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Mini Review

The Type I and II Secretory Systems in Gram-Negative Bacteria: A Brief Overview

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Abstract

Bacteria utilize a multitude of methods to secretion of specific substrate across phospholipid membranes. These bacterial strategies can play many roles in promoting bacterial process, from enhancing pathogenesis to bacterial response to environmental condition. The secretion in bacterial species transfer and release performance substance such as proteins, enzymes and metabolites to environment. The process of secretion plays an important role in the performance and compatibility with environment. Many bacteria use dedicated protein secretion systems to secrete from the cytosol into environment. In general, bacterial protein secretion systems classified to different classes, there are at least six specific secretory systems in Gram-negative bacteria. In this study, we review type I and II secretory systems of Gram-negative bacteria.

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Introduction

Secretion in prokaryotic cells

The prokaryotic cells release a chemical substance in process of the secretion; contrary to excretion of cellular waste products, secreted substance maybe have specific performance. Secretory systems in bacterial species transfer and release performance substances such as proteins, enzymes and metabolites to environment. The process of secretion plays an important role in the performance and compatibility with environment.

Secretion in Gram negative bacteria

There are at least six specific secretory systems in Gram-negative bacteria; many secretory proteins have a role in bacterial pathogenesis [1]. Gram-negative bacteria secrete toxins and enzymes by various secretory pathways to the extracellular environment. All systems can identify their substrate and facilitate their secretion without disrupt membranes. Secretory pathways in Gram negative bacteria sometimes used a two-step process. In the first phase, molecular machine (Sec) transfer substrate of inner membrane into periplasmic space. In the second stage, the substrate is passed from the outer membrane, but some used an one-step process without Sec for direct discharge to the outside of bacteria [2].

Secretory system type I (T1SS)

Secretory system type I, such as ABC transporter in other organisms but because Gram negative bacteria have two layer membrane, other proteins exist in system [3, 4]. These proteins provide additional relationship between inner and outer membranes and cross substrate through the outer membrane. Secretory system Type I is a simple system consists only of three subunits including ATP-binding cassette (ABC) protein, membrane fusion protein (MFP) and outer membrane protein (OMP).

Type I secretory system transfer molecules such as ions, drugs and proteins in various sizes up to 900 kDa [5]. Type I secretion system remove the non-protein substrates such as beta-glucan and polysaccharides.

Also this system crosses proteins in one step without the machine Sec. T1SS include ABC protein, membrane fusion protein in inner membrane and pore-forming proteins in outer membrane. Type I secretory system in *Escherichia coli* is formed of proteins HlyB, HlyD and TolC (Fig. 1) [6].

Each complex of three proteins detects usually one or a group of similar proteins, ABC (HylB) responsible for detecting protein substrate. The protein substrate is detected through a secretory signal at C-terminal of the substrate; Protein without signal peptide is not removed. There are limitations in terms of size and structure to discharge. Many proteins that are removed by the type I secretory system contain repeats of glycine. The repeated regions involved in folding of proteins.

ABC proteins transport large number of substrate using of ATP. These proteins have a transmembrane domain in membrane and hydrophilic cytoplasmic domain that contain ATP binding site. ABC Protein (HlyB) and membrane fusion protein (HlyD) form a complex. HlyD has a hydrophobic domain in inner membrane, but most of it is placed in the periplasmic space [7-9].

TolC placed in the outer membrane and form a trimer complex. The outside diameter of ternary complex is 5.8 nm, and has an internal acavity that represent the protein conducting channel. In addition, a large part of TolC expanded into periplasmic space. Only when the substrate connected to complex HlyD, HlyB, complex interacts with the pore TolC. After removing the substrate, complex HlyD-HlyB with TolC can be separated. Re-formation of

protein complexes, does not need to ATP but requires the proton gradient (Fig. 2) [10,11].
Each subunit of TolC is composed of four beta sheets. The beta sheets together create a membrane beta barrel. Several alpha helices remove the beta barrel into periplasmic space and create a 100 Å tunnel. These observations confirm secretion of the cell substrate in a single step without the intermediary periplasmic space. The interaction of secretory signal with nucleotide-binding domain of protein ABC induce conformation changes and cause hydrolysis of ATP in nucleotide binding domain. Further conformation changes driven by the energy of ATP and allow movement substrate in the T1SS [12].
ABC component in T1SS divide into two groups, a group are dedicated to transport large proteins and another responsible for transport small proteins and peptides. ABC proteins have two hydrolyser ATP domains in the cytosol and tow transmembrane domains.
Recent studies identified some examples of the type I secretory system in interactions plant microbe with their hosts. Secretion of AvrXa21 in rice pathogenic bacteria (*xanthomonas oryzae*) need to the type I excretory system that contain RaxB, RaxA and RaxC [13]. Phylogenetic analyzes have shown that the RaxB operates as ABC proteins and it is equivalent to HlyB in *E. coli*. It seems that AvrXa21 consisting of a small sulfated polypeptides that are secreted by the type I secretory system and recognized by the host plant. The pathogen factors such as metalloprotease secreted through type I secretory system pathogenic *Pseudomonas* species and *Xanthomonas*. Also, some of the proteins involved in biofilm formation are released by this system. In addition, the type I secretory system can secrete exopolysaccharide to the outside of the bacteria [14].

Type II secretory system (T2SS)

Proteins that are released through type II secretion system must enter to the periplasmic space through Sec or Tat systems at the start of the transition [15]. When proteins enter to periplasmic space, they pass of outer membrane through a multi-subunit complex (between 12 to 14 subunits). In addition, 10 to 15 proteins in inner and outer membranes make a complete secretory apparatus. The role and function is not known of these proteins. Type IV Pili of Gram negative bacteria derive of the type II secretion system with modified proteins [16].

Protein secretion of the cell membrane to the periplasmic space

Two systems are responsible for the protein secretion of the inner membrane into the periplasmic space: the Sec (general system) and Tat. The components of Sec system exist in all living organisms, from prokaryotes to developed eukaryotes. Most organisms have a copy of the Sec system, but a Tat system exists only in some bacteria [17].
Sec system formed of trimer complex of Sec Y, E, G and it is a protection and comprehensive system [18]. Inclusiveness of Sec systems indicate that this system is fundamental method of protein secretion in all cells. Recently, it has been shown that Sec secretion system together with translation (co-translation) are responsible for secretion of several large proteins which secrete by the type V secretory system of outer membrane and they have the unusual signal [17].
Sec secretory machine identifies a hydrophobic signal peptide at the N-terminal of the protein substrates and transfer unfold proteins into periplasmic space. Sec system secrete proteins into periplasmic space using energy of ATP and proton gradient [19, 20].

Figure 1. Type I secretory system. Substrates in Type I secretory system lack the signal peptide in N- terminal, but instead has a Sec signal peptides in C-terminal of protein which is not deleted in the end. In order to direct passage substrate to the extracellular environment, during secretion, MFP interacts with ABC and outer membrane channel-forming protein (OMP). OMP and MFP operate as trimer. ABC provides the energy required for secretion by hydrolysis of ATP [6].

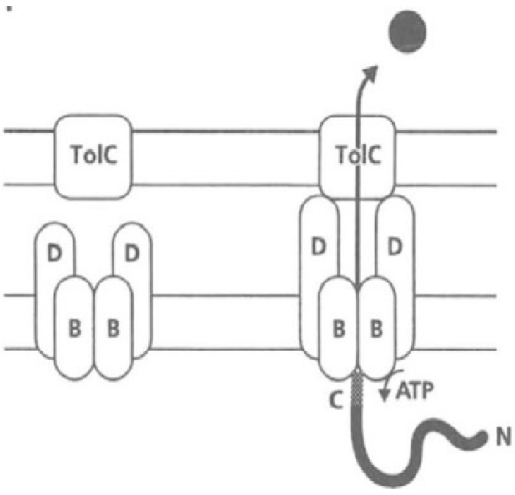

Figure 2. The formation of Type I secretory channels: HlyB and HlyD create together a sustainable complex in inner membrane. When a substrate attitude with HlyB. Then HlyD interact to the outer membrane channel (TolC) and cause direct secretion of proteins, after protein secretion, HlyD separate of TolC channels [10].

Hetrotrimer SecY/SecE/SecG is formed protein conducting pore in Sec secretory system. All proteins transfer to translocase hetrotrimer using of hydrolysis of ATP by two methods. The first method is specialized for membrane proteins and proteins contain hydrophobic signal peptide. Signal recognition particle (SRP) binds to signal peptide of these proteins group after initiation of translation when signal peptide come out of translation apparatus and the translation stop [21]. Signal recognition particle transfer translation apparatus together proteins to translocase by receptor of FtsY, then the translation resume and the translocase transfer translating protein to periplasmic space [22]. The second method, secreted proteins in the end stage of translation is identified by chaperons such as SecB. This chaperon transfer secretion protein to translocase by SecA [23-25].

Tat secretory system in *E. coli* is composed of four transmembrane proteins, three proteins (TatABC) are located in a single operon but another is located in different locus in the genome. Investigations of genome has shown which Tat standard system is formed of TatC and two homolog molecule of TatA [26].

Bacillus subtilis strain has two sets genes for Tat system which are adjusted individually. Homologous families of Tat doesn't in all bacteria and also doesn't in yeasts and developed eukaryote organisms [17]. Tat secretory machine has specific motif (SRRxFLK) in N-terminal of proteins contain of cofactors and transfer folded proteins to periplasmic space. This secretory machine use of the energy from proton gradient [14].

Proteins secretion of periplasmic space to environment by T2SS machine

Type II secretion system or general secretion system is a two stage system which used generally by Gram-negative bacteria. Proteins that are substrates of this system have a signal peptide at the N-terminal, which leads them to the Sec machine in inner membrane and transfer to periplasmic space, then proteins fold correctly in Periplasmic space [27]. The correct fold is prerequisite for transition from the outer membrane. The signal peptide may be a three-dimensional signal and depend on the formation of 3D protein structure [28, 29]. The secretion of folded proteins from membrane need to very large pore contain multi subunit channel, the molecular machine which is very complex and is composed of 12 to 15 different proteins (Fig. 3) [20].

Conclusion

Bacterial utilize a multitude of methods to secretion specific substrate across phospholipid membranes. These bacterial strategies can play many roles in promoting bacterial process, from enhancing pathogenesis to bacterial response to environmental condition. The secretion in bacterial species transfer and release performance substance such as proteins, enzymes and metabolites to environment. The process of secretion plays an important role in the performance and compatibility with environment. As we know, proteins may be transferred out of the bacterial cytoplasm through a variety of mechanisms, in other hand, most of biotechnological products are proteins that express in Gram-negative bacteria hosts (including *E. coli*) and mostly of them secreted protein and secreted by type I, II secretory systems, for this reason, the study of type I, II secretory systems has been an important focus in biotechnology. We are preparing and reviewing on other methods of bacterial secretion that will be available in near future.

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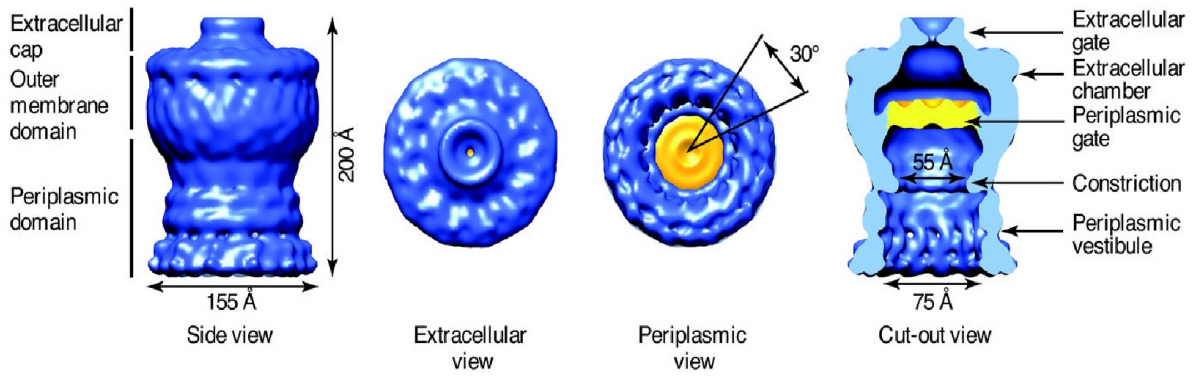

Figure 3. SEM image of type II secretion secretin system of *Vibriocholerae* (GspD). The Secretins consisting of 12 identical polypeptide chain and makes channel in the outer membrane for secretion. In the side view, three domains from top to bottom can be distinguished: extracellular cap, membrane domain and the periplasmic domain. The longitudinal incision of channel shows: extracellular gate and extracellular chamber, periplasmic gate and periplasmic vestibule [20].

References

1. Green, E.R., Mecsas, J., Bacterial secretion systems – an overview. *Microbiol Spectr*, 2016, Vol. 1, pp. 1-8.
2. Koster, M., Bitter, W., Tommassen, J. Protein secretion mechanisms in Gram-negative bacteria. *Int J Med Microbiol: 2001,* Vol. (4–5), pp. 325–331.
3. Thomas, S., Holland, I.B., Schmitt, L. The type 1 secretion pathway - the hemolysin system and beyond. *Biochim Biophys Acta*, 2014, Vol. 8, pp. 1629–1641.
4. Delepelaire, P. Type I secretion in Gram-negative bacteria. *Biochim Biophys Acta*, 2004, Vol. (1–3), pp. 149–161.
5. Kanonenberg, K., Schwarz, C.K.W., Schmitt, L. Type I secretion systems - a story of appendices. *Res Microbiol*, 2013, Vol. 6 , pp. 596–604.
6. Valentin, K. Substrate-triggered recruitment of the TolC channel-tunnel during type I export of hemolysin by *Escherichia coli*. *Mol Biol*, 2001, Vol. 3, pp. 501–510.
7. Linton, K.J., Higgins, C.F. The Escherichia coli ATP-binding cassette (ABC) proteins. *Mol Microbiol*, 1998, Vol. 1, pp. 5–13.
8. Pohl, A., Devaux, P.F., Herrmann, A. Function of prokaryotic and eukaryotic ABC proteins in lipid transport. *BBA Mol Cell Biol Lipid*, 2005, Vol. 1, pp. 29–52.
9. Decottignies, A., Goffeau, A. Complete inventory of the yeast ABC proteins. *Nature Genet*, 1997, Vol. 2, pp. 137–145.
10. Buchanan, S.K. Type I secretion and multidrug efflux: transport through the TolC channel-tunnel. *TIBS,* 2001, Vol. 1, pp. 3–6.
11. Yamanaka, H., Nomura, T., Fujii, Y., Okamoto, K. Need for TolC, an *Escherichia coli* outer membrane protein, in the secretion of heat-stable enterotoxin I across the outer membrane. *Microb Pathog*, 1998, Vol. 3, pp. 111–120.
12. Bleves, S., Viarre, V., Salacha, R., Michel, G.P.F., Filloux, A., & Voulhoux, R. Protein secretion systems in *Pseudomonas aeruginosa*: a wealth of pathogenic weapons. *IJMM*, 2010, Vol. 8, pp. 534–543.
13. da Silva, F.G., Shen, Y., Dardick, C., Burdman, S., Yadav, R.C., de Leon, A.L., Ronald, P.C. Bacterial genes involved in type I secretion and sulfation are required to elicit the rice Xa21-mediated innate immune response. *MPMI*, 2004, Vol. 4, pp. 593–601.
14. Tseng, T.T., Tyler, B. M., Setubal, J. C. Protein secretion systems in bacterial-host associations, and their description in the gene ontology. *BMC Microbiol*, 2009, Vol. 1, pp.23-45
15. Natale, P., Brüser, T., & Driessen, A.J.M. Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane--distinct translocases and mechanisms. *Biochim Biophys Acta*, 2009*,* Vol. 9, pp. 1735–1756.
16. Sauvonnet, N., Vignon, G., Pugsley, A.P., Gounon, P. Pilus formation and protein secretion by the same machinery in Escherichia coli. *EMBO*, 2000, Vol. 10, pp. 2221–2228.
17. Pallen, M.J., Chaudhuri, R.R., Henderson, I.R. Genomic analysis of secretion systems. *Curr Opin Microbiol*, 2003. Vol. 5.
18. Collinson, I. The structure of the bacterial protein translocation complex SecYEG. *Biochem Soc Trans*, 2005 ,Vol. 6, pp. 1225–1230.
19. Schulze, R.J., Komar, J., Botte, M., Allen, W.J., Whitehouse, S., Gold, V.A.M., Collinson, I. Membrane protein insertion and proton-motive-force-dependent secretion through the bacterial holo-translocon SecYEG-SecDF-YajC-YidC. *Proc Natl Acad Sci USA*, 2014, Vol. 13, pp. 4844–4849.
20. Nijeholt, J.A.L., Driessen, A.J.M. The bacterial Sec-translocase: structure and mechanism. *Phil Trans R Soc B*, 2012, Vol. 367, pp. 1016–1028.
21. Luirink, J., Sinning, I. SRP-mediated protein targeting: structure and function revisited. *Biochim Biophys Acta*, 2004, Vol. 1–3, pp. 17–35.
22. Sijbrandi, R., Urbanus, M.L., ten Hagen-Jongman, C.M., Bernstein, H.D., Oudega, B., Otto, B.R., Luirink, J.x. Signal recognition particle (SRP)-mediated targeting and Sec-dependent translocation of an extracellular *Escherichia coli* protein. *J Biol Chem*, 2003, Vol. 7, pp. 4654–4659.
23. Papanikou, E., Karamanou, S., Economou, A. Bacterial protein secretion through the translocase nanomachine. *Nature Reviews*, 2003, Vol. 11, pp. 839–851.
24. Lenz, L.L., Portnoy, D.A. Identification of a second Listeria secA gene associated with protein secretion and the rough phenotype. *Mol Microbiol*, 2002, Vol. 4, pp. 1043–1056.
25. Hartl, F.U., Lecker, S., Schiebel, E., Hendrick, J.P., Wickner, W. The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. *Cell*, 1990, Vol. 2, pp. 269–279.
26. Robinson, C.,Bolhuis, A. Tat-dependent protein targeting in prokaryotes and chloroplasts. *BBA Mol Cell Res*, 2004, Vol. 5, pp.135–147.
27. Korotkov, K.V., Sandkvist, M., Hol, W.G.J. The type II secretion system: biogenesis, molecular architecture and mechanism. *Nat Rev Microbiol,* 2012, Vol. 5, pp. 336–351.
28. Mogensen, J.E., & Otzen, D.E. Interactions between folding factors and bacterial outer membrane proteins. *Mole Microbiol*, 2005, Vol. 2, pp. 326–346.
29. Korotkov, K.V., Gonen, T., Hol, W.G.J. Secretins: dynamic channels for protein transport across membranes. *TIBS*, 2011, Vol. 8, pp. 433–443.