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Secretins revealed: structural insights into the giant gated outer membrane portals of bacteria

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The acquisition and evolution of customized and often highly complex secretion systems allows Gram-negative bacteria to efficiently passage large macromolecules across both inner and outer membranes and, in some cases, that of the infected host. Essential to the virulence and ultimate survival of the many pathogenic species that encode them, secretion systems export a wide variety of effector proteins and DNA as well as the downstream extracellular filaments of the secretion apparatus themselves. Although these customized secretion systems differ in their cytosolic and inner membrane components, several commonly rely on the secretin family of giant pores to allow these large substrates to traverse the outer membrane. Recently, several near-atomic resolution cryo-EM secretin structures have unveiled the first insights into the unique structural motifs required for outer membrane localization, assembly, hallmark ultrastable nature, spontaneous membrane insertion, and mechanism of action — including the requisite central gating needed to prevent deleterious passage of periplasmic contents to the extracellular space.

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Introduction

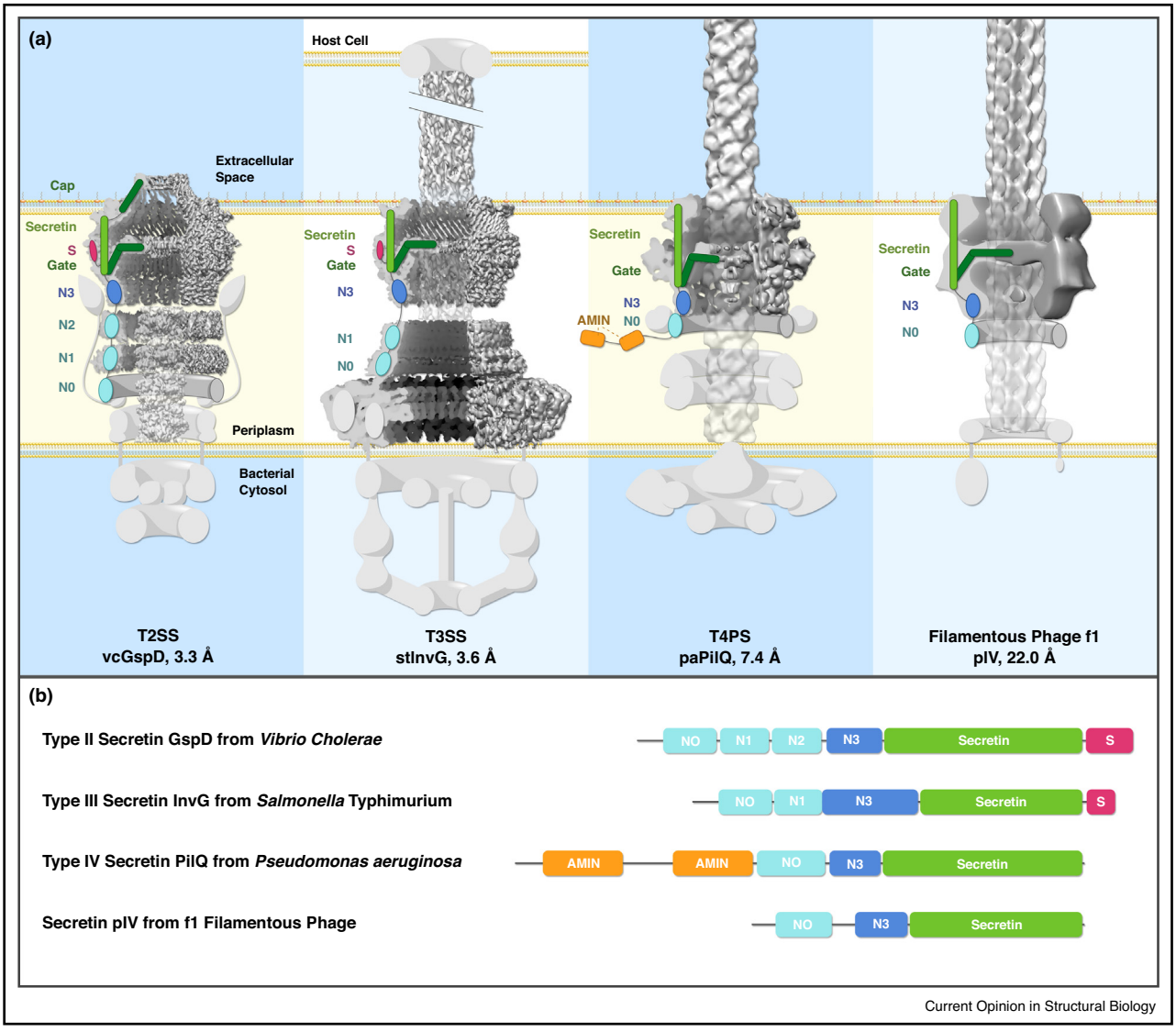
While the inner and outer membranes of the Gram-negative bacterial envelope provide vital structural support and environmental protection, they pose a substantial barrier for transport into and out of the cytosol. As bacteria rely on extracellular secretion for survival and virulence, they have evolved complex protein secretion systems to transport specific substrates through their multi-layered envelope. Many of these envelope-spanning nanomachines share a conserved outer membrane channel, termed the secretin pore. Research interest in secretin pores was first ignited when d'Enfert *et al.* found that knockout of the T2SS secretin PulD prevented secretion of pullalanase, a saccharide debranching enzyme [1]. PulD was later revealed to share sequence homology in its C-terminal region (termed the secretin domain) with outer membrane proteins from other bacterial species and secretion systems, coining the secretin family of proteins [2,3]; members of this family share a remarkable stability to temperature, denaturing agents, and detergents [4–6]. These massive, necessarily gated outer membrane portals are essential for secretion of folded substrates in the type II secretion system (T2SS) [7], the needle of the type III secretion system (T3SS) and subsequent virulence effectors passaged therein [8], the pilus of the type IV pilus system (T4PS) [9], and filamentous phage [10] (see Figure 1).

It has been long recognized by primary sequence analysis and supporting genetic/low resolution EM data that secretins from diverse secretion systems and bacterial species have a similar apparent domain organization [6,11]. They are made up of a single polypeptide, wherein a proposed 12–15 copies oligomerize into a >1 MDa outer membrane (OM) pore [12–14]. The secretin domain is the most stringently conserved region, encompassing the C-terminal half of the protein and harboring a propensity for high β-sheet character [2]. T2SS and T3SS secretins from specific bacterial strains also encode a small C-terminal motif called the S domain, where cognate chaperone-like pilotin proteins can bind to facilitate assembly and/or OM localization of the pore [15,16]. The most diverse sequence of the secretin protein is the periplasmic N-terminal region, which is comprised of a variable number of globular domains; these have evolved to suit the specific function unique to each system [17–19]. This review will provide a summary of the recently established structural architecture in secretin pores that now illuminate the role of these conserved sequences and the significance for potential mechanisms of pore assembly and function.

Recent first insights into secretin structure

The secretin family has historically been recalcitrant to high resolution structural study, with understanding of secretin architecture limited to low resolution molecular envelope EM reconstructions from various species and systems [13,18,20–25] and the crystal structures of isolated, monomeric N-terminal modular domains [26–30]. However, enabled by the remarkable revolution in single-

Figure 1



Overview of secretin-reliant bacterial secretion systems. **(a)** Schematic of the T2SS, the T3SS, the T4PS and the f1 phage system. Gram-negative bacteria rely on the T2SS for export of fully-folded proteins across the outer membrane [7]. This export is made possible through the secretin pore, which has a sufficiently wide diameter to enable passage of folded substrates. A defining feature of the T2SS is its periplasmic pseudopilus, a dynamic filament of oligomerized pilin, which is involved in mechanically pushing substrate through the periplasm and out of the cell. The T2SS is commonly found in pathogenic bacteria, where it secretes toxins and hydrolases such as cholera toxin from *V. cholerae* and heat-labile enterotoxin from enterotoxicogenic *E. coli* (ETEC). The T3SS is found in pathogenic and symbiotic Gram-negative bacteria, where it is capable of injecting proteins that specifically target and modulate various cellular functions directly into the host cytoplasm [8]. Also called the injectisome, it resembles a molecular syringe, with protein ring assemblies traversing the inner and outer membranes while an extracellular hollow needle and translocon pore create a continuous channel from the bacterial cytoplasm through to that of the host cell. The secretin pore encompasses the needle, anchoring it through the OM. The T3SS is essential to the virulence of many clinically relevant pathogens, including *Salmonella enterica* serovar Typhi (typhoid fever), *Vibrio cholera* (cholera), enteropathogenic *Escherichia coli* (EPEC; food and water borne disease), *Shigella dysenteriae* (severe diarrheal disease), *Bordetella pertussis* (whooping cough), *Chlamydia trachomatis* (sexually transmitted disease) and *Pseudomonas aeruginosa* (serious nosocomial infections of immunocompromised patients as well as lung infections in cystic fibrosis patients). T4PS are multi-functional systems characterized by long, thin pilin filaments that extend into the extracellular space [9]. Their functions are often essential to the virulence of Gram-negative pathogens, and include surface motility, host-cell adhesion, biofilm formation, and double stranded DNA uptake. There are two broad sub-types of T4PS: T4aPS, better characterized and used mainly for twitching motility; and T4bPS, found almost exclusively in intestinal pathogens such as *Vibrio cholerae* and EPEC. The T4aPS protects the pilus in the periplasm with a series of concentric protein rings, and the pilus is extruded through the outer membrane by the secretin. The f1 filamentous phage also uses a secretin pore for extrusion from the Gram-negative host [10]. This enables exiting from the host without lysis, allowing the virus to continuously produce phage particles within the same cell. The secretion system used by the phage is relatively simple, made up of only two inner membrane proteins that interact with the secretin pore. Some filamentous phages lack a secretin homologue instead relying on host secretins such as CTX*ø* phage, which hijacks the T2SS secretin in *V. cholerae* for secretion. **(b)** Specific domain organization of each secretin illustrated in (a).