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REVIEWS

Review

The Role of EMC during Membrane Protein Biogenesis

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Ten years ago, high-throughput genetic interaction analyses revealed an abundant and widely conserved protein complex residing in the endoplasmic reticulum (ER) membrane. Dubbed the ER membrane protein complex (EMC), its disruption has since been found to affect wide-ranging processes, including protein trafficking, organelle communication, ER stress, viral maturation, lipid homeostasis, and others. However, its molecular function has remained enigmatic. Recent studies suggest a role for EMC during membrane protein biogenesis. Biochemical reconstitution experiments show that EMC can directly mediate the insertion of transmembrane domains (TMDs) into the lipid bilayer. Given the large proportion of genes encoding membrane proteins, a central role for EMC as a TMD insertion factor can explain its high abundance, wide conservation, and pleiotropic phenotypes.

Highlights

Membrane proteins are topologically, structurally, and biophysically diverse and constitute ~20% of protein coding genes in all organisms. Their membrane insertion, folding, and assembly with other proteins are processes essential for all organisms.

The EMC is an abundant protein complex in the ER of eukaryotes. Its disruption has pleiotropic phenotypes in all organisms examined. The phenotypes often impact the abundance or localization of membrane proteins.

Among the proteins impacted by EMC loss are tail-anchored proteins and GPCRs. In both cases, EMC mediates the insertion of a transmembrane domain near the N terminus (for GPCRs) or C terminus (for tail-anchored proteins).

EMC also interacts with other types of membrane proteins transiting through the ER and might participate in either their folding or assembly by serving as a chaperone.

Discovery of a Conserved ER Membrane Protein Complex

The ER is the largest membrane system of eukaryotic cells and plays central roles in the biogenesis and quality control of proteins, lipid synthesis and storage, calcium storage and homeostasis, several metabolic reactions, and the egress of cargoes into the secretory pathway [1]. The goal of describing these essential cellular processes in molecular terms has inspired high-throughput studies in yeast aimed at defining all genes required to maintain ER homeostasis [2,3]. Functional relationships between genes were inferred by systematically clustering them based on similarities in their patterns of aggravating versus alleviating genetic interactions. Genes within a cluster typically participate in a shared pathway, thereby providing a parts list for that pathway's subsequent mechanistic dissection. These pioneering studies filled out pathways where the function of one or more components were known and illuminated new protein complexes of unknown function. However, it has been nontrivial to designate functions for new complexes in instances where no subunit had already been assigned definitively to a specific molecular process.

For example, the genetic clustering and physical association of three poorly understood gene products (named Arr4, Mdm39, and Rmd7 at the time) strongly implicated them in a common process [3]. Although hierarchical clustering of these three genes near vesicular trafficking genes hinted at a related function, the molecular process mediated by this new complex was unclear. Once the mammalian homolog of Arr4 (termed TRC40) was shown to directly mediate tail-anchored (TA) membrane protein targeting [4,5], the phenotypes could be explained as secondary consequences of impaired TA protein biogenesis [6]. The three genes are now established to be part of a broader GET pathway (termed the TRC pathway in mammals), with Get3 (formerly Arr4) mediating TA protein targeting to a receptor formed by Get1 (Mdm39) and Get2 (Rmd7) at the ER [7].

The EMC is another widely conserved complex that was initially identified in high-throughput genetic interaction studies in yeast [2]. Posing an even greater challenge than the GET complex,

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EMC function was not immediately apparent because most of its subunits had never been studied, none of them had been directly associated with a specific molecular process, and sequence analysis revealed no clear biochemical activities for any subunits or domains within them. Since that time, numerous phenotypes across different organisms have indirectly suggested that EMC influences the biogenesis, quality control, and trafficking of many membrane proteins [8–16]. Recent studies show that EMC is an insertase [17,18] and potential chaperone [19] for TMDs. In this review, we discuss how this assignment of a molecular function to EMC provides a mechanistic framework that begins to explain the various and complex phenotypes observed upon its disruption.

EMC Inserts a Subset of TA Membrane Proteins

EMC is a widely conserved protein complex of the ER [20] and is generally composed of nine subunits (Box 1). Two EMC subunits are cytosolic proteins that are tightly bound to the remaining seven integral membrane subunits that collectively contain 12 TMDs (Figure 1). Although it was apparent at the time of the initial discovery of EMC in yeast [2] and mammals [13] that it is needed for optimal ER homeostasis, a molecular function for EMC remained elusive for many years. The disruption of EMC subunits in a variety of model organisms from yeast to mouse revealed its particular importance for numerous membrane proteins (Table 1) but did not reveal the biological process(es) in which EMC participated (Box 2). A key advance on this front came with the recent discovery that EMC can serve as a membrane insertase for TA membrane proteins [17].

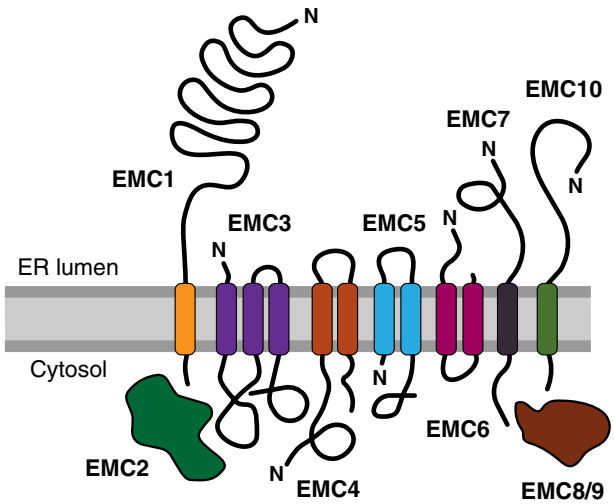
TA proteins are defined as membrane proteins whose sole TMD is located within ~50–70 amino acids of the C terminus [21]. One pathway for TA protein insertion into the ER is the widely conserved GET pathway, known as the TRC pathway in mammals [7]. The convergence of several observations led to the hypothesis that EMC may also mediate insertion of a subset of TA pro-

Box 1. EMC Composition and Conservation

EMC was originally defined in the yeast *Saccharomyces cerevisiae* as six genes (named *EMC1–EMC6*) whose protein products co-purified as a complex [2]. Each EMC subunit showed similar patterns of alleviating and aggravating genetic interactions with other genes encoding ER resident proteins. Most notably, loss of EMC enhanced the phenotypic cost associated with ERAD mutants (i.e., an aggravating genetic interaction), similar to the phenotype of strains overproducing ERAD substrates. This suggested that one consequence of EMC disruption is the generation of misfolded proteins in the ER, consistent with the observation of a mildly activated unfolded protein response in some EMC-disrupted yeast strains.

Parallel functional and proteomic studies of factors involved in ERAD in cultured mammalian cells also uncovered EMC as a set of ten ER-resident proteins (named EMC1–EMC10) that co-associate with each other [13]. Six of the mammalian EMC components correspond to yeast EMC1–EMC6. Later analysis [12,20] showed that mammalian EMC7 and EMC10 have homologs in yeast (Sop4 and YDR056C, respectively, subsequently renamed EMC7 and EMC10). Although yeast EMC7 and EMC10 did not cluster genetically with EMC1–EMC6, they are apparently all part of a single physical complex [2]. EMC8 and EMC9 are ~40% identical to each other and appear to be the result of a relatively recent gene duplication [20]. Although the *S. cerevisiae* ortholog (if any) of EMC8/9 is not known, most species contain an *EMC8* gene. Thus, EMC in most eukaryotes can be considered to probably contain nine subunits: EMC1–EMC7, EMC8/9, and EMC10 (see Figure 1 in main text).

Based on high-throughput proteomic studies [77,78] and the amount recovered after purification [17], EMC abundance seems to be roughly comparable with the Sec61 translocon in both yeast and mammalian cells. Subunit stoichiometry estimates based on stained gels of purified mammalian EMC suggest that each subunit is equimolar with one copy per ~250–300 kD complex [17,18]. Sequence analysis indicates that EMC contains 12 TMDs distributed among its seven relatively small integral membrane subunits, which associate tightly with the cytosolic subunits EMC2 and EMC8/9. Only EMC1, EMC7, and EMC10 have appreciable lumenal domains that collectively account for ~140 kD of mass. Individual knockdown experiments in mammalian cells suggest that loss of EMC1, EMC2, EMC3, EMC5, or EMC6 strongly impair the integrity of the remaining subunits, suggesting that they may form the 'core' of EMC [15]. By contrast, EMC4, EMC7, and EMC10 may be more peripheral because their knockdowns are less disruptive to the remaining complex.



	Human	MW (kDa)	Yeast	MW (kDa)
EMC1	KIAA0090	112	YCL045C	87
EMC2	TTC35	35	YJR088C	34
EMC3	TMEM111	30	YKL207W	28
EMC4	TMEM85	20	YGL231C	21
EMC5	MMGT1	15	KRE27	16
EMC6	TMEM93	12	YLL014W	12
EMC7	C11orf3	26	Sop4	27
EMC8	COX4NB	24	–	–
EMC9	FAM158a	23	–	–
EMC10	HSM1	27	YDR056C	23

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Figure 1. Endoplasmic Reticulum (ER) Membrane Protein Complex (EMC) Subunits and Topology. The predicted topology is shown for the nine widely conserved subunits of human EMC. Interactions between the subunits, which are depicted in their numerical order, should not be inferred from this diagram. The original names and molecular weights (MW) of the human and yeast (*Saccharomyces cerevisiae*) genes for each subunit are indicated below the diagram.

teins. First, HHpred analysis of Get1 suggested that it is part of a larger family that includes EMC3, Oxa1, and YidC [22]. A heterodimer of Get1 and Get2 directly mediates TA protein insertion at the ER [23–25]. Because Oxa1 and YidC also mediate TMD insertion (in mitochondria and bacteria, respectively) [26], this sequence analysis provided the first hint that EMC might act similarly.

The second clue came from the long-standing observation that the GET/TRC pathway does not mediate insertion of all TA proteins. For example, the TMD of cytochrome b5 (Cb5) did not interact effectively with the mammalian Get3 homolog TRC40 [4]. While Cb5 and other low-hydrophobicity TMDs are capable of unassisted insertion into liposomes *in vitro* [27,28], the molecular basis of their specificity for the ER membrane in cells has long been unclear. The third clue came from the then-unpublished observation that Cb5 and another low-hydrophobicity TA protein, squalene synthase (SQS), were under-represented in cells lacking an intact EMC [15]. These clues motivated the study of EMC in TA protein insertion.

Direct analysis of the relationship between TMD hydrophobicity and engagement of the TRC pathway suggested that roughly half of the human genome's ~235 ER-destined TA proteins

Table 1. Phenotypes Associated with EMC Disruption in Organisms and Cells

Subunit and species[a]	Mutation[b]	Phenotype(s)	Protein(s) affected[c]	Refs
Hs EMC1	Splice site[d]	Global dev. delay, cerebellar atrophy	ND	[90]
Hs EMC1	T82M G471R Het. G868R P874R fs[e]	Global dev. delay, cerebellar atrophy	ND	[91]
Hs EMC1	A144T	Non-syndromic retinitis pigmentosa	ND	[92]
Mm EMC3	cKO (lung)[f]	Neonatal lethality; respiratory distress; unfolded protein response	ABCA3, SP-B, SP-C	[14]
Mm EMC10	KO	Viable; sperm dysfunction; infertility	Na^+/K^+ ATPase	[16]
Mm EMC10	KO	Viable; mild behavioral effects	ND	[93]
Mm EMC10	KO	Viable; delayed angiogenesis after myocardial infarction	ND	[94]
Dr EMC3	TKV ➔ I[g]	Lethal (larval stage 10 dpf); light-independent photoreceptor degeneration	Rhodopsin	[35]
Dm EMC3, EMC1, EMC8/9	KO	Lethal (late larval instar); retinal degeneration; unfolded protein response	Rhodopsin, Na^+/K^+ ATPase, TRP	[9]
Ce EMC6	Hypomorph and KDs	Embryonic lethality; growth retardation, low brood size; unfolded protein response	Acetylcholine and GABAA receptors	[11]
Hs EMC5/6[h]	KO	Cholesterol auxotrophy; impaired cholesterol storage	TA proteins (various); GPCRs (various); SOAT1	[15,17,18]
Hs EMC2, EMC4[i]	KD	Stress response activation	~30 membrane proteins	[19]
Hs EMC	KD and KO	Impaired dengue and Zika virus replication	ND	[81]
Hs EMC	KD	Impaired West Nile virus cytotoxicity	ND	[85]
Hs EMC1	KD	Impaired ER-to-cytosol entry of SV40	ND	[84]
Hs EMC2	KD	Reduced level of CFTRΔF598	CFTRΔF508	[12]
Hs EMC6	KD	Impaired autophagosome formation	ND	[86]
Sc EMC1–EMC6	KO	Unfolded protein response	ND	[2]
Sc EMC[j]	KO	Lipid homeostasis and ER–mitochondria interactions	ND	[8]
Sc EMC	KO	Reduced resistance to oligomycin mediated by mutant Yor1ΔF transporter	Yor1ΔF	[12]
Sc EMC	KO	Altered cell surface expression	Mrh1	[10]
Sc EMC7	KO	Prolonged ER residence, improved folding, and reduced vacuolar degradation	Mutant versions of Pma1 and Ste2	[80]

dpf, days post-fertilization; KD, knock-down; KO, knockout, ND, not determined.
[a]Species abbreviations: Hs, *Homo sapiens*; Mm, *Mus musculus*; Dm, *Drosophila melanogaster*; Dr, *Danio rerio*; Ce, *Caenorhabditis elegans*; Sc, *Saccharomyces cerevisiae*.
[b]Unless otherwise indicated, mutated alleles are homozygous (or in haploid yeast cells).
[c]The indicated proteins were affected in their expression, localization, or both.
[d]Splice site mutation causing intron 11 retention resulting in translation up to codon 404 followed by 42 intronic codons and premature termination. A second R401W mutation was also detected.
[e]This frameshift at codon 874 results in 21 out of frame amino acids before a stop codon.
[f]Inducible knockout selectively in lung epithelial cells generated by mating mice containing floxed EMC3 alleles with mice expressing Cre recombinase driven by a Shh promoter.
[g]This mutation replaces residues 138–140 (TKV) with an Isoleucine.
[h]CRISPR-mediated knockout in cultured HEK293 and U2OS cells of either EMC5 or EMC6, both of which were documented to cause nearly complete loss of all the other subunits.
[i]Acute depletion using CRISPR-i.
[j]The phenotype was only observed when multiple EMC subunits were deleted simultaneously.

Box 2. The Pleiotropic Consequences of EMC Disruption

Because the original discoveries of EMC in both yeast [2] and mammals [13] were linked to ERAD, it has long been suspected that the function of EMC is somehow related to maintaining protein homeostasis at the ER. Perturbed ER homeostasis (and hence, unfolded protein response induction) in EMC-null cells [2] could feasibly be explained by a defect in protein maturation, quality control, or export from the ER. Consistent with this idea, zebrafish containing mutant EMC3 were noted years before to cause light-independent photoreceptor degeneration, which is typically caused by excessive retention of the membrane protein rhodopsin in the ER [35,79].

Several phenotypes in EMC subunit mutants have since reinforced this initial idea of perturbed membrane protein homeostasis (see Table 1 in main text). This includes decreased cell surface expression or increased ER retention of Mrh1, Yor1, and Pma1 in yeast [10,12,80], acetylcholine receptor in worms [11], several membrane proteins, including rhodopsin, in flies [9], and ABCA1 in mouse alveolar cells [14]. Despite this seeming concordance, EMC subunit deletions were also associated with altered ER–mitochondria interactions in yeast [8], resistance to multiple viruses in mammalian cells [81–85], and altered autophagosome formation [86]. Although a cohesive picture does not emerge from these studies, several of these examples impact unrelated membrane proteins with multiple TMDs. This observation has propagated the idea that EMC specifically impacts multipass membrane proteins.

These phenotypic studies left the molecular function of EMC as a matter of speculation. Although EMC1 in isolation was observed to directly interact with SV40 to stabilize an intermediate in capsid translocation from the ER to the cytosol [84], it was unclear whether this reflected a normal EMC function in cells. Similarly, the effects of overexpressing EMC4 [87] or EMC5 [88] on oxidative stress sensitivity and Mg^{2+} transport, respectively, may be unrelated to the function of the complete EMC. Thus, until recently, one could conclude only that EMC has some basic function in ER homeostasis and that EMC disruption impacts a range of physiologic processes linked to membranes and membrane proteins [20]. It is noteworthy, particularly in light of the biochemical function of EMC as an insertase, that a similarly diverse set of phenotypes in 'membrane-dependent pathways' was described for GET genes [89] prior to their functional assignment in mediating membrane protein insertion [4–6].

(including SQS and Cb5) cannot bind TRC40 with maximal efficiency [17]. The TMDs that cannot engage TRC40 were also the same TMDs whose insertion was impaired upon EMC deletion. Hence, SQS, containing one of the least hydrophobic TMDs, was completely dependent on EMC for insertion and could not use the TRC pathway. Purified EMC reconstituted into synthetic liposomes effectively mediated SQS insertion, establishing a direct role for EMC as a TMD insertase [17].

Two additional points are noteworthy. First, while the TMDs that require EMC are of low hydrophobicity, there is no reason to believe that EMC can only insert low-hydrophobicity TMDs. Those of higher hydrophobicity are simply captured by TRC40 in the cytosol and never encounter EMC. Recent experiments on non-TA proteins (discussed later) suggest that even high hydrophobicity TMDs can be inserted by EMC [18]. Second, although some substrates such as Cb5 have long been observed to insert into protein-free liposomes [27,28], EMC may provide ER specificity and mediate sufficiently rapid insertion to avoid aggregation, inappropriate interactions, and degradation in a crowded cellular environment. Consistent with this idea, Cb5 levels are reduced in EMC knockout cells [15].

How might EMC mediate TA protein insertion? This key reaction involves the transfer of a hydrophobic segment of ~15–20 amino acids from the aqueous cytosol to the membrane interior with concomitant translocation of the C-terminal flanking domain (Figure 2A). The translocated domain is invariably short (usually less than ~50 amino acids) and unstructured, while the cytosolic domain can be long and folded, and typically contains a net positive charge within ten residues of the TMD. Experiments with hydrophobic peptides demonstrate that partitioning of a TMD into a hydrophobic environment is an energetically favored reaction [29,30] that can even drive transbilayer movement of 100 or more amino acids [28]. The main barrier to this reaction is the

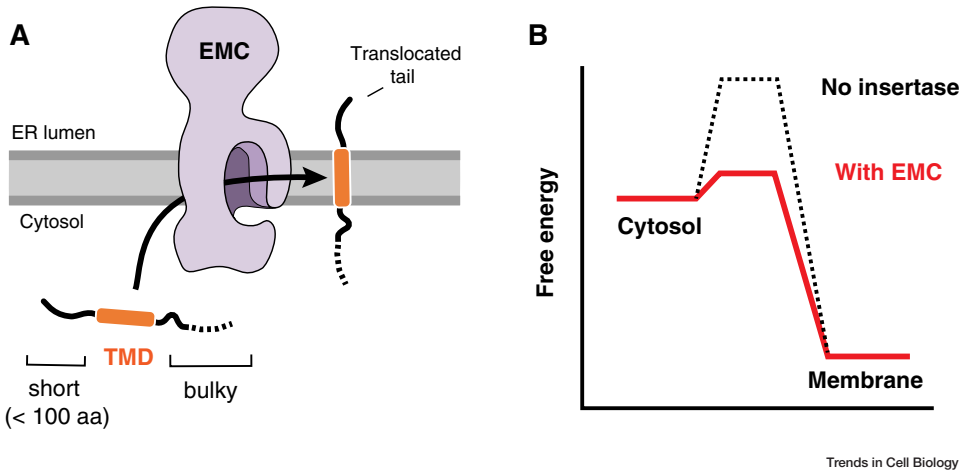

Figure 2. Model for Transmembrane Domain (TMD) Insertase Reaction Mediated by Endoplasmic Reticulum (ER) Membrane Protein Complex (EMC). (A) The substrate is a membrane protein containing a TMD (orange) flanked by a short unstructured domain and a larger bulky domain. EMC is proposed to provide a path for the TMD between the aqueous cytosol and hydrophobic membrane interior. (B) Hypothetical energy diagram of the insertion reaction without and with EMC. A high energetic barrier in the absence of EMC is imposed by the hydrophilic head groups of membrane lipids. TMD insertion via EMC bypasses this barrier more easily, thereby speeding the insertion reaction. aa, amino acid.

hydrophilic head groups of membrane lipids that must be bypassed to initially give a TMD access to the membrane core.

Presumably, EMC mediates TA protein insertion by reducing this energetic barrier (Figure 2B). This might be facilitated by a conduit within EMC similar to current models for TMD insertion by YidC [26,31], the Sec61 translocon [32,33], and the Get1/Get2 complex [24,34]. Such a model would readily reconcile why such an insertion reaction can be unassisted under certain experimental conditions, at least for some substrates that do not aggregate rapidly, but can be stimulated and speeded by EMC. The ability of substrates to insert at least partially in the absence of an insertase helps to explain why EMC is not essential for viability in yeast [2] or cultured mammalian cells [17] but essential for multicellular organisms [9,11,14,35,36] or even individual cells under nonoptimal growth conditions [15]. The improved efficiency and decreased off-pathway products (such as aggregation) presumably impart sufficient selective pressure to acquire and maintain insertases even if the reaction does not strictly require it. Future structural and biochemical studies of EMC will be needed to define the precise path a TMD takes into the membrane and compare its mechanism with other insertase complexes.

EMC Can Act Co-translationally to Insert N-Terminal TMDs

The TA protein insertase function for EMC suggested two hypotheses to explain how EMC disruption affects the levels or localization of various non-TA membrane proteins. TA proteins that use EMC for insertion could be essential factors in other membrane-related processes like biogenesis (e.g., Sec61β [37]), degradation (e.g., Ubc6 [38]), or trafficking (e.g., SNAREs [39]). Alternatively, the insertase activity may have a broader substrate range, thereby directly impacting biogenesis of other membrane proteins. While the two models are not mutually exclusive, recent experiments provide support for the second idea.

Among the proteins observed to be impaired by EMC loss in flies was the G protein-coupled receptor (GPCR) rhodopsin [9]. Rhodopsin was retained in the ER of photoreceptor cells lacking EMC, suggesting that the impairment was relatively early in its maturation. Consistent with these

observations, several GPCRs expressed exogenously in cultured mammalian cells showed ~50% or more reduction at a post-translational stage [18]. Cell-free translation of one such GPCR (β1AR) demonstrated ~50% reduced maturation to a folded conformation in ER microsomes lacking EMC (ΔEMC) compared with wild type microsomes. Parallel analysis of several types of model proteins argued against a general defect in the core processes of translocation, membrane insertion, signal peptide processing, or glycosylation. These observations provided the first direct evidence that EMC impacts a subset of non-TA proteins at the initial biogenesis step [18].

The inability to achieve a correctly folded state in ΔEMC microsomes was traced to a failure in the co-translational insertion of the first TMD of β1AR in the correct N_{exo} topology (N terminus in the ER lumen). Analysis of the first TMDs of several other GPCRs showed that they too were at least partially impaired in correct N_{exo} insertion into microsomes lacking EMC [18]. By contrast, several proteins whose co-translational translocation is initiated with cleavable signal sequences or TMDs in the opposite N_{cyt} orientation (i.e., N terminus facing the cytosol) were expressed normally in cultured cells and unimpaired *in vitro* when translated in the presence of ΔEMC microsomes. Reconstitution experiments showed that purified EMC in liposomes was sufficient to mediate co-translational insertion of the first TMD of β1AR in the N_{exo} topology, but was inactive in co-translational insertion of an N_{cyt} TMD or translocation via an N-terminal signal sequence. These experiments suggested that in addition to post-translational TA protein insertion, EMC mediates co-translational insertion of N-terminal TMDs in the N_{exo} topology [18].

At first glance, it may seem paradoxical that EMC inserts TMDs of two opposite orientations in two seemingly different contexts: post-translational N_{cyt} TMDs of TA proteins and co-translational N_{exo} TMDs of non-TA membrane proteins. However, if one looks beyond topology, it becomes apparent that the biochemical insertion reaction involved in both types of substrates is essentially identical. As depicted in Figure 3, both TA proteins and N_{cyt} TMDs contain a relatively short (~50 amino acids or less) unstructured translocated domain, a nontranslocated side that is bulky (which is effectively the ribosome for N_{exo} TMDs being inserted co-translationally), and TMD flanking charges that favor basic residues on the nontranslocated side. Thus, the role of EMC in both cases is to provide a path for the hydrophobic TMD into the membrane, with the energy gained from this transition offsetting the cost of moving a short unstructured region to the *trans* side of the lipid bilayer.

There may be additional situations when hydrophobic domains are inserted into the membrane from the cytosol with little or no soluble domain being translocated across the membrane. For example, the SNARE protein Stx17 is thought to be anchored via two closely spaced moderately hydrophobic TMDs with a short lumenal loop and both termini facing the cytosol [40]. The coupled insertion of two such closely spaced TMDs as a unit in a hairpin configuration is similar in many ways to the insertion reaction for a single TMD depicted in Figure 2: an energetically favored cytosol-to-membrane transition that effectively 'drags' a small hydrophilic region across the lipid bilayer. A similar coupled insertion reaction might also be needed for closely spaced TMDs during co-translational insertion of many multipass membrane proteins. For example, insertion of the closely spaced third and fourth TMDs within human MDR1 (an ABC transporter) only occurs after both TMDs have fully emerged from the ribosome on the cytosolic side of the membrane [41]. It is attractive to speculate that EMC can facilitate such two-TMD insertion reactions, especially in cases where the individual TMDs are poorly recognized by the Sec61 complex [42]. Such a role might provide one explanation for why multiple members of the ABC transporter family have been observed to be impacted by EMC deletion [12,14,19]. The molecular basis of this impairment warrants mechanistic investigation.

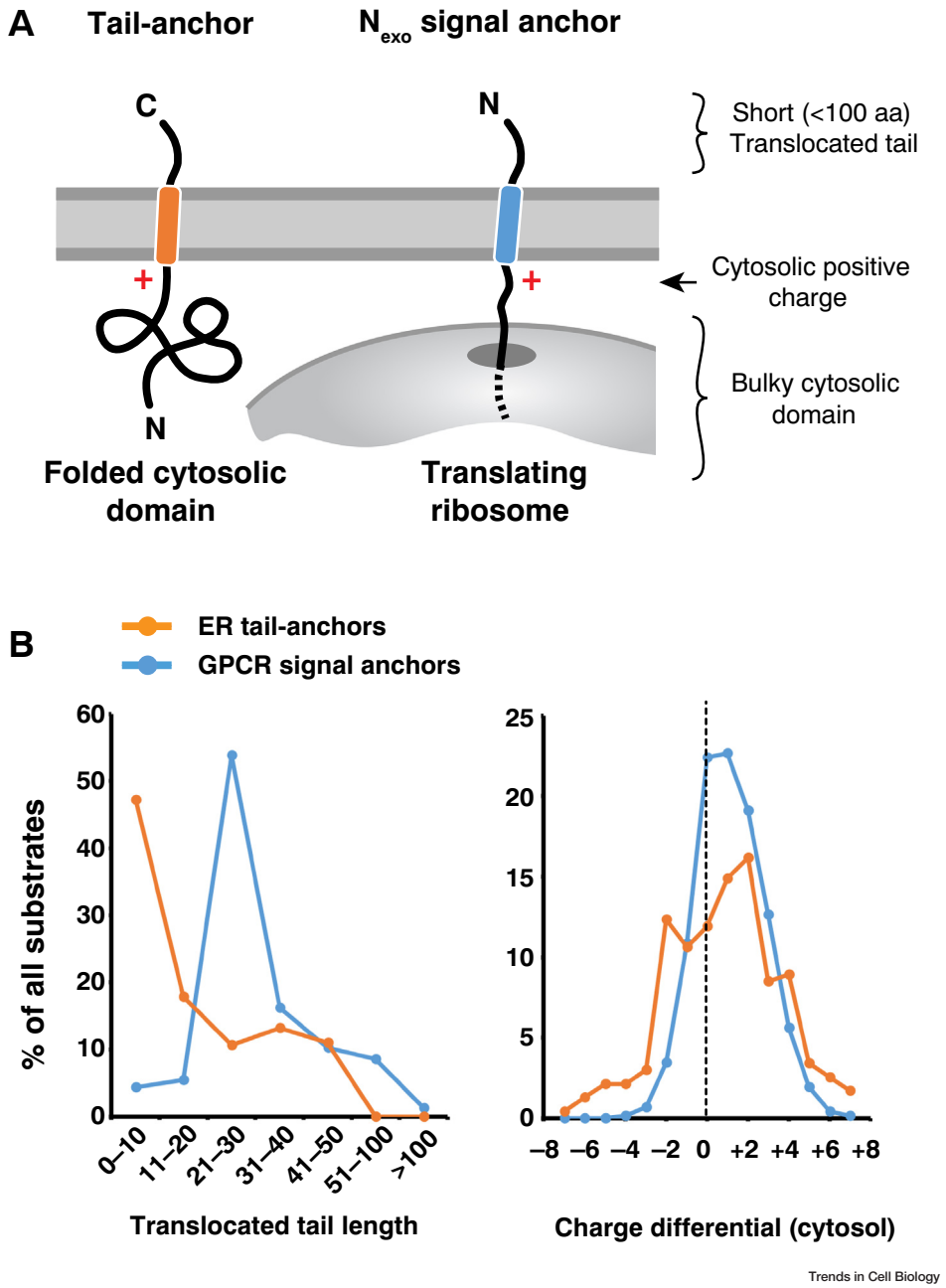

Figure 3. Similarity of Post- and Co-translational Endoplasmic Reticulum (ER) Membrane Protein Complex (EMC) Substrates. (A) Diagram of a C-terminal tail-anchor (orange) and N_{exo} signal anchor (blue), illustrating that they both have short translocated tails, bulky cytosol-facing domains, and a preference for cytosolic positively charged amino acids (aa) flanking their transmembrane domains. The N_{exo} signal anchor is shown as a co-translational intermediate because this is the step at which membrane insertion occurs. (B) Histograms of all human ER-targeted tail-anchored proteins (orange, n = 235) and N_{exo} signal anchors from G protein-coupled receptors (GPCRs) (blue, n = 728). The left graph shows the length distributions for the translocated tail; the right graph shows the charge differential (cytosol–lumen) of the ten flanking residues on either side of the transmembrane domain. Note that for both tail-anchors and N_{exo} signal anchors, translocated tails are usually less than 50 amino acids and favor a net positive cytosolic flanking charge.

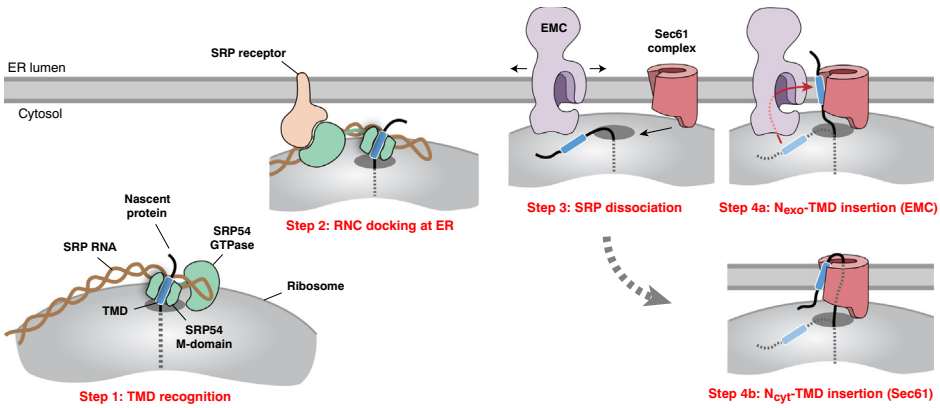

Figure 4. Early Steps in Co-translational Membrane Protein Topogenesis. Model depicting the key steps leading from initial signal anchor recognition in the cytosol to insertion at the endoplasmic reticulum (ER) membrane. As the transmembrane domain (TMD) emerges from the ribosome, it is recognized by SRP, a ribonucleoprotein containing an RNA scaffold (brown) bound to SRP54 (green) and five other protein subunits (not depicted for clarity). The M-domain of SRP54 binds and shields the TMD (step 1). The GTPase domain of SRP54 mediates ER targeting via its interaction with the SRP receptor. This interaction causes the GTPase domain to bind a different site on the SRP RNA, freeing space near the ribosome exit tunnel (step 2). When GTP is hydrolyzed by SRP and SRP receptor, the complex dissociates and releases the TMD (step 3). At this point, the TMD is available for insertion, and the Sec61 docking site near the ribosome exit tunnel is unoccluded. Depending on features of the TMD and flanking regions, the TMD inserts in either the N_{exo} or N_{cyt} topology. N_{exo} insertion would be mediated by ER membrane protein complex (EMC), after which the TMD resides close to the lateral gate of the Sec61 complex at the ribosome exit tunnel (step 4a). N_{cyt} insertion is mediated by the Sec61 complex, after which the TMD is similarly positioned at the lateral gate. In both cases, the endpoint is an engaged Sec61 complex docked at the ribosome exit tunnel and ready to accommodate downstream TMD(s). RNC, ribosome-nascent chain.

How Do EMC and Sec61 Cooperate?

The steps preceding and following EMC-mediated N_{exo} TMD insertion remain to be investigated directly, but a plausible model can be proposed from the available data (Figure 4). Shortly after emergence from the ribosome, the N_{exo} TMD of β1AR engages the M-domain of SRP [18], as expected from the substrate range [43,44] and mechanism of TMD binding [45,46] of SRP (Figure 4, step 1). The GTPase domain of SRP54 will then interact with the GTPase domain of the alpha subunit of SRP receptor (SR), mediating targeting of the ribosome-nascent chain (RNC) to the ER [47,48]. The SRP–SR interaction causes the GTPase domain complex to bind a distal site on SRP RNA, freeing the eventual Sec61 binding site [49–51]. However, the M-domain remains close to the ribosome exit tunnel, sterically hindering Sec61 binding (Figure 4, step 2). This short-lived intermediate presumably rearranges when SRP and SR hydrolyze their bound GTPs, causing SRP to dissociate from SR and release the TMD.

The order of events following SRP release from SR are unclear, but are speculated to be coordinated with docking of the RNC on the cytosolic loops of the Sec61 translocon [50,51]. The ribosome–Sec61 interaction 'primes' the Sec61 complex for accepting a substrate by slightly cracking its lateral gate [52]. At the same time, EMC would be sampling the vicinity of the RNC due to the high abundance and rapid diffusion of EMC. We therefore posit that the hydrophobic domain released by SRP at the ER membrane has access to both Sec61 and EMC at this crucial moment (Figure 4, step 3). The choice of insertion route (EMC versus Sec61) and orientation (N_{exo} versus N_{cyt}) would be determined by the biophysical properties of the hydrophobic domain and flanking regions.

In vitro insertion experiments of a range of substrates in ΔEMC microsomes (which contain normal levels of Sec61 and all known associated factors) reveal that EMC is fully dispensable for insertion of cleavable signal sequences and N_{cyt} TMDs [18]. This is consistent with the observation that purified Sec61 complex in proteoliposomes can mediate N_{cyt} insertion [53] and structural analysis of N_{cyt} hydrophobic domains engaged at the Sec61 lateral gate [54,55]. By contrast, most N_{exo} TMDs show at least some insertion deficiency in ΔEMC microsomes [18], with some examples being almost entirely incapable of insertion. Conversely, all tested N_{exo} TMDs showed nearly unimpaired insertion into proteoliposomes immunodepleted of Sec61 complex [18].

These results suggest that EMC handles N_{exo} TMDs (Figure 4, step 4a) while Sec61 handles signal sequences and N_{cyt} TMDs (Figure 4, step 4b). This dichotomy precisely matches the observation that Sec61 inhibitors potently block signal sequences and N_{cyt} TMDs [56], but are ineffective against N_{exo} TMDs [57,58]. While EMC does not seem capable of any co-translational N_{cyt} insertion, Sec61 can mediate N_{exo} TMD insertion with varying efficiencies. Substrate mutagenesis experiments show that Sec61 is most permissible for short TMD length, high hydrophobicity, and flanking cytosolic basic residues. This explains why the N_{exo} TMD from the model protein leader peptidase inserts effectively into proteoliposomes containing purified Sec61 complex [59]. Remarkably, simply extending this TMD by three amino acids was sufficient to make it strongly EMC-dependent for its successful insertion [18].

After N_{exo} insertion by EMC, the TMD would necessarily be near to Sec61 due to the tight binding of Sec61 to the ribosome [60]. Because the lateral gate of Sec61 is partially cracked by ribosome binding [52] and most TMDs have at least some polar residues, the membrane-inserted TMD or signal sequence may preferentially dock at the lateral gate regardless of its orientation (Figure 4, step 4a versus 4b). This would explain the long-observed crosslinking of signal sequences, N_{exo} TMDs, and N_{cyt} TMDs with Sec61 immediately after insertion [59,61]. Docking of the newly inserted TMD at the lateral gate would place it in an ideal position to facilitate insertion of downstream TMDs of a multipass membrane protein [62–64]. Reconstitution experiments suggest that the second TMD of β1AR requires Sec61 for insertion [18]. Furthermore, if the first TMD of β1AR is preceded by a signal sequence or an N_{cyt} TMD, the full β1AR no longer needs EMC for biogenesis. Thus, at least for β1AR (and three other GPCRs that have been tested), the requirement of EMC is limited to initiating accurate topogenesis of the first N_{exo} TMD, after which the remaining TMDs are presumably inserted via the Sec61 complex [18].

Does EMC Have Chaperone or Triage Functions?

At present, direct biochemical analysis of EMC function has illustrated its capacity to insert a C-terminal TMD in the context of a TA protein or an N-terminal N_{exo} TMD of non-TA membrane proteins. However, the identification of yeast and mammalian EMC interacting partners [13,19], proteomic analyses of proteins impacted by EMC loss in cultured mammalian cells [15,19], and possible EMC proximity with nascent membrane proteins in yeast [19] all suggest additional role(s) for EMC. These additional roles remain poorly explored for now, but may help to explain the observation that many proteins affected by EMC are neither TA proteins nor N_{exo}-containing membrane proteins [9,11,12,15,19].

The insertase activity of EMC suggests that like the bacterial insertase YidC [31], EMC probably has a cavity capable of binding a TMD within the plane of the membrane. Such a property may impart on EMC a chaperone-like capacity toward TMDs analogous to similar proposals for YidC [65–68]. Evidence for this idea comes from three types of studies. First, EMC has been shown to physically associate by co-immunoprecipitation with several cell surface membrane proteins, including mammalian ABCA3 [14] and yeast Fks1, Fks2, Mrh1, and Pma1 [19],

presumably during their transit through the ER. Second, proximity-specific ribosome profiling suggests that yeast EMC might transiently be close to ribosomes synthesizing a subset of multipass membrane proteins, including Fks1, Fks2, and Pma1 [19]. Third, a subset of membrane proteins are reduced in cells lacking EMC [9–12,14,19].

One model that links these observations posits that EMC aids in the folding of multipass membrane proteins by acting as an intramembrane chaperone for TMDs [19]. Although attractive, it remains to be determined whether EMC indeed interacts via substrate TMDs or whether folding of any of its interacting substrates is impaired in the absence of EMC. While it has been assumed that increased degradation of EMC clients is synonymous with a misfolded state, this remains to be tested. An alternate model is that EMC acts as a 'guardian' [69] that protects a subset of membrane proteins (regardless of folding status) from promiscuous degradation during their ER transit.

The two models are not mutually exclusive, and a parsimonious hypothesis might be a hybrid model in which EMC serves a triage function. Indeed, EMC has been observed to co-immunoprecipitate with factors involved in ER-associated degradation (ERAD) on the one hand [13] and substrate-specific chaperones on the other [19]. The interaction with overexpressed ERAD factors such as Hrd1 and Derlin must be interpreted with some caution because it was not clear whether they were engaging EMC as substrates during their maturation or as fully mature and functional proteins. If the latter proves to be true, the interaction of EMC with seemingly opposing factors (chaperones versus ERAD components) might suggest a role in mediating triage of nascent proteins to degradation should maturation fail [70,71]. In this model, nascent membrane proteins that engage EMC (either directly or indirectly via substrate-specific chaperones) for an excessively long time would be more likely to transfer to ERAD factors for degradation. This is analogous to protein triage by the Bag6 complex, a large cytosolic scaffolding complex involved in TA protein targeting, that can interact with both biosynthetic and degradation factors [70,72,73]. The relatively large lumenal and cytosolic domains of EMC, which may be unnecessary for an insertase function, could function as adaptors for chaperones and ERAD machinery.

Concluding Remarks and Future Perspectives

The very broad expression, wide conservation, high abundance, and essentiality of EMC in many organisms have long suggested that EMC functions in a core process associated with the ER. Although the full extent of EMC functions remain to be elucidated, the available data indicate a central role in the biogenesis of membrane proteins. We argue that EMC mediates its functions via its insertase activity, its potential to act as a TMD chaperone, and its capacity to interface with the other core ER processes of protein degradation and maturation. Thus, the view that emerges is that an ancient insertase module [22] may have been elaborated with several peripheral subunits that additionally provide EMC with chaperone and triage activities. Such a complex role would help explain the range of different proteins and biological processes impacted by its absence.

A number of important issues warrant investigation (see Outstanding Questions). Although reconstitution studies have demonstrated an insertase role for TMDs that explains the corresponding insertion defect in cells [17], the molecular basis for this activity remains to be elucidated. While some insights can certainly be gleaned from biochemical studies, as has been the case for the Get1/Get2 complex, YidC, and Sec61, a full understanding will necessarily require structural information combined with structure-guided mutational analysis. It will be interesting to see the extent to which the EMC mechanism is shared with evolutionarily related (e.g., YidC and Get1/Get2)

and divergent (e.g., Sec61 and mitochondrial translocase) insertion factors. Presumably, the critical feature in each case is a gated conduit that connects the aqueous and membrane phases.

Another goal will be to rigorously define the ER-resident factors that functionally interact with EMC. At present, there are varying degrees of evidence for engagement of Sec61, ERAD factors, and substrate-specific chaperones. Once the molecular details of these putative interactions are established, they can be selectively disrupted to understand their functional relevance. Of particular importance will be investigation of the putative EMC–Sec61 interaction. The current data support a model where EMC and Sec61 work sequentially during the biogenesis of a GPCR, with EMC playing a critical role in initiating topogenesis of the first TMD and Sec61 mediating insertion of downstream TMDs. How this sequential function is coordinated remains unclear.

In a broader sense, the range of substrates directly handled by EMC needs to be defined. The studies thus far have provided an initial analysis based on proteomics in cultured cells and *ad hoc* observations from several systems, but it remains unclear which proteins are direct EMC substrates and which are indirect consequences. Proximity-based studies such as selective ribosome profiling to monitor co-translational interactions [74], rapid biotinylation of direct EMC clients [75], or substrate trapping via photo-crosslinker incorporation into EMC [76] hold promise on this front, but will require considerable time resolution because most interactions are likely to be short-lived.

Outstanding Questions

Does EMC mediate insertion of TMDs other than those close to the N or C terminus? If so, what are the features of such internal TMDs that warrant the function of EMC? One attractive model is the insertion by EMC of pairs of closely spaced individually poor TMDs.

Does EMC have chaperone functions separate from its insertase function? If so, what feature(s) of a substrate protein does EMC protect, and how does this aid biogenesis? At present, the direct roles of EMC during biogenesis of most putative substrates is unknown.

What is the structure of EMC and how does this explain its biochemical activities? Does the membrane-embedded domain provide a path from the cytosol into the membrane interior, and is this path gated? What are the functions of the large lumenal and cytosolic domains?

How does EMC work with the Sec61 translocon during biogenesis of multipass membrane proteins? Do these complexes interact, and what steps are mediated by each complex?

EMC has been found to interact with other putative chaperones and ERAD factors. How are these interactions mediated and regulated, and do they facilitate substrate triage between biogenesis and degradation?

Are there other ER membrane insertases and chaperones dedicated to membrane protein biogenesis? Membrane proteins are incredibly diverse in structure and biophysical properties, but the biogenesis of only a few relatively simple examples have been studied.

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