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Douglas I. Johnson
University of Vermont
Department of Microbiology & Molecular Genetics
Burlington, VT, USA

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This book is dedicated to my loving parents Ian J. Johnson and Carolyn H. Johnson, who nurtured and supported my life and my career, and to my amazing children Ian, Erin, and Lauren, who have made my life worth living.

Preface

Bacterial pathogens have been purveyors of human disease and death throughout history, and their virulence factors play critical roles in the pathogen–host interactions that lead to this morbidity and mortality. Virulence factors can influence the ability of bacterial pathogens to enter human hosts, to grow and divide within different host niches, to cause host cell damage, and to evade the innate and adaptive host defense systems. Many of these factors are conserved between different genera and species, but each bacterial pathogen has its own unique "toolkit" of virulence factors that is essential for its survival and pathogenicity. A detailed knowledge of a pathogen's virulence toolkit is essential to understanding its disease-causing capabilities, and it may open up new anti-virulence therapeutic paradigms in the future that are geared toward treating pathogen-specific bacterial infections. These anti-virulence strategies would target the action of specific virulence factors and bypass the classical antibiotic routes that kill both pathogenic bacteria and beneficial human microbiota indiscriminately. Importantly, these antibody-based and small molecule-based approaches may provide critical diagnostic and therapeutic advantages in the rapidly approaching "post-antibiotic" age of increased levels of bacterial antibiotic resistance.

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About the Author

Douglas I. Johnson Received his B.S. degree in chemistry from Miami University in 1978 and his Ph.D. in biochemistry from Purdue University in 1983. His doctoral research with Dr. Ronald Somerville focused on the isolation of new mutants that affected the transcriptional regulation of amino acid biosynthesis in *E. coli*. Postdoctoral work with Dr. John Pringle at the University of Michigan centered on control of the yeast *Saccharomyces cerevisiae* cell cycle, with the initial discovery of the Cdc42 GTPase and its role in regulating the actin cytoskeleton. Dr. Johnson's research in the Department of Microbiology and Molecular Genetics at the University of Vermont extended the analysis of Cdc42 in *S. cerevisiae*, *Schizosaccharomyces pombe*, and the pathogenic yeast *Candida albicans*. In addition, anti-virulence studies with small molecule inhibitors of the budded-to-hyphal transition virulence determinant in *C. albicans* were undertaken. Dr. Johnson's primary teaching responsibilities are introductory microbiology and infectious disease and advanced clinical microbiology, which provided the impetus for writing this book.

Chapter 1
Bacterial Virulence Factors

What is a pathogen? What is a virulence factor? At one time, these were relatively straightforward questions to address. During the late nineteenth century, when Pasteur and Koch were developing the germ theory of disease, a pathogen was simply defined as a microorganism that was capable of causing disease in a host (only human hosts will be considered in this book). Pioneers in the burgeoning field of clinical microbiology showed that life-threatening diseases such as anthrax, diphtheria, tetanus, and tuberculosis were caused by single bacterial pathogens. These primary pathogens were capable of causing unique disease symptoms in a healthy host, as defined by the satisfactory fulfillment of Koch's Postulates. In addition, macromolecular factors (e.g., exotoxins, endotoxin, capsules) were shown to be responsible for inducing these specific disease symptoms. These so-called virulence factors were thought to be inherently present in pathogenic bacteria but not present in nonpathogenic bacteria.

While these simple cause-and-effect relationships were adequate early on to explain the pathogenicity of a number of major bacterial pathogens, it has become clear over the last 40 years that many infectious diseases are not due to the actions of a single primary pathogen and its virulence factors. Disease symptoms associated with the majority of bacterial infections are actually due to host tissue damage that results from either an overreaction or under-reaction of the host immune system to the pathogen. The concept that bacteria are either pathogenic or nonpathogenic ignores this essential interplay between a pathogen and its infected host and the ability of pathogens to modulate the host innate and adaptive immune responses to its advantage. Thus, the qualitative nature of pathogenicity and the quantitative measurement of virulence for a specific bacterial pathogen are only meaningful in the context of host–pathogen interactions and are not inherent qualities of pathogens versus nonpathogens (Casadevall and Pirofski 1999, 2001, 2014; Falkow 1997; Finlay and Falkow 1997; Kubori and Nagai 2016). As Casadevall and Pirofski wrote: "virulence is predicated on the variable nature and outcome of host-microbe interaction, rather than on either microbe- or host-based characteristics" (Casadevall and Pirofski 2001). This concept is no more evident than in the virulence of

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opportunistic pathogens, which are microorganisms that can cause disease in immunocompromised individuals but not in immunocompetent individuals. Not surprisingly, the appreciation of opportunistic pathogens and the central role of host–pathogen interactions in infectious disease coincided with the dramatic rise in the immunocompromised population, due in large part to chemotherapeutic immune suppression in organ transplantations and cancer treatments and in acquired immune suppression associated with HIV/AIDS. This appreciation has also led to a reevaluation of the concepts of pathogenicity, virulence, and virulence factors.

The truism that bacteria are only pathogens if they have a susceptible host, and the understanding of the importance of interplay between pathogens and host defense systems has led to a shift in the qualitative definition of pathogenicity. Instead of the ability to cause disease in a host, pathogenicity is now considered the ability to cause damage to a host (Casadevall and Pirofski 1999). Many bacterial pathogens have the ability to induce damage to host cells and tissues (i.e., changes in cellular homeostasis), but it is usually the response of the immune system to either the pathogen and/or the host damage that leads to the signs and symptoms of disease. This damage is either through a direct response to the presence of certain bacterial components termed pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), or through direct stimulation and/or suppression of innate and adaptive immune responses by bacterial virulence factors. This new definition of pathogenicity also allows for better insight into the immune system-dependent damage associated with opportunistic pathogens and their virulence factors.

With this reevaluation of pathogenicity also comes the need to reexamine the quantitation of virulence. Historically, virulence has been measured in many ways, but the most common was the ability of the pathogen to cause death of the infected host (i.e., an irreparable change in homeostasis). The lethal dose (number of microorganisms) required to kill half of infected hosts (LD_{50}) was a relatively simple endpoint measurement that could be used to compare the virulence of multiple pathogens against a common host. This absolute measurement was useful for primary pathogens that could induce host death, but it did not take into account alternative damage endpoints or the role of host defense systems in damage, especially those associated with opportunistic pathogens. In addition, LD_{50} measurements were subject to the genetic vagaries of the susceptible host utilized in the assay. Casadevall has recently proposed the more useful concept of pathogenic potential (PP) to quantify a pathogen's virulence (Casadevall 2017). PP takes into account (i) variable damage to the host, (ii) bacterial inoculum needed for host damage, (iii) level of mortality associated with an infection, (iv) disease communicability, (v) incubation times prior to disease symptoms, and (vi) toxicity of primary pathogens. PP promises to be an important new means to quantify the myriad of pathogenic properties that go into the measurement of a pathogen's virulence.

With revised concepts of pathogenicity and virulence, the categorization of bacterial factors that contribute to virulence has become muddied. Not only can a virulence factor be an inherent component of a pathogen that causes damage to host cells and/or tissues (e.g., exotoxins), but it also can be a molecule or structure (e.g.,

capsule, biofilm) that enables the pathogen to evade or modulate host defense systems to its replicative advantage. Included in this list of virulence factors are adherence factors that enhance the ability of a pathogen to resist host fluid flow, attach to specific target cells, and potentially invade those target cells. Invasion and survival within host cells is a special property of certain so-called intracellular pathogens (both obligate and facultative) that requires additional virulence factors not usually found in extracellular pathogens. Bacterial capsules and biofilms, which can protect pathogens from host defenses, such as phagocytosis or complement-mediated lysis, are critical to the ability of certain pathogens to disseminate from initial infection sites, often leading to life-threatening systemic diseases. Likewise, molecules that specifically modulate the expression or function of the host immune system, leading to a hyper- or hypo-immune response, would also be considered important virulence factors. Factors that enhance the metabolic capability of pathogens will not be addressed in this book except for factors that facilitate essential iron acquisition, an important virulence determinant for bacteria within the human host. Finally, transcriptomic and proteomic studies have clearly shown that the differential expression of bacterial virulence factors plays a critical role in the ability of a pathogen to induce host damage in response to both environmental and host signals.

Each of the abovementioned categories of virulence factors is discussed in this chapter, focusing on their general characteristics and functions. Subsequent chapters address specific bacterial pathogens and their associated virulence factors. Pathogens will initially be sorted by Gram-stain phenotype (Gram positive, Gram negative, no Gram stain), followed by an alphabetical sorting by genus. Within each chapter, a brief description of the pathogen's genome, morphology, speciation, and disease manifestations is presented, followed by a detailed listing of the known or hypothesized factors found within each virulence category. Non-exhaustive bibliographies accompany each chapter, allowing the reader to delve deeper into the specifics of each pathogen. The last chapter, which provided the initial impetus for writing this book, focuses on the emerging concept of devising antibacterial strategies based on counteracting specific virulence determinants, as opposed to the wholesale destruction of bacteria by antibiotics. This anti-virulence approach may help overcome the drastic worldwide rise in antibiotic-resistant pathogens by not targeting the destruction of the pathogen, which leads to reproductive selective pressure to become resistant to the antibiotic, but by blocking virulence mechanisms that may not be under selective pressure. Only by understanding the structure and function of virulence factors produced by specific pathogens can these new pathogen-specific therapeutic paradigms be envisioned and developed.

Adherence Factors (Adhesins)

Adhesins play essential roles in binding to host epithelial and endothelial cells, interactions with host mucosal layers and components of the extracellular matrix (ECM) that surround host cells, and in biofilm formation (Kline et al. 2009;

Pizarro-Cerda and Cossart 2006; Ringot-Destrez et al. 2017; Stones and Krachler 2015; Vengadesan and Narayana 2011). Intimate attachment to host cells and structures is crucial for protection from the shear forces associated with host fluid flow, especially in the gastrointestinal and urogenital tracts. These interactions are also critical for the initial colonization of host tissue and subsequent invasion of susceptible host cells. Binding can be non-specific to "sticky" host components, such as mucins, or it can be host cell specific. This specific binding is mediated through host cell receptors and is the key initial step in tissue tropism, leading to disease manifestations in different host locales. Beyond adherence, binding of adhesins to host cell receptors can also trigger intracellular signaling pathways that can lead to morphology changes, immune activation, and apoptosis. It is important to note, however, that adherence can be a double-edged sword: while binding to non-phagocytic cells can lead to advantageous colonization, binding to phagocytic cells, such as neutrophils, macrophage, and dendritic cells, can lead to phagocytosis and pathogen destruction.

Most pathogens express several different types of adhesins, but not all adhesins are expressed by all pathogenic species within a specific genus. The myriad of adhesins expressed on the bacterial cell surface makes it difficult to assign a specific virulence function to a specific adhesin in vivo. In addition, the structure and function of adhesins greatly depend on the structure of the pathogen's cell surface itself (i.e., Gram-positive vs. Gram-negative cell walls; capsule vs. non-capsule). The outer surface of Gram-positive bacteria contains a thick mesh-like peptidoglycan layer comprised of polymers of the polysaccharides *N*-acetylglucosamine and *N*-acetylmuramic acid that are extensively cross-linked via interpeptide bridges. As such, Gram-positive adhesins are usually attached directly to the outermost peptidoglycan layer (see sortases below), or if attached to the underlying cell membrane, they must be able to span the peptidoglycan layer. In contrast, the outer surface of Gram-negative bacteria is composed of a thin peptidoglycan layer that is separated by a periplasmic space from a surrounding outer membrane comprised of assorted lipids, lipoproteins, porins, and the tripartite lipopolysaccharide (LPS) layer. LPS is inserted into the outer membrane through a lipid A moiety, which is linked to polymers of complex core polysaccharides and O-antigen polysaccharides. These extended LPS structures are quite immunogenic and can be highly variable between pathogens, even within the same species. To help evade the host immune response, the LPS polysaccharides and lipid A of many pathogens can undergo phase and/or antigen variation (see Immune Evasion below). Based on these structures, Gram-negative adhesins can be LPS components themselves, or they can be attached to the outer membrane or to the underlying cell membrane and able to span the peptidoglycan layer, periplasmic space, and outer membrane. Not surprisingly, Gram-negative pathogens have evolved numerous protein secretion systems that facilitate the synthesis, transport, and assembly of adhesins across these different structural barriers (see Protein Secretion Systems below).

Certain major pathogens, such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae,* contain a large capsule structure (glycocalyx, slime layer) surrounding their outer surfaces (Fig. 1.1). These self-produced

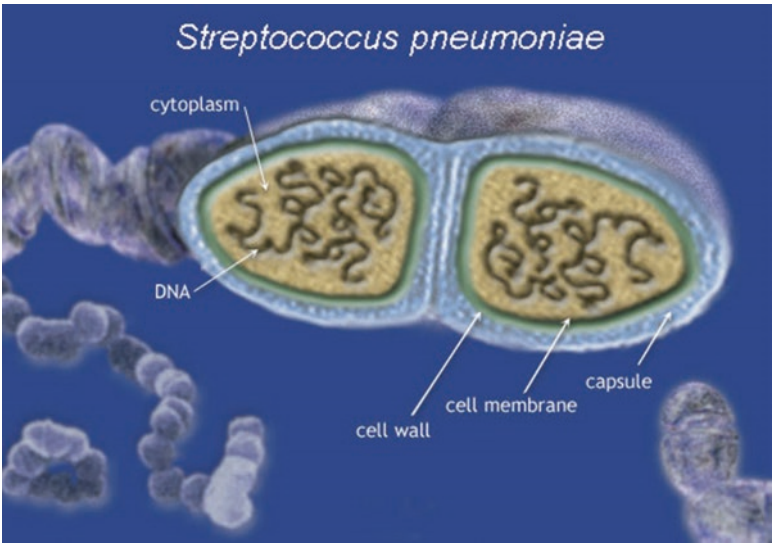


Fig. 1.1 *Streptococcus pneumoniae* capsule (Courtesy of the National Foundation for Infectious Diseases)

structures are quite variable in their lipid, polysaccharide, and protein composition. In addition to their primary function in blocking phagocytosis and complement-mediate lysis (see Immune Evasion below), capsules can act as adherence factors themselves, or they can sterically block the action of adherence factors such as LPS and adhesins.

Fimbrial Adhesins

Adhesins can be categorized structurally as either fimbrial or afimbrial. Fimbrial adhesins (fimbriae, pili) are long filamentous structures extending outward from the cell surface (Fig. 1.2). Fimbriae can have different morphologies, including the long thin P pili (pyelonephritis-associated pilus) and type I fimbriae, the bundled rope-like type IV fimbriae that are capable of retraction, and the thin aggregative curli fimbriae. The fimbrial rigid rods can contain a central channel (Fig. 1.2c–e, dark central thread), and end in a flexible, spring-like tip structure (Fig. 1.2d, e; arrow-heads) that contains the tip adhesin protein(s) needed for adherence. Gram-negative fimbriae are usually attached directly to the outer membrane or the cell membrane, whereas Gram-positive fimbriae, of which there are considerably fewer examples, are usually attached directly to the peptidoglycan layer.

Fimbriae are either homopolymeric or heteropolymeric protein structures that have complex assembly mechanisms (Fig. 1.3) (Kline et al. 2009; Pizarro-Cerda and Cossart 2006; Sauer et al. 2000). Multiple copies of the structural filament

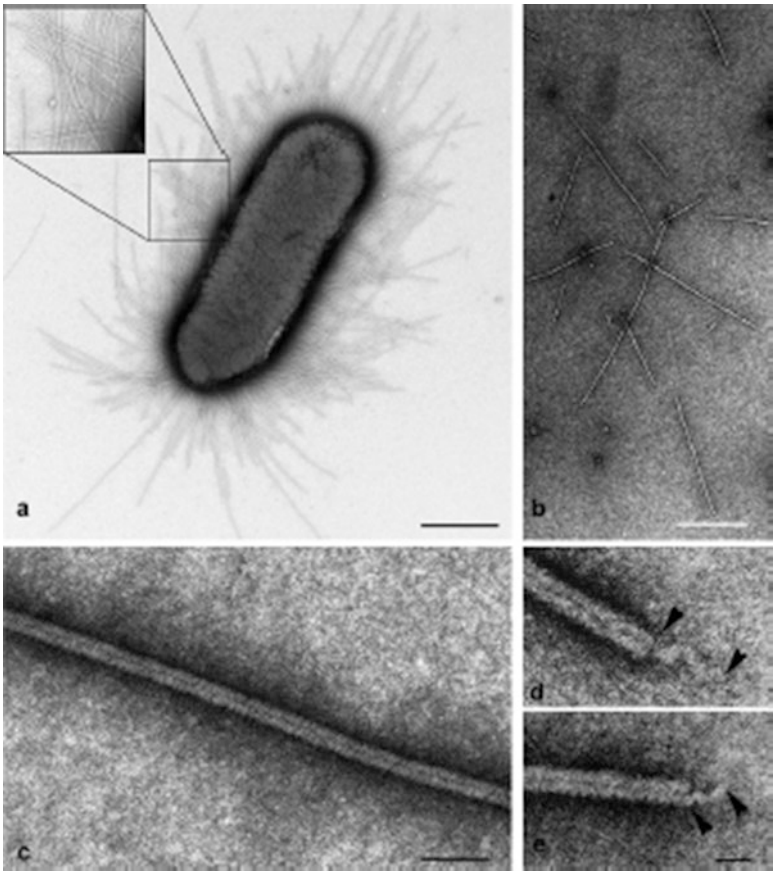


Fig. 1.2 *E. coli* P pili (From: Hahn et al. 2002)

protein (e.g., P pili PapA) are transported through the outer membrane and polymerized from their base. The adherence specificities of these fimbriae are primarily mediated by tip adhesins, which bind to different host cell surface receptors, such as the sugar moieties of glycolipids (PapG of P pili) and glycoproteins (FimH of type I fimbriae), the peptide domains within other cell surface proteins (PilC of type IV fimbriae), and mucin glycoproteins within the mucosal layer. The presence of specific receptors on host cell surfaces determines the binding and subsequent disease tropism of these fimbriae-containing pathogens. For instance, PapG binds to the α−D-galactopyranosyl-(1-4)−β−D−galactopyranoside sugar moiety present in surface glycolipids within upper urinary tract cells.

The chaperone–usher assembly pathway is used for the assembly of Gram-negative fimbriae found associated with gastrointestinal and urogenital pathogens, such as the P pili associated with uropathogenic *E. coli* (UPEC), type I fimbriae associated with UPEC and diffusely adherent *E. coli* (DAEC), and Afa/Dr adhesins associated with UPEC and DAEC (Fig. 1.3) (Busch et al. 2015; Sauer et al. 2000).

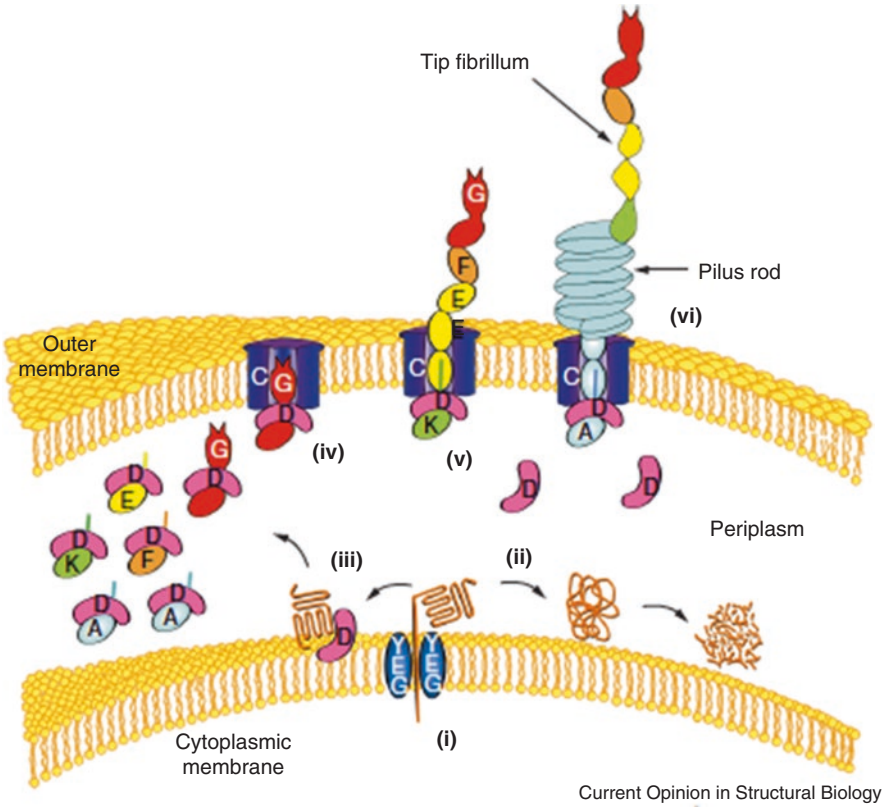


Fig. 1.3 Chaperone–usher P pili assembly (From: Sauer et al. 2000)

Since the ultimate site of fimbrial assembly is the outer membrane, chaperone proteins are needed to transport the filament and tip adhesin proteins through an usher transmembrane protein embedded in the outer membrane. Each fimbria has its own set of transport and assembly proteins (e.g., P pili Pap proteins, type I fimbriae Fim proteins; Fig. 1.3), which are usually encoded within gene clusters that are under transcriptional control. Unlike chaperone–usher fimbriae, type IV fimbriae are assembled within the inner cell membrane and extruded through the outer membrane. This assembly mechanism allows for fimbrial retraction/extension, which is important for the twitching motility exhibited by pathogens expressing type IV fimbriae as well as their adherence function (Giltner et al. 2012).

Fimbriae have been found in an increasing number of Gram-positive pathogens (Vengadesan and Narayana 2011). The paradigm for Gram-positive fimbriae is the Spa family of *C. diphtheriae*. These fimbriae are composed of only three subunits: the homopolymeric filament protein, the tip adhesin, and an accessory protein that binds to the shaft of the filament. Attachment of the fimbriae to the peptidoglycan cell wall is mediated through a sortase-based mechanism (Mazmanian et al. 1999; Ton-That and Schneewind 2003, 2004). Sortases are cysteine transpeptidases that

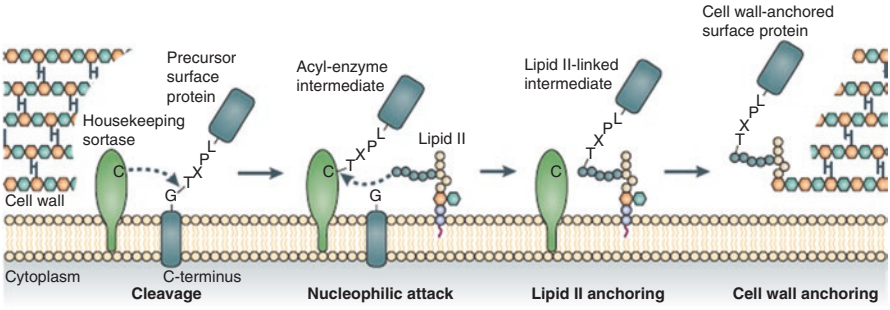


Fig. 1.4 Sortase mechanism (From: Hendrickx et al. 2011)

catalyze the covalent linkage of fimbriae, as well as other afimbrial adhesins, to the pentapeptide crossbridges found within the lipid II component of the peptidoglycan layer (Fig. 1.4) (Clancy et al. 2010; Hendrickx et al. 2011; Spirig et al. 2011). Sortases recognize proteins that have a carboxyl-terminal cell wall sorting signal (CWSS) containing the amino acids LPXTG or its variants. There are four major sortase classes with varying substrates and CWSSs: class A (SrtA in *S. aureus*) function as housekeeping sortases for many outer surface afimbrial adhesin proteins (see below), class B (SrtB) play a role in iron acquisition, class C (SrtC) are used for fimbriae attachment, and class D are utilized within spore-forming bacteria.

Afimbrial Adhesins

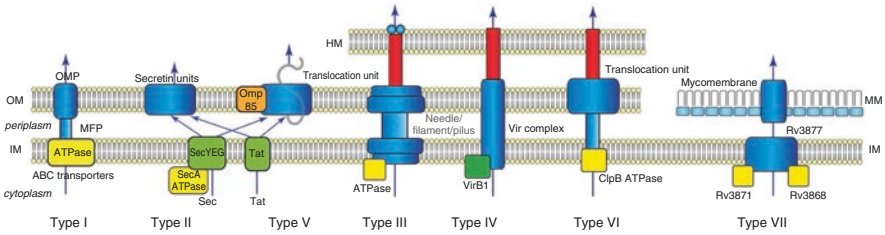
There are a plethora of afimbrial adhesins used by Gram-positive and Gram-negative pathogens. These proteins do not form filamentous structures and are attached either to the Gram-negative outer membrane or to the Gram-positive peptidoglycan layer. They usually do not extend a substantial distance away from the cell surface; hence, their binding can be sterically blocked by the presence of a capsule. Afimbrial adhesins have the differential capacity to bind to (i) specific host cell receptors, (ii) glycosylated mucins within the mucosal layer, (iii) ECM components, and/or (iv) soluble host proteins such as plasminogen, fibrinogen, and certain antibodies (IgA, IgG). Host cell receptors are usually glycosylated integral membrane proteins, including cadherins, integrins, selectins, and CD (cluster of differentiation) proteins such as CEACAMs (carcinoembryonic antigen-related cell adhesion molecules). While the mucosal layer is one of the first lines of defense against invading pathogens, some afimbrial adhesins as well as fimbriae and flagella can bind to the highly glycosylated mucin proteins within the host mucosal layer (Ringot-Destrez et al. 2017). This binding aids in the protection of the pathogen from host fluid flow stresses, but it does render the pathogen sensitive to expulsion via mucociliary escalators.

Gram-positive pathogens, such as *S. aureus* and *S. pyogenes*, utilize MSCRAMM (microbial surface components recognizing adhesive matrix molecules) proteins to bind to ECM components, such as type I and type IV collagens, elastin, fibronectin, proteoglycans, and laminin. MSCRAMMs are anchored to the peptidoglycan cell wall using a sortase-based mechanism and interact with ECM components through specific protein domains. Several MSCRAMMS can bind to multiple ECM components, such as fibronectin-binding proteins [FnBPs; (Henderson et al. 2011)], laminin-binding proteins [LnBPs; (Singh et al. 2012)], and collagen-binding proteins [CnBPs; (Singh et al. 2012)]. Fibronectin, laminin, and collagens can interact directly with host cell receptors, such as multiple integrin molecules, thereby linking the bound pathogen to the host cell through the bridging ECM component. These interactions can also stimulate various host cell processes, including actin cytoskeletal rearrangements, gene transcription, and immune responses (Finlay and Falkow 1997; Stones and Krachler 2015). *S. aureus* and other Gram-positive pathogens also express MSCRAMMs that can bind to soluble host proteins, such as fibrinogen and plasminogen, thereby affecting host coagulation pathways.

Protein Secretion Pathways

In addition to the sortase and chaperone–usher secretion mechanisms described above, Gram-positive and Gram-negative pathogens have developed additional mechanisms by which fimbrial and afimbrial adhesins can be transported and attached at the cell surface. These protein secretion pathways are also used to deliver bacterial proteins and toxins into the host environment or directly into host cells across one (inner cell membrane), two (Gram-negative outer membrane), and/or three (host membrane) hydrophobic phospholipid bilayers (Abby et al. 2016; Costa et al. 2015; Green and Mecsas 2016; Tseng et al. 2009). Both Gram-positive and Gram-negative pathogens can transport proteins into and across the inner cell membrane using either the SecYEG translocase machinery, which is part of the general secretion (SecDEFGY) pathway for unfolded proteins, or the twin arginine translocation (Tat) pathway for folded proteins. The Sec pathway is powered by ATP hydrolysis using the SecA ATPase. The translocated proteins can either remain in the inner membrane, which is mediated by a signal recognition particle (SRP)-specific signal sequence, or they can be transported into the periplasmic space or outside the cell using a SecB-specific signal sequence. However, secreted proteins in Gram-negative pathogens must transit through the outer membrane in order to reach the external environment. This added hydrophobic barrier necessitates the use of additional secretion systems.

There are six major protein secretion systems utilized by Gram-negative pathogens to bypass the outer membrane and host cell membranes (Fig. 1.5): type 1 secretion system (T1SS), type 2 secretion system (T2SS), type 3 secretion system (T3SS), type 4 secretion system (T4SS), type 5 secretion system (T5SS), and type 6 secretion system (T6SS). An additional type 7 secretion system (T7SS; ESX) has



Summary of known bacterial secretion systems. In this simplified view only the basics of each secretion system are sketched. HM: Host membrane; OM: outer membrane; IM: inner membrane; MM: mycomembrane; OMP: outer membrane protein; MFP: membrane fusion protein. ATPases and chaperones are shown in yellow.

Fig. 1.5 Protein secretion systems (From: Tseng et al. 2009)

been identified in *Mycobacterium* spp. and other Gram-positive pathogens that contain a heavily lipidated hydrophobic mycomembrane barrier beyond the peptidoglycan layer. It should be noted that not all Gram-negative pathogens express all six secretion systems and that the proteins secreted through these systems vary dramatically between genera (Abby et al. 2016).

T1SS uses ATP hydrolysis by ATP-binding cassette (ABC) transporters to passage proteins through a membrane fusion protein (MFP) linked to an outer membrane protein (OMP). This one-step process can secrete polypeptides up to 1000 amino acids and is Sec- and Tat-independent (Thomas et al. 2014). Examples of T1SS substrates include exotoxins (*B. pertussis* pertussis toxin PTx, adenylate cyclase toxin CyaA), RTX toxins [uropathogenic *E. coli* (UPEC) HlyA hemolysin, *V. cholerae* RtxA], adhesins (*S. enterica* SiiE), proteases (*P. aeruginosa* AprA), and heme-binding proteins (*P. aeruginosa* HasA).

T2SS uses a two-step process in which proteins transit the inner membrane in a Sec- or Tat-dependent process, and the secreted proteins fold in the periplasmic space prior to passage through an outer membrane secretin pore (Korotkov et al. 2012). T2SSs are structurally related to the machinery used to assemble type IV fimbriae. T2SSs are used for the transport of many exoproteins, including proteases, lipases, and phosphatases. Examples of T2SS substrates include *V. cholerae* cholera toxin, enterotoxigenic *E. coli* (ETEC) LT toxin, and the *P. aeruginosa* virulence factors ExoA (exotoxin A), PlcH (hemolytic phospholipase C), LasA (staphylolysin), LasB (pseudolysin elastase), PrpL (protease IV), AprA (aeruginolysin), ChiA (chitinase), and NanH (neuraminidase).

T3SS (Deng et al. 2017), T4SS (Kubori and Nagai 2016), and T6SS (Cianfanelli et al. 2016; Hachani et al. 2015) use one-step processes to directly inject bacterial proteins (effectors) into host cells. The assembly and function of these secretion systems involve a conserved set of proteins that create macromolecular structures spanning the bacterial inner and outer membranes and the host cell membrane. Each system secretes its own set of effectors, which vary dramatically between pathogens. Regardless, the primary function of all of these effectors is to manipulate host cell processes, including signal transduction pathways, actin cytoskeletal rearrange-

ments, intracellular vesicle transport and stability, and host immune responses. T3SS "injectisomes," which are structurally related to the flagellar apparatus, are used by many Gram-negative pathogens to deliver effectors into a wide variety of host cells. Actin dynamics, mitogen-activated protein kinase (MAPK) signaling, and nuclear factor-κB (NF-κB)-based inflammasome activation are modulated by enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), *P. aeruginosa*, and *V. cholerae* T3SS effectors. *Y. pestis* Yop effectors also affect these signaling pathways as well as facilitating intracellular persistence within macrophage. The intracellular pathogens *C. trachomatis, S. enterica*, and *S. flexneri* require T3SS effectors to invade and persist within the vacuolar system of host cells.

T4SS, which are structurally related to DNA conjugation systems, have the ability to transport DNA, DNA-protein complexes, and protein effectors across membranes. *N. gonorrhoeae* uses a T4SS to acquire virulence genes through horizontal gene transfer mechanisms. *L. pneumophila* uses the Icm/Dot T4SS to inject ~330 effector proteins that affect multiple host processes, including vesicle trafficking, autophagy, host protein synthesis, host inflammatory response, macrophage apoptosis, and host cell egress. *H. pylori* uses the Cag T4SS to insert effectors that modulate the host immune response. The contact-dependent T6SS uses a phage-tail-spike-like injectisome structure to deliver effectors not only to host cells but also to competitor bacterial species, thereby giving pathogens competitive advantages within certain host growth niches. T6SS effectors have been shown to function in adherence (*E. coli, C. jejuni, V. parahaemolyticus*), host cell invasion (*E. coli, C. jejuni, S. enterica, P. aeruginosa, Y. pseudotuberculosis*), actin dynamics (*E. coli, V. cholerae*), and host immune responses (*K. pneumoniae, V. parahaemolyticus*).

T5SS use a unique two-step process in which the substrates promote their own secretion; i.e., they contain their own β-barrel domain that forms a pore in the outer membrane with the help of the BAM β-barrel assembly machinery (Leyton et al. 2012). The first step for these so-called autotransporters utilizes the Sec-dependent machinery to translocate across the inner membrane. There are three subclasses of T5SS based on the second step of the secretion mechanisms. T5aSS substrates are single polypeptides that contain amino-terminal Sec-specific signal sequences and passenger domains and a carboxyl-terminal β-barrel domain, which forms the pore through which the passenger domain is translocated. Depending on the function of the passenger domain polypeptide, it can either be anchored in the outer membrane through the β-barrel domain (e.g., adhesins) or be secreted after cleavage from the β-barrel domain (e.g., toxins, exoenzymes). T5bSS (a.k.a., two-partner secretion; TPS) consists of two polypeptides: one polypeptide contains the β-barrel domain, and the other polypeptide is secreted. T5cSS [a.k.a., trimeric AT adhesin (TAA) system] consists of three polypeptides, which together form the β-barrel pore. Examples of T5SS effectors include adhesins (*B. pertussis* FHA, pertactin, and BapC, *E. coli* AIDA-I and Ag43, *H. influenzae* Hia, HWM1, and HWM2, *S. flexneri* IcsA, *Y. enterocolitica* YadA), proteases (*N. gonorrhoeae* and *N. meningitidis* IgA protease, *S. flexneri* SepA), and toxins (*H. pylori* VacA).

Host Cell Invasion and Growth

While entry of most pathogens into host cells results in their destruction, certain bacterial pathogens have the ability to actively invade and replicate within non-phagocytic and/or phagocytic cells. Obligate intracellular pathogens, such as *Chlamydia* spp. and *Rickettsia* spp., can only replicate inside host cells, primarily because they are metabolically crippled. Facultative intracellular pathogens, including enteroinvasive *E. coli* (EIEC)/*Shigella* spp., *Francisella* spp., *Legionella* spp., *Listeria* spp., *Mycobacterium* spp., *Neisseria* spp., *Nocardia* spp., *Salmonella* spp., and *Yersinia* spp., can replicate inside and outside host cells. The ability to invade non-phagocytic cells, such as mucosal epithelial cells or bloodstream endothelial cells, facilitates the bypass of host barriers and protects pathogens from host defense systems such as antibody-mediated opsonophagocytosis and complement-activated lysis. The ability to persist within phagocytic cells, which usually requires additional virulence determinants not found in extracellular pathogens, provides protection and also facilitates bloodstream and lymphatic dissemination of pathogens. These pathogens, which include *M. tuberculosis* and *L. pneumophila*, are passively phagocytized into macrophage using host cell machinery.

Invasion into non-phagocytic cells is usually accomplished through either a zipper mechanism or a trigger mechanism (Fig. 1.6) (Pizarro-Cerda and Cossart 2006;

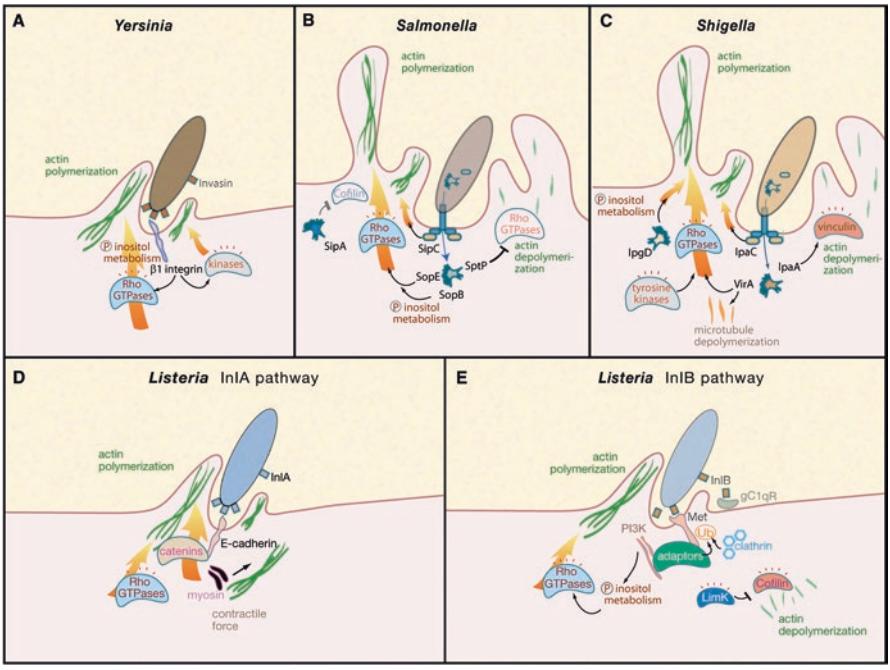


Fig. 1.6 Cell invasion mechanisms (From: Pizarro-Cerda and Cossart 2006)