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Neglected but amazingly diverse type IVb pili

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Abstract

This review provides an overview of current knowledge concerning type IVb pili in Gram-negative bacteria. The number of these pili identified is steadily increasing with genome sequencing and mining studies, but studies of these pili are somewhat uneven, because their expression is tightly regulated and the signals or regulators controlling expression need to be identified. However, as illustrated here, they have a number of interesting functional, assembly-related and regulatory features.

Keywords: Type IVb pilus; BFP; TCP; R64 plasmid; Flp pilus

1. Introduction

Type IV pili (Tfp) are the most widespread appendages emanating from the bacterial cell envelope and protruding out to the extracellular environment. They participate in adhesion, cell–cell interactions, autoaggregation, DNA exchange and motility. Tfp consist of oligomerized pilin subunits assembled through a dedicated type IV piliation machinery that has evolved from a progenitor common to the type II secretion system. The Tfp machineries span the bacterial envelope and their complexity depends on whether an outer membrane is present (Gram-negative species) or absent (Gram-positive species). Tfp have been classified into two types — type IVa and type IVb — based on the features of their pilins. However, type IVb pili (Tfpb) live "in the shadow of the Tfpa celebrity" and their existence is commonly ignored. We therefore decided "to do justice" to Tfpb in this review, despite the complexity of this undertaking. Some Tfpb are not particularly neglected, often being considered together with Tfpa in previous studies, but their characteristics, other than their type IV pilin features, have been little investigated, as was also put forward in a very recent review (Burrows, 2012). Type IV pilins are processed before oligomerization by a prepilin peptidase, a polytopic inner membrane enzyme of the Tfp assembly machinery. Type IVb pilins were initially distinguished from type IVa pilins on the basis of the length of the N-terminal leader peptide removed by the prepilin peptidase. Type IVa pilins have a short (5–6 residues) leader peptide, whereas type IVb pilins commonly have a longer (13–30 residues) leader peptide, the final mature pilin being either longer (180–208 residues) or considerably shorter (40–50 residues) than mature type IVa pilins (150–160 residues) (Table 1). Tfpb also have other features in common, including their genetic organization. They are encoded by a limited number of genes clustered into operons that are probably acquired by horizontal transfer in some species. Tfpb are also tightly regulated, not associated with motility and have assembly machineries with similar characteristics.

Seven different types of Tfpb pili have been identified in Gram-negative bacteria: the BFP (bundle-forming pilus) of *Escherichia coli*, the TCP (toxin-coregulated pilus) of *Vibrio cholerae*, the R64 plasmid thin pilus of *E. coli*, the Flp (fibril-associated protein) pilus of *Aggregatibacter actinomycetemcomitans*, the Cpa pilus of *Caulobacter crescentus*, the Lng or longus pilus of *E. coli* and the Cof or CFA/III pilus of *E. coli*. The genes encoding type IVb piliation machineries,

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Table 1
Comparison of the features of the various type IVb pilins.

Name of the major pilin	BfpA	TcpA	PilS	Flp	PilA	LngA	CofA
Size (aa) and mw (kDa)	193/20.3	224/23.5	221/21.3	75/7.99	59/6.0	236/25.2	238/25.3
Length of the mature form (aa)	180	199	181	49	45	206	208
Aa^{+1}	L	M	W	V	A	M	M
Modification of Aa^{+1}	Methylation	Methylation	Unknown modification	Glycosylation?	Not identified	Methylation	Methylation
Pair of cysteines at C-terminus	yes	yes	yes	no	no	yes	yes
Type of prepilin peptidase	GI/PilD-like	GI/PilD-like	GII	GII	GII	GI/PilD-like	GI/PilD-like
Prepilin peptidase	BfpP	TcpJ	PilU	TadV	CpaA	LngP	CofP

including the gene encoding the pilin subunit to be assembled, are all clustered into operons (Fig. 1). This property is not shared by genes encoding type IVa piliation machineries, which are often scattered throughout the genome. Tfpa biogenesis requires 40 genes, whereas type IVb operons comprise about a dozen genes (from 11 to 14) that are sufficient for assembly of the corresponding Tfpb. The *cpa* locus of *C. crescentus* comprises only seven genes (*pilA-cpa* to *cpaF*), which are highly homologous to the first seven genes of the *tad* operon (Skerker and Shapiro, 2000), plus a distant gene, *pleA*, encoding a lytic transglycosylase required for Cpa pilus assembly (Viollier and Shapiro, 2003). This strongly suggests that they were acquired secondarily or that they can be transmitted to bacterial species that previously lacked them and that they have coevolved (Giron et al., 1997). This is exemplified perfectly by the R64 pilus, for which the coding operon may be present on a plasmid or within a pathogenicity island in the bacterial genome (Kim and Komano, 1997; Carter et al., 2010), depending on the bacterial species. The *tcp*, *tad* and *cpa* operons are chromosomally encoded, whereas the *lng*, *cof* and *bfp* operons are present on 70 kb, 55 kb and the EPEC adherence factor (EAF) virulence plasmids, respectively, in enterotoxigenic *E. coli* (ETEC) or enteropathogenic *E. coli* (EPEC).

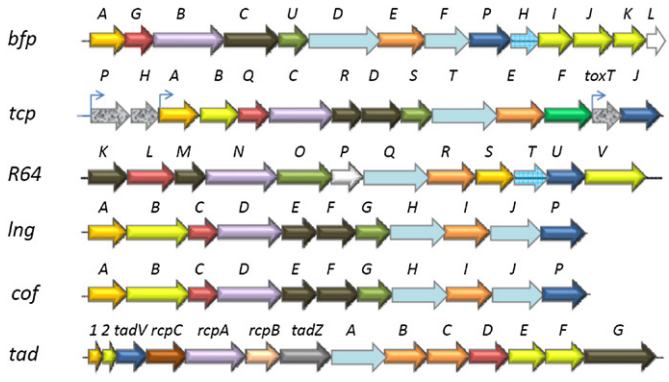

Fig. 1. Genetic organization of type IVb loci: *bfp* from EPEC, *tcp* from *V. cholerae*, R64 from *E. coli*, *lng* from *E. coli*, *cof* from *E. coli*, *tad* from *A. actinomycetemcomitans*. Color codes have been attributed to genes with products thought to have identical functions. In addition to the components of the piliation machinery, genes encoding regulatory proteins are also shown. Major pilin subunit, dark yellow; minor pilin subunit, greenish yellow; secretin, violet; accessory or gating protein, red; IM bitopic protein, khaki; IM polytopic protein, orange; ATPase, sky blue; periplasmic protein, gray; secretin dynamic-associated protein, apple green; peptidoglycan-associated bacterial lytic transglycosylase, mid-blue; prepilin peptidase, dark blue; additional OM-localized proteins, brown and light orange (flesh color); unknown, white.

We aim here to review Tfpb functions, the architecture of their type IVb piliation machineries and the regulation of their expression, focusing on the BFP, TCP, R64 pilus and Flp pilus.

2. Functions

Tfpb fulfill various functions in self-aggregation, the structuring of biofilms, adhesion to eukaryotic host cells, secretion, conjugation (delivering endogenous DNA) and as receptors for phages (acquisition of exogenous DNA). Thus far, no Tfpb-dependent motility has been demonstrated, except for the longus pilus of ETEC, which has recently been shown to be involved in twitching motility (Mazariego-Espinosa et al., 2010). It has been suggested that BFP retraction may also confer motility but other functions have been proposed for it, as detailed below.

2.1. BFP

Enteropathogenic *E. coli* (EPEC) is a major cause of pediatric infectious diarrhea throughout the developing world, and typical EPEC strains carry a large ~80-kb enteroadherence factor (EAF) plasmid encoding the bundle-forming pilus (BFP). The 14 genes of the *bfp* operon are sufficient to allow BFP assembly when expressed under the control of an artificial promoter in a laboratory *E. coli* host (Stone et al., 1996). BFP are responsible for the development of EPEC microcolonies on tissue culture cell monolayers, a phenomenon called localized adherence (LA) phenotype (Andrade et al., 1989), in which they create a network of fibers binding the individual organisms together (Giron et al., 1991). This pilus contributes to EPEC virulence, as shown by studies in volunteers. BfpF-mediated (BfpF is one of the two ATPases of the Bfp machinery) energy-dependent pilus retraction leads to dispersal (Bieber et al., 1998) and to tight-junction disruption, by bringing the bacterium into contact with host cell surfaces, facilitating the timely and effective introduction of bacterial effectors into the host cell via the type III secretion apparatus (Zahavi et al., 2011). BFP and other bacterial agonists appear to act as key determinants of intestinal inflammation, eliciting the production of cytokines, such as IL-8 and CCL_{20} (Edwards et al., 2011).