PERSPECTIVES: STRUCTURAL BIOLOGY

How Chaperones Protect Virgin Proteins

David Eisenberg

Molecular chaperones are proteins found in all cells that help other proteins to fold and assemble. The duty of one family of these chaperones is to coordinate the assembly of pilin protein subunits into adhesive pili—the rodlike structures that enable pathogenic Gram-negative bacteria such as *Escherichia coli* to adhere to and colonize host tissues. The chaperones bring the subunits one by one to a large channel protein in the bacterial outer membrane called the usher. Here the pilins are released and become attached to each other to form the pilus, which is composed of a thick rod connected to a thin tip (the fibrillum) by adaptor proteins.

Two reports in this issue of *Science* (*1, 2*) lift a corner of the great veil that has obscured the action of these bacterial chaperones. Choudhury *et al.* (*1*) and Sauer *et al.* (*2*) present the crystal structures of the FimC and PapD chaperones bound to their respective pilin subunits, which form the type 1 and type P pili of uropathogenic strains of *E. coli*. It has not escaped the notice of the investigators that these chaperone-subunit structures immediately suggest an elegant mechanism for the intricate assembly of pili.

Since the pioneering experiments of Anfinsen earlier this century, biochemists have believed that it is simply the order of the amino acids in a protein that determines how it folds into a three-dimensional structure and assembles into complexes with other proteins. Yet Anfinsen realized that this simple "thermodynamic hypothesis ... is open to many refinements ... Another large molecule (for example, an antibody, another protein, or possibly even the same protein) could influence the folding process by intermolecular interactions" (*3*). Nearly 40 years after Anfinsen proposed his thermodynamic hypothesis, we now know that there are more than 20 families of molecular chaperones that assist in the noncovalent assembly of protein structures (*4*). What is more, there are many cases where interaction of a protein molecule with another of its kind influences the structure of both (*5*).

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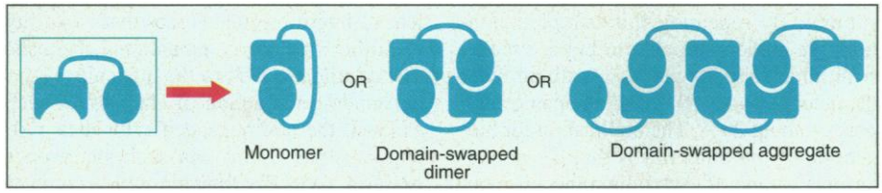
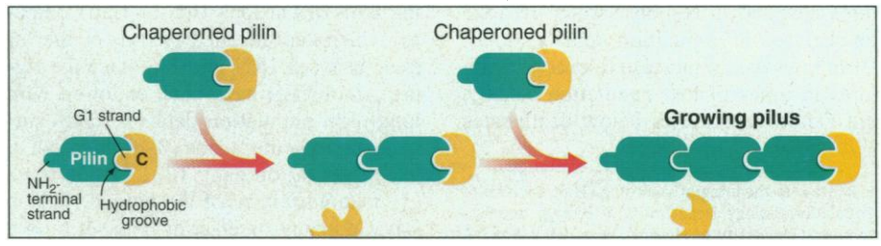
Imagine the frustration that a newly synthesized (virgin) protein feels as it seeks its thermodynamically stable structure in the crowded milieu of a cell (see top figure). If there are many partially folded copies of the same protein close to each other, domains of two adjacent proteins may fit together, forming a 3D domain-swapped dimer. This dimer has domain interfaces that are identical to those of the protein monomer but with a very different conformation in the protein chain. Under certain circumstances this domain swapping could continue indefinitely to form an extended insoluble aggregate, a state frequently found by protein chemists attempting to purify and refold proteins. It has always been assumed that the main function of molecular chaperones is to somehow protect folding proteins from associating prematurely and forming insoluble aggregates. But how do they do this? The mechanism of this protection has been intensely studied (*6–8*). An impressive x-ray structure exists for the bacterial chaperone GroE, and there are many ideas about how it might protect newly synthesized protein chains from associating with each other as they fold, although no definitive mechanism has yet been identified (*9, 10*).

Both the Choudhury and Sauer reports present the crystal structure of a complex between a bacterial molecular chaperone and its associated pilin subunit. In both structures, the pilin domain (which enables one pilin subunit to bind to the next) has an immunoglobulin-like fold composed of antiparallel β strands. However, the final β strand is missing from the fold, exposing a hydrophobic groove on the surface of the pilin subunit. Into this groove fits the β strand (the G1 strand) of the molecular chaperone, which complements the pilin structure, apparently resulting in the formation of a stable, water-soluble complex. The authors call this process "donor strand complementation" (complementation being the formation of a stable complex by the interaction of two molecules). Both structures offer graphic pictures of a molecular chaperone at work: The chaperone complements the molecule that it is protecting and prevents it from prematurely coupling with other molecules. The chaperone carries the pilin subunit to the large pore protein (the usher) where the pilin subunit is released by the chaperone and becomes attached to the end of the growing pilus rod (see bottom figure).

Whether complementation between chaperone and protected protein is a general phenomenon remains to be seen. But both sets of authors report donor strand complementation, even though the chaper-



Folding foibles. A newly synthesized (virgin) protein molecule in the process of folding (**left**) seeks a thermodynamically stable structure. Among possible low-energy structures that it may seek are a monomer, a 3D domain-swapped dimer, and a 3D domain-swapped aggregate (**right**).



The benefits of a chaperone. A chaperoned pilin protein molecule is added to a growing pilus (the adhesive structure that enables bacteria to bind to host cells). The pilin molecule to be added is not able to couple with the growing pilus until the chaperone (**c**) is displaced by another pilin molecule. The chaperoned pilins can be complemented by a different molecule (the chaperone) or the same molecule (another pilin).

one-pilin structures they examined were different. Sauer *et al.* (*2*) describe a complex between the PapD chaperone of *E. coli* and the PapK subunit of the P pilus assembly. PapK is an adaptor pilin subunit that connects the PapA subunits of the rod with the fibrillum composed of PapF, G, and H subunits. In contrast, Choudhury and colleagues (*1*) studied the structure of the FimC chaperone complexed with the FimH pilin component of type 1 pili. FimH has both a pilin domain to bind to its fellow pilins in the fibrillum and an adhesion domain that enables it to bind to the mannose sugars of host tissue surface glycoproteins.

Both reports offer atomic models of how the pilus rod is assembled. In these models, the pilin subunit is no longer complemented by the G1 strand of the chaperone; rather, each pilin subunit is complemented by the amino-terminal strand from another pilin subunit. By repeating this complementation, the authors are able to build a model with three subunits per turn, with an outer diameter of about 70 Å and an open central pore of about 20 Å. The implication for biogenesis of the pilus rod is that the amino-terminal strand of each pilin molecule, containing several hydrophobic side chains, replaces the hydrophobic side chains temporarily donated by the G1 strand of the chaperone. Thus, in the final pilus structure, every pilin subunit completes the immunoglobulin-like fold of the neighboring subunit (see bottom figure, previous page). During assembly of the pilus, the chaperone of a chaperoned pilin must be displaced by another pilin.

A complication that the authors encountered in building their pilus models is that in order to create the rod-like structure, the amino-terminal strand of each pilin subunit has to be oriented into the next molecule antiparallel to the neighboring F β strand, whereas the chaperone's β strand is parallel to the F strand in both crystal structures. Thus, if their compelling model for pilus assembly is correct, the complementation of the pilus subunit occurs by two different β strands (its own and the chaperone's), lying in opposite directions. This is a problem of intermolecular forces that is worthy of further study by computational chemists. The dilemma is akin to the difficulty in understanding the action of chaperones such as GroE that are nonspecific for their protein substrates. How can such chaperones perform a specific function with a range of substrate proteins?

The study by Choudhury *et al.* (*1*) raises another point. In the FimC–FimH chaperone-pilin complex, the FimH subunit actually contains two domains: the immunoglobulin-like domain that is complemented by the chaperone and a second sugar-binding domain. This sugar-binding domain—also built from antiparallel β-strands—contains a pocket into which a sugar analog can fit snugly. This presumably marks the sugar-binding site that enables the pilus to latch on to its host cell.

Thus, these two crystal structures illuminate much about how chaperones protect virgin proteins and how the pili of bacteria are assembled.

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PERSPECTIVES: ECOLOGY

A Tale of Big Game and Small Bugs

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For much of this century ecologists have puzzled over the fluctuations in numbers of animal and plant populations in the wild (*1*). Although usually irregular, these changes in population density can be remarkably cyclical, occurring repeatedly between well-defined upper and lower boundaries. Early theoretical ecologists proposed that populations could be regulated by a density-dependent feedback mechanism in which birth and death rates changed in response to an increase or decrease in population density (*2*, *3*). Birth rates would increase to exceed death rates in times of low population density, but would decrease to below death rates when population numbers were high. A central element of this feedback mechanism is the ability of the birth and death rates to operate with different lag times, potentially resulting in a cyclical rise and fall in population density.

By analyzing data on changing population densities in various organisms over many years, ecologists can address the causes and consequences of population dynamics. Thanks to the excellent bookkeeping skills of Canada's Hudson Bay Company, which kept detailed records of the fur trade between 1821 and 1939 (see the figure), ecologists have been endowed with long-term population data for many animals throughout Canada (*1*, *4*). Stenseth *et al.*, reporting on page 1071 of this issue (*5*), take advantage of the unique Hudson Bay Company time-series data set (and a second time series, from 1921 to the present, compiled by Statistics Canada, a government agency that keeps detailed statistics on forestry, agriculture, trade, etc.) to analyze population fluctuations in the Canadian lynx (*Lynx canadensis*). They found that the dynamics of lynx populations could be grouped according to three geographical regions of Canada that differed in climate and proposed that external factors such as weather had an influence on lynx population density. In a companion paper on page 1068, Turchin *et al.* (*6*) analyzed fluctuations in the population density of the southern pine beetle (*Dendroctonus frontalis*)—a pest responsible for destroying large tracts of forest in the southern United States (see the figure). In contrast to the lynx data, their time-series analysis—supported by field experiments—showed that predation was a crucial factor in determining the rise and fall of the beetle population. In both of these studies, the time-series analyses were critical to understanding the dynamics of the mammalian and insect populations.

Since the 1950s, ecologists have proposed that populations are limited either by extrinsic factors—such as weather, especially extremes of cold, drought, or rainfall—or by intrinsic factors—such as birth and death rates, or interactions with other species (prey, predators, or parasites) (*1*, *3*, *7*, *8*). The intrinsic factor hypothesis postulates that current and past population densities reflect the variations in renewal of the population, a view supported by the Stenseth and Turchin findings.

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