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Minireview

Bacterial protein translocase: a unique molecular machine with an army of substrates

Anastassios Economou*

Institute of Molecular Biology and Biotechnology-FORTH and Department of Biology, University of Crete, P.O. Box 1527, Iraklio, Crete GR-711 10, Greece

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Abstract Secretion of most polypeptides across the bacterial plasma membrane is catalyzed by the Sec protein translocase. This complex molecular machine comprises a flexible transmembrane conduit coupled to a motor-like component and displays four activities: (a) it is a specific receptor at its cytoplasmic side for all secretory polypeptides, (b) it converts metabolic energy from ATP and proton gradients into mechanical motion, (c) it prevents substrates from folding *in statu translocanti* and (d) it binds and releases short segments of the polymeric substrate sequentially. Combination of these activities allows translocase to move processively along the length of the substrate. Substrates are thus gradually expelled from the membrane and are released for subsequent extracytoplasmic folding.

Key words: SecYEG; SecA; Protein translocase; Secretion; ATPase; Signal peptide

1. Introduction

The cell is a membrane-bound chamber filled with an aqueous milieu that is immensely crowded with two thirds of the proteome. The remaining third of cytoplasmically synthesized proteins escape to extracytoplasmic locations. Such hydrophilic proteins can be fully secreted to the cell surface or to the surrounding environment and be hydrolytic enzymes, cytolytic toxins, adhesins, growth factors, hormones or antibodies. In addition, some of the extracytoplasmic proteins that contain hydrophobic patches get trapped in the membrane where they acquire their native functional state. Such proteins become membrane transporters, ion channels, lipid biosynthesis enzymes, environmental sensors, flagellar components, cell division regulators or energy-converting machines.

The chemistry of biological macromolecules is catalyzed by proteinaceous nanomachines that utilize energy. Modern cells harbor a large variety of membrane surfaces with varying lipid and protein content that define different compartments and enclose different populations of specialized resident enzymes. Moreover, some secreted or surface-anchored proteins mediate specialized interactions with different types of cells. Accordingly, evolution has selected several (at least six in the bacterial domain alone) protein secretion machines termed translocases or translocons. Translocases operate under similar principles but frequently utilize dissimilar components. One fascinating translocase built with components that are largely conserved throughout life is the Sec translocase, the enzyme that is responsible for most of the essential housekeeping secretion needs of the cell [1–5]. In satisfying this function, the translocase catalyzes secretion of hundreds of different substrates and hence displays an astonishing degree of substrate promiscuity. Not surprisingly, Sec translocases are essential for life. The bacterial counterpart of the family was the first translocase to be characterized in substantial molecular detail and will be the focus of the present review.

All the essential and most, if not all, regulatory components of the bacterial translocase are known and have been biochemically isolated. Attesting to this, protein secretion has been successfully reconstituted in vitro from purified components with efficiencies approaching those of the complete membrane [6]. Correct subcellular localization of secretory and membrane polypeptides requires that they avoid folding in the cytoplasm and that they reach and recognize the translocase correctly. These goals are achieved by use of bar-code signals on the secretory substrate that are specifically recognized by cytoplasmic chaperones with an affinity for the translocase. As a result, premature folding of secretory proteins in the cytoplasm is prevented, the substrate latches on to the translocase and is pumped to the outside world.

Synthesis of available experimental data offers a preliminary glimpse of bacterial protein targetting and translocation as a three-stage reaction comprising: targeting, membrane translocation and release [2,4]. Here I will place particular emphasis on current understanding of the second stage of the reaction as it has emerged during the past few years from studies carried out using *Escherichia coli*. In what concerns translocation, microbial genomics has proven *E. coli* to be a true prototype bacterium. The essential features and genes described in *E. coli* have been conserved in all of the bacterial genomes currently known.

2. The mechanical parts

The translocase core comprises three subunits essential for catalysis and life: the polytopic membrane proteins SecY and SecE and the peripheral membrane protein SecA [1–4]. The core is organized in a membrane domain containing a tight dimeric complex of SecY and SecE [7] with a more peripheral and mobile domain comprising the SecA protein, a subunit unique to the Bacteria. Half of the cellular SecA exists as a

*Fax: (30)-81-391166.
E-mail: aeconomo@imbb.forth.gr

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soluble enzyme in the cytoplasm. The anionic phospholipids phosphatidylglycerol and cardiolipin although not essential for life or translocation [8] nevertheless play an important role in SecA activation, correct substrate membrane interaction and SecYEG stability [9].

Additional proteins with regulatory or undefined roles were shown to associate with the translocase core. SecG, an exchangeable [7], auxiliary [10] subunit, is a small membrane protein not present in all of the bacteria (T. Samuelsson, personal communication). The SecD and SecF subunits form a subcomplex together with a small uncharacterized protein YajC and are important for translocation particularly when SecG is deleted [11]. YidC, a polytopic plasma membrane protein [12], copurifies with the translocase under SecYEGDF overexpression conditions [13]. YidC is widespread among several organisms and is homologous to Oxa1, a protein functioning in the absence of the Sec translocase to catalyze mitochondrial cytochrome *c* oxidase and ATP synthase membrane assembly [14] and for chloroplast insertion of a light harvesting complex subunit [15]. YidC was shown to be important for insertion and integration of at least one membrane polypeptide in *E. coli* [13].

No molecular machine can be really understood without its three-dimensional plan. However, the difficulty in obtaining crystals of membrane proteins makes their structure determination at atomic resolution a slow process. As a result, only low resolution images of translocase subunits and complexes are currently available. In two studies employing electron microscopy, up to three SecYE dimers [16] or four SecYEG trimers [17] were proposed to assemble in a ring-shaped structure built around a putative $\sim$1.5–5 nm pore in the middle. SecA was modelled by small angle X-ray scattering as an elongated 15 nm particle [18]. When SecA is bound to SecYEG, the shape of the SecYEG ring and of the presumed pore appears altered [17]. One remarkable hypothesis derived from the work of Manting et al. [17] is that SecA may nucleate formation of SecYEG tetramers, thereby scaffolding the assembly of translocase holoenzyme. This possibility would explain the lack of exposure of SecYEG-assembled SecA to the lipid bilayer [19,20].

3. Substrate, meet thy translocase

Successful subcellular communication is the result of productive protein–protein interactions. In the secretory pathway, this is achieved through the recognition of peptide tags located on the secretory substrate by appropriate binding sites on chaperone proteins [1,3,4]. Peptide tags can be short amino-terminal sequences of defined physicochemical properties [21]. Such signals, called signal or leader peptides, are specifically recognized on nascent chains by a highly conserved and essential ribonucleoprotein chaperone termed signal recognition particle (SRP) [22]. Short stretches with aromatic and positively charged residues in the mature part of the secretory chain can be recognized by the SecB chaperone [23]. SRP is particularly important for targeting of a subset of polytopic membrane proteins [24–26] by virtue of its recognition of very hydrophobic stretches.

Eventually, ternary complexes formed by chaperone–substrate interactions mediate substrate targeting to the membrane at SecA [27,28]. SRP associates with its membrane-bound receptor FtsY that has an affinity for acidic phospholipids [29], while SecB binds specifically to a C-terminal sequence in SecA [28]. Moreover, the signal peptide [21] and the mature domain [30] of the secretory chain are directly recognized by SecA bound to SecYE. Upon initiation of translocation, the mature part of the preprotein is transferred from SecB to SecA and SecB is no longer needed and is expelled [28,30]. Similarly, the signal peptide is no longer necessary and is proteolytically removed by leader peptidase [31]. The precise mechanism by which SRP-targeted substrates are handed onto the translocase is poorly understood and remains an exciting question for future research. In vitro experiments suggested that one SRP substrate can insert in the membrane in the absence of SecA [26]. In contrast, another in vitro study demonstrated that Ffh, the SRP proteinaceous subunit, enhances SecA–substrate interaction and binds to SecA [32]. Finally, a large-scale proteomics study in *Bacillus subtilis* showed that almost all of the secretory polypeptides that are affected in Ffh mutations are also affected by SecA mutations [33].

4. Energy input and its regulation

Arrival of substrates to SecY-bound SecA sets the translocase machine in motion. This is powered by ATP and electrochemical (proton motive force; PMF)-derived energy and is physically converted to mechanical work primarily through conformational changes in the SecA [2,34,35] and probably SecY proteins [36]. SecA is dimeric and each protomer comprises two primary domains: the ATPase N-domain and the dimerization C-domain [37] that also targets SecA to SecYEG [38]. The N-domain contains a classical nucleotide-binding domain (NBD1-carrying Walker boxes A and B) essential for SecA-mediated catalysis. A second hypothetical low affinity site NBD2 is also essential and probably regulates NBD1 ATP hydrolysis [37]. The C-domain contains a conserved and essential sequence termed intramolecular regulator of ATP hydrolysis (IRA). When IRA is disrupted, association of the C-domain with the N-domain is abrogated [37]. Remarkably, this interaction allows IRA to function as a molecular switch, suppressing vain ATP hydrolysis in the cytoplasm when SecA is not doing translocation work, but permitting ATP hydrolysis at the membrane only after SecA has bound to SecYEG. ATP-derived energy is essential for translocation while the contribution of the PMF is increasing the reaction rates [39].

5. The translocase machine in motion

The central mechanistic element of translocase is that SecA uses energy from ATP to undergo membrane insertion and deinsertion proposed to operate in multiple repeated cycles [2,34,35,37]. This model proposes four distinct reaction steps for the translocation of hydrophilic secretory proteins.

5.1. Turning off the IRA switch

Binding of SecA to the membrane at SecYEG relieves IRA-mediated suppression on the N-domain ATPase activity [37]. In addition, SecA becomes primed for SecB and preprotein binding [3,28,30]. The fact that the IRA switch lies within the SecY-binding region of the C-domain led to the proposal that SecY regulates switching through localized conformational changes [37]. Precise IRA switching imparts order on the catalytic steps.

5.2. SecA and substrate membrane co-insertion

SecA inserts spontaneously and stably in the membrane at SecYEG [19,34,35,38,40–43] but it is the binding energy of ATP at NBD1 that stabilizes what appears to be an even more integral membrane state [34,35,43]. SecA membrane topologies have only been studied with coarse biochemical probes that give frequently contradicting results and are not understood in any molecular detail (see discussion in [4]). Phospholipids have not been detected in the immediate viscinity of membrane-inserted SecA [19,20] and SecA is very hydrophilic, suggesting that ATP-driven SecA membrane penetration may take place largely within a proteinaceous environment. The SecYEG ring [16,17] may supply appropriate and sufficient surfaces and with sufficient flexibility to envelope the large SecA dimer [18]. SecA membrane insertion drives reversion of SecG topology and this change was proposed to facilitate SecA cycling [44].

Kinetically coincident with SecA insertion, short stretches of $\sim$25 aminoacyl residues of the substrate also enter the membrane plane in two distinct steps [39,45]. At this stage, secretory substrates are threaded through translocase and in proximity to SecA and SecY [46], and are prevented from sliding back through the stabilization of inserted SecA by the regulatory subunits SecDF [11,35,47]. SecA was proposed to physically mediate the forward movement of substrates [34] but the molecular details remain totally elusive. A first step in dissecting these events is the determination of the preprotein substrate binding site on SecA (Baud, C. and Economou, A., unpublished results).

5.3. Substrate release and SecA deinsertion

Hydrolysis of ATP causes substrate release from liposome-bound SecA and it was extrapolated that the same happens during translocation [39]. In this case, segments of the polymeric substrate released from SecA are stably associated with SecYEG and take the form of transmembrane intermediates [39,46]. This steric trapping underscores translocase processivity on hydrophilic substrates [2]. Interestingly, hydrolysis of ATP at NBD1 was shown by chase experiments to drive SecA membrane deinsertion [34] thus allowing recycling of the enzyme (for an alternative view, see [42]). Deinsertion may be facillitated by the acquisition by SecA of the ADP state where the N- and C-domains acquire a closer, more compact association [37]. The rate of the deinsertion subreaction was shown in an elegant recent study to be enhanced in the presence of the PMF [48], explaining the enhancing effect that PMF has in the translocation reaction rates overall.

5.4. The PMF takes over

After the substrate is half-way threaded through the translocase, the PMF alone can complete translocation if SecA is biochemically removed or inactivated [39]. Experiments by Nouwen et al. [36] have identified SecY mutations that allow translocation in the absence of the PMF. These two observations suggest that, as is the case for several membrane transporters, the PMF affects SecY conformation or assembly and thereby has an indirect effect on the translocation of the substrate.

5.5. Repeated SecA membrane cycling

A single ATP-binding and hydrolysis event seems sufficient to promote one SecA insertion stroke and limited substrate translocation and SecA deinsertion. However, productive ongoing translocation leads to multiple ATP turnovers and is important for final periplasmic folding of the substrates [4,39]. Multiple rounds of SecA insertion/deinsertion were therefore proposed to be required for complete translocation [34]. Whether one SecA molecule catalyzes the translocation of one chain and whether ATP expenditure is stoichiometric to substrate translocation is not yet known. Real-time, non-destructive biophysical measurements would be necessary to test this important aspect of the model.

6. Concluding remarks

Protein secretion is a multi-stage reaction occurring in the user-unfriendly environment of the membrane. Nevertheless, a combination of biochemical and genetic approaches has already yielded the complete inventory of mechanical parts of the secretion machine, its accessories and the general operational conditions. Eagerly awaited atomic structures of the components have begun appearing and tools for biophysical and ultrastructural studies have been developed. The combination of the two SecA mechanical strokes essentially allows translocase to 'walk' along the length of the polymeric substrate in a fascinating model of enzymatic processivity [2,4]. SecA oscillations, the dynamic relationship of the regulatory subunits with the core and the apparent assembly of the core 'on demand', paint the picture of translocase as an astonishingly flexible membrane protein. This plasticity may explain the amazing ability of translocase to accommodate for large aminoacyl polymers, to prevent their folding, to move along them and to occasionally allow them to escape laterally to the lipid bilayer.

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