Polar N-terminal Residues Conserved in Type 2 Secretion Pseudopilins Determine Subunit Targeting and Membrane Extraction Steps during Fibre Assembly

Javier Santos-Moreno[1,2,3,4,5], Alexandra East[6], Ingrid Guilvout[7,8], Nathalie Nadeau[7], Peter J. Bond[9,10], Guy Tran Van Nhieu[2,3,4,5] and Olivera Francetic[7,8]

*1 - **Université Paris Diderot (Paris 7) Sorbonne Paris Cité,** 11 Place Marcelin Berthelot, 75231 Paris, France*
*2 - **Laboratory of Intercellular Communication and Microbial Infections,** CIRB, Collège de France, 75231 Paris, France*
*3 - **Institut National de la Santé et de la Recherche Médicale (Inserm) U1050,** 75231 Paris, France*
*4 - **Centre National de la Recherche Scientifique (CNRS),** UMR7241, 75231 Paris, France*
*5 - **MEMOLIFE Laboratory of Excellence and Paris Sciences et Lettres,** 75231 Paris, France*
*6 - **Centre for Molecular Informatics,** Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK*
*7 - **Laboratory of Macromolecular Systems and Signalling,** Institut Pasteur, Department of Microbiology and CNRS ERL6002, 25 rue du Dr Roux, 75724 Paris, Cedex 15, France*
*8 - **Biochemistry of Macromolecular Interactions Unit,** Department of Structural Biology and Chemistry, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris, Cedex 15, France*
*9 - **Bioinformatics Institute (A*STAR),** 30 Biopolis Str, #07-01 Matrix, Singapore 138671, Singapore*
*10 - **Department of Biological Sciences,** Faculty of Science, National University of Singapore, 5A Engineering Drive 1, Singapore 117411, Singapore*

***Correspondence to Olivera Francetic:** Institut Pasteur, 28 rue du Dr Roux, 75724 Paris, Cedex 15, France.*
ofrancet@pasteur.fr.
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Abstract

Bacterial type 2 secretion systems (T2SS), type 4 pili, and archaeal flagella assemble fibres from initially membrane-embedded pseudopilin and pilin subunits. Fibre subunits are made as precursors with positively charged N-terminal anchors, whose cleavage *via* the prepilin peptidase, essential for pilin membrane extraction and assembly, is followed by N-methylation of the mature (pseudo)pilin N terminus. The conserved Glu residue at position 5 (E5) of mature (pseudo)pilins is essential for assembly. Unlike T4 pilins, where E5 residue substitutions also abolish N-methylation, the E5A variant of T2SS pseudopilin PulG remains N-methylated but is affected in interaction with the T2SS component PulM. Here, biochemical and functional analyses showed that the PulM interaction defect only partly accounts for the PulG^{E5A} assembly defect. First, PulG^{T2A} variant, equally defective in PulM interaction, remained partially functional. Furthermore, pseudopilus assembly defect of *pulG(E5A)* mutant was stronger than that of the *pulM* deletion mutant. To understand the dominant effect of E5A mutation, we used molecular dynamics simulations of PulG^{E5A}, methylated PulG^{WT} (MePulG^{WT}), and MePulG^{E5A} variant in a model membrane. These simulations pointed to a key role for an intramolecular interaction between the pseudopilin N-terminal amine and E5 to limit polar interactions with membrane phospholipids. N-methylation of the N-terminal amine further limited its interactions with phospholipid head-groups to facilitate pseudopilin membrane escape. By binding to polar residues in the conserved N-terminal region of PulG, we propose that PulM acts as chaperone to promote pseudopilin recruitment and coordinate its membrane extraction with subsequent steps of the fibre assembly process.

Introduction

Prokaryotes build diverse surface appendages and protein transport systems to colonize their niche and acquire nutrients. Some of the most ancient and versatile prokaryotic nanomachines that mediate these functions belong to the type 4 filament (Tff) superfamily [1]. These conserved membrane

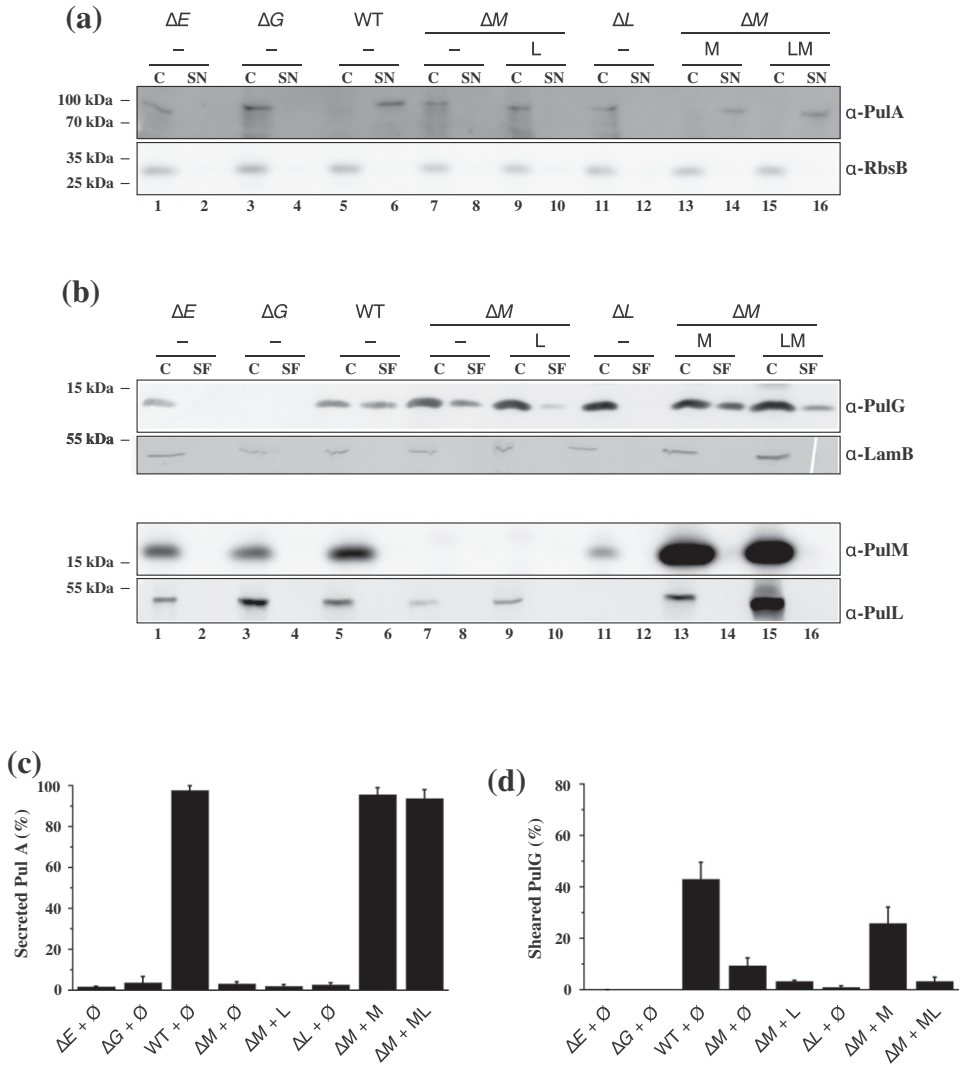

Fig. 1. The role of PulM in T2SS function. (a) PulA secretion assay in near-chromosomal expression conditions using *Escherichia coli* PAP5299 co-transformed with pCHAP8185 (all *pul* genes, WT) or its derivatives containing single non-polar *pul* gene deletions as indicated by a single letter code: Δ*E* (pCHAP8200), Δ*G* (pCHAP8184), Δ*M* (pCHAP8496), or Δ*L* (pCHAP8251), and with compatible pSU19 (-) or its derivatives carrying indicated *pul* genes: L (pCHAP8258), M (pCHAP1353), or LM (pCHAP8843). The amount of pullulanase PulA in 0.015 OD_{600} units of cell extracts (C) and culture supernatants (SN) was assessed by Western blot. Immunodetection of the periplasmic ribose-binding protein RbsB in 0.03 OD_{600} units served as a lysis control. Molecular weight (Mw) markers and lane numbers are shown. (b) PulG pilus assembly assay of *E. coli* PAP7460 overexpressing the *pul* genes from the same plasmids as in (a). Cell (C) and sheared pili (SF) fractions from an equivalent of 0.05 OD_{600} units were separated on Tris-Tricine SDS-PAGE, transferred onto nitrocellulose membranes, and probed with antibodies against PulG, LamB, PulM, and PulL. Mw markers and lane numbers are shown. (c) Quantification of the percentage of secreted PulA (mean + SD) from three independent experiments as the one in (a). Ø indicates empty vector. (d) Quantification of the percentage of sheared PulG (mean + SD) from three independent experiments like the one in (b). Ø, empty vector.

complexes use ATP-derived energy to drive the assembly of flagella (archaella) and pili in archaea [2–4] and to build type 4 pili (T4P) and T2SS pseudopili in bacteria. Illustrating the diversity of Tff functions, T4P, thin bacterial surface fibres, mediate adherence, aggregation, motility, protein transport and DNA uptake [1,5]. In T2SSs, found in Gram-negative (or diderm) bacteria, short periplasmic pseudopilus fibres promote protein transport from the periplasm across the outer membrane [6–8].

Bacterial Tffs are helical polymers of repeating subunits of the major pilin or pseudopilin, which may also contain one or more minor subunits that modulate the fibre assembly and function [1]. These subunits are made as membrane-embedded precursors, with an N-terminal cytoplasmic prepeptide, followed by a