylate cyclase provide a means to understand the relations between structure and function of these cellular components.—GINA BARI KOLATA