Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Type V secretion: From biogenesis to biotechnology ☆

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ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 1 November 2013
Accepted 13 November 2013
Available online xxxx

Keywords:
Autotransporter
Autotransporter secretion
Two-partner secretion
Outer membrane
Protein folding
Surface display
Biotechnological application

ABSTRACT

The two membranes of Gram-negative bacteria contain protein machines that have a general function in their assembly. To interact with the extra-cellular milieu, Gram-negatives target proteins to their cell surface and beyond. Many specialized secretion systems have evolved with dedicated translocation machines that either span the entire cell envelope or localize to the outer membrane. The latter act in concert with inner-membrane transport systems (*i.e.* Sec or Tat). Secretion via the Type V secretion system follows a two-step mechanism that appears relatively simple. Proteins secreted via this pathway are important for the Gram-negative life-style, either as virulence factors for pathogens or by contributing to the survival of non-invasive environmental species. Furthermore, this system appears well suited for the secretion of biotechnologically relevant proteins. In this review we focus on the biogenesis and application of two Type V subtypes, the autotransporters and two-partner secretion (TPS) systems. For translocation across the outer membrane the autotransporters require the assistance of the Bam complex that also plays a generic role in the assembly of outer membrane proteins. The TPS systems do use a dedicated translocator, but this protein shows resemblance to BamA, the major component of the Bam complex. Interestingly, both the mechanistic and more applied studies on these systems have provided a better understanding of the secretion mechanism and the biogenesis of outer membrane proteins. This article is part of a Special Issue entitled: Protein Trafficking & Secretion.

1. Introduction

The secretion systems in Gram-negative bacteria that are classified as Type V comprise until now the subclasses Types Va-e [1,2]. These five subclasses share structural features, in that they include for transport a β-barrel protein or domain that is embedded in the outer membrane. Moreover, they all depend on the Sec complex for translocation across the inner membrane, whereas the Bam complex in the outer membrane contributes to the translocation of the secreted protein to the cell surface. The Sec complex plays a generic role in the transport of soluble proteins to the periplasmic space and the insertion of integral inner membrane proteins into the membrane. The Bam complex facilitates the folding of outer membrane proteins into a β-barrel conformation and their insertion into the outer membrane. Both complexes are reviewed in detail elsewhere in this issue of BBA [Chapters 5, 6 and 13 this issue of BBA]. Of the Type V subclasses, Type Va, the classical monomeric autotransporters, and Type Vb, the two-partner secretion (TPS) systems, have been studied in greatest depth and will be the focus of this review. The other subclasses are Type Vc comprising the trimeric autotransporters [3], Type Vd comprising the patatin-like autotransporters with a distinct C-terminal transport domain that resembles the translocation unit of the TPS system [4] and Type Ve, which comprises the intimin/invasin family of proteins that resemble classical autotransporters, but with their domains in reversed order [5,6]. Autotransporters are found in all Gram-negative bacterial genera, but not in all species of which genome sequences are available [7]. They are multi-domain proteins (Fig. 1A; [8,9]) that include a signal peptide at the N terminus for targeting to the Sec machinery to mediate inner-membrane translocation. During translocation, the signal peptide is cleaved off, the matured protein is released into the periplasm and the C-terminal β-domain inserts into the outer membrane. During or after its insertion the β-domain facilitates outer membrane translocation of the passenger domain which in the precursor protein is located between the signal peptide and the β-domain. For this reason the β-domain is also called translocator domain. Passenger translocation proceeds from C- to N-terminal direction [10] in a hairpin conformation through the translocation channel and both the insertion of the

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0167-4889/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbamcr.2013.11.006

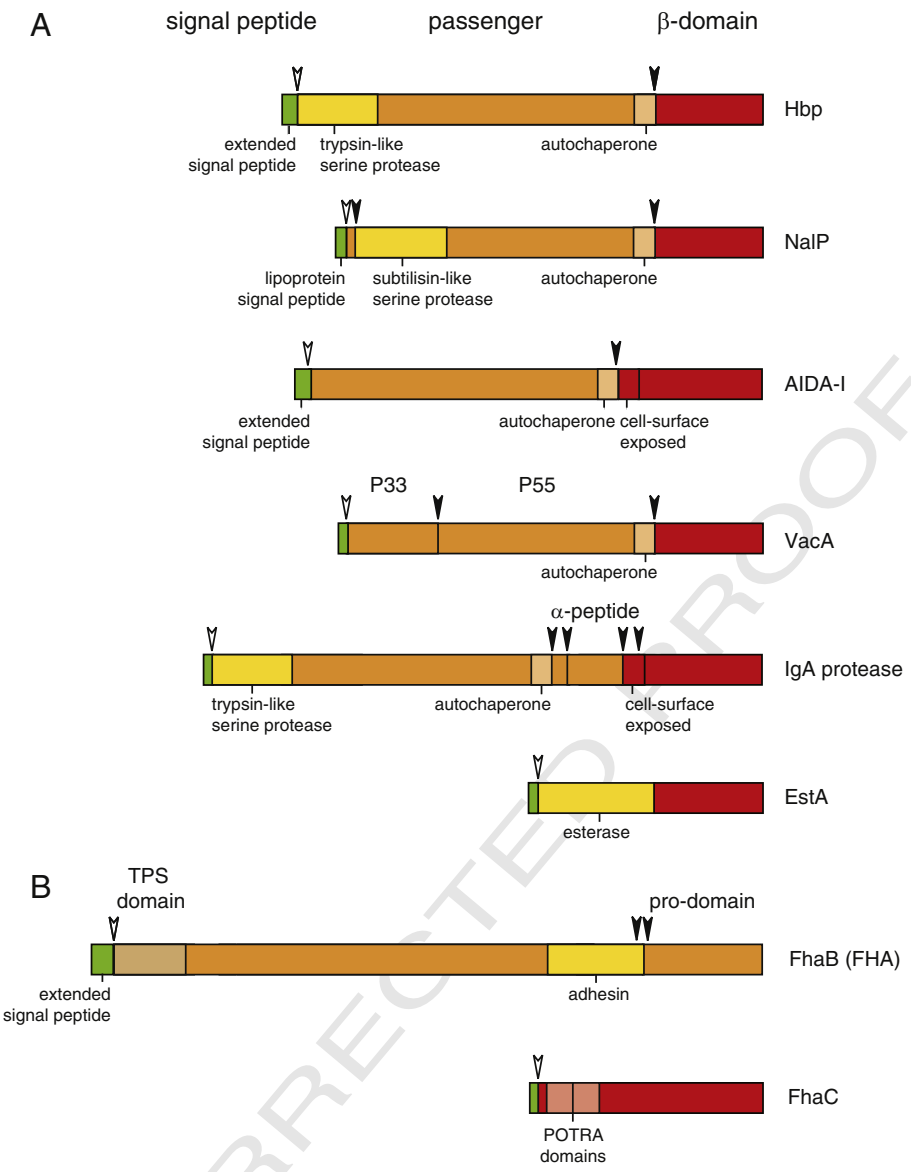

Fig. 1. Schematic representation of the domain organization of autotransporters and the TpsA and TpsB proteins of TPS systems. (A) Autotransporters show a general tripartite domain organization with a signal peptide (green), a passenger (yellow-orange) and a β-domain (red). (B) Domain organization of the canonical FHA TPS system, with the secreted FhaB (TpsA; yellow-orange), which is processed into the adhesin FHA after translocation to the cell surface. Its TPS domain involved in recognition of FhaC is indicated in pale orange. FhaC (TpsB; red) functions as transporter of FhaB in the outer membrane, with the periplasmic POTRA domains indicated in pale red. Both proteins carry a signal peptide (green). Indicated above the proteins are the names of subdomains (in VacA, IgA protease and FhaB), indicated below the proteins are specific features that are discussed in the review (functional subdomains in yellow). The open arrowheads indicate the signal peptidase cleavage sites; the closed arrowheads indicate sites where proteolytic cleavages occur after translocation to the cell surface.

β-domain and the translocation of the passenger require the active involvement of the Bam complex. A similar involvement of the Bam omplex has also been described for the trimeric autotransporters (type Vc) [11] and type Ve systems [5,12]. At the cell surface, most autotransporter passengers are proteolytically cleaved and then either remain attached to the cell surface via non-covalent interactions, or are released into the extracellular milieu. The passenger domains vary in sequence and length and carry functional subdomains that are invariably involved in interaction with the environment. Some autotransporters are post-translationally modified; *e.g.* the AIDA-1 adhesin of *Escherichia coli* is glycosylated by a dedicated glycosyltransferase that is active in the cytoplasm [13]. Another example is the NalP protease of *Neisseria meningitidis* which is lipid-modified during its transfer across the cell envelope [14].

The mechanism of translocation across both membranes and the involvement of the Sec and Bam complexes will be discussed in this review. A more detailed understanding of the molecular details of this process is required to improve the performance of autotransporters as carriers for secretion or surface display of recombinant proteins [15,16]. Current roadblocks for these applications will be discussed.

Unlike autotransporters, TPS systems consist of two proteins: a secreted protein generically named TpsA and an outer-membrane inserted transport protein, TpsB (Fig. 1B; [17,18]). The TpsA and TpsB proteins both include an N-terminal signal peptide for Sec-mediated transport across the inner membrane. Upon arrival in the periplasm, TpsB inserts into the outer membrane as a 16-stranded β-barrel with a large periplasmic domain that includes two POTRA motifs (for polypeptide transport associated domains). The TpsB proteins show homology to the BamA protein, the major component of the Bam complex [19]. The TpsA protein, after cleavage of the signal peptide, carries at its N terminus a conserved domain called the TPS domain that targets TpsB in the outer membrane. Upon recognition, secretion of TpsA across

Please cite this article as: P. van Ulsen, et al., Type V secretion: From biogenesis to biotechnology, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamcr.2013.11.006