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Our attempt to repeat this experiment at the Valley of Ten Thousand Smokes in Alaska, the still-hot site of the largest eruption on Earth in this century, ran afoul when the Department of Interior objected to "mechanized" research in the national park containing the site. (*8*). A bold proposal to drill the seething throat of White Island Volcano in New Zealand failed to surmount obstacles of safety and funding (9). Another project is in an early planning stage as a result of an international symposium in Shimabara, Japan, in May of this year: a proposal to drill to the conduit of Unzen Volcano (*10*), active from 1991 to 1995 in an eruption that did $2 billion of damage and took 44 lives.

Successful volcano drilling projects must carefully address issues of safety, funding, environmental protection, property ownership, subsurface targeting, directional drilling in an extreme environment, and public relations. Each of these issues has been overcome at one time or another in other scientific drilling projects, but they have yet to be tackled together in an international project at a single, well-watched, recently active volcano. When they are, the results will permanently change textbook depictions of how volcanoes work and will greatly improve the basis for eruption predictions. Such efforts may ultimately allow drilling into the source chamber itself, sampling live magma quenched in situ. These samples could perhaps answer the age-old question of how the magma "still" works, that is, how magmas as sluggish as molten granite, the key ingredient of continental crust, fractionate so rapidly and cleanly.

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CELL BIOLOGY

Death by Lethal Injection

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Gram-negative bacteria of the genus *Yersinia* cause human diseases that range in severity from distressful gastroenteritis to the horrific Black Death. The success of the Yersiniae in these nefarious endeavors requires that all three of the responsible species overcome the sophisticated defense mechanisms devised by a vigilant host. For example, the Yersiniae must kill macrophages quickly, before these voracious phagocytes devour them. To combat this huge and powerful enemy, these bacteria use a fiendishly clever molecular injection device. When the macrophage contacts these bacteria, a necessary prelude for bacterial engulfment, a specialized secretion channel is opened that allows direct transfer of certain toxic proteins from the bacterial cytoplasm to the cytoplasm of the mammalian cell (see figure, right). These toxic proteins, termed Yops (*Yersinia* outer proteins), effectively paralyze and incapacitate the defender, allowing the bacteria to escape and continue to grow and multiply (*1*). This scenario is analogous to the bite of a poisonous insect or snake inflicted on a predator, except that the contestants are single cells, one prokaryotic and one eukaryotic.

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The discovery of this molecular injection device 4 years ago sparked intense interest, but several key questions remain. One of these is to discover how Yops are marked for injection. On page 1140 of this issue, Anderson and Schneewind (*2*) adapt the logic of a classic experiment in molecular genetics (*3*) to provide compelling evidence for a novel targeting signal or "zip code" within the messenger RNA (mRNA) that specifically tags Yops for secretion.

Most proteins targeted for translocation from the bacterial cytoplasm are made initially in precursor form with a signal sequence of 15 to 30 amino acids located at the amino-terminus. This signal targets the precursor to a complex of Sec (Secretion) proteins that physically moves the precursor across the cytoplasmic membrane. During the translocation reaction a special pro-

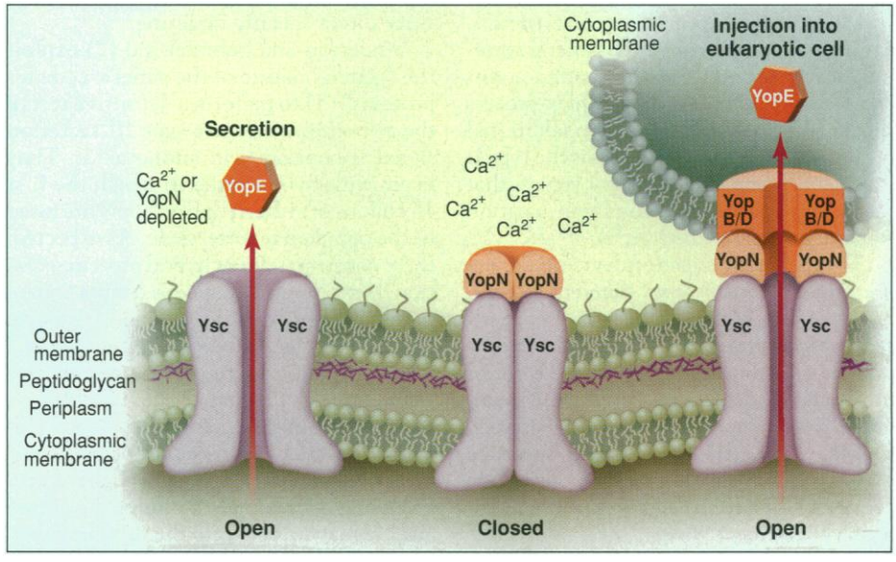

ILLUSTRATION: K. SUTLIFF

The Ysc type III secretion system. The apparatus (**center**) is normally kept closed by YopN, which acts as a cork. The system can be opened either by removing Ca^{2+} or YopN, allowing secretion of Yops (**left**). A microinjection device is formed upon contact of Yops with a eukaryotic cell (**right**). Then Yops pass through the type III system and YopB/D directly into the cytoplasm of the eukaryotic cell. [Adapted from (*1*)]

tease, termed signal or leader peptidase, removes the signal sequence. Signal sequences and Sec proteins are conserved throughout the biological kingdoms. Accordingly, this secretion system is sometimes called the general secretion pathway (*4*). Despite the absence of primary sequence identity, signal sequences share common physical and structural properties, and they are easily recognized by the cell and by the experimenter (*5*).

In Gram-positive bacteria, proteins that are translocated by the general secretion pathway are effectively secreted into the environment. However, Gram-negative bacteria have an outer membrane (see figure), and in these organisms, proteins translocated by the general secretion pathway end up in the periplasm. Gram-negative bacteria can and do secrete proteins into the environment, but with few exceptions this requires additional gene products or a completely different cellular machinery. The machinery for Yops is sometimes called a type III system to distinguish this mechanism from other major routes of protein secretion.

The simplest Gram-negative secretion system, at least in terms of number of components, is the type I system in which proteins are not made in precursor form, they have no signal sequence, and they are secreted directly from the cytoplasm to the environment by a specialized machinery consisting of two cytoplasmic and one outer membrane proteins. Although a precise targeting signal has not yet been identified, it seems to lie near the carboxyl-terminus of the secreted protein within the last 100 amino acids (*6*).

Many other proteins are first translocated to the periplasm by the general secretion pathway and then transported across the outer membrane by a complex process that requires more than a dozen additional proteins. This two-step process is the type II secretion system. The signal or signals that target proteins for the second translocation step are not understood (*4*, *6*).

The molecular injection device in the figure (right) is really two different protein translocation systems. The actual type III system is composed of 22 Ysc (Yop secretion) proteins and translocates Yops directly across the bacterial cytoplasmic and outer membranes (see figure, left). In the laboratory, this type III system can be made to secrete Yops in the absence of a partner eukaryotic cell, most simply by depleting the growth media of calcium (*1*).

The Yop proteins are not made in precursor form, they have no signal sequence, and they can be classified in three functional groups. First, the effector Yops (for example, YopE) are proteins that are injected into, and subsequently kill, the eukaryotic cell. YopB, and perhaps YopD, form the second translocation system that transports the effector Yops into the eukaryotic cell. YopN is special, in that it forms a "cork" that plugs the Ysc type III system. Mutants that lack this cork secrete the remaining Yops continuously, regardless of calcium concentrations or the presence or absence of a eukaryotic cell (*1*).

Proteins delivered by this microinjection device must contain two targeting signals. The first directs secretion from the bacterial cytoplasm via the Ysc type III system, and the second directs entry into the eukaryotic cell via YopB/D. Because in the laboratory the type III system can work without the eukaryotic cell, the type III secretion signal can be analyzed independently.

A standard method for detecting targeting signals (*7*) requires fusing bits of a particular *yop* gene to a reporter gene that specifies a reporter function that can be easily detected. By comparing which hybrid genes direct reporter secretion, and which do not, one can deduce the minimum *yop* gene sequence required. Results of such molecular cutting and pasting showed that targeting signals are contained at the very beginning of the *yop* gene. This is reminiscent of signal sequences, and accordingly it was reasonable to propose that targeting signals were contained at the amino-terminal end of the Yop, within the first 15 to 17 amino acids (*1*). In marked contrast to signal sequences, however, there is no common feature shared by amino acid sequences at the beginning of the 12 known Yop proteins. How could sequences that are so diverse direct specific targeting?

Anderson and Schneewind (*2*) exploit the "general nature of the genetic code for proteins" (*3*) to perform a definitive test of the hypothesis that the type III secretion signal is contained in amino acids. They begin with hybrid genes in which the first 15 codons of either YopE or YopN are fused to the common reporter gene. As expected, these constructs direct hybrid protein secretion. Next, they introduce two mutations, a frameshift (for example, they add or remove a DNA base pair) after the start codon and a compensating suppressor frameshift (for example, they remove or add a DNA base pair) at the fusion joint after codon 15. These two frameshift mutations cause minor changes in the DNA and the corresponding mRNA, but they alter the amino acid sequence between them completely. Secretion is strikingly unaffected. Therefore, we must conclude that the secretion signal is not contained in the Yop amino acid sequence. Rather, the signal must lie in the Yop messenger RNA instead.

The consequences of this prediction are remarkable. If this type III secretion signal lies in the Yop mRNA, then some Ysc protein must recognize and deliver Yop mRNA to the secretion apparatus. Moreover, translation and secretion must be coupled. If synthesis occurred before delivery, then no secretion could occur. Anderson and Schneewind (*2*) propose a model in which the 5′ end of Yop mRNA folds into a structure that prevents translation initiation. Interaction with the secretion machinery would simultaneously relieve this block and promote secretion. Data supporting this attractive model are presented, but additional studies are required to exclude alternative explanations for these results. Now that researchers know to look at the mRNA, more details about this secretion signal should be rapidly forthcoming.

An understanding of the type III secretion system is important for several reasons. First, many different bacteria use these systems to enhance their virulence in different ways (*1*, *8*). Detailed knowledge of this secretion mechanism could suggest novel targets for new antibacterial agents. Second, the biotechnology industry has long sought an efficient method for using bacteria to produce and secrete proteins. The proteases produced by Gram-positive bacteria complicate their use. In Gram-negative bacteria the outer membrane remains a formidable barrier for the GSP, and type I and II systems promote secretion of heterologous proteins poorly, if at all. Type III secretion systems seem to work well (*1*, *2*, *9*, *10*). Moreover, hybrid constructs that contain the 5′ end and at least the first 50 codons of *yopE* can direct not only reporter secretion from the bacteria, but translocation into a eukaryotic cell as well (*10*). This ability to deliver specific proteins into eukaryotic cells could open new vistas for research, industry, and medicine.

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