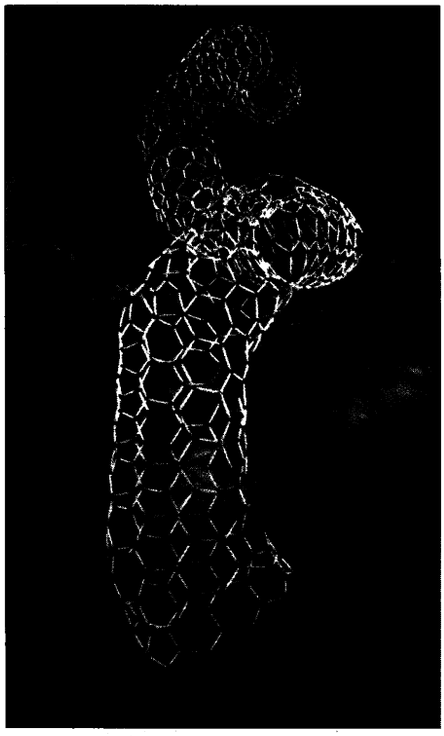

Fig. 3. Two helix turns of the same coiled, polygonized tubule as seen more longitudinally. [Figures courtesy of A. A. Lucas, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium]

the intersection of like-sized tubes at 30° could be accomplished by introducing a five-membered ring on the outer point of contact and a seven-membered ring at the inner point. Inspired by this model, Amelinckx *et al.* deduced that their structures could, in fact, be derived from periodic pentagon-heptagon arrays that served as joints for straight tubes. In calculations of structural stability, Ihara *et al.* (*12*) had deduced that curvature could be accomplished by an appropriate array of pentagons and heptagons in the hexagonal mesh and had predicted that some structures would be favored over others.

The mechanisms of tube growth, like those responsible for fullerene growth, have long stimulated the imaginations of those dealing with such structures. Although much remains to be learned, Amelinckx *et al.* have provided considerable food for thought, at least with respect to the kinetics of the process. In their report, they have described "extrusion" of carbon from the metal particle as though it were a continuous medium, considering the kinds of shapes that could be produced. They have examined straight tube extrusion from a circular starting configuration under the assumption that growth proceeds from the contact area with the metal particle. The more interesting geometries that they discuss involve greater quantities of carbon being supplied at one part of the tube base than another, resulting in a curved tube, and the effects of noncircular contact areas with the particle.

Future investigations, both theoretical and experimental, must resolve issues related to the interaction of the carbon atoms with the catalyzing particle at the point of contact. From early studies of filament growth, it was concluded that carbon atoms dissolve in the catalyst and diffuse through it to the point where the tube is grown (*13*). It is fascinating to imagine the nucleation of a tube on a metal surface, that is, considering the movement of carbon atoms and the pathways that lead them to produce chains from which the first complete rings form. The assembly of this ring must reflect surface interactions with the particle and, as Amelinckx *et al.* (*3*) conclude, this structure defines the orientation of the nascent tube. The motion of carbon atoms as they pop through the surface or diffuse to the growth point would make a most stimulating video, although the time scale of such processes is not amenable to even the fastest imaging probe.

As Baker, one of the seminal figures in carbon research, recently noted, "filamentous carbon is a material which fulfills the axiom that one man's garbage is another man's treasure" (*13*), referring to the fact that carbon deposits can lead to undesirable performance in many applications. It continues to be the case that carbon structures offer exciting new perspectives into the state of matter, now with nanoscale dimensions.

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Phage Assembly: A Paradigm for Bacterial Virulence Factor Export?

Marjorie Russel

Because they are so genetically and biochemically tractable, bacterial viruses (bacteriophages) have long been used as tools for investigating fundamental biological processes—identifying signals, proteins, and mechanisms that govern replication, transcription, and translation. Now, analyses of protein-protein interactions that occur during assembly of filamentous phage are likely to be relevant to a complex cell biological question—how many bacterial pathogens export the proteins that enable them to interact with and exploit their plant and animal hosts? The unexpected parallels between these processes confirm the power of basic research in one area to fertilize another and, perhaps, to generate useful ideas.

At least three distinct systems mediate extracellular export of proteins in Gram- negative bacteria. Two of them are complex, with 12 to 14 different proteins required in the Type II systems of plant pathogens like *Erwinia* and *Klebsiella oxytoca* (*1*) and probably 13 or more Ysc proteins in the Type III system exemplified by pathogenic *Yersiniae* (*2*). Each contains a protein with an adenosine triphosphate (ATP)–binding motif, but the constituents of these two export systems are otherwise unrelated, with one notable exception. The exception is a protein also homologous to an essential morphogenetic protein (pIV) of filamentous phage. A third system present in *Haemophilus influenzae* may mediate export and assembly of a cell surface structure, the "transformasome"; transformation in these bacteria requires a homolog of pIV, as well as proteins that are unrelated to the other Type II or III proteins (*3*). Filamentous phage also encode an essential morphogenetic protein (pI) with an ATP-binding motif.

Several bacteria export virulence factors by means of the Type III set of proteins, even though they interact with their eukaryotic hosts quite differently to cause disease (*4*). Thus, pathogenic *Yersiniae* (including Y. *pestis*, the agent of the plague or

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Black Death) adhere to the surface of phagocytes and inactivate the phagocytic process through the action of at least two exported bacterial proteins (Yops). YopE is expressed on contact with a suitable target (HeLa cells in this case) and is secreted directly from the bacteria into the HeLa cell cytoplasm, where it leads to depolymerization of the actin microfilament network (*5*). Other Yops, including a tyrosine phosphatase (which resembles corresponding enzymes of eukaryotic origin from which it might have derived), are also likely exported directly into the target cell cytoplasm where they may act by disrupting signal transduction (*6*). *Shigella flexneri* undergoes a different interaction with its host to cause dysentery; these bacteria are taken up in phagolysosomes and must exit from this vacuole into the cytoplasm to cause disease. Nonetheless, the set of *mxi/spa* genes necessary for export of virulence (Ipa) proteins is homologous to the *ysc* genes (*7*). By using a clever scheme that allowed mutant *Shigella* to escape from the phagolysosome, Zychlinsky and co-workers (*8*) identified IpaB as a cytotoxic protein necessary for apoptotic killing of macrophages. In *Salmonella typhimurium*, the *ysc*-related *inv* genes are necessary for its invasive properties (*9*). Large appendages transiently form on the surface of *S. typhimurium* on contact with cultured epithelial cells; certain *inv* mutants (including *invG*, the *gene IV* homolog) are defective in the appearance or disappearance of these structures and are unable to enter epithelial cells (*10*). Type III genes (including a *gene IV* homolog) have also been identified in several plant pathogens, where they are required to export Harpin, a protein that elicits rapid local necrosis in nonhost plants (the hypersensitive response) (*11*, *12*).

Type II export systems also occur in both plant and animal pathogens. In *Erwiniae*, which cause soft-rot disease in plants, degradative enzymes (such as pectinases and cellulases) are exported. In animal pathogens like *Neisseria gonorrhoeae* a Type II system mediates export and assembly of type 4 pili (*13*), cell surface structures necessary for adherence to eukaryotic cells. Despite the similarity of the Type II systems, even close relatives (*Erwinia chrysanthemi* and *carotovora*, *Pseudomonas aeruginosa* and *putida*) cannot export one another's enzymes (*14*, *15*). *Pseudomonas aeruginosa* contains two sets of Type II genes, *xcp* genes required for export of lipases and other enzymes and *pil* genes for assembly of type 4 pili (*16*, *17*). The major plant pathogens—*Pseudomonas*, *Xanthomonas*, and *Erwinia*—contain both Type II and Type III systems (*18*, *19*).

Some functional differences between the two systems are known. Enzymes exported by the Type II system are synthesized with conventional signal sequences, and their externalization also requires Sec functions (*1*). They are secreted into the periplasm (the default location when the export proteins are absent) and must fold properly to be exported (*20*, *21*). No simple signal capable of directing them across the outer membrane has been found (*22*). By contrast, proteins exported by the Type III system lack a classical presequence; a short, amino-terminal sequence appears to be necessary and sufficient to target proteins for export (*4*). These proteins presumably remain in the cytoplasm when export is blocked.

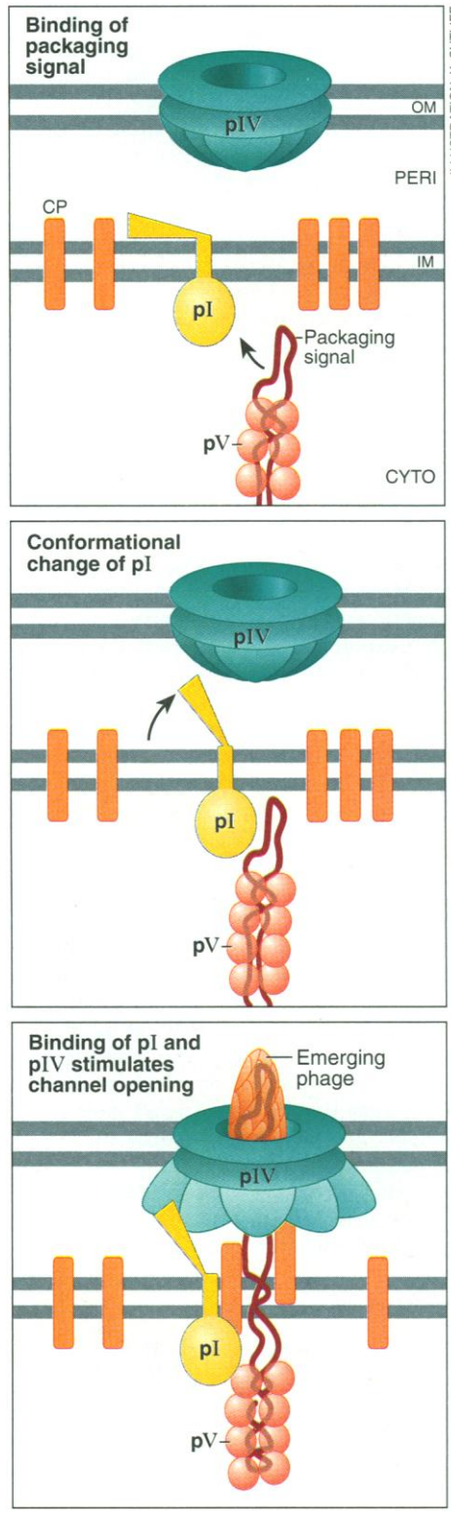

ILLUSTRATION: K. SUTLIFF

Proposed triggering of pIV channel opening during filamentous phage assembly. Association between the packaging signal in phage single-stranded DNA and the cytoplasmic domain of pI (*top*) causes a conformational change in the periplasmic domain of pI (*middle*). This allows the periplasmic domains of pI and pIV to associate (*bottom*), triggering pIV channel opening and allowing the nascent phage particle—the phage DNA, newly released from the DNA-binding protein (pV) and coated with coat proteins (CP)—to assemble and exit.

Most of the Type II export proteins are integral inner membrane proteins (*23*), and only one, the pIV homolog, is in the outer membrane. This preponderance of essential inner membrane proteins is puzzling for an export system that appears to translocate periplasmic substrates across the outer membrane. Although it is widely assumed that the export proteins in both systems form an "export organelle," no evidence is yet available. Little is known of the specific functions of the export machinery proteins except that in the Type II system, one component cleaves and methylates four others. These four proteins are similar to pilin and might form a pilus-like structure as part of the secretion apparatus (*24*). The sole cytoplasmic protein is membrane-associated and contains an essential nucleotide-binding motif (*25*, *26*). Several Type III machinery components have putative membrane-spanning domains, and the pIV homolog is in the outer membrane (*7*, *27*).

Similarities between pIV family members (currently four phage and 16 bacterial proteins) extend over 200 to 300 amino acids in the carboxyl-terminal portions of the proteins. Multiple sequence alignments highlight a number of better conserved blocks within this region in which the conserved residues are hydrophobic amino acids, glycines, and prolines. Within the best conserved, 60–amino acid block, one glycine and one proline are invariant, and substitution at either renders pIV nonfunctional for phage assembly (*28*). The amino-terminal 200 to 500 residues of the Type II, Type III, and pIV family members are unrelated, and within each group sequence variation is greatest in this region.

The striking relation between these bacterial and phage proteins probably reflects the similarity in the processes in which they participate. Filamentous phage (f1, M13, and many others) resemble pili structurally and morphologically, and they, too, are exported from the bacterium as they are assembled. The phage coat proteins are transfered from the cytoplasmic membrane to cover the single-stranded DNA genome of the

phage as the nascent particle is extruded. Infected bacteria are not killed, but continue to grow and divide and release phage.

Two morphogenetic proteins (pI and pIV) are essential for particle formation. Because pIV is an outer membrane protein, whereas the phage components are cytoplasmic (the single-stranded DNA, coated with multiple copies of pV, the phage-encoded DNA binding protein) or in the inner membrane (the coat proteins), pIV might form a phage-conducting channel. A similar suggestion has been made for the Type II homologs. If so, these channels must be very large, much larger than the 11 Å diameter of the trimeric porin channels that allow passive diffusion of small (600 daltons) molecules across the outer membrane into the periplasm (*29*). Filamentous phage are 65 Å in diameter, type 4 pili are 54 Å, and some of the exported bacterial proteins are large (130 kD) and fold in the periplasm before export across the outer membrane. No direct evidence for such a channel has yet been obtained in any system, but the recent finding that pIV forms a homomultimer is an important first step. The pIV extracted from cell membranes with nonionic detergents is a complex composed of 10 to 12 monomers (*30*). The bacterial homologs are likely to form multimers as well, because pIV forms mixed multimers with some of these homologs (*30*). Both pIV and these homologs induce transcription of an *Escherichia coli* operon (*psp*) that promotes bacterial survival under extreme conditions (*31*, *32*), which further points to a commonality of action.

The second phage-encoded morphogenetic protein (pI) spans the inner membrane once, leaving a large, amino-terminal domain that contains the ATP-binding motif in the cytoplasm (*33*). Genetic evidence suggests that this domain recognizes the packaging signal in phage DNA (*34*, *35*) and that the periplasmic domain of pI interacts with the periplasmic, amino-terminal portion of pIV (*36*, *37*). This latter interaction, and a genetically defined interaction between pI and the major coat protein, can account for the failure of pIV (or pI) from one filamentous phage to substitute for another. Because a simple diffusion channel of the requisite size would render *E. coli* exquisitely sensitive to membrane perturbants (*38*, *39*)—and pIV does not—if a pIV channel exists, it must be gated. The inferred interaction between the periplasmic domains of pI and pIV suggests a way that channel opening might be gated to initiation of phage assembly. Binding the packaging signal to the cytoplasmic portion of pI could trigger a conformational change in the periplasmic portion of pI that enables it to associate with the exposed domain of pIV (that is, the gate). This association could stimulate channel opening and allow particle elongation and export. Newly isolated gene IV mutants with substitutions at an invariant glycine do render *E. coli* highly sensitive to membrane perturbants (*28*); they may form constitutively open pIV channels.

Many of the ideas about how pI and pIV function in filamentous phage assembly and export remain to be tested, and their applicability to the bacterial export systems further explored. Is phage assembly a related but simpler export process, or does it require the participation of additional host proteins? Recently discovered Type II export proteins in *E. coli* (*40*) are potential candidates. Do the variable, amino-terminal domains of the bacterial homologs of pIV provide the remarkable specificity of the export systems? Do these domains interact with another component of the export machinery that serves a function analogous to the membrane-spanning and periplasmic domain of pI? What special features of the *Yersinia* export system (or of its pIV homolog, YscC) enable it to translocate some (or all) Yops across bacterial and target cell membranes? Answering these questions will go a long way toward explaining the details of the remarkable miniature machines that mediate both phage and bacterial pathogenesis.

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