The Sec61 complex is the major protein translocation channel of the endoplasmic reticulum (ER), where it plays a central role in the biogenesis of membrane and secretory proteins. Whilst Sec61-mediated protein translocation is typically coupled to polypeptide synthesis, suggestive of significant complexity, an obvious characteristic of this core translocation machinery is its surprising simplicity. Over thirty years after its initial discovery, we now understand that the Sec61 complex is in fact the central piece of an elaborate jigsaw puzzle, which can be partly solved using new research findings. We propose that the Sec61 complex acts as a dynamic hub for co-translational protein translocation at the ER, proactively recruiting a range of accessory complexes that enhance and regulate its function in response to different protein clients. It is now clear that the Sec61 complex does not have a monopoly on co-translational insertion, with some transmembrane proteins preferentially utilising the ER membrane complex instead. We also have a better understanding of post-insertion events, where at least one membrane-embedded chaperone complex can capture the newly inserted transmembrane domains of multi-span proteins and co-ordinate their assembly into a native structure. Having discovered this array of Sec61-associated components and competitors, our next challenge is to understand how they act together in order to expand the range and complexity of the membrane proteins that can be synthesised at the ER. Furthermore, this diversity of components and pathways may open up new opportunities for targeted therapeutic interventions designed to selectively modulate protein biogenesis at the ER.

Introduction

Integral membrane proteins are often anchored into their host membrane via one or more hydrophobic polypeptide segments, or transmembrane domains (TMDs), that span the entire width of the phospholipid bilayer. These so-called ‘transmembrane’ proteins (TMPs) represent ~ 25% of human genes, are diverse
in structure and perform a plethora of essential cellular functions [1]. The endoplasmic reticulum (ER) is a major site for the biogenesis of such integral membrane proteins, acting as their entry point into the secretory pathway, an elaborate network tasked with the synthesis, folding and transport of both membrane and secretory proteins (Fig. 1A) [2,3]. Given the molecular crowding of the cytosol and the biophysical constraints of the lipid bilayer, most TMPs enter a dedicated ER targeting pathway(s) as soon as an appropriate subcellular targeting signal has emerged from the ribosome. Upon arrival at the ER, these nascent polypeptides are threaded into and across its membrane via specialised protein translocation channels that typically act concomitantly with translation [4].

Amongst these ER translocation channels, the heterotrimeric Sec61 complex (α, β, γ subunits), or Sec61 ‘translocon’ [5], is the principal protein-conducting channel through which secretory proteins are fully translocated across the ER membrane. TMPs also access the Sec61 complex, however, in contrast to secretory proteins, they are only partially translocated, with their TMD(s) exiting the Sec61 complex via a lateral gate. This enables stable membrane integration and thereby constrains their membrane topology as they navigate the secretory pathway towards the plasma membrane. Single-span TMPs can be grouped into different types based on their structural features and the location of their N and C termini relative to the ER membrane (Fig. 1B). Here, we will classify them as type I, type II or type III TMPs and tail-anchored (TA) proteins (Fig. 1B; [6]). Based on the features of their first TMD, this characterisation can, in principle, also be extrapolated to multi-span TMPs (type I-like, type II-like, type III-like). However, given that the membrane insertion of multiple TMDs is not necessarily sequential and may also be co-operative [7,8], such an approach may be of limited use when trying to understand the biogenesis of multi-span TMPs.

It is increasingly clear that TMP biogenesis at the ER is a substrate-selective and mechanistically diverse process that involves a range of molecular machines well beyond the canonical Sec61 translocon [9]. Herein, we review the rapidly expanding field of co-translational membrane protein biogenesis at the mammalian ER; that is, when membrane insertion is concomitant with ribosomal polypeptide synthesis. Focussing on the mechanisms of ER targeting, together with protein translocation across, and TMD insertion into, the ER membrane, where relevant, we draw upon molecular details obtained in bacterial and yeast systems so as to gain prospective insight into mammalian mechanisms of co-translational TMP biogenesis that are yet to be fully elucidated.

**ER membrane targeting: the SRP-delivery system**

Within the arsenal of ‘accessory components’ employed by the Sec61 complex [9], the signal recognition particle-(SRP) and its ER membrane-localised cognate binding partner, the SRP receptor, constitute the first key players that are encountered by the majority of proteins destined for the secretory pathway. Together, these complexes mediate protein targeting to the ER [4], typically by virtue of an N-terminal hydrophobic stretch of amino acids [13], or signal sequence, that acts as a ‘molecular postcode’ and, in many cases, is cleaved [11] from the newly synthesised polypeptide once it is committed to membrane translocation and/or insertion.

Not all polypeptides that are destined for the ER are equipped with a so-called cleavable N-terminal signal sequence (Fig. 1B). Hence, in the case of type II and III TMPs their hydrophobic N-terminal TMD(s) act as ‘signal-anchor’ sequences, emulating the functions of N-terminal ER signal sequences and targeting nascent TMPs to the ER prior to their integration into the membrane bilayer [7,8]. Thus, whether cleavable or not, these hydrophobic regions within TMPs act as ‘signal flares’, efficiently recruiting and interacting with the SRP at an early stage during the synthesis of the nascent polypeptide. Hence, the SRP-delivery system predominantly operates co-translationally, targeting a range of structurally diverse single- and multi-span TMP clients to the ER for co-translational membrane insertion. Notable exceptions include TMPs whose ER targeting and integration occurs after protein synthesis is completed (post-translationally), as best exemplified by the TA proteins (cf. Fig. 1B) [14–16]. In yeast, the proteome-wide effects of rapid SRP depletion suggest it is essential for the efficient ER targeting of TMPs utilising their TMDs as signal-anchor sequences [17]. In contrast, SRP is only required for the ER delivery of ~14% of yeast proteins with cleavable N-terminal signal sequences [17]. Likewise in bacteria, SRP is essential for membrane targeting of inner-membrane proteins utilising signal-anchor sequences. However, it is dispensable for the targeting of many secreted precursor proteins with N-terminal signal sequences [18,19]. Although it is generally assumed that SRP plays a wider role in the ER targeting of proteins that bear N-terminal signal sequences in mammalian cells, proteome-wide analyses that directly test this hypothesis are presently lacking.
Fig. 1. Accessing the secretory pathway via Sec61: ‘many hands make light work’ (A) Newly synthesised secretory proteins and TMPs are targeted to and translocated into the ER lumen in order to enter the secretory pathway (green arrow). Mature proteins that have progressed through the Golgi apparatus are then delivered to the plasma membrane (PM) where they may be either incorporated or secreted (red arrow). (B) Representative structures of four classes of single-span TMPs: type I TMPs are equipped with an N-terminal signal sequence (s.s.), a lumenally translocated N terminus and a stop-transfer sequence (ST) which acts as the TMD; type II and type III TMPs do not possess an N-terminal signal sequence and have a signal-anchor sequence (SA) and, respectively, translocate their C and N termini into the ER lumen; tail-anchored (TA) proteins are topologically and structurally similar to type II TMPs, but their extremely short C-terminal region necessitates that their insertion into the ER occurs post-translationally. (C) The Sec61 complex can call on a diverse repertoire of additional cellular machineries to facilitate various aspects of its role in co-translational TMP biogenesis including: ER targeting (top left inset), Sec61 channel gating (top right inset), TMD insertion and TMD folding/assembly (bottom left inset). Additional events, such as N-linked glycosylation (via OST, oligosaccharyltransferase complex), signal sequence cleavage (via SPC, signal peptidase complex) and ER chaperone-mediated luminal folding (see BiP, binding immunoglobulin protein; Grp94; PDI, protein disulphide isomerase; ERp57; CRT, calreticulin), are also coupled to the actions of the Sec61 translocon (bottom right inset), and we direct the reader to recent articles that review these processes [9–12]. Schematics are illustrative only and are not drawn to scale.
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C. Co-translational SRP-mediated ER targeting & SGTA

Criteria for SGTA recruitment:
1) inefficient translational stalling AND/OR
2) a short linker between two targeting signals

Signal handover, TMD insertion, SRP/SGTA dissociation & recycling

Proteasomal degradation of ubiquitinated nascent chains?

D. ER Targeting: SRP vs. the hSnd2 & TRC40 pathways

N-terminal TMDs

C-terminal TMDs

Redundancy

Redundancy
In mammals, the cytosolic targeting factor SRP is a multimeric complex of six protein subunits assembled onto a core 7S RNA (Fig. 2A) [20–22], and is first recruited by signal sequences/anchors from within the ribosomal exit tunnel [23,24]. As the exit tunnel typically shields the first ~ 40 amino acid residues of nascent polypeptide chains from the cytosol [25], such early SRP recruitment is presumed to occur via ribosome-nascent chain (RNC)-induced structural rearrangements within the actively translating ribosome [26]. Once recruited, SRP binds to the ribosome at a location that is partly occupied by the nascent polypeptide-associated complex (NAC); a co-translational chaperone that enhances SRP-dependent targeting by increasing the fidelity of signal sequence recognition by SRP [27] and preventing the promiscuous interaction of ribosomes with Sec61 [28]. Thus, co-translationally bound and NAC-regulated SRP [27] is suitably poised to ‘scan’ and engage ER signal sequences/anchors as soon as they emerge from the ribosome [22,24,29,30].

Cellular levels of SRP are significantly lower than the near stoichiometric concentrations of ribosomes and NAC [27]. Thus, if an ER targeting signal is not encountered quickly, SRP rapidly dissociates from the RNC complex, effectively cycling on and off ribosomes in search of a substrate signal sequence [31]. As the exit tunnel typically shields the first ~ 40 amino acid residues of nascent polypeptide chains from the cytosol [25], such early SRP recruitment is presumed to occur via ribosome-nascent chain (RNC)-induced structural rearrangements within the actively translating ribosome [26]. Once recruited, SRP binds to the ribosome at a location that is partly occupied by the nascent polypeptide-associated complex (NAC); a co-translational chaperone that enhances SRP-dependent targeting by increasing the fidelity of signal sequence recognition by SRP [27] and preventing the promiscuous interaction of ribosomes with Sec61 [28]. Thus, co-translationally bound and NAC-regulated SRP [27] is suitably poised to ‘scan’ and engage ER signal sequences/anchors as soon as they emerge from the ribosome [22,24,29,30].

Cellular levels of SRP are significantly lower than the near stoichiometric concentrations of ribosomes and NAC [27]. Thus, if an ER targeting signal is not encountered quickly, SRP rapidly dissociates from the RNC complex, effectively cycling on and off ribosomes in search of a substrate signal sequence [31]. In the case of timely SRP engagement by an ER signal sequence/anchor, a process that may be enhanced by certain nonoptimal, ‘translation slowing’ mRNA codons located downstream of the signal sequence coding region [32], ribosomal translation is transiently stalled by the Alu domain of SRP [33,34; see Fig. 2B, left]. This translational stalling effectively maintains the nascent chain in a ‘translocation competent’ state during the time window available for successful ER delivery, as dictated by the limiting number of SRP receptor targeting sites [33].

Once at the membrane, and coordinated by the concerted actions of two GTPases, the signal sequence binding subunit of SRP (SRP54) and the membrane-tethered alpha subunit of the SRP receptor (SRα), the now quiescent SRP-RNC complex engages the SRP receptor [35]. Complex formation between SRP and its receptor leads to repositioning of both SRP54 and SRα relative to the SRP RNA from the so-called ‘proximal’ site to an alternative ‘distal’ site (Fig. 1B). This generates a ‘prehandover complex’, where the Sec61 binding site of the ribosome that was previously occluded by SRP54 now becomes accessible, whilst also blocking GTP hydrolysis by SRP54 and SRα. Subsequent arrival of this complex at the Sec61 translocon triggers handover of the ribosome and nascent chain from the SRP/SRP receptor complex concomitantly with GTP hydrolysis by SRP54 and SRα. Post-handover, and following the opening of the Sec61 translocon (see Gating of the Sec61 complex), translation is resumed as membrane translocation and/or membrane insertion of the nascent polypeptides takes place (Fig. 2B, right) and SRP is recycled for additional rounds of ER targeting [22].

Since the ‘signal hypothesis’ was postulated [36–39], the mammalian SRP-delivery system outlined above...
has been extensively characterised [22,40]. Neverthe-
less, novel aspects of SRP-mediated co-translational
delivery to the ER continue to emerge. For example, it
has been suggested that SGTA [41], a cytosolic quality
control component involved in the post-translational
targeting of TA proteins to the ER [14,41–43] also
contributes to co-translational ER targeting. Hence,
SGTA may facilitate the biogenesis of TMPs contain-
ing two or more closely spaced hydrophobic signals by
binding prematurely exposed TMDs that do not
recruit the substoichiometric SRP [44] (Fig. 2C). In
this way, the actions of SGTA would complement the
ER targeting role of SRP by shielding potentially vul-
nerable TMDs in the nascent polypeptide from inap-
propriate, and possibly damaging, interactions [44].
Such recruitment of SGTA thereby protects nascent
membrane proteins from potential proteasomal degra-
dation until they engage the Sec61 complex and initi-
ate membrane insertion (Fig. 2C) [44]. Exactly how
SGTA is recruited to and associates with the ribosome
and/or other ribosome-associated chaperones such as
NAC [45], why it does not compete with SRP despite
its comparative cytosolic abundance (~ 1 μM versus
~ 5–10 nM) [43,46] and how it is released upon delivery
to the ER membrane are all questions that remain to
be answered.

Despite the range of precursor proteins that are
catered for by the SRP-delivery system, at least two
other ER targeting pathways are operational in the
cytosol: a mammalian version [16] of the co-transla-
tional, SRP-independent or, ‘SND’ pathway first iden-
tified in yeast [47]; and a post-translational route for
TA protein biogenesis known as the TRC40 pathway
in mammals [14]. Whilst these pathways function in
parallel, they are also most likely overlapping and/or
partially redundant in terms of their substrate speci-
ficity. In principle, SRP typically caters for signal
sequences/anchors that are located at or near the N
terminus of nascent polypeptides, SND favours signal
anchors that are more central and TRC40 deals with
C-terminal tail anchor sequences (Fig. 2D) [17,47,48].
Only one mammalian orthologue of the three compo-
nents which make up the SND pathway in yeast has
been identified to date [16,47]. However, this compo-
nent, known as TMEM208 or hSnd2 [49], has been
implicated in the Sec61-mediated biogenesis of short
secretory proteins [50] and single- and multi-span
TMPs [16,51,52], with the wider hSnd2/SND targeting
pathway able to compensate for an absence of the
SRP or TRC40/GET pathways in yeast and mammals
[16,47,51]. The partial redundancy of these three ER
targeting pathways (see [17,47]), which most likely
allows cells to efficiently target membrane proteins
under a wide range of physiological conditions and/or
external stresses, probably explains why the hSnd2/
SND pathway remained undiscovered for so long [48].

Whilst bacteria typically contain only one location,
the inner membrane, to which newly synthesised pro-
teins are delivered, mammalian SRP must correctly
discriminate between the multiple membrane systems
that are accessible from the eukaryotic cytosol. When
compared to its bacterial equivalent, mammalian SRP
displays not only greater structural complexity
(Fig. 2A) but also increased functional complexity, as
evidenced by its early recruitment to the nascent chain,
regulation by the NAC complex and capacity to
induce a translational arrest. When combined with the
‘fail-safe’ option provided by SGTA recruitment, we
speculate that this additional complexity of eukaryotic
SRPs has most likely evolved to enhance the specificity
of, and lengthen the window for, nascent chain target-
ing to the ER membrane; a feat of increasing import-
ance for TMPs containing multiple TMDs located
after the initial ER signal sequence that may even
require additional rounds of SRP-mediated targeting
to the ER [17,19,53].

We further suggest that in the event that SRP fails to
engage the TMD of a protein client, whether as a con-
sequence of a nonfunctional SRP-delivery pathway, a
more C-terminal location of its ER targeting signal or
for some other reason, the hSnd2/SND pathway pro-
vides an alternative and/or additional system to ensure
that co-translational protein clients continue to be tar-
geted to the ER membrane. We anticipate that uncover-
ing the mechanistic details of the hSnd2/SND pathway
will help to delineate the extent of the substrates that it
caters for, why SRP ‘loses’ its ability to engage TMDs
as a polypeptide chain extends and whether the hSnd2/
SND pathway integrates with the SRP-dependent deliv-
ery pathway at the ER membrane.

Gating of the Sec61 complex

Following signal sequence/anchor-mediated SRP-de-
dependent delivery and transfer of RNCs to the Sec61
complex (cf. Fig. 2), these hydrophobic targeting sig-
als perform a second key action to fulfil their role as
an ‘ER-entry tag’: they must open the Sec61 channel.
Organised into two distinct halves, with TMDs 1–5
and 6–10 surrounding a central pore (Fig. 3A), the
hourglass-shaped conduit of Sec61α appears empty on
the cytosolic side of the ER membrane whereas a ring
of hydrophobic residues, known as the ‘plug domain’,
seals the ER lumenal side of the pore [55,56,57], effec-
vively preventing the free movement of small molecules
across the Sec61 translocon when inactive (see [57]).
Opening of the Sec61 channel involves three major steps. First, the channel is primed to open via the docking of RNCs to the Sec61 complex (Fig. 3Ai). Secondly, docking-induced conformational changes ‘crack’ the cytosolic end of the lateral gate (comprised by TMDs 2, 3, 7 and 8) which facilitates the engagement and intercalation of the signal sequence/anchor between TMDs 2 and 7. Thirdly, the signal sequence/anchor-mediated displacement of TMD2 finally results in movement of the plug domain and opening of the lateral gate (Fig. 3Aiii, supp. [5,55,56,58,64]). In situ studies of the native Sec61 translocon suggest that the lateral gate can be opened by ribosome binding alone, even in the absence of a nascent chain [58,59], although in the case of bacterial SecYEG (Fig. 3A) [59] the presence of a signal sequence on a nascent chain enhances opening of the equivalent lateral gate [60]. These conformational changes enable co-translational movement of the growing polypeptide chain across the membrane and into the ER lumen (Fig. 3Aiii, iv, supp. [5,55,56]). In contrast to these actively translocated hydrophilic regions of polypeptide, hydrophobic targeting signals are laterally inserted into the lipid bilayer via the lateral gate. In the case of N-terminal signal sequences, they are cleaved from the nascent chain by the signal peptidase peptidase [61]. In contrast, signal-anchor sequences form a stable membrane tether for the newly synthesised TMPs. Following translation termination and the exit of newly synthesised polypeptides from the channel, the ribosome dissociates from the Sec61 complex and the Sec61α plug domain returns to its original position (Fig. 3Av).

The central role of the Sec61 translocon during the biogenesis of secretory proteins, type I and type II TMPs has been well established for many years (Fig. 3A, B; see Membrane insertion via the EMC for type III TMPs). However, there is now a growing body of evidence that signal sequences can provide an additional, as yet poorly defined, level of control during membrane translocation [62]. Highly diverse in terms of their hydrophobicity, length, charge and specific amino acid composition [8,13,63], ER targeting signals appear to regulate the opening, or ‘gating’ of the Sec61 translocon [62], particularly since SRP effectively caters for targeting signals seemingly irrespective of their intrinsic ability to gate the translocon which, in some cases, is ‘inefficient’ and ‘slow’ (Fig. 3C) [64,65].

The defining structural feature of an archetypically efficient and ‘strongly gating’ signal sequence appears to be that its core h-region (Fig. 3D) is of sufficient hydrophobicity to successfully engage with, and insert ‘head-on’ into, the Sec61 translocon (Fig. 3A, stage ii) and then subsequently re-orientate to form a hairpin conformation within the channel (Fig. 3A, stage iii). During both of these apparently discrete stages, the RNC is subject to a distinct force that pulls the nascent chain away from the ribosome (Fig. 3Ci) [64]. In contrast, if an ‘inefficient’ signal sequence is appended to the same polypeptide, the nascent chain experiences a single, weaker pulling force that reflects its inability to successfully engage the translocon and undergo in-channel re-orientation (Fig. 3Ci) [64]. We speculate that hydrophobic signal-anchor sequences, some of which can also reorient inside the Sec61 translocon [68], will be subject to pulling forces comparable to those experienced by signal sequences during their membrane insertion at the ER [65,69]. Likewise, it seems plausible that the profiles and strength of the pulling forces experienced by signal sequences and/or signal anchors may be influenced by the drivers and determinants of signal orientation/TMD topology, such as the ‘positive-inside’ rule, the degree of N-terminal folding and, perhaps, even the lipid composition of the bilayer [7,8].

In cases where inefficient signal sequences (see [70]) and/or signal anchors require extra help to gate the translocon, the Sec61 complex may employ ‘gating assistants’. Hence, the tetrameric TRAP (translocon-associated protein) complex, composed of α, β, γ and δ subunits [71–73], and/or the Sec62/Sec63 proteins [74] (Fig. 3D), contribute to the second pulling event that occurs during secretory protein translocation (Fig. 3Cii) [75]. Despite subtle differences in the features that facilitate their recruitment (Fig. 3D), these gating assistants exhibit a common propensity to assist signal sequences/anchors that are of lower hydrophobicity [72,74] and/or contain clusters of positive charge in regions of polypeptide that must be translocated [74–76]. Furthermore, given that different gating assistants can contribute to the efficient translocation of the same protein substrate, whether a secretory protein or TMP (Fig. 3Ei, Tables 1–4) [74], and cellular levels of TRAP-β are upregulated following depletion of Sec62 [74], it seems likely that the TRAP complex and Sec62/Sec63 perform overlapping, but nonidentical, functions during Sec61-mediated protein translocation [62].

Such a notion is supported by the behaviour of the mammalian prion protein (PrP) during its ER translocation [75] where TRAP is required for both signal sequence engagement with Sec61 and its in-channel inversion, whereas Sec62/63 only influence the latter event, and to a lesser extent [75] (Fig. 3ci). Thus, it appears that Sec62/63 supports the translocation of
ER membrane protein biogenesis

A. Sec61-mediated co-translational translocation

B. TMP Insertion

C. Signal sequence force pulling profiles

D. TRAP vs. Sec62/63-dependent Features

E. Classification of TRAP and/or Sec62/63-dependent proteins
polypeptides with suboptimal ER targeting features that may first be recognised by the cytosolic portion of TRAP [76], prior to TRAP-mediated opening of the Sec61 complex [72,75–78]. Previous studies established that Sec62/63 contribute strongly to Sec61 gating during the post-translational translocation of certain substrates, including short secretory proteins [50]. However, structural studies strongly suggest that in order to stabilise RNCs that co-translationally engage the Sec61 translocon, either alone [5], or in its TRAP-assisted mode [78], the Sec62/63 complex must adopt a different conformation to its posttranslational one [9,79–81].

By subjecting a broader range of TRAP- and Sec62/63-dependent clients of the Sec61 translocon [72,74] to detailed force-pulling studies [64,65,69,75,76], it should be possible to discover mechanistic detail about these gating assistants that is currently lacking. Currently unanswered questions include: is the constitutively Sec61-bound TRAP complex [78] the only ‘gating assistant’ capable of exerting a pulling force on RNCs (see [75]); can the Sec62/63 complex compensate for the loss of TRAP-mediated assistance; is TRAP- and/or Sec62/63-assisted translocation used to regulate the flux of protein substrates through the Sec61 translocon [72,74]? Likewise, although the translocating chain-associated membrane (TRAM1) protein is closely associated with the active Sec61 translocation complex [7,8], a recent ‘global’ analysis suggests that it is not a gating assistant [82]. Rather it seems that TRAM1 may facilitate the egress of hydrophobic regions from the Sec61 lateral gate into the phospholipid bilayer [82], a possibility that clearly merits further exploration. Answering these questions may also help us to finally reconcile the long-standing enigma surrounding the broad sequence diversity that is seen across ER targeting signals [13].

Membrane insertion via the EMC

Analogous to the recruitment of ‘gating assistants’ by ER targeting signals that the Sec61 complex finds challenging, the multisubunit ER membrane complex (EMC) (Fig. 4A) provides a membrane insertase for TMDs that also appear to be more ‘demanding’ of the ER translocon [83,84]. The EMC was first implicated in membrane protein biogenesis when gene disruption of its subunits was found to have pleiotropic effects on the expression of multi-span TMPs in several species [85–88]. Multi-span TMPs were subsequently found to be significantly enriched amongst putative EMC-dependent protein clients (Fig. 4A, Table 5) [89,90], and the EMC was also identified as an ER membrane insertase that can facilitate the post-translational insertion of certain TA proteins [15,91]. In light of several recent structural studies, we now have a better

Fig. 3. ‘With a little help from my friends’: TRAP and/or Sec62/63-assisted gating of Sec61. (A) Schematics of the heterotrimeric Sec61 complex (α, β, γ) and the regulation of Sec61α via its plug domain and lateral gate during the co-translational translocation of secretory proteins [5,9]. Schematics are not drawn to scale. (B) Models for the Sec61-mediated insertion of type I and type II single-pass TMPs. (Bi) For type I TMPs, a cleavable N-terminal signal sequence enters the Sec61 translocon ‘headfirst’ and is then inverted allowing the subsequent stop-transfer sequence to become laterally inserted as a TMD with an NαβCγ topology [88]. (Bii) The NαβCγ topology of type II TMPs necessitates that they are membrane inserted in the opposite orientation; this may be achieved either by the ‘headfirst’ insertion of a signal-anchor sequence followed by its inversion within the Sec61 translocon (shown in brackets) or via a ‘hairpin integration’ mechanism whereby the signal-anchor engages the translocon in a looped conformation [8]. (C) Schematic overview of the pulling forces experienced by; (Ci) the protein substrate preprolactin equipped with its normal, archetypically strong, signal sequence (PPL, black solid line) versus its replacement with an inefficient signal sequence (*PPL, red dashed line; see [64]); (Cii) the Prion protein (PrP) in control cells (red solid line) and cells depleted of the Sec62/63 (purple dashed line) or the TRAP complex (green dashed line) [75]. (D) TRAP- and/or Sec62/63-dependent cleavable signal (orange) or signal-anchor (yellow) sequences typically include reduced hydrophobicity in the signal and/or clusters of positive charge in the early mature domain [72,74,75]. Cleavable signal sequences are N-terminal and typically composed of three regions: a polar n-region that facilitates signal sequence insertion/inversion at the ER translocon (hatched orange segment), a central hydrophobic h-region that is recognised by SRP (plain orange) and a polar C-terminal region that contains the site for signal sequence cleavage (dotted orange). Other features of ER signal sequences that necessitate a Sec61-‘gating assistant’ include a high glycine/proline content (TRAP complex) [72], a longer core h-region (Sec62/63 [74]) and regions of decreased polarity (Sec62/63; [74]). Whilst favouring different groups of clients, the roles of the TRAP complex and Sec62/63 may be partially redundant. (E) Classification of membrane and secretory protein clients of Sec62, Sec63 and TRAP based on previous global studies [72,74]. (Ei) Proteins that were negatively affected by the absence/depletion of one or more of Sec62, Sec63 and TRAP were classified (see Eii) as secretory proteins, single-span TMPs (type I, II, III), TA protein, undefined or multi-span TMPs (type I-like, type II-like, type III-like, undefined) based on their features and the topology of their first TMD. The proportion of the putative clients that belong to each of these groups is shown as a percentage of the total number of proteins (n) that were negatively affected in each case: Sec62 (n = 84), Sec63 (n = 56) and TRAP (n = 61) (Tables 1–4). Proteins that do contain an ER targeting sequence and any subunits of Sec61 ‘gating assistant’ were discounted from the analysis. Figure 3D has been reproduced from Ref. [62].
understanding of how the evolutionarily conserved EMC acts in concert with the Sec61 complex to perform two important, yet apparently discrete, roles during co-translational TMP biogenesis [92–95]. Firstly, the EMC acts as a membrane insertase that enables the stable integration of certain types of TMD into the lipid bilayer [92–95]. Hence, following SRP-dependent delivery to the ER (cf. Fig. 2), membrane proteins destined to assume a type III orientation do not employ the canonical Sec61 translocon [97]. Rather, following engagement of the SRP receptor, these nascent type III TMPs can uniquely access the membrane insertase activity of the EMC [96,97, our unpublished data]; an action that may potentially be assisted by the Sec61 complex acting as a ribosomal docking site and/or via Sec61-stimulated release of ribosome-nascent chains from the SRP receptor (see [98]). Secondly, the EMC acts as a chaperone/holdase for multi-span TMPs with TMDs that contain suboptimal features [99–101].

Whilst some multi-span TMPs have cleavable ER targeting signals (cf. Fig. 1B), many employ signal-anchor sequences to enable their SRP-dependent delivery to the ER. These signal-anchor sequences form the first TMD and, as for single-span TMPs (cf. Fig. 1B), this sequence can be inserted into the ER membrane either with its N terminus remaining in the cytosol (type II-like, see Fig. 4) or with its N terminus translocated into the lumen (type III-like, see Fig. 4). When a type III-like multi-span TMP is truncated to enable the membrane insertion of its first TMD to be studied in isolation, its integration can be mediated by the EMC alone [96]. Hence, TMDs that assume a type III orientation, either in the context of a single-span TMP or as the first TMD of a multi-span TMP, employ the EMC for the membrane insertion. The ability of type III and type III-like TMPs to access the membrane insertase capacity of the EMC also provides a molecular level explanation of their unusual capacity to bypass the otherwise extremely potent blockade of the Sec61 translocon that can be achieved using small molecule inhibitors such as ipomoeassin-F and mycolactone [97,102–105]. We also note that type III and type III-like TMPs appear to show a reduced dependence on Sec61 translocon gating assistants when compared to obligate Sec61 clients (Fig. 3E, Table 5, cf. secretory proteins, type I and type II TMPs versus type III TMPs), further supporting their use of an alternative site for translocation into and across the ER membrane.

Whilst the precise molecular mechanisms that enable the integration of type III and type III-like TMDs via the EMC remain to be determined, a conserved hydrophilic vestibule formed by the EMC3, EMC4 and EMC6 subunits within the cytosolic side of the bilayer is most likely its \textit{de facto} insertase site [92–95]. Like other members of the Oxa1 ‘superfamily’ of membrane protein biogenesis factors [106,107], EMC3 is a structural homologue of YidC [95], a bacterial insertase which acts downstream of SRP and whose flexible cytosolic domains transiently contact SecY (a Sec61\textsubscript{a} Table 1. Negatively affected secretory proteins and TMPs following Sec61 and accessory factor depletion; analysis of data presented in Ref. [74]. Putative Sec63 clients that were identified in cells transiently depleted of Sec63 but not in Sec63 knockout cells are denoted in brackets (X).

| Uniprot ID | Protein name | Protein class | Single/Multi | Sec61A1 | Sec62 | Sec63 | TRAP | TMD number |
|-----------|--------------|---------------|--------------|---------|-------|-------|------|------------|
| Q10589    | BST2         | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| Q9H6E4    | CCDC134      | Secretory     | N/A          | X       | X     | X     |      | 0          |
| O75603    | CLN5         | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| Q96HD1    | CRELD1       | Type I-like   | Multi        | X       | X     | X     |      | 2          |
| Q9UB54    | DNAJB11      | Secretory     | N/A          | X       | X     | (X)   |      | 0          |
| Q9UM22    | EPDR1        | Secretory     | N/A          | X       | X     | (X)   |      | 0          |
| Q96AY3    | FKB10        | Secretory     | N/A          | X       | X     | X     |      | 0          |
| P06280    | GLA          | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q14554    | PIA5         | Secretory     | N/A          | X       | X     | (X)   |      | 0          |
| Q15262-2 | PFTK         | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q9NXG6    | P4HTM        | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| Q00584    | RNASET2      | Secretory     | N/A          | X       | X     | (X)   |      | 0          |
| Q99470    | SDF2         | Secretory     | N/A          | X       | X     | (X)   |      | 0          |
| Q9H173    | S1L1         | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q08357    | SLC20A2      | Type III-like | Multi        | X       | X     | X     |      | 12         |
| Q9ULF5    | SLC9A10      | Type III-like | Multi        | X       | X     | X     |      | 7          |
| Q15533    | TAPBP        | Type I        | Single       | X       | X     | X     |      | 1          |
| Q15582    | TGFBI        | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q9Y9A6    | TMEDS        | Type I        | Single       | X       | X     | (X)   |      | 1          |
Table 2. Negatively affected secretory proteins and TMPs in Sec62 knockout HeLa cells; analysis of data presented in Ref. [74]. Protein substrates with an undefined topology of the 1st TMD (Uniprot) are in red.

| Uniprot ID | Protein name | Protein class | Single/Multi | Sec61A1 | Sec62 | Sec63 | TRAP | TMD number |
|------------|--------------|---------------|--------------|---------|-------|-------|------|------------|
| P11117     | ACP2         | Type I TMP    | Single       | X       | X     |       |      | 1          |
| D14672     | ADAM10       | Type I TMP    | Single       | X       | X     |       |      | 1          |
| P78536     | ADAM17       | Type I TMP    | Single       | X       |       |       |      | 1          |
| P20933     | AGA          | Secretory     | N/A          | X       |       |       |      | 0          |
| Q9NRZ7     | AGPAT3       | Type II-like   | Multi        | X       |       |       |      | 2          |
| Q9NRZ5     | AGPAT4       | Undefined     | Multi        | X       | X     |       |      | 4          |
| Q8N855     | ARL6IP6      | Undefined     | Multi        | X       | X     |       |      | 3          |
| Q816V6     | BPHL         | Secretory     | N/A          | X       |       |       |      | 0          |
| Q10589     | BST2a        | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| O61228     | B3GALTL      | Type II TMP   | Single       | X       |       |       |      | 1          |
| P54289     | CACNA2D1     | Type I TMP    | Single       | X       |       |       |      | 1          |
| Q8NF28     | CADM4        | Type I TMP    | Single       | X       | X     |       |      | 1          |
| O43852-1   | CALU         | Secretory     | Single       | X       |       |       |      | 0          |
| O43852-4   | CALU         | Secretory     | Single       | X       |       |       |      | 0          |
| Q9H6E4     | CCDC134a     | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q9BVS9     | CHD1         | Secretory     | N/A          | X       |       |       |      | 0          |
| O75503     | CLN5         | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| Q9H8M5     | CNNM2        | Type III-like | Multi        | X       | X     |       |      | 4          |
| P12109     | COL6A1       | Secretory     | N/A          | X       |       |       |      | 0          |
| P16870     | CPE          | Secretory     | N/A          | X       |       |       |      | 0          |
| Q9H9G5     | CPVL         | Secretory     | N/A          | X       |       |       |      | 0          |
| Q96H01     | CRELD1a      | Type II-like  | Multi        | X       | X     | X     |      | 2          |
| P07858     | CTSB         | Secretory     | N/A          | X       |       |       |      | 0          |
| P07339     | CTSF         | Secretory     | N/A          | X       |       |       |      | 0          |
| O9JRB2     | CTZ          | Secretory     | N/A          | X       |       |       |      | 0          |
| O9JBS4     | DNAJ111a     | Secretory     | N/A          | X       | X     | (X)  |      | 0          |
| Q12117     | DNAJ3C       | Secretory     | N/A          | X       |       |       |      | 0          |
| O9UM22     | EPDR1a       | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q9N078     | ERAP1        | Type II TMP   | Single       | X       |       |       |      | 1          |
| Q9ED21     | ERLEC1       | Secretory     | N/A          | X       |       |       |      | 0          |
| O9BS26     | ERP44        | Secretory     | N/A          | X       |       |       |      | 1          |
| O75063     | FAM20B       | Type II TMP   | Single       | X       | X     |       |      | 1          |
| P98173     | FAM9A        | Secretory     | N/A          | X       |       |       |      | 0          |
| P26885     | FKB2P        | Secretory     | N/A          | X       |       |       |      | 0          |
| O9Y600     | FKB7P        | Secretory     | N/A          | X       |       |       |      | 0          |
| Q96AY3     | FKB10p       | Secretory     | N/A          | X       | X     |       |      | 0          |
| Q9NW9M8    | FKB14        | Secretory     | N/A          | X       |       |       |      | 0          |
| P10253     | GAA          | Secretory     | N/A          | X       |       |       |      | 0          |
| Q14697-2   | GANAB        | Secretory     | N/A          | X       |       |       |      | 0          |
| O92820     | GGH          | Secretory     | N/A          | X       |       |       |      | 0          |
| P06280     | GLAa         | Secretory     | N/A          | X       | X     | X     |      | 0          |
| P08236     | GUSB         | Secretory     | N/A          | X       |       |       |      | 1          |
| P23229     | ITGA6        | Type I TMP    | Single       | X       |       |       |      | 1          |
| Q14573     | ITPR3        | Type II-like  | Multi        | X       |       |       |      | 6          |
| Q724H8     | KDELRC2      | Secretory     | N/A          | X       |       |       |      | 0          |
| P49257     | LMAN1        | Type I TMP    | Single       | X       | X     |       |      | 1          |
| Q9U106     | LNEPS        | Type II TMP   | Single       | X       | X     |       |      | 1          |
| Q8TDW0     | LRRRC8C      | Type II-like  | Multi        | X       | X     |       |      | 4          |
| Q17RY6     | LY6K         | Secretory     | N/A          | X       | X     |       |      | 0          |
| Q14165     | MLNC         | Type I TMP    | Single       | X       |       |       |      | 1          |
| Q13724     | MOGS         | Type II TMP   | Single       | X       |       |       |      | 1          |
| P17050     | NAGA         | Secretory     | N/A          | X       |       |       |      | 0          |
| P13591     | NCAM1        | Type I TMP    | N/A          | X       | X     |       |      | 1          |
| Q8TEM1     | NUP210       | Type I TMP    | Single       | X       |       |       |      | 1          |
orthologue) during membrane protein insertion [108,109]. By analogy with YidC, it may be envisaged that an equivalent cytosolic region of EMC3, for example the methionine-rich C1 loop and/or C terminus [95], might somehow selectively capture type III and/or type III-like TMDs and direct them towards the EMC insertase site. We additionally speculate that, as for YidC [110,111], positively charged regions within one or more cytosolic domains may promote RNC binding and thereby enable co-translational insertion via the EMC. Like YidC and SecYEG in bacteria [110], the EMC may support co-translational membrane insertion both alone and when acting in concert with the Sec61 complex. In the latter case, this would provide a flexible site for the membrane insertion of multi-span proteins containing closely spaced TMDs with distinct requirements for Sec61 and EMC-mediated integration (Fig. 4B; see also [83]).

The role of the EMC during the co-translational biogenesis of multi-span TMPs is not strictly limited to protein clients whose first TMD is type III-like (Fig. 4A), additionally extending to the stabilisation and/or insertion of downstream TMDs [99–101,112,113] irrespective of the orientation of the first TMD [89,90]. The decisive feature for the EMC-dependence of these multi-span TMPs is reduced hydrophobicity and/or increased polarity or charge, as further evidenced by the ability to create artificial EMC dependency by introducing polar/charged residues into a TMD [90,99,100]. Likewise, the observation that a multi-span TMP containing such a suboptimal TMD is diverted into a pre-emptive ribosome quality control pathway in the absence of a functional EMC [114] implicates the EMC in a chaperone-like protective role akin to, but distinct from, that of the Sec61 translocon (Fig. 4C) [115].

Informed by structural and functional studies [92–97], we propose that, together, the Sec61 complex and the EMC provide a flexible hub for co-translational membrane insertion which can effectively mitigate the potentially error-prone biogenesis of a diverse range of client TMPs. We anticipate that future studies will

| Uniprot ID | Protein name | Protein class | Single/Multi | Sec61A1 | Sec62 | Sec63 | TRAP | TMD number |
|-----------|--------------|---------------|--------------|---------|------|------|------|------------|
| Q9UHG3    | PCYOX1       | Secretory     | N/A          |         | X    | X    | (X)  | 0          |
| Q14554    | PDIA5       | Secretory     | N/A          | X       | X    | (X)  | 0    |
| Q92508    | PIEZO1      | Undefined     | Multi        | X       |      |      |      | 36         |
| Q95427    | PIGN         | Type II-like  | Multi        | X       |      |      |      | 15         |
| Q8TEQ8    | PIGO         | Undefined     | Multi        | X       |      |      |      | 14         |
| Q8NBL1    | POGlut1      | Secretory     | N/A          |         | X    |      |      | 0          |
| P42785    | PRCP         | Secretory     | N/A          |         | X    |      |      | 0          |
| Q13162    | PRDX4        | Secretory     | N/A          |         | X    |      |      | 0          |
| P14314    | PRKCSH       | Secretory     | N/A          |         |      |      |      | 0          |
| Q13308-6  | PTK7         | Type I TMP   | Single       |         |      |      |      | 1          |
| P10586    | PTFRF        | Type I TMP   | Single       |         |      |      |      | 1          |
| P23470    | PTFRG        | Type I TMP   | Single       |         | X    | X    |      | 1          |
| Q15262-2  | PTKRκ        | Secretory     | N/A          |         | X    | X    |      | 0          |
| Q9NXG6    | P4HTMκ       | Type II TMP  | Single       |         | X    | X    |      | 1          |
| Q15293    | RCNI         | Secretory     | N/A          |         |      |      |      | 0          |
| Q00584    | RNASET2κ     | Secretory     | N/A          |         | X    | X    | (X)  | 0          |
| Q9HB40    | SCPEP1       | Secretory     | N/A          |         |      |      |      | 0          |
| Q99470    | SDF2κ        | Secretory     | N/A          |         | X    | X    | (X)  | 0          |
| P07093    | SERPIN2      | Secretory     | N/A          |         |      |      |      | 0          |
| P51688    | SGSH         | Secretory     | N/A          |         |      |      |      | 0          |
| Q9H173    | SIL1κ        | Secretory     | N/A          |         | X    | X    | (X)  | 0          |
| Q08357    | SLC20A2κ     | Type III-like | Multi        |         | X    | X    |      | 12         |
| Q9ULF5    | SLC39A10κ    | Type I-like   | Multi        |         | X    | X    |      | 7          |
| Q15533    | TAPBPκ       | Type I TMP   | Single       |         | X    |      |      | 1          |
| Q9Y3A6    | TMED5κ       | Type I TMP   | Single       |         | X    | X    | (X)  | 1          |
| Q14556    | TOR1A        | Secretory     | N/A          |         |      |      |      | 0          |
| Q8NFQ8    | TOR1AIP2     | Type II TMP  | Single       |         |      |      |      | 1          |
| Q8NBZ7    | UX51         | Type II TMP  | Single       |         | X    | X    |      | 1          |
| Q9ULK5    | VANGL2       | Type I-like   | Multi        |         | X    |      |      | 4          |
| Q9BWQ6    | YIPF2        | Type I-like   | Multi        |         | X    |      |      | 5          |

*Protein substrates that were also negatively affected following Sec61 depletion.
Table 3. Negatively affected secretory proteins and TMPs in Sec63 knockout HeLa cells; analysis of data presented in Ref. [74]. Protein substrates with an undefined topology of the 1st TMD (Uniprot) are in red.

| Uniprot ID | Protein name | Protein class | Single/Multi | Sec61A1 | Sec62 | Sec63 | TRAP | TMD number |
|------------|--------------|---------------|--------------|---------|-------|-------|------|------------|
| Q9NRZ5     | AGPAT4       | Undefined     | Multi        | X       | X     |       |      | 4          |
| Q9H6U8     | ALG9         | Type III-like | Multi        | X       |       |       |      | 8          |
| Q9HDC9     | APMAP        | Type II TMP   | Single       |         | X     |       |      | 1          |
| Q8N655     | ARL6IP6      | Undefined     | Multi        | X       | X     |       |      | 3          |
| P15848     | ARSB         | Secretory     | N/A          | X       |       |       |      | 0          |
| P96194-7  | ATP2C1       | Type II-like  | Multi        | X       |       |       |      | 10         |
| Q10589     | BST2a        | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| Q7KYR7     | BTN2A1       | Type I TMP    | Single       |         |       | X     |      | 1          |
| Q8NFZ8     | CADM4        | Type I TMP    | Single       | X       | X     |       |      | 1          |
| Q9H6E4     | Ccdc134a     | Secretory     | N/A          | X       | X     | X     |      | 0          |
| Q4G010     | Ccsmst1      | Type I TMP    | Single       |         |       | X     |      | 1          |
| P13967     | CD59         | Secretory     | N/A          | X       |       |       |      | 0          |
| Q8TC2Z     | CD99L2       | Type I TMP    | Single       |         |       | X     |      | 1          |
| O75503     | CNL5a        | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| P16909     | CLU          | Secretory     | N/A          | X       |       |       |      | 0          |
| Q9H8M5     | CNNM2        | Type III-like | Multi        | X       |       |       |      | 4          |
| Q9BT09     | CNpy3        | Secretory     | N/A          | X       |       |       |      | 0          |
| Q6NB5J     | COLGALT1     | Secretory     | N/A          | X       |       |       |      | 0          |
| P12109     | COL6A1       | Secretory     | N/A          | X       | X     |       |      | 0          |
| P16879     | CPE          | Secretory     | N/A          | X       | X     |       |      | 0          |
| Q96HO1     | CRELD1a      | Type I-like   | Multi        | X       | X     | X     |      | 2          |
| P81605     | DCD          | Secretory     | N/A          | X       |       |       |      | 0          |
| P52429     | DGKE         | Undefined     | Single       |         |       | X     |      | 1          |
| O9BWV60    | Elov1        | Undefined     | Multi        |         |       | X     |      | 7          |
| O75063     | Fam20B       | Type II TMP   | Single       | X       |       | X     |      | 1          |
| P06280     | GLAa         | Secretory     | N/A          | X       | X     |       |      | 0          |
| O68CQ7     | Glt8D1       | Type II TMP   | Single       | X       |       | X     |      | 1          |
| Q70UQ0     | Ikbp         | Type II TMP   | Single       |         |       | X     |      | 1          |
| A1L0T0     | IlvBL        | Undefined     | Single       |         |       | X     |      | 1          |
| P53708     | Itga8        | Type I TMP    | Single       |         |       | X     |      | 1          |
| O8TD0W     | Lrrc8C       | Type II-like  | Multi        | X       | X     |       |      | 4          |
| Q6NSJ5     | Lrrc8E       | Type II-like  | Multi        | X       | X     |       |      | 4          |
| Q17RY6     | Ly6k         | Secretory     | N/A          | X       | X     |       |      | 0          |
| Q9UKM7     | Mann1B1      | Type II       | Single       |         |       | X     |      | 1          |
| Q8N659     | MarvelD2     | Type II-like  | Multi        |         |       | X     |      | 6          |
| Q10469     | Mgat2        | Type II TMP   | Single       |         |       | X     |      | 1          |
| P13591     | Ncam1        | Type I TMP    | Single       |         |       | X     |      | 1          |
| Q8N5Y8     | Parp16       | Type II TMP   | Single       |         |       | X     |      | 1          |
| P23470     | Ptprg        | Type I TMP    | Single       |         |       | X     |      | 1          |
| Q15262-1   | Ptprk        | Type I TMP    | Single       |         |       | X     |      | 1          |
| Q15262-2   | Ptprk*       | Secretory     | N/A          | X       | X     | X     |      | 0          |
| P02753     | Rbp4         | Secretory     | N/A          | X       |       |       |      | 0          |
| Q08357     | Scl20a2a     | Type III-like | Multi        | X       | X     | X     |      | 12         |
| P46977     | Stt3a        | Type II-like  | Multi        |         |       | X     |      | 13         |
| Q66K14     | Tbc1d9b      | Undefined     | Single       |         |       | X     |      | 1          |
| Q9PP2C     | Tmem181      | Undefined     | Multi        |         |       | X     |      | 9          |
| Q62XV5     | Tmtc3        | Undefined     | Multi        |         |       | X     |      | 9          |
| Q8NC27     | Unc80        | Undefined     | Multi        |         |       | X     |      | 4          |
| Q8NBZ7     | Uxs1         | Type II TMP   | Single       | X       | X     | X     |      | 1          |
| Q9ULK5     | Vangl2       | Type II-like  | Multi        | X       | X     | X     |      | 4          |
| O9BWVQ6    | Yipf2        | Type II-like  | Multi        | X       | X     | X     |      | 5          |

*Protein substrates that were also negatively affected following Sec61 depletion.
Table 4. Negatively affected secretory proteins and TMPs in siRNA-mediated TRAP depleted HeLa cells; analysis of data presented in Ref. [72]. Protein substrates with an undefined topology of the 1st TMD (Uniprot) are in red.

| Uniprot ID | Protein name | Protein class | Single/Multi | Sec61A1 | Sec62 | Sec63 | TRAP | TMD number |
|------------|--------------|---------------|--------------|---------|-------|-------|-------|------------|
| P11117     | ACP2         | Type I TMP    | Single       | X       | X     |       |       | 1          |
| Q14672     | ADAM10       | Type I TMP    | Single       | X       | X     |       |       | 1          |
| Q9BRK6     | ADPKG        | Secretory     | N/A          |         |       |       |       | 0          |
| Q9NV15-2   | ANO10        | Type II-like  | Multi        | X       |       |       |       | 8          |
| Q9H6X2     | ANTXR1       | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q9BXK5     | BCL2L13      | Undefined     | Single       | X       |       |       |       | 1          |
| P08962     | CD63         | Type II-like  | Multi        | X       |       |       |       | 4          |
| Q8N129     | CNPY4        | Secretory     | N/A          | X       |       |       |       | 0          |
| P08572     | COL4A2       | Secretory     | N/A          | X       |       |       |       | 0          |
| Q75629     | CREG1        | Secretory     | N/A          | X       |       |       |       | 0          |
| Q00622     | CYR61        | Secretory     | N/A          | X       |       |       |       | 0          |
| P16103     | DAD1         | Type II-like  | Multi        | X       |       |       |       | 3          |
| P39656     | DDOST        | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q15121     | DEGS1        | Undefined     | Multi        | X       |       |       |       | 6          |
| P00533     | EGFR         | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q9LM22     | EPDR1a       | Secretory     | N/A          | X       | X     |       |       | 0          |
| P02751     | FN1          | Secretory     | N/A          | X       |       |       |       | 0          |
| Q88CQ7     | GLT8D1       | Type II TMP   | Single       | X       |       |       |       | 1          |
| Q9VW58-2   | GFR107       | Type II-like  | Multi        | X       |       |       |       | 7          |
| P98356     | GUSB         | Secretory     | N/A          | X       |       |       |       | 0          |
| Q6TC9      | HM13         | Type III-like | Multi        | X       |       |       |       | 9          |
| P56937     | HSD17B7      | Type III TMP  | Single       | X       |       |       |       | 1          |
| P09069     | IGF1R        | Type I TMP    | Single       | X       |       |       |       | 1          |
| P06756     | ITGAV        | Type I TMP    | Single       | X       |       |       |       | 0          |
| Q8WB1      | ITPRIP       | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q08380     | LGALS3BP     | Secretory     | N/A          | X       |       |       |       | 0          |
| Q12907     | LMAN2        | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q9UQ6      | LNPEP        | Type II TMP   | Single       | X       |       |       |       | 1          |
| Q643R3     | LPCAT4       | Undefined     | Multi        | X       |       |       |       | 2          |
| Q9H0J3     | MAGT1        | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q8NH65     | MDSPD2       | TA protein    | Single       | X       |       |       |       | 1          |
| P15941     | MUC1         | Type I TMP    | Single       | X       |       |       |       | 1          |
| P54802     | NAGLU        | Secretory     | N/A          | X       |       |       |       | 0          |
| Q969V3     | NCLN         | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q9UMX5     | NENF         | Secretory     | N/A          | X       |       |       |       | 0          |
| Q99S19     | NEU1         | Secretory     | N/A          | X       |       |       |       | 0          |
| Q5JFE7     | NOMO2        | Type I TMP    | Single       | X       |       |       |       | 1          |
| H0Y65     | N/A          | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q9EM52     | OMA1         | Undefined     | Single       | X       |       |       |       | 1          |
| Q9UVB2     | SEL1L        | Type I TMP    | Single       | X       |       |       |       | 1          |
| Q13214     | SEMA3B       | Secretory     | N/A          | X       |       |       |       | 0          |
| Q9H173     | SILA         | Secretory     | N/A          | X       | X     |       |       | (X)        |
| P11116     | SLC2A1       | Type II-like  | Multi        | X       |       |       |       | 12         |
| Q8TB61     | SLC38B2      | Undefined     | Multi        | X       |       |       |       | 9          |
| Q9ULF5     | SLC39A10a    | Type III-like | Multi        | X       | X     |       |       | 7          |
| P04920     | SLC4A2       | Type II-like  | Multi        | X       |       |       |       | 11         |
| P35610     | SOAT1        | Type II-like  | Multi        | X       |       |       |       | 9          |
| Q15005     | SPSC2        | Type II-like  | Multi        | X       |       |       |       | 2          |
| Q8T752     | STT3B        | Type II-like  | Multi        | X       |       |       |       | 13         |
| Q15682     | TGFBIa       | Secretory     | N/A          | X       |       |       |       | 0          |
| P55061     | TMBIM6       | Type II-like  | Multi        | X       |       |       |       | 7          |
| Q9Y3A6     | TMED5a       | Type I TMP    | Single       | X       | X     |       |       | (X)        |
| Q6UW68     | TMEM205      | Undefined     | Multi        | X       |       |       |       | 4          |
| A0PV6W6    | TMEM223      | Undefined     | Multi        | X       |       |       |       | 2          |
establish how individual TMDs are directed to either
the EMC or Sec61 complex as appropriate, how the
EMC governs TMD release into the membrane and
further explore the regulation and potential interplay
between the insertase activity of the EMC and its role
as a chaperone/holdase. Given that EMC disruption
negatively affects the levels of several secretory pro-
teins [89], some of which also require Sec62 and/or
TRAP (Fig. 4A) [72,74], we speculate that Sec61
gating assistants and/or the EMC may also exert
some, as yet undefined, regulatory role during Sec61-
mediated co-translational translocation. Likewise, how
the actions of this Sec61/EMC membrane insertion
hub are co-ordinated with other recently identified
TMD insertases/assemblases such as TMCO1 and the
PAT complex (see New routes for insertion and fold-
ing: TMCO1 and the PAT complex) and various mem-
brane protein complexes responsible for co-
translational modifications including N-glycosylation
(for a review see [9]) is a fascinating question.

### New routes for insertion and folding: TMCO1 and the PAT complex

TMCO1 belongs to the same family of membrane pro-
tein insertases as EMC3/YidC (see [95,106,107]), and it
can transiently associate with the ribosome-bound
Sec61 complex [117]. In addition to the Sec61 complex,
TMCO1 associates with CCDC47 [118] and the Nica-
lin-TMEM147-NOMO complex [119] to form a higher
order collective referred to as the ‘TMCO1 translocon’
(Fig. 4A), which is implicated in the biogenesis of mul-
tispan TMPs [117]. Like the EMC, the TMCO1
translocon appears widely conserved and its disruption
leads to reduced cellular fitness [120] and various organi-
smal phenotypes [117,121]. Thus, although the precise
biochemical function of the TMCO1 translocon
remains unclear, structural and functional analogy
with the EMC suggest that it may integrate insuffi-
ciently hydrophobic TMDs alone. Alternatively, given
its association with the active Sec61 complex, it might
assist the ‘core’ Sec61 translocon with the membrane
insertion of suboptimal TMDs and/or help to shield
newly integrated TMDs during the biogenesis and
assembly of multi-span TMPs [117]. As with the EMC,
we anticipate that a fuller understanding of TMCO1
protein clients, together with high-resolution structures
of Sec61-TMCO1 bound RNCs, will be required to
provide a unifying model for the concerted actions of
the Sec61 and TMCO1 complexes. Despite clear paral-
lels between the EMC and TMCO1 complex, including
their potential dual activities as both a TMD insertase
and chaperone/holdase [92,117], one feature firmly sets
them apart; subunits of the TMCO1 translocon do not
stably associate in the absence of ribosomes [117].
Thus, unlike the EMC, the TMCO1 translocon
appears to exist as a short-lived entity that transiently
assembles and disassembles according the needs of the
Sec61 complex and the RNCs that it is presented with.
The availability of TMCO1 subunits for transient
assembly into the TMCO1 translocon may also be reg-
ulated by ER calcium levels. Hence, TMCO1 subunits
homotetramerise to form calcium release channels in
response to critically high levels of ER lumenal cal-
cium, but rapidly disassemble once calcium levels are
restored [121].

Structural evidence that the EMC has distinct TMD
chaperone and TMD insertase activities is only begin-
ning to emerge [92]. However, a protein complex that
acts as a bona fide chaperone for TMDs that have
exited the Sec61 translocon has recently been identi-
fied, firmly establishing the physiological necessity of
such components [122]. The existence of the PAT com-
plex was first apparent from in vitro studies of multi-
span TMP biogenesis, which characterised a component named PAT10 [123]. Following its lateral
exit from the Sec61 translocon, the first TMD of a
model multi-span TMP was shown to next encounter
PAT10 and remain associated with this component as
subsequent TMDs from the same nascent multi-span
TMP were integrated via the Sec61 complex [123–125].
Only now do we know that PAT10 is in fact a protein

| Uniprot ID | Protein name | Protein class | Single/Multi | Sec61A1 | Sec62 | Sec63 | TRAP | TMD number |
|-----------|--------------|---------------|--------------|---------|-------|-------|------|-------------|
| Q8N2U0    | TMEM256      | Type III-like  | Multi        | X       | 2     |       |      |             |
| Q14773    | TPP1         | Secretory     | N/A          | X       | 0     |       |      |             |
| Q15629    | TRAM1        | Type II-like   | Multi        | X       | 8     |       |      |             |
| Q13454    | TUSC3        | Type I-like    | Multi        | X       | 4     |       |      |             |
| Q992X9    | TWSG1        | Secretory     | N/A          | X       | 0     |       |      |             |
| Q579L3    | WLS          | Type II-like   | Multi        | X       | 8     |       |      |             |
| P41221    | WNT5A        | Secretory     | N/A          | X       | 0     |       |      |             |

*Protein substrates that were also negatively affected following Sec61 depletion.
called Asterix, which forms an obligate heterodimer with CCDC47 that has been termed the PAT complex [122] (Fig. 5B). Most significantly, the PAT complex chaperones the assembly of multi-span TMPs, acting after their TMDs are inserted into the membrane but before protein folding is complete (Fig. 5C) [122]. Furthermore, whilst the substrate-binding Asterix subunit co-translationally engages membrane inserted TMDs with charged/polar residues that are exposed to the lipid bilayer, the PAT complex may remain associated with client TMDs even after translation termination, effectively shielding suboptimal TMDs until they are correctly packed into a natively structured multi-span TMP [122–124]. Interestingly an earlier genome-wide...
screen had implicated both Asterix (also known as WDR83OS) and hSnd2 (see ER membrane targeting: the SRP-delivery system) in the biogenesis of multi-span TMPs [52].

Significantly, both the apparent preference of the PAT complex for TMDs of a more hydrophilic nature and its ability to engage TMDs irrespective of their transmembrane orientation [122,123] mirror the chaperone/holdase activity of the EMC (cf. Fig. 4). Furthermore, at least some multi-span TMPs that are dependent on the insertase activity of the EMC also require the PAT complex in order to assume a native conformation [122]. However, the EMC does not compensate for loss of the PAT complex, whilst the dependence of a TMP client on the PAT complex is unaffected if the EMC is bypassed during membrane insertion [122], suggesting that any functional redundancy between the two complexes is limited. Rather, it seems likely that the molecular basis for TMD recognition by each complex is sufficiently distinct that specific protein clients are able to access the chaperone activity of one complex whilst being precluded from engaging with the other (Fig. 5C). In the case of TMDs that are inserted via the Sec61-containing TMCO1 translocon, one possibility is that hydrophilic TMDs may be sequentially handed over to the PAT complex until the assembly of a multi-span TMP is complete (Fig. 5C) [126]. In this scenario, the interaction of CCDC47 with the Sec61-bound ribosome near its exit tunnel could provide an important physical link that enables client TMDs to access the substrate-binding Asterix subunit of the PAT complex. However, it should be noted that at present there is no direct evidence that TMCO1-associated CCDC47 is also in complex with Asterix [117,126].

Whether or not Asterix is an as yet unidentified component of the TMCO1 translocon, or specific to a distinct PAT complex (Fig. 5A,B), is an urgent question that needs to be addressed. Likewise, determining how specific TMDs are directed to the PAT complex, how it helps these proteins assemble into a native fold and how correctly folded TMPs are eventually released, are all key steps towards fully understanding exactly how these recently identified Sec61 assistants contribute to the biogenesis of multi-span TMPs. Given that CCDC47 was named calumin on the basis of its calcium binding properties [127], and that calcium levels affect the homomerisation state of the TMCO1 subunit [121], the possibility that calcium levels might influence the biogenesis of multi-span TMPs via CCDC47 and/or the TMCO1 subunit should also be considered (for a review see [128]).

Concluding remarks: where do we go from here?

Over the past few years, our understanding of the molecular machineries that can be recruited by, and in at least one case completely bypass, the core Sec61 complex has skyrocketed. Various studies have redefined the roles of cytosolic components, including NAC and SGTA, during co-translational TMP biogenesis at the ER, discovered new routes for TMD insertion via the EMC and TMCO1 translocon, and identified the

Fig. 4. The role of the EMC in co-translational integration: two sides of the same coin (A) Schematics of the human EMC depicting its tripartite organisation in the ER membrane: a basket-shaped cytosolic region comprised of EMC2 and either of the functional paralogues EMC8 or EMC9; a membrane spanning core containing both gated and lipid-filled membrane cavities; and an L-shaped ER lumenal domain comprised of EMC1, EMC7 and EMC10 [92,94,95]. The insertase site formed by EMC3/EMC4/EMC6 is near the cytosolic vestibule, whilst the hydrophobic cleft may have a role in client TMD capture [92,94,95]. EMC subunits identified as structurally integral are based on deletion studies of individual subunits which destabilise the wider EMC complex [116] and the classification of EMC subunits relies on structural studies and/or topology prediction software, with the topology of EMC4 remaining ambiguous and that of EMC6 dependent on its assembly with EMC5 [92,94,95]. (Aii) The types of potential client proteins that were negatively affected using a mass-spectrometry-based proteomic approach in EMC2, EMC4 and EMC6 knockout HeLa cells are indicated [89,90]. The percentage of each substrate class is shown (2022) 6835–6862 © 2021 The Authors. The FEBS Journal published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies
Table 5. Negatively affected secretory proteins and TMPs in EMC2, EMC4 or EMC6 knockout HeLa cells; analysis of data presented in Refs [89,90]. Protein substrates with an undefined topology of the 1st TMD (Uniprot) are in red, whilst those that are negatively affected by Sec62, Sec63 or TRAP depletion are in blue. Hydrophobicity values (ΔGapp) were calculated using https://dgpred.cbr.su.se/index.php?p=home [67]. Polar/charged amino acid residues: N, Y, T, S, D, E, R, K, H.

| Uniprot ID | Protein | Localisation | Protein Class | Single/TMD Number | Signal/TMD Sequence | ΔGapp (ss/TMD1) | Polar/charged AAs (ss/TMD1) | Sec62/63/TRAP-dependent? |
|------------|---------|--------------|---------------|-------------------|--------------------|----------------|-----------------------------|--------------------------|
| Q9UBR2     | CATZα   | Lysosome     | Secretary     | N/A               | ss: MARRPGWPRLVLL VLAGAAGOG | 2.23           | 4                           | N/A                      |
| Q9UM22     | EPDR1α  | Lysosome     | Secretary     | N/A               | ss: MPGAPRLTVPG A GLGALLGLG WATL CGLC SLGAV | 1.67           | 5                           | Sec62, TRAP              |
| Q9220      | GHβ     | Lysosome     | Secretary     | N/A               | ss: MASPGCLGLGGC AA ASLELS | -0.28          | 4                           | Sec62                   |
| P13284     | GILTA   | Lysosome     | Secretary     | N/A               | ss: MTLSPLLLFLPPLL LLLD VPTAAVQA | 1.19           | 5                           | N/A                      |
| P10619     | PPGBα   | Lysosome     | Secretary     | N/A               | ss: MIRA APPPLFLLL LLLLLLV SWAGSRE A | -1.84          | 5                           | N/A                      |
| Q9BRK5     | SDF4α   | Golgi        | Secretary     | N/A               | ss: MVWPPWAASM RS WGPL IGLAC CLW LGAV LLMDAS A | 2.59           | 4                           | N/A                      |
| Q96470     | SGPL1   | ER           | Type III      | Single 1          | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| P37268     | FDFT1β  | ER           | Type III      | Single 1          | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q9N3C2     | GDE1β   | PM           | Protein       | Single 1          | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q9UJG1     | MSPD1α  | ER, Golgi    | Undefined     | Multi 2           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q96S56     | CLCC1β  | ER, Golgi,  | Type III-like | Multi 3           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q9UH04     | BAP29   | ER           | Type III-like | Multi 3           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| P51572     | BAP31α  | ER           | Type III-like | Multi 3           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q96605     | CD9     | PM           | Type III-like | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q6NSJ5     | LRC8E   | ER           | Type III-like | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q5B2F2     | SGMR2α  | ER, Nucleus  | Type III-like | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| P30408     | T4S1α   | Undefined    | Type III-like | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| P27449     | VATL    | Undefined    | Type III-like | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q15126     | EBβ     | ER, Nucleus  | Undefined     | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Q96H6F5    | ZDHHC6  | ER           | Undefined     | Multi 4           | ss: MA GRPGW PRL VLL VLAGA AGOG | 2.23           | 4                           | N/A                      |
| Uniprot ID | Protein | Localisation | Protein Class | Single/Multi | TMD Number | Signal/TMD Sequence | ΔGapp (ss/TMD1) | Polar/charged AAs (ss/TMD1) | Sec62/63/TRAP-dependent? |
|------------|---------|--------------|---------------|--------------|-------------|---------------------|----------------|-----------------------------|---------------------------|
| Q9GZM5     | YIPF3b  | Golgi        | Type II-like  | Multi        | 5           | LGPLMLVFVLAIIHLGMKT | 0.03           | N/A                         | N/A                       |
| Q9UN00     | ABCG2a  | Mitochondria | Type II-like  | Multi        | 6           | IAQIVTVGLVLGAIYFGL | -1.28          | N/A                         | N/A                       |
| P55061     | B11     | ER           | Type II-like  | Multi        | 6           | KYVASFALCMFVAAAGAYVV| 0.43           | N/A                         | N/A                       |
| Q14643     | ITPR1   | ER           | Type II-like  | Multi        | 6           | LRLTLDCLQVYHTTFPI | 1.1            | 7                           | N/A                       |
| O43688     | PLPP2a  | PM           | Undefined     | Multi        | 6           | WVFVLDVLCLLVASLPAILT | -1.8           | 3                           | N/A                       |
| Q96HH6     | TMM19   | Undefined    | Undefined     | Multi        | 6           | MVTNIVLSSICISLAFWISM | -2.58          | 5                           | N/A                       |
| P61073     | CXCR4a  | Endosome, Lysosome, PM | Type II-like | Multi        | 6 | TIYSIIFLTGNGLVILVMGY | -0.18           | 6                           | N/A                       |
| O60535     | FZD6    | PM           | Type III-like | Multi        | 7           | MFTLLTCIFLPLLRGHSLF | 0.03           | 5                           | N/A                       |
| Q4KM02     | ANO6a   | PM           | Type II-like  | Multi        | 8           | GYYTQMLLLLAAVGVACFLGYLY | -2.08          | 6                           | N/A                       |
| P43003     | EAA1a   | PM           | Type II-like  | Multi        | 8           | AFVLLTAVWGTILGFTLR | -1.35          | 5                           | N/A                       |
| Q93050     | VP11    | Undefined    | Type II-like  | Multi        | 8           | APYTIATFPFLAVMFQDF | 0.97           | 4                           | N/A                       |
| Q13488     | VP33    | Undefined    | Type II-like  | Multi        | 8           | VALQQLFLPTAAAYTCVSR | 2.31           | 6                           | N/A                       |
| Q96S97     | MYADMa  | Undefined    | Undefined     | Multi        | 8           | LLRLLQCVSTCAVSLSVAWSVGAW | 0.06           | 6                           | N/A                       |
| Q8WWI5     | CTL1a   | Mitochondria, PM | Type II-like | Multi        | 9           | IPWLLFILFCGMSGICGFSIA | -3.41          | 1                           | N/A                       |
| P23634     | ATB4a   | PM           | Type II-like  | Multi        | 10          | VTLILEIAILILVLSLYFYR | -0.82          | 6                           | N/A                       |
| P98196     | AT11A   | Endosome, ER, PM | Type II-like | Multi        | 10          | FRVVFYFLIFLVLIDT | -0.16          | 7                           | N/A                       |
| Q9HD20     | AT131a  | ER           | Type II-like  | Multi        | 10          | RLALLRLTVLPFAGLLYPAWL | -4.24          | 5                           | N/A                       |
| P04920     | B3A2a   | Undefined    | Type II-like  | Multi        | 10          | LDVLSEVPVVRFLFLLLG | 2.65           | 3                           | N/A                       |
| P51790     | CLCN3   | Endosome, Golgi | Type II-like | Multi        | 10          | AWSGLVLTGGLGSGAALAGLI | 0.74           | 4                           | N/A                       |
| Q8WA5     | CTL2    | Undefined    | Type II-like  | Multi        | 10          | IICCFLILAIGVAYVAGIIAWT | -2.41          | 2                           | N/A                       |
| P20020     | AT2B1a  | PM           | Type II-like  | Multi        | 11          | VTLILEIAAILSLGLSYF | 0.05           | 5                           | N/A                       |
| Uniprot ID | Protein | Localisation | Protein Class | Single/ Multi TMD Number | Signal/TMD Sequence | ΔGapp (ss/TMD1) | Polar/charged AAs (ss/TMD1) | Sec62/63/TRAP-dependent? |
|------------|---------|--------------|---------------|--------------------------|---------------------|-----------------|----------------------------|---------------------------|
| Q9H2H9     | S38A1a  | PM           | Type II-like  | Multi 11                 | LAFLAANTGILLFLVLLTSVTLL | -2.57           | 5                          | N/A                       |
| Q8WUX1     | S38A5a  | PM           | Type II-like  | Multi 11                 | LAYAMAHGTVFLALLCLALL | -3.24           | 3                          | N/A                       |
| Q9H7F0     | AT133a  | Undefined    | Defined       | Multi 11                 | LAIVSLVGCSGFLPLLLYLWML | -2.39           | 3                          | N/A                       |
| Q60503     | ADCY9   | PM           | Type II-like  | Multi 12                 | RRFLFYALYSFAAIFVSYFAV | -2.47           | 7                          | N/A                       |
| Q58K74     | AG10A   | ER           | Type II-like  | Multi 12                 | FSAALSCFVLYCLFSAFSRAL | -0.67           | 7                          | N/A                       |
| P30825     | CTR1    | PM           | Type II-like  | Multi 12                 | TFDLVALGVSTLGAVYVLA  | 1.53            | 5                          | N/A                       |
| Q965L1     | DIRC2   | Lysosome     | Type II-like  | Multi 12                 | VYGRRLVLPLLFLLAFQVQLVV | -1.94           | 5                          | N/A                       |
| P31641     | SC6A6b  | PM           | Type II-like  | Multi 12                 | FVLSVAGGFVGLGNVRFPYLCY | 1.91            | 5                          | N/A                       |
| O15439     | MRP4    | Undefined    | Defined       | Multi 12                 | CYVWKSFLVGLGFTLEESA  | 2.39            | 8                          | N/A                       |
| Q96508     | PIE201   | Endosome, PM | Defined       | Multi 12                 | LLAACLRSGLSLLLYLLFLLLL | -3.17           | 4                          | N/A                       |
| Q96783     | SL9A7   | Endosome, GoGi | Defined     | Multi 12                 | RLLLLFLVLGGLRVAAA   | 0.17            | 2                          | N/A                       |
| Q9NB15     | S43A3b  | Undefined    | Defined       | Multi 12                 | LLTGGLLCLGFGVLFGWPSLV | -0.23           | 3                          | N/A                       |

*Proteins identified via a mass-spectrometry proteomics-based approach using EMC2 and EMC4 knockout cells [89].; bThose that were also identified in EMC6 knockout cells [90].
Fig. 5. TMCO1 translocon and PAT complex: one or two more Sec61-assistants for multi-span TMPs? (A) Schematics of the TMCO1 translocon and the topologies and domain structures of each subunit [117]: TMEM147 is the core subunit of the Nicalin-TMEM147-NOMO complex [119] which, when assembled into the Sec61-TMCO1-RNC complex (NOMO not depicted), lines a lipid cavity at the centre of this transient complex. (B) The CCDC47 and Asterix subunits of the PAT complex form a stable heterodimer [122], but its precise relationship to the TMCO1 translocon, if any, is unclear [126]. Based on its proximity to the ribosomal exit tunnel [117], CCDC47 may be able to sense hydrophilic TMD residues and recruit Asterix into the wider TMCO1 complex. Alternatively, the PAT complex may function as an independent entity, closely associated with the Sec61 complex [123], that shields and assembles TMDs following their initial membrane insertion [122]. (C) A snapshot of multi-span TMP biogenesis at the ER. Following SRP-dependent targeting, TMPs may access the ER membrane via a Sec61-mediated pathway, a Sec61/EMC-mediated pathway or a TMCO1 translocon-mediated pathway. Irrespective of the initial mechanism of TMD integration in the ER membrane, the PAT complex can associate with membrane inserted TMDs that would otherwise expose polar residues to the lipid bilayer, and chaperone them until they can be assembled with other TMDs to form a stable multi-span TMP.
PAT complex as a TMD chaperone and assemblase. Likewise, we now understand that Sec62, Sec63 and the TRAP complex modulate and enhance the capabilities of the core Sec61 translocon in order to expand its client base. When all of these elements are taken together, they provide an amazingly flexible platform that is capable of synthesising an incredibly diverse and challenging range of secretory and TMP clients. On the basis of our current understanding, we propose that the Sec61 complex provides the central component of this flexible platform, acting as a dynamic hub for membrane translocation at the ER. This begs the question as to how alternative membrane insertion pathways or particular Sec61-assistants are engaged by different client TMPs and how their actions can be co-ordinated; a feat that becomes increasingly complex when one considers the recent finding that both homomeric and heteromeric TMP complexes can begin their assembly co-translationally [129,130].

Another important contribution to our increased knowledge and understanding of TMP biogenesis has been the discovery and characterisation of small molecules and toxins, which selectively inhibit the Sec61 translocon [131–133]. Hence, via the study of individual proteins [6,97,104] together with a global proteomics-based approach [103], it was the resistance of type III and type III-like TMPs to such compounds which revealed a previously unanticipated level of complexity that was incompatible with the prevailing models of TMP biogenesis [7,8,83]. Given that many of the TMP clients of the Sec61 complex are drug targets [133], Sec61 inhibitors are promising candidates for therapeutic development; particularly since they appear well tolerated in vivo [134–137] and have demonstrated promising analgesic [134], antibacterial [138], anti-inflammatory [135], antitumour [136] and antiviral [139–141] activity.

As evidenced by studies of proteins from influenza and SARS-CoV-2 viruses, the antiviral activity of these compounds typically relies on their host-targeted inhibition of the canonical Sec61 translocon, effectively blocking the biogenesis of important viral proteins at the host cell ER [97,103]. Dengue and Zika viruses likewise co-opt the TMP biosynthetic machinery of the host cell ER [141], whilst cell-based studies of influenza, HIV and dengue have firmly established proof of concept for the inhibition of viral growth and propagation through the selective perturbation of Sec61-mediated protein translocation [139]. Thus, Sec61 inhibitors may provide one route for developing much needed broad-spectrum agents that can be mobilised against many different viruses [142]. Likewise, the discovery of the EMC, TMCO1 translocon and PAT complex make them valid candidates for developing complementary small molecule inhibitors which target the biogenesis of specific classes and/or groups of TMPs at the ER. In short, as we gain more insight into the components, pathways and molecular mechanisms utilised by our cells to create functional membrane proteins, this knowledge will in turn present us with new and exciting opportunities to modulate these processes for the benefit of human health [143,144].

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**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

SO’K, MRP and SH wrote and edited the manuscript. The work mentioned as “our unpublished data” in the section entitled "Membrane insertion via the EMC" and the legend to Figure 4 has now been accepted for publication in Communications Biology.

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