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Review

Organization of the native ribosome–translocon complex at the mammalian endoplasmic reticulum membrane

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ABSTRACT

Background: In eukaryotic cells, many proteins have to be transported across or inserted into the endoplasmic reticulum membrane during their biogenesis on the ribosome. This process is facilitated by the protein translocon, a highly dynamic multi-subunit membrane protein complex.
Scope of review: The aim of this review is to summarize the current structural knowledge about protein translocon components in mammals.
Major conclusions: Various structural biology approaches have been used in synergy to characterize the translocon in recent years. X-ray crystallography and cryoelectron microscopy single particle analysis have yielded highly detailed insights into the structure and functional mechanism of the protein-conducting channel Sec61, which constitutes the functional core of the translocon. Cryoelectron tomography and subtomogram analysis have advanced our understanding of the overall structure, molecular organization and compositional heterogeneity of the translocon in a native membrane environment. Tomography densities at subnanometer resolution revealed an intricate network of interactions between the ribosome, Sec61 and accessory translocon components that assist in protein transport, membrane insertion and maturation.
General significance: The protein translocon is a gateway for approximately one third of all synthesized proteins and numerous human diseases are associated with malfunctioning of its components. Thus, detailed insights into the structure and molecular organization of the translocon will not only advance our understanding of membrane protein biogenesis in general, but they can potentially pave the way for novel therapeutic approaches against human diseases.

1. Introduction

In eukaryotic cells, many proteins have to be translocated across or inserted into the endoplasmic reticulum (ER) membrane. The precursors of these proteins typically carry hydrophobic N-terminal signal sequences or transmembrane helices of 20–30 amino acids in length [1], which commit them to the 'secretory pathway' [2]. Upon emergence from the ribosomal tunnel exit during translation, this hydrophobic protein stretch is bound by the signal recognition particle (SRP), a universally conserved RNA–protein complex abundant in the cytosol [3]. Association of SRP to the ribosome nascent chain (RNC) complex stalls protein synthesis and targets the RNC complex to the ER membrane [4], where it binds to the ER-resident SRP receptor (SR). When the RNC complex is transferred to the ER translocon [5], a multisubunit complex located in the ER membrane, SRP is released into the cytosolic pool. Alternatively, synthesis of secretory and membrane proteins can be initiated on ribosomes already bound to the ER translocon, bypassing SRP-dependent recruitment [6].

Co-translational protein transport, membrane insertion and maturation via the translocon require concerted action of various associated cofactors and enzymes. The functional core of the mammalian translocon is formed by the universally conserved protein-conducting channel Sec61, which facilitates protein transport across or protein insertion into the ER membrane. These basic functions are complemented by accessory translocon components, which have been found to be physically associated with Sec61 using biochemical methods [7–9]. They either assist the protein-conducting channel Sec61 or

Abbreviations: CET, cryo-electron tomography; EM, electron microscopy; ER, endoplasmic reticulum; ES, expansion segment; FIB, focused ion beam; OST, oligosaccharyl–transferase; RNC, ribosome nascent chain complex; SPC, signal peptidase complex; SRP, signal recognition particle; SR, SRP receptor; TRAM, translocating chain-associated membrane protein; TRAP, translocon associated protein.

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facilitate maturation of nascent chains by covalent modifications and chaperone-like functions. This accessory machinery is not entirely conserved in the different kingdoms of life and it also varies to small extent for different eukaryotic species. Prominent nascent-chain-modifying translocon components are the signal peptidase complex (SPC) [10], cleaving off signal peptides from translocating or translocated secretory proteins, and the oligosaccharyl–transferase (OST) complex, catalyzing one of the most common covalent protein modifications in eukaryotic cells: co-translational N-glycosylation [11–13]. Other commonly detected components of the ER translocon include the translocating chain-associated membrane protein (TRAM) [14] and the translocon-associated protein (TRAP) complex [9]. Their roles are not understood in detail, yet. Recent studies suggest that TRAM may assist Sec61 in membrane protein insertion [15], whereas TRAP may adopt a chaperone-like function in helping to assemble the correct topology for polytopic membrane proteins [16]. Recently, mutations in human TRAP subunits were observed to result in congenital disorders of glycosylation [17], suggesting a further role for TRAP. The Hsp40 co-chaperones Sec63 [18–22] and ERj1 [23,24] associate with the translocon and recruit the lumenal Hsp70 chaperone BiP to both the Sec61 complex and incoming polypeptides, thereby supporting Sec61 in its gating as well as in the transport of nascent proteins across the membrane. The ER-resident lectin-like chaperone calnexin assists in maturation and oligomerization of secretory and membrane glycoproteins and may associate with the core translocon, at least transiently or in a substrate-specific manner [25–27].

Membrane-association and the transient nature of many inter-subunit interactions have long hampered detailed structural investigation of the ER translocon. Even a precise definition of the invariant 'core' components of this dynamic assembly has been elusive, not to speak of its overall architecture and the detailed functional mechanism of its components. Technical advances in structural biology techniques, most notably in the field of cryo-electron microscopy (EM), have propelled our understanding of protein biogenesis at the ER in the last few years, which we discuss in this review.

2. Architecture and variability of the mammalian ER translocon complex

2.1. Cryo-electron tomography defines the ER translocon

Being a multi-subunit membrane protein complex, the complete translocon is difficult to study in isolation, e.g., by X-ray crystallography or single particle cryo-EM. When solubilized from their native membrane and interaction partners, many translocon components become disordered and often completely dissociate from Sec61, favoring their structural study in a native context. Cryo-electron tomography (CET) in combination with subtomogram analysis is uniquely suited to study the structure of large membrane-embedded and -associated complexes in a natural membrane environment [28]. This approach provided first insights into the overall structure of the native mammalian ribosome-bound translocon in isolated rough ER (rER) vesicles [29]. Further studies using siRNA-mediated gene silencing to manipulate the translocon composition in human cells prior to CET analysis allowed to determine the main components of the ER translocon: Sec61, TRAP and OST were localized in the native translocon [30]. Benefitting from developments in automated tomography data acquisition, image processing and, in particular, direct detector technology, a subtomogram average of the native ribosome-bound mammalian translocon could be refined to subnanometer resolution (Fig. 1A), revealing secondary structure elements for many parts of the translocon [31], including the protein-conducting channel.

2.2. Compositional heterogeneity of the translocon

CET studies on isolated rER vesicles from mammalian cells have characterized TRAP as a strictly stoichiometric component of the mammalian translocon, whereas OST was determined to be a substoichiometric constituent [30]. These studies also indicated that the stoichiometric OST ratio is cell-type dependent: whereas the occupancy is approximately 70% in canine pancreatic microsomes and microsomes isolated from several other cell types specialized in protein secretion, only 35% of translocon complexes contained OST in microsomes isolated from HeLa or HEK cells. Analysis of translocon complexes within undisturbed vitrified HeLa cells thinned by focused ion beam (FIB) milling showed essentially the same OST abundance as in microsomal preparations from HeLa cells [32], indicating that the OST distribution observed in rER vesicles is physiological. It is unclear, whether OST associates only transiently with the translocon for specific translocation phases and substrates, or whether different stable translocon types co-exist in the cell. Using cell-free protein transport assays, it was recently observed that initiation of translation strengthens the association of OST to Sec61 [18], which would support the former model. However, in these experiments, ribosome-bound translocon complexes were detergent-solubilized and separated using native gel electrophoresis, which both affects protein-protein interactions. Indeed, the relative spatial distribution of OST-containing and OST-free translocon complexes observed in CET studies, e.g. within separate polyribosomes, does not reveal any conclusive patterns *in vivo* [32]. Thus, a systematic screening of OST abundance in non-solubilized native translocon complexes, ideally using CET, would be essential to clarify the modulation of OST interaction by initiation of translation.

Efforts to locate the position of biochemically verified translocon components other than TRAP or OST using CET and siRNA-based gene silencing to manipulate translocon composition in HeLa cells were not successful. Although Western blotting indicated nearly quantitative depletion of the respective target components, subunit silencing did not yield significant density differences to wild type samples for the SPC [30], the SRP receptor, the Sec62/Sec63 complex or the Hsp40 co-chaperone ERj1. All silenced translocon components possessed substantial (>30 kDa) lumenal or cytosolic segments, which should have been detected in the CET studies (for comparison: the lumenal part of the TRAP complex is ~50 kDa). The absence of significant difference density suggests that these translocon components are indeed significantly underrepresented in the average ribosome-bound translocon complex. Thus, they are likely recruited transiently, possibly in a substrate-specific manner, to the ribosome-bound translocon.

In summary, Sec61 and TRAP appear to be strictly stoichiometric components of the mammalian ER translocon, while OST is present in varying intermediate ratios. Almost all other ER translocon subunits are likely to bind transiently, resulting in strongly substoichiometric abundance on average. Only TRAM, which has no lumenal or cytosolic domains large enough to be identified unambiguously in CET maps, remains a candidate for stoichiometric binding to Sec61 and TRAP.

3. Structure and function of Sec61 in the ER translocon

3.1. Structures of the ribosome-free protein-conducting channel

The overall structure of the hetero-trimeric protein-conducting channel Sec61 has first been revealed by X-ray crystallographic structures of isolated prokaryotic homologs (SecY complex) [33–35]. The high sequence conservation of the protein-conducting channel indicated that its architecture is universally conserved, which was confirmed by a number of subsequent cryo-EM studies (see below). The central Sec61α subunit consists of ten transmembrane helices, arranged in two pseudo-symmetrical N- and C-terminal halves around a central

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