involve some sort of transposable genetic element capable on the one hand of allowing or preventing pilus synthesis and on the other of altering the quality of the pilin produced (presumably by altering only the C-terminal end of the pilus protein). It is intriguing that a set of equal-sized (160 base pair) *Hae*II restriction fragments can be detected both within and adjacent to the pilus-coding region of the MS11 genome. Whether these are part of some invertible sequence controlling pilus expression remains to be seen. It is also unclear whether post-translational modification of envelope components has any role in mediating the changes in gonococcal surface properties.

Nevertheless, the observation that chromosome rearrangements can affect virulence is a very important step towards understanding the extremely complex genetic and phenotypic interactions between this important pathogen and its human host. □

Bacterial toxins and cyclic AMP

from Simon van Heyningen

It has been known for several years that cholera toxin and the very similar protein toxin of some strains of *Escherichia coli* enter intoxicated cells and activate the intracellular adenylate cyclase that catalyses the formation of cyclic AMP from ATP. In cholera patients, this increase in cyclic AMP in gut cells is the chief cause of the massive diarrhoea that is characteristic of the disease. Several recent papers have shown that other toxins also increase the cyclic AMP concentration. In view of the demonstrated connection between adenylate cyclase and cholera, it is surprising that, until recently, little attention had been given to the possibility that the soluble adenylate cyclases secreted by a number of bacteria play a role in virulence. (These cyclases differ from the mammalian enzymes in that they are more active, soluble, heat stable and are not usually regulated by guanine nucleotides.)

A picture is now emerging, however, as a result of recent work, particularly that by Leppla[1], on the toxins of *Bacillus anthracis* which causes anthrax. Three different proteins produced by this organism can be purified: protective antigen (PA or factor II), lethal factor (LF or factor III) and oedema factor (EF factor I). None of these has much biological effect by itself, but combinations of PA with either of the others do: PA and EF produce a skin oedema, and PA and LF are lethal.

Leppla guessed that EF might have a similar action to that of cholera toxin because it has similar effects on vascular permeability in rabbit skin and on the morphology of Chinese Hamster Ovary (CHO) cells. Accordingly, he showed that PA and EF together greatly increased the cyclic AMP concentration in CHO and other cultured cells — however this was not due to increased activity of the adenylate cyclase in the cells, but to enzymatic activity of EF itself. When EF was tested *in vitro*, it was found to be a highly active cyclase, but only in the presence of a CHO cell lysate. The lysate could be replaced by calmodulin (known to be an activator of many adenylate cyclases) and the activity of the enzyme varied with calcium ion concentration in a manner characteristic of calmodulin-dependent systems. In whole cells, EF was active only in the presence of PA which presumably functions in some way to aid EF entry into the cell. (It must also have this function with the other protein toxin, LF, which is not a cyclase.) EF was thus the first example of a bacterial cyclase that enters eukaryotic cells and is activated by a eukaryotic protein. The relevance of all this to anthrax is not clear — the mixture of PA and LF has generally been thought to be the lethal agent, and they may mask the effects of adenylate cyclase during the disease.

Bacillus anthracis is not the only pathogen that secretes adenylate cyclase. *Bordetella pertussis*, responsible for whooping cough, also produces a highly active adenylate cyclase that is heat stable and stimulated by calmodulin[2]. Confer and Eaton[3] have shown that this enzyme can be taken up by phagocytic cells and generate cyclic AMP. Human neutrophils incubated with extracts from *Bordetella* prepared by urea extraction followed by dialysis showed a striking increase in intracellular cyclic AMP concentration, and the cyclase activity in the cells was heat stable, showing that it must have come from the bacterium. How it got into the cells is not clear — purified bacterial cyclase is very active but only with broken cells. A temperature-dependent process is involved, as well as other factors secreted by *Bordetella* (perhaps analagous to the PA of anthrax toxin).

What is the function of the increase in cyclic AMP? It is possible it is used by the invading bacteria to supress many of the normal functions of phagocytes, including bacterial killing, and so allow the bacteria to survive longer in a hostile environment. It may also explain the impaired host defence, for example the absence of fever and frequent secondary bacterial pneumonias, found in whooping cough.

Toxin production by *Bordetella pertussis* is complicated and confusing. As well as the extracellular cyclase, the bacterium secretes as many as twenty other antigens. One of these, known as 'pertussis toxin', or islet-activating protein, because there is good evidence that it causes the main harmful effects of whooping cough, has many biological effects, such as potentiation of the immune response, induction of lymphocytosis and sensitization to histamine, which may also be due to an increase in cyclic AMP. However the toxin itself is not a cyclase — it works, like cholera toxin, by activating the cyclase of the intoxicated cell. ATP and NAD^+ are required in broken cell preparations and, like cholera and diphtheria toxins, pertussis toxin catalyses the transfer of ADP-ribose from NAD^+ to a receptor protein[4]. This receptor protein has a molecular weight of 41,000 and is presumably part of the adenylate cyclase complex, but it is not the same protein as the one ADP-ribosylated by cholera toxin. Thus *Bordetella pertussis* secretes both an adenylate cyclase of its own and a protein that activates another cyclase. Perhaps the 'toxin' is responsible for the overt symptoms of disease and the cyclase for the ability of the bacteria to survive.

It is remarkable that bacteria can produce proteins that have such similar properties to eukaryotic cyclases and that can be activated by a eukaryotic protein — calmodulin — which is not known to occur in bacteria. It suggests the proteins might have originated in some eukaryotic cyclase which the bacteria have picked up during the course of their evolution.

The same suggestion has been made of the toxins that catalyse ADP-ribosylation. Some recent work suggests that the hormone thyrotropin can increase ADP-ribosylation of several proteins, including the substrate of cholera toxin, in thyroid cell membranes at the same time as adenylate cyclase activity[5]. It seems that the hormone does not catalyse the reaction directly, but increases the activity of endogenous ADP-ribosyl transferases which are known to be present and are presumably part of the normal control process. Cholera, pertussis and other opportunistic toxins are thus subverting this process to their own ends. Since cholera and pertussis toxins ADP-ribosylated different proteins, the system must be a very complicated one. □

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