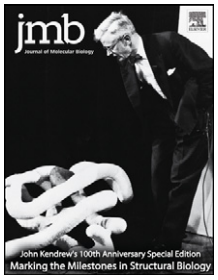
Review



Structural and Molecular Biology of a Protein-Polymerizing Nanomachine for Pilus Biogenesis

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http://dx.doi.org/10.1016/j.jmb.2017.05.016
Edited by I B. Holland

Abstract

Bacteria produce protein polymers on their surface called pili or fimbriae that serve either as attachment devices or as conduits for secreted substrates. This review will focus on the chaperone–usher pathway of pilus biogenesis, a widespread assembly line for pilus production at the surface of Gram-negative bacteria and the archetypical protein-polymerizing nanomachine. Comparison with other nanomachines polymerizing other types of biological units, such as nucleotides during DNA replication, provides some unifying principles as to how multidomain proteins assemble biological polymers.

Introduction

Pili (or fimbriae) are widespread protein polymers that form organelles at the surface of Gram-negative and -positive bacteria and archaea. In Gram-negative bacteria, depending on their assembly mechanisms, they can be categorized into five different classes: (i) chaperone–usher (CU) pili, (ii) curli, (iii) type IV pili, (iv) type V pili, and (v) type IV secretion pili [1]. Pili of the first four classes are mostly used for adhesion, host recognition, and/or biofilm formation, while pili of the fifth class may be used as a conduit for secretion of effector proteins or single-stranded DNAs [2–5]. All five categories have defined structures and defined mechanisms of assemblies mediated by distinct and sometimes extremely elaborated pilus biogenesis mechanisms: the machineries responsible for producing type IV pili and type IV secretion pili are very large, composed of tens of components assembling in various stoichiometries to form multimegadalton complexes in size embedded in both the inner and outer membranes [1,6,7]; those producing CU pili and curli are much simpler both in composition and size and are located at the outer membrane [8–11]. As a consequence of their location, while the double-membrane-embedded nanomachines are able to use ATP from the cytoplasm, the outer-membrane-embedded systems cannot, as there is no ATP in the periplasm, the compartment between the inner and outer membranes. Thus, these systems must energize the assembly process in different ways.

Investigations of pilus-producing machineries have provided mechanistic details, but none has yet yielded as much information as those that have focused on the CU pathway of pilus biogenesis. This is because this system has proved more amenable than most to structural, biochemical, and biophysical characterization. In this review, I describe how successive breakthroughs have led to detailed mechanistic insights on this relatively simple molecular machine capable of polymerizing protein subunits and secreting the resulting polymer.

Type P and 1 Pili

Type P and 1 pili have served as model systems for CU pili. These filaments are elaborated by strains of *Escherichia coli* able to infect the urinary tract of higher eukaryotic hosts including humans. They play important roles in the process of infecting the host bladder and kidney by mediating attachment of the bacterium to these organs' epithelia [12–14].

P pili are elaborated from six types of subunits encoded by the *pap* cluster in *E. coli*. In a P pilus,

these subunits appear in a defined order, starting at the tip with the PapG subunit, a protein termed "adhesin", which binds Galα-1,4-Galβ receptors lining the kidney epithelium, followed successively by one copy of the PapF subunit, 5–10 copies of the PapE subunit, 1 copy of the PapK subunit, circa 1000 copies of the PapA subunit, and finally 1 copy of PapH at the pilus base. PapG to PapK forms a rather short, flexible part termed "the tip fibrillum", while the numerous PapA subunits form a helically wound polymer of 3.3 subunits per turn, termed a "rod" and forming most of the 2-μm pilus [3,15–19] (Fig. 1). A type 1 pilus is composed of four subunits encoded by the *fim* cluster in *E. coli*. Its tip fibrillum is shorter than that of P pili, being composed of one copy each of only three subunits, the adhesin FimH at the tip, followed by the FimG subunit, and the FimF subunit. Its rod is similar to that of P pili, containing circa 1000 subunits of FimA, also helically wound with a similar number of subunits per turn [3,15,16] (Fig. 1). FimH binds D-mannosylated receptors, such as uroplakins, found on the surface of the bladder epithelium [20,21]. Thus, while P pili are thought to mediate the attachment of uropathogenic *E. coli* to the kidney to cause pyelonephritis, type 1 pili mediate the attachment of bacteria to the bladder to cause cystitis.

All pilus subunits, except the tip adhesins, fold into a single domain termed "pilin" domain that serves as "lego" block for assembly [22,23]. The tip adhesins are the exception as they are composed of two domains, an N-terminal "adhesin" domain, which mediates receptor interaction, and a C-terminal pilin domain that is used as lego block for assembly [23,24]. While adhesins usually exhibit unremarkable lectin folds, pilin domains have a singular structure consisting of a C-terminally truncated Ig fold: regular Ig folds include seven strands, A to G, folding into a common β-sandwich; however, pilus subunits lack strand G [22,23] (Fig. 2).

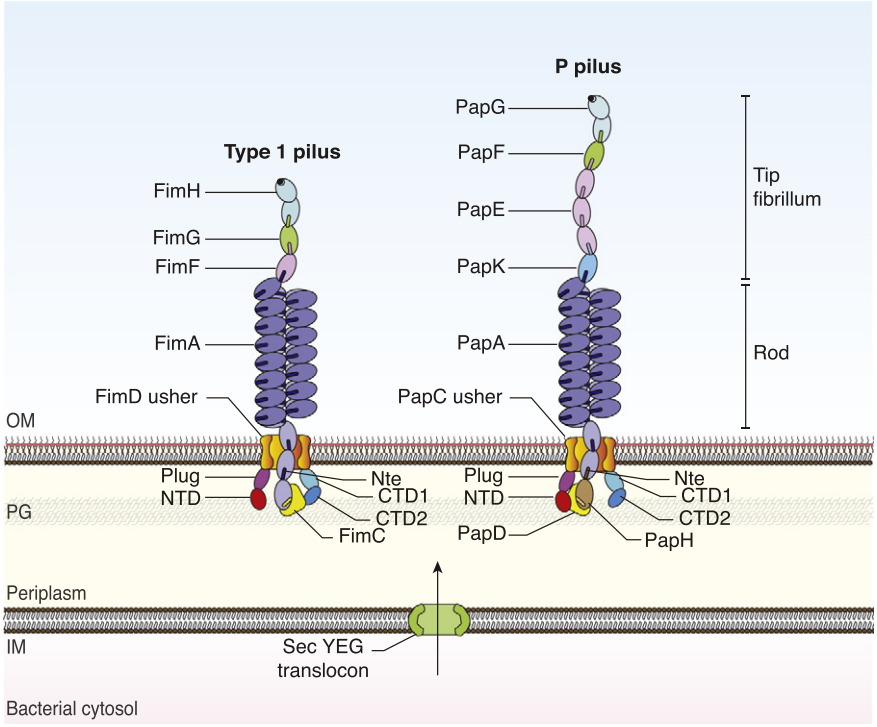

Fig. 1. Schematic diagram of P and type 1 pili. A schematic diagram of a type 1 and a P pilus is shown. Subunits and usher domains in each system are labeled and color-coded differently. This color-coding is used consistently throughout the review. The black dot at the tip of FimH and PapG indicates the receptor-binding site. P and type 1 pili are archetypal pili composed of six (PapG, F, E, K, A, and H) and four (FimH, G, F, and A) known subunits, respectively, assembling in a defined order. Each subunit traverses the inner membrane using the SecYEG translocon, at the exit of which the subunit is received by the periplasmic chaperone PapD or FimC for P and type 1 pili, respectively. The chaperone assists in subunit folding by donating one of its own strands to the C-terminally truncated Ig fold of the subunit to form stable binary chaperone–subunit complexes. These complexes are then ferried for assembly to the usher, an outer membrane protein composed of five domains, NTD, plug, pore, CTD1, and CTD2. The usher orchestrates subunit polymerization by catalyzing the substitution of the chaperone strand by the N-terminal sequence (or Nte) of the subunit next in assembly (Ntes are indicated here in short thick dashes).