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

Co-translational protein targeting to the bacterial membrane ☆

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ARTICLE INFO

Article history:
Received 10 August 2013
Received in revised form 9 October 2013
Accepted 16 October 2013
Available online xxxx

Keywords:
Protein targeting
SecYEG
GTPases
Molecular recognition and regulation
Ribosome

ABSTRACT

Co-translational protein targeting by the Signal Recognition Particle (SRP) is an essential cellular pathway that couples the synthesis of nascent proteins to their proper cellular localization. The bacterial SRP, which contains the minimal ribonucleoprotein core of this universally conserved targeting machine, has served as a paradigm for understanding the molecular basis of protein localization in all cells. In this review, we highlight recent biochemical and structural insights into the molecular mechanisms by which fundamental challenges faced by protein targeting machineries are met in the SRP pathway. Collectively, these studies elucidate how an essential SRP RNA and two regulatory GTPases in the SRP and SRP receptor (SR) enable this targeting machinery to recognize, sense and respond to its biological effectors, i.e. the cargo protein, the target membrane and the translocation machinery, thus driving efficient and faithful co-translational protein targeting. This article is part of a Special Issue entitled: Protein trafficking & Secretion.

1. Overview of protein targeting in bacteria

A major challenge for all cells is to correctly transport newly synthesized proteins from the cytosol, where they are initially synthesized, to their final cellular destination. In the 1970s, Günter Blobel postulated that newly synthesized proteins carry intrinsic signals, termed signal sequences, that encode information about their cellular location [1]. This finding spawned a new era in cell biology. In subsequent years, the signal sequences for various organelles including the endoplasmic reticulum (ER), nucleus, mitochondria and chloroplasts were identified. Targeting factors were also identified that recognize these distinct signal sequences and mediate the delivery of the substrate proteins to their respective target membranes [2].

Despite the lack of sub-cellular organelles, bacterial cells also contain distinct sites to which newly synthesized proteins must be correctly localized, including the plasma membrane and the extracellular space. Additional destinations in Gram-negative bacteria include the periplasmic space and the outer membrane. Across all bacterial species, the major protein trafficking route involves the transport of newly synthesized membrane and secretory proteins from the cytosol to the plasma membrane. As often occurs in microorganisms, bacteria have evolved multiple pathways for the targeted delivery of these proteins (Fig. 1) [3,4].

Protein targeting in bacteria can be divided into two major routes: (a) Post-translational pathways, in which the nascent protein is completely synthesized and released from the ribosome prior to targeting (Fig. 1, route 1); (b) the co-translational pathway, in which the targeting and translocation of the nascent cargo protein is coupled to its ongoing synthesis by the ribosome (Fig. 1, route 2). Co-translational targeting is preserved throughout evolution and is the major pathway for targeting all secretory and membrane proteins to the endoplasmic reticulum in higher eukaryotes. In contrast, most secretory proteins in bacteria are targeted to the plasma membrane via post-translational mechanisms (Fig. 1, route 1). Why bacteria have evolved these different mechanisms remains unclear. It has been suggested that since protein translation is slower than translocation, it is beneficial to uncouple these pathways in rapidly growing organisms, like bacteria and yeast, to fully utilize the limited number of SecYEG translocation channels, a major translocon in the bacterial inner membrane [5]. Additional targeting mechanisms may have also evolved to accommodate specific substrates unable to use the Sec translocon (e.g. Tat pathway, see below).

Co-translational targeting is carried out by a universally conserved ribonucleoprotein complex, the Signal Recognition Particle (SRP) (Fig. 1, route 2a), which primarily mediates the targeted delivery of ribosomes translating integral membrane proteins and some periplasmic proteins to the Sec translocon (SecYEG in bacteria, Sec61p in eukaryotes) at the plasma membrane [6]. Here, a continuous channel is formed from the ribosome exit tunnel to the SecYEG translocation pore, allowing the nascent protein to be directly released into the membrane. The co-translational mode of targeting ensures that proteins containing highly hydrophobic transmembrane domains are sequestered from the aqueous environment of the cytosol and thus protected from misfolding or aggregation.

While SecYEG is the main site for protein insertion, other translocation machineries are often found to participate in membrane protein

☆ This article is part of a Special Issue entitled: Protein trafficking & Secretion.

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http://dx.doi.org/10.1016/j.bbamcr.2013.10.013

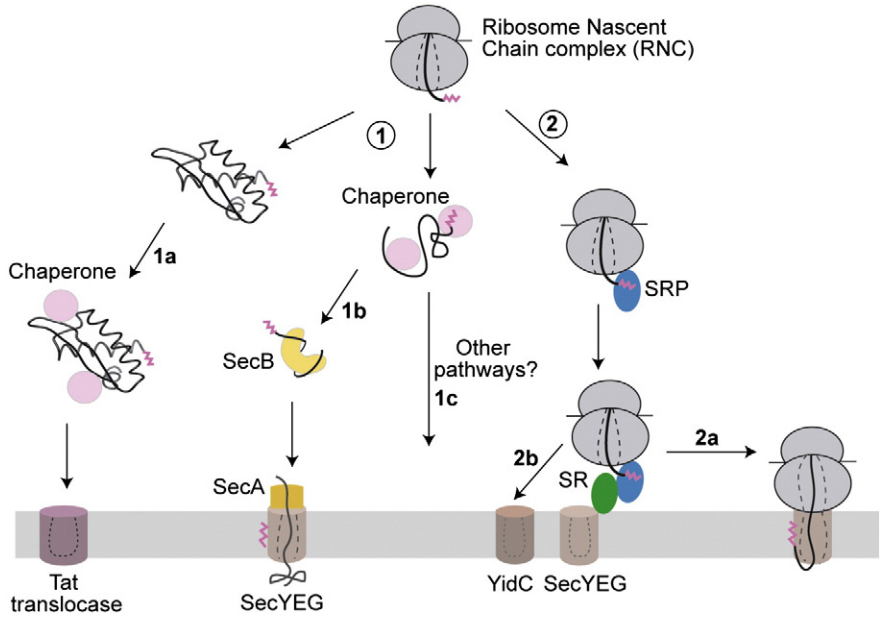

Fig. 1. A schematic depiction of various targeting pathways for delivering proteins to the bacterial inner membrane. Newly synthesized proteins with N-terminal targeting sequences (magenta) can be targeted either post-translationally (route 1) or co-translationally (route 2). Post-translational targeting (route 1) involves targeting of the nascent protein either in a fully folded state via the Tat pathway (1a) or in an unfolded state via the chaperone SecB and the ATPase SecA (1b). Both pathways may also involve general chaperones (pink) that maintain the proteins in a translocation-competent state. The co-translational targeting pathway (route 2), which primarily handles inner membrane proteins in bacteria, is mediated by the signal recognition particle (SRP, blue) and its receptor (SR, green) (2a). Both SecA (yellow) and SRP deliver proteins to the SecYEG protein-conducting channel and may co-operate in the translocation of membrane proteins with large periplasmic domains. Translating ribosomes may also be directly delivered to the YidC translocase (2b), which may either act independently or in conjunction with SecYEG. Whether additional pathways exist for the targeting of substrates, such as tail-anchored proteins, remains to be determined (1c). The same color scheme is maintained throughout the paper.

insertion in bacteria. The most notable of these is the non-homologous YidC translocon [7], which is essential in bacteria and is also found in organelles derived from them. *In vivo*, YidC appears to exist in two pools: one that is tightly associated with SecYEG and assists in the integration of polytopic membrane proteins [8–11], and another that acts independent of SecYEG to mediate the integration of several multi-spanning membrane proteins [12–14]. Targeting to YidC (Fig. 1, route 2b) is thought to occur via the SRP pathway, although SRP-independent mechanisms may also be involved [15]. Although YidC has been shown to bind translating ribosomes [16,17], the mechanism by which YidC mediates insertion of its substrates is not well understood [14,18].

Post-translational targeting of many periplasmic, outer membrane, and secretory proteins to SecYEG is carried out by the chaperone SecB, which captures newly synthesized substrate proteins in a translocation-competent state and delivers them to the ATPase SecA. SecA tightly associates with SecYEG and inserts the unfolded substrate protein across it using ATP-driven conformational changes (Fig. 1, route 1b) [3,4,19]. Other general chaperones, such as trigger factor (TF), may also be involved in maintaining the nascent polypeptides in a translocation-competent unfolded state. Recent reports suggest that SecA can also associate with ribosomes bearing the SecM nascent chain, raising the intriguing possibility that post-translational targeting machineries could also exert some of their actions co-translationally [20].

In an alternative targeting route, a subset of secretory proteins may be translocated in a completely folded state. This may be essential for substrate proteins that fold quickly, require cytosolic co-factors for maturation, or are multi-protein complexes in which only one subunit has a signal sequence. Substrates for this pathway have a twin arginine motif in their signal sequence and are translocated via the Tat translocon, composed of TatA, TatB and TatC subunits (Fig. 1, route 1a) [21]. How the substrate proteins, which presumably fold in the cytosol, are targeted to and translocated across the membrane by this pathway remains a mystery [22].

In addition to these pathways, there may be other mechanisms for targeting proteins to the bacterial membrane (Fig. 1, route 1c). For example, bacteria contain several proteins with putative C-terminal transmembrane domains (called tail-anchored proteins) that lack an N-terminal targeting sequence [23]. The mechanism by which these proteins are targeted to the membrane is not known. In a radically distinct mechanism, targeting could also precede translation and may instead rely on cis-acting elements in the TM-encoding regions of the mRNA [24]. The detailed mechanisms for targeting of these substrates have not been elucidated.

Despite the diversity of trafficking pathways, protein targeting can be divided into three key steps that are common to all pathways: recognition of substrates in the cytosol, their delivery to the target membrane, and passage through the membrane. The SRP pathway embodies these general principles and has served as a paradigm for understanding the molecular basis of protein localization in all cells. In this review, we focus on key events in the bacterial SRP pathway and highlight recent advances in our understanding of co-translational protein targeting.

2. SRP-mediated co-translational targeting

Although the size and composition of SRP varies significantly across species, the bacterial SRP contains the essential ribonucleoprotein core of SRP. Bacterial SRP can replace its more complex eukaryotic homologues to carry out efficient protein targeting to the endoplasmic reticulum [25,26], highlighting the remarkable evolutionary conservation of this pathway. As such, the much simpler bacterial SRP has served as a model system to understand the fundamental molecular mechanisms and energetic principles of this targeting machine in both prokaryotic and eukaryotic cells.

Bacterial SRP is comprised of the protein Ffh (a homologue of SRP54, the only evolutionarily conserved protein component of eukaryotic SRP) bound to a 4.5S SRP RNA [25,26]. Ffh has two functional domains connected by a flexible linker: a C-terminal M-domain, which contains the binding site for the SRP RNA and the signal peptide [27–30]; and an NG-domain composed of an N-terminal N-domain packed tightly against a central G-domain. The helical N-domain binds the ribosomal

Please cite this article as: I. Saraogi, S. Shan, Co-translational protein targeting to the bacterial membrane, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamcr.2013.10.013