Annual Review of Biochemistry

The Function, Structure, and Origins of the ER Membrane Protein Complex

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Annu. Rev. Biochem. 2022. 91:8.1–8.28

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

https://doi.org/10.1146/annurev-biochem-032620-104553

Keywords

endoplasmic reticulum, membrane proteins, protein translocation, protein folding, chaperone, transporter

Abstract

The endoplasmic reticulum (ER) is the site of membrane protein insertion, folding, and assembly in eukaryotes. Over the past few years, a combination of genetic and biochemical studies have implicated an abundant factor termed the ER membrane protein complex (EMC) in several aspects of membrane protein biogenesis. This large nine-protein complex is built around a deeply conserved core formed by the EMC3–EMC6 subcomplex. EMC3 belongs to the universally conserved Oxa1 superfamily of membrane protein transporters, whereas EMC6 is an ancient, widely conserved obligate partner. EMC has an established role in the insertion of transmembrane domains (TMDs) and less understood roles during the later steps of membrane protein folding and assembly. Several recent structures suggest hypotheses about the mechanism(s) of TMD insertion by EMC, with various biochemical and proteomics studies beginning to reveal the range of EMC's membrane protein substrates.

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1. INTRODUCTION

Transmembrane proteins populate essentially all biological membranes. They are the primary means to communicate and transfer material across the mostly impermeable barrier formed by the membrane. This central role explains why membrane proteins account for approximately one-fourth of all protein coding genes in any organism (1). They have essential functions in transmembrane signaling, metabolite and ion transport, cell adhesion, lipid biosynthesis, and many other biological activities (2). The insertion of newly made proteins into a membrane is an ancient and fundamental process carried out by all living organisms. Accordingly, widely conserved core machineries tasked with this process arose in the last universal common ancestor (3–5).

Transmembrane proteins can span the membrane as either α-helices or β-barrels. The insertion of β-barrel proteins is mediated by a machinery that is qualitatively different from that used for α-helical membrane protein insertion (reviewed in 6, 7). Membrane-spanning α-helices are known as transmembrane domains (TMDs). They are typically between 15 and 30 amino acids long with predominantly hydrophobic side chains (1, 8, 9). In the earliest life forms, TMDs would have inserted unassisted into a membrane owing to their hydrophobicity. Although unassisted TMD insertion can occur in vitro on the basis of well-established physical chemical principles (10–12), insertion in cells is facilitated by transporters (sometimes called insertases) (13–16).

The last universal common ancestor had membrane protein transporters from at least two families, SecY (14) and Oxa1 (15), that may have shared an Oxa1-like ancestor (4). Members of both families have been retained in the bacterial and archaeal plasma membranes and the evolutionarily related eukaryotic endoplasmic reticulum (ER) (5, 14, 17). The inner membranes of endosymbiont organelles (mitochondria and plastids), which are evolutionarily related to the

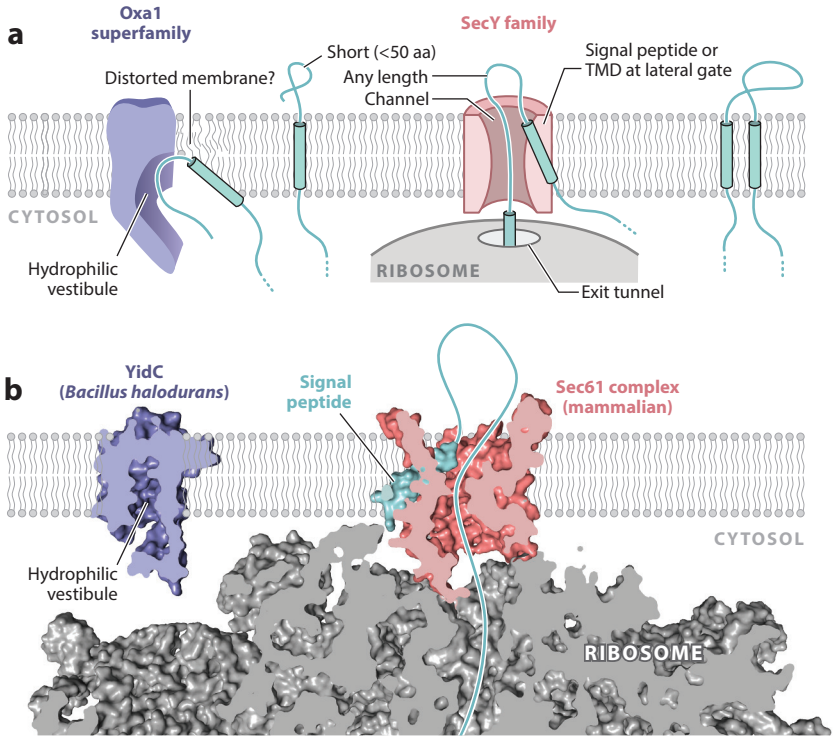

Figure 1

(*a*) Simplified cutaway diagrams of the Oxa1 superfamily and SecY family. The first contains a partial channel (or hydrophilic vestibule), and the second has a complete channel. The membrane adjacent to the hydrophilic vestibule might be locally distorted and thinned to facilitate translocation of the substrate. (*b*) Structures of the *Bacillus halodurans* YidC (PDB ID: 3WO6) and the mammalian Sec61 complex opened by a signal (based on PDB IDs 3JC2 and 3J7R). Note that YidC is turned by 90° from the diagram in panel *a* to better show its vestibule, and the Sec61 complex is turned by 180° to show continuity of its translocation channel with the polypeptide tunnel inside the ribosome. Abbreviation: PDB ID, Protein Data Bank identifier.

bacterial plasma membrane, can also contain members of both families (18–20), although SecY has typically been lost from the mitochondria of most species. Members of the Oxa1 family are membrane protein transporters (15, 19), whereas members of the SecY family are transporters for both secretory and membrane proteins (14, 21).

Membrane protein transporters serve two functions. First, they (or their associated proteins) serve as either direct or indirect receptors for their TMD substrates. In the simplest case, this targeting step can be a direct interaction between the substrate TMD and the transporter. More typically, a targeting factor first recognizes and shields the TMD in the cytosol, and it is then delivered to the transporter for membrane insertion. Protein targeting therefore serves to prevent aggregation of hydrophobic substrates in the cytosol and, in eukaryotes, also prevents inappropriate substrate delivery to the wrong membrane. Protein targeting pathways have been reviewed elsewhere (22, 23).

The second function of transporters is to provide a conduit that reduces the energetic barrier to translocation of the hydrophilic segment of polypeptide that flanks the TMD (**Figure 1**). In the SecY family, this conduit is an aqueous membrane-spanning channel capable of translocating long segments of polypeptides. In the Oxa1 superfamily, the conduit is a partial channel (sometimes

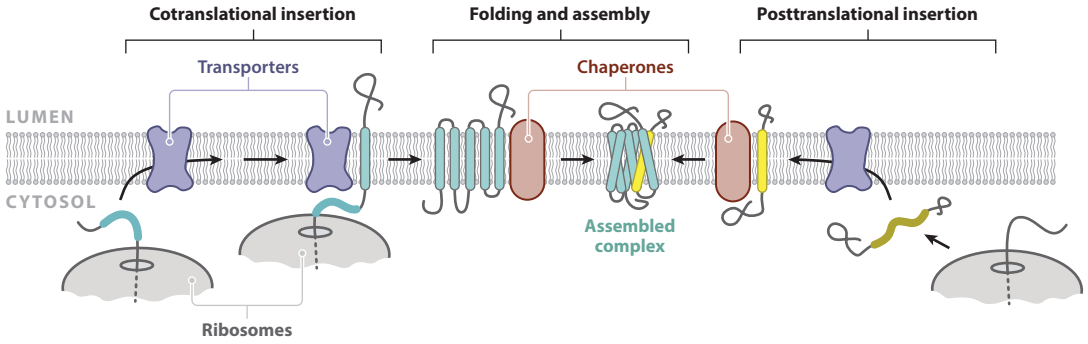

Figure 2

Schematic of co- and posttranslational membrane protein insertion and posttranslational assembly, showing steps where transporters and chaperones are likely to function. The identities of the transporters and chaperones are unspecified in this diagram. During cotranslational insertion (*left*), a translating ribosome is targeted to the membrane, typically via a targeting factor (such as SRP, not depicted) and its receptor (not depicted). The TMD(s) of the substrate is then inserted by the aid of a transporter (such as a member of the Oxa1 superfamily or SecY family) that facilitates translocation of the substrate's soluble domain(s) across the membrane. During posttranslational insertion (*right*), the substrate is fully synthesized in the cytosol before targeting to a transporter (such as a member of the Oxa1 superfamily) for insertion. Targeting factors (not depicted) prevent substrate aggregation in the cytosol. Chaperones in the membrane protect an unassembled TMD until it associates with other TMDs in the final folded structure. Abbreviations: SRP, signal recognition particle; TMD, transmembrane domain.

termed a hydrophilic vestibule or membrane-embedded hydrophilic groove) whose translocation capacity is limited to relatively short polypeptide segments (typically less than ~50 amino acids). The short translocated domain can either be the polypeptide terminus adjacent to a TMD or the loop between two closely spaced TMDs. The conduits in both SecY and Oxa1 have a lateral gate or opening through which the substrate can pass into the lipid phase.

Most membrane proteins contain more than one TMD, either within the same polypeptide or as part of obligate complexes with the TMDs of other membrane proteins (2, 24). Thus, after insertion, a separate step of TMD–TMD interaction is needed to complete membrane protein folding and assembly into its final structure. The TMDs of essentially all multi-TMD complexes have nonhydrophobic amino acids that facilitate TMD interactions (25–27). TMDs of moderate to low hydrophobicity may need to be temporarily stabilized within the membrane until they interact with their TMD partner(s). Chaperones that act within the membrane are thought to serve this temporary stabilization function (28, 29).

Thus, the crux of membrane protein biogenesis involves two posttargeting steps (**Figure 2**): (*a*) TMD insertion into the lipid bilayer, which is transporter-mediated and can occur either cotranslationally or posttranslationally, and (*b*) postinsertion assembly of multi-TMD bundles within the lipid bilayer, which might require chaperones. An abundant and widely conserved protein complex termed the ER membrane protein complex (EMC) has been implicated in both processes. This review discusses the initial discovery of EMC and the path that led to its assignment as a membrane protein transporter of the Oxa1 superfamily. Recent structures of EMC suggest possible mechanisms by which it mediates TMD insertion, and various functional analyses implicate EMC in a broader range of functions during membrane protein folding and assembly.

2. THE EARLY HISTORY OF THE ER MEMBRANE PROTEIN COMPLEX

Unlike many proteins uncovered through the course of investigating a biological process, EMC emerged from a hypothesis-free large-scale analysis of genetic interaction networks in the yeast

Saccharomyces cerevisiae (30). This section traces how EMC was first defined, the initial links between EMC and membrane protein homeostasis, and how investigation of tail-anchored (TA) membrane protein insertion by the GET (guided entry of TA protein) pathway provided an unexpected insight that ultimately led to the discovery of a key EMC function.

2.1. Epistasis Maps Reveal New Protein Complexes

In the early 2000s, the capacity to perform large-scale genetic perturbations in yeast led to various efforts aimed at the systematic elucidation of protein function. This included genome-wide analysis of protein localization and abundance, physical and genetic interactions, and phenotyping (31–34). One strategy that proved particularly fruitful was epistasis analysis (35). In this method, null or hypomorphic alleles of a set of genes are crossed with a library containing null (or hypomorphic) alleles of all genes. The double mutants are then analyzed by a quantitative readout such as growth or stress pathway activation. Each gene has a pattern of epistatic relationships with other genes; the key point is that genes with similar patterns of epistasis tend to have similar functions (e.g., they are all part of a single pathway).

Using this strategy with growth as a readout, three poorly understood genes clustered together near other genes with roles in membrane trafficking (35). The protein products of these genes formed a complex. The genes were (re)named Get1, Get2, and Get3, and at that time, they were thought to play a role in membrane trafficking (hence, their original name of Golgi-to-ER trafficking) (**Figure 3**). Later, using the same approach but with a reporter of ER stress (30), the GET gene cluster was again observed (with two more genes that were renamed Get4 and Get5). In addition, a cluster of six uncharacterized genes whose protein products form a complex was also reported. These were named Emc1 through Emc6, with EMC standing for ER membrane protein complex (**Figure 3**).

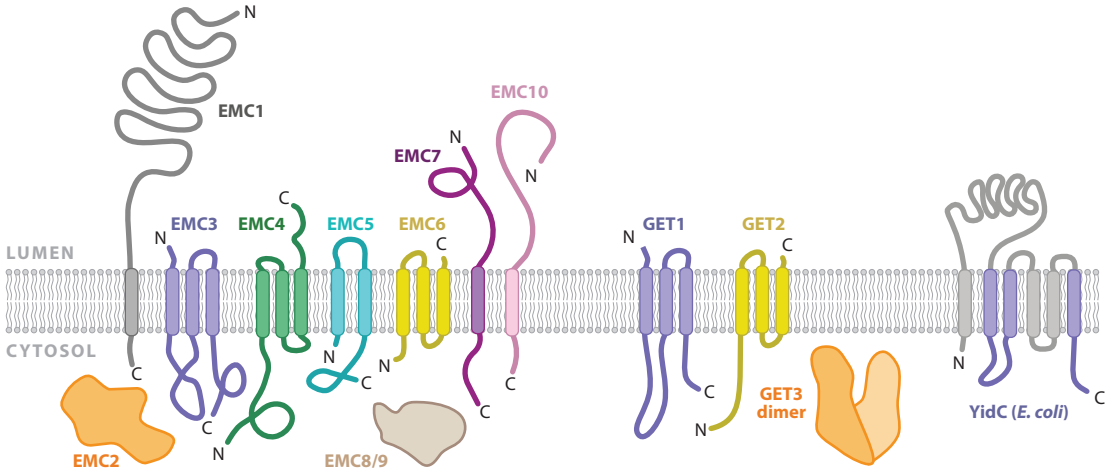

Figure 3

Topology diagrams of EMC subunits, GET subunits, and *Escherichia coli* YidC. The use of matching colors for multiple proteins indicates noteworthy relationships. The three lavender-colored TMDs in YidC are structurally and evolutionarily related to the three-TMD proteins GET1 and EMC3. GET2 is structurally similar to EMC6 and may be evolutionarily related. Although GET3 and EMC2 are unrelated proteins, they interact with the equivalent cytosolic domains of GET1 and EMC3, respectively. Abbreviations: EMC, ER membrane protein complex; ER, endoplasmic reticulum; GET, guided entry of TA protein.

2.2. EMC Is Linked to Membrane Protein Homeostasis

At the time of their respective discoveries, the biochemical functions of the GET and EMC components were unknown, and it was certainly not suspected that they share an evolutionary and functional relationship. EMC was, however, linked very early on to membrane protein homeostasis because its pattern of epistatic relationships was most similar to that of a yeast strain overexpressing a misfolded membrane protein (30). Furthermore, Emc3 had been found some years before as a gene whose mutation causes red blindness in zebrafish, evidently due to an early deficiency in the membrane protein rhodopsin (36).

A later genetic analysis of the surface expression of the yeast ATP binding cassette (ABC) transporter Yor1 also found a link to EMC (37). Here, each of the EMC subunits was needed for surface expression of a mutant Yor1 whose correct folding is particularly sensitive to perturbation. Because Sop4 behaved very similarly to the EMC subunits in this experiment, and because Sop4 had copurified with EMC previously (30), it could now be considered a bona fide subunit of this complex. Mutants in EMC subunits emerged in both worms (38) and flies (39) using assays dependent on the acetylcholine receptor and rhodopsin, respectively. In flies, several other multipass membrane proteins (that is, proteins with multiple TMDs) were impacted by disruption of EMC subunits (39).

Parallel studies in mammals examining the physical interaction network of proteins involved in ER-associated degradation (ERAD) also identified EMC as an complex containing ten proteins named EMC1 through EMC10 (40). In this work, three ERAD-related proteins (Derlin1, Derlin2, and UBAC2) were identified as interactors with EMC subunits. Together with the findings that EMC disruption induces the unfolded protein response (UPR) and that EMC is induced by UPR activation (30, 38–40), these results firmly linked EMC to the maintenance of ER homeostasis via an effect on membrane proteins. Consistent with a housekeeping function, the phenotypes of EMC mutants in metazoans are severe and affect highly diverse biological pathways (reviewed in detail in 41).

It is noteworthy that multipass membrane proteins consistently featured in these studies. Whether EMC participated in their insertion, folding, degradation, or trafficking was mostly unclear, but most models at the time, including a comprehensive review of EMC phylogeny and function (42), favored an early step in biogenesis. Based on the bioinformatics analysis in this study, EMC was already considered a nine-protein complex in most species, with EMC8 and EMC9 being paralogs in vertebrates (**Figure 3**). Some fungi, such as *S. cerevisiae*, lack EMC8 and therefore contain an eight-protein complex. EMC2, EMC8, and EMC9 are cytosolic proteins, whereas the others are integral membrane proteins, each containing between one and three TMDs. EMC1 was noted to have a very large and conspicuous ER lumenal domain. None of the proteins were recognized at the time to have homologs to proteins of known function, leaving the biochemical activity of EMC a matter of speculation.

2.3. The GET Transporter Is Related to EMC

At the time of their discovery, deletion strains lacking Get1, Get2, or Get3 were found to have pleiotropic phenotypes that included trafficking defects (35), glycosylation and cell wall defects (43), mitochondrial fragmentation (44), sporulation and meiosis defects (45), and other defects (46). However, beyond the knowledge that Get3 is a cytosolic ATPase (46) and that Get1 and Get2 were membrane proteins of the early secretory pathway (35), the biochemical functions of these proteins were unknown.

This changed with the discovery that the mammalian homolog of Get3 is a targeting factor for TA membrane protein insertion at the ER (47, 48). Initially named TRC40 (TMD recognition complex of 40 kDa) but now widely called GET3, this protein was shown to specifically recognize

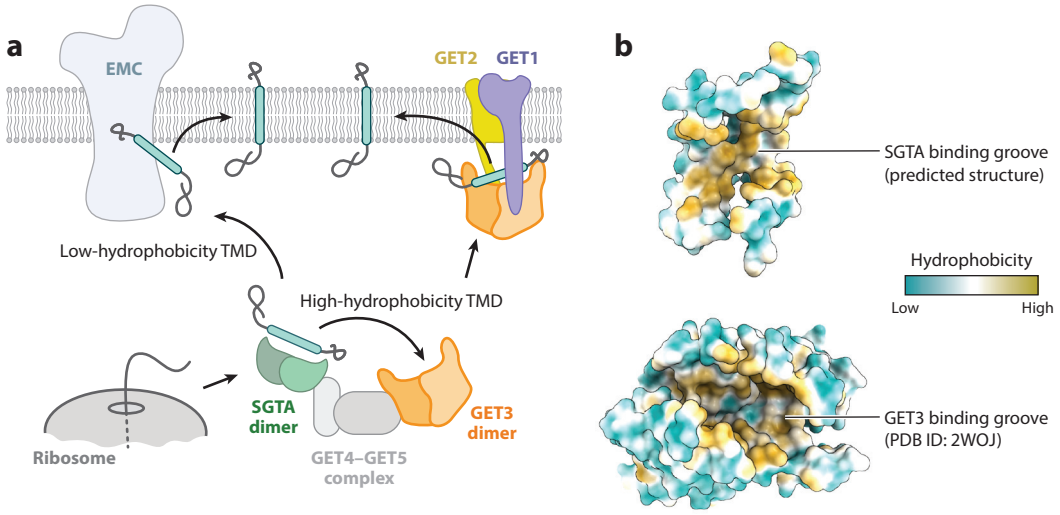

Figure 4

(*a*) Simplified schematic of the GET pathway showing substrate capture by SGTA, handover to GET3 via a bridging complex containing GET4 and GET5, and targeting to the GET1–GET2 complex. Low-hydrophobicity TMDs do not transfer to GET3 and instead dissociate from SGTA and engage EMC. (*b*) The relatively shallow substrate-binding groove of SGTA [predicted by AlphaFold2 (137); see also 81, 82] compared to the deeper groove in GET3 (PDB ID: 2WOJ). SGTA functions as a dimer, so two domains may engage substrate. Abbreviations: EMC, endoplasmic reticulum membrane protein complex; GET, guided entry of tail-anchored protein; PDB ID, Protein Data Bank identifier; SGTA, small glutamine-rich tetratricopeptide repeat-containing protein alpha; TMD, transmembrane domain.

the TMDs of TA proteins in the cytosol and release them at a then-unknown receptor at the ER membrane. An ATPase-deficient mutant of GET3 had a dominant-negative effect and inhibited TA protein insertion (47). Even at that time, it was suspected that Get3's interaction partners Get1 and Get2 might be the ER-resident receptor and TMD insertase (49). Curiously however, at least one relatively low-hydrophobicity TA protein, cytochrome b5 (Cb5), which had earlier been shown to be capable of unassisted insertion into membranes in vitro (50), neither interacted with GET3 nor was inhibited by the GET3 dominant-negative mutant (47).

The puzzling observation with Cb5 notwithstanding, the availability of GET3 as a molecular and genetic handle shifted the focus toward mechanistic dissection of this new membrane protein insertion pathway. The Get1–Get2 complex was shown to be the receptor for Get3 (51), additional earlier-acting components were identified (30, 52, 53), and the major steps in TA protein insertion were biochemically reconstituted with purified factors (54–58). Parallel studies identified the mammalian homologs (with some differences) (59–62), and most of the pleiotropic phenotypes were ascribed to a deficiency in TA protein insertion. For example, impaired membrane trafficking is due to impaired insertion of SNAREs, a prominent family of TA proteins (51, 63).

What has emerged (reviewed in detail in 16, 64) is a cascade of cytosolic factors that begins at the ribosome and culminates with TMD loading into a deep hydrophobic binding pocket of GET3 (**Figure 4*a***). This targeting complex of GET3 and TA protein then engages the GET1–GET2 complex at the ER (56, 57, 65). GET1 triggers TA protein release from GET3, and the TMD of the TA protein is inserted via the six-TMD membrane domain of the GET1–GET2 complex (66). The key result that the GET1–GET2 complex serves as a transporter and not simply a receptor highlighted its membrane domain as one of the remaining mysteries of this pathway.

At that time, sensitive homology-detection methods based on profile hidden Markov models (67) were becoming more widely used outside of computational biology. Application of this search method to GET1 identified Oxa1, YidC, Alb3, EMC3, and TMCO1 as high-confidence hits. This was striking for two reasons. First, Oxa1 of the mitochondrial inner membrane, YidC of the bacterial plasma membrane, and Alb3 of the chloroplast inner membrane were known to be membrane protein transporters that share a common evolutionary origin (19). Second, the finding that GET1, EMC3, and TMCO1 might be related to this family indicated that it might not have been lost during eukaryogenesis, when the prokaryotic plasma membrane gave rise to the intracellular compartments of the secretory pathway, including the ER.

Although this observation would not be published for several years (17), it motivated the Keenan group to pursue a putative protein transporter function for TMCO1 and the Hegde group to pursue EMC. As described in Sections 4.1 and 4.2, recent structures have now shown that GET1, TMCO1, and EMC3 are indeed part of the Oxa1 superfamily of membrane protein transporters (reviewed in 68).

3. EMC MEDIATES MEMBRANE PROTEIN INSERTION

Indirect evidence had long indicated that TA protein insertion in vitro could proceed by more than one pathway (69), including the unassisted mechanism noted for Cb5 (50). Furthermore, GET pathway components are nonessential in yeast despite the essentiality of many TA proteins (35), also indicating the existence of some alternative insertion mechanism(s). The observation that GET1 might be related to EMC3 provided an attractive candidate for this alternative route. Particularly intriguing was the observation that EMC subunits are synthetic lethal with GET subunits (30, 70). This section describes the discovery that EMC mediates membrane protein insertion, how its substrates differ from the GET pathway, and why this difference might influence the mechanism of substrate targeting to these two transporters.

3.1. Transmembrane Domain Hydrophobicity Influences the Insertion Pathway

Although not published until some years later (71), a key early observation was the finding in proteomic experiments that mammalian cells lacking EMC showed markedly reduced levels of squalene synthase (SQS, also called FDFT1) and Cb5. Both are TA proteins (although that was not entirely clear for SQS based on predictions) whose TMDs are particularly low in hydrophobicity. Analysis of TA protein insertion into ER microsomes in vitro showed a clear positive correlation between TMD hydrophobicity and the ability to stably associate with GET3 (52, 72, 73). The requirement for high hydrophobicity matched the overall length, depth, and hydrophobicity of the substrate-binding groove in GET3 (**Figure 4*b***).

Consistent with the interaction analysis, inhibition of the GET pathway using a GET2-derived peptide that blocks targeting failed to inhibit insertion of TA proteins with low-hydrophobicity TMDs (73). Importantly, the GET-independent TA protein SQS could be made GET-dependent simply by increasing its TMD hydrophobicity. The GET-independent pathway was evidently not unassisted because trypsin pretreatment of ER microsomes abolished their capacity to accommodate TA protein insertion. These experiments revealed another protein-mediated insertion pathway for low-hydrophobicity TMDs.

The sequence similarity between GET1 and EMC3, the observation of reduced SQS in EMC-deficient cells, and the synthetic lethality of EMC and GET components made EMC a uniquely strong candidate for this alternative pathway. Mammalian cells lacking EMC showed strongly reduced insertion of an SQS-based reporter, and ER microsomes from these cells showed impaired insertion selectively of GET-independent TA proteins. Purified EMC reconstituted into synthetic

liposomes supported TA insertion, arguing for a direct role in this process. Importantly, trypsin digestion of such proteoliposomes inactivated the insertion activity, illustrating a specific role for cytosolically exposed regions. Thus, EMC finally had at least one specific function in mediating the insertion of low- and medium-hydrophobicity TMDs (73).

For reasons that are not clear, particularly high hydrophobicity TMDs such as that from the SNARE protein VAMP2 cannot be inserted effectively by a non-GET pathway (54), perhaps explaining why membrane trafficking is most strongly impaired in GET deletion strains (35). The majority of TA proteins contain TMDs of intermediate hydrophobicity that seem to be compatible with both the GET and EMC pathways. This probably explains why loss of either GET or EMC is tolerated reasonably well at the single-cell level, albeit with constitutively activated stress pathways(s) indicative of protein misfolding (30, 35, 73–75).

In addition to these two pathways, a role for the signal recognition particle (SRP) in TA protein targeting has long been suggested on the basis of in vitro experiments (76). However, a systematic photocrosslinking analysis of direct TMD interactors in the cytosol did not find an appreciable crosslink to SRP (73). A fourth potential route, called the SRP-independent targeting pathway, has been defined genetically as a GET pathway backup (70, 75), but this has not been examined in mechanistic detail. The GET and EMC pathways currently represent the two best-defined insertion routes for TA proteins, with the range of naturally occurring TMD hydrophobicity evidently accommodated between them.

It is worth asking how one reconciles the earlier finding that some TA proteins such as Cb5 can insert unassisted in vitro (50, 77). The simplest explanation is that due to different experimental conditions, the earlier studies had relatively minimal off-pathway reactions to compete with insertion. Such reactions might include aggregation or inappropriate interactions with irrelevant factors. This is analogous to how a protein that can fold successfully in dilute conditions is strongly disfavored from folding when aggregation is a competing outcome (78, 79). As such competing reactions become extreme, as is probably the case in the highly crowded cellular environment, factors that facilitate the favored outcome (e.g., EMC) or disfavor off-pathway reactions (e.g., chaperones) become increasingly important.

3.2. Tail-Anchored Protein Targeting to EMC

Whereas GET3 is a dedicated targeting factor that delivers TA protein substrates to the GET1–GET2 complex, an analogous factor for EMC has not been found. An initial attractive idea was that perhaps EMC's conserved cytosolic subunit (EMC2) might cycle between a cytosolic and a membrane-bound state, similar to GET3. However, cells do not seem to contain a free population of EMC2, which was tightly associated with the remaining subunits (71, 73). A crosslinking strategy to find cytosolic interaction partners of the SQS TMD found that the two most prominent binding proteins were calmodulin (CaM) and SGTA (small glutamine-rich tetratricopeptide repeat-containing protein alpha) (73). Both of these factors can reversibly and dynamically bind TMDs to prevent their aggregation, with CaM requiring Ca^{2+} (at levels normally found in the cytosol) to do so.

Both CaM and SGTA were previously shown to bind TMDs (and in the case of CaM, cleavable signal peptides as well) (52, 54, 55, 80). SGTA in particular is noteworthy because it was well established to engage GET pathway TMDs and transfer them to GET3 via a bridging complex containing GET4 and GET5 (**Figure 4*a***) (52, 54, 72). Low-hydrophobicity TMDs engage the binding groove of SGTA, which is predicted to be shallower and to bind substrates more dynamically than that of GET3 (81, 82), but do not transfer to the high-hydrophobicity GET3 binding groove (**Figure 4*b***). Thus, the handover to GET3 seems to be the key branchpoint

between eventual insertion by EMC versus the GET1–GET2 complex (52). The dynamic binding of TMDs to SGTA combined with the high abundance of CaM probably explains why the latter was also a prominent crosslinking partner of weakly hydrophobic TMDs.

The SQS TMD in complex with either CaM or SGTA can support TA protein insertion into ER microsomes in an EMC-dependent manner (73). However, neither CaM nor SGTA has been found to be an interaction partner of EMC, arguing against either factor having a direct targeting function. Instead, a model has been proposed in which TA proteins are always binding and releasing from these (and perhaps other) abundant TMD-binding factors to prevent their aggregation. In the periods of release, the TMD can potentially engage EMC for insertion. Consistent with this idea, preventing TMD release from CaM by artificially raising Ca^{2+} levels also precludes its membrane insertion (73).

Targeting to EMC is therefore postulated to occur via direct substrate–EMC interaction, a model with modest support from the finding that crosslinks exist between a TMD and either EMC3 (83) or EMC2 (84). It is possible that a weak or dynamic interaction between CaM or SGTA with the cytosolic domain of EMC has been overlooked. This still needs to be carefully examined to determine whether a more directed targeting and handover reaction mediates TA protein targeting to EMC. The most attractive model would be one where SGTA is the targeting factor, thereby uniting the GET and EMC pathways for TA protein insertion.

3.3. Cotranslational Transmembrane Domain Insertion by EMC

Even before EMC was defined as a complex, a mutant in EMC3 (then known as pob) had been identified in zebrafish as a cause of red blindness (36). Later, deletion of the *Drosophila* EMC3 homolog was also shown to result in strongly impaired rhodopsin levels (39). In both cases, the defect was thought to be a posttranslational problem in production of cell surface functional rhodopsin, leading to its degradation. Rhodopsin is a G protein–coupled receptor (GPCR); these receptors comprise the single largest family of membrane proteins and have a wide variety of functional roles (85).

With the knowledge that EMC has TMD-insertase activity, a potential role in the insertion of GPCRs was investigated in cell-based and in vitro systems (86). As in the zebrafish and fly systems, biogenesis of a model GPCR, the β1-adrenergic receptor (β1AR), was impaired by ∼50% in EMC-deficient cells and when translocated into EMC-deficient ER microsomes in vitro. Notably, proteins previously established to be translocated or inserted by the Sec61 complex (the eukaryotic SecY complex) were not affected by the loss of EMC. By analyzing progressively earlier intermediates in β1AR insertion, the defect could be traced to insertion of the first TMD.

Most GPCRs have a short (typically less than ∼50 amino acids) translocated N terminus that precedes their first TMD. This TMD directs both SRP-mediated targeting and initiates GPCR insertion into the membrane. Such TMDs with dual targeting and insertion functions are known as signal anchors (87, 88). Depending on their context (especially flanking charged residues), signal anchors can be inserted in either the N_{cyt} topology (with the N terminus facing the cytosol) or the opposite N_{exo} topology (88–91). Analysis of the β1AR signal anchor showed that in the absence of EMC, approximately half of the nascent chains inserted in the N_{cyt} topology rather than the correct N_{exo} topology (86).

The only known machinery for signal anchor insertion at the ER was the Sec61 complex (13, 21, 91). Strikingly, biochemical depletion of the Sec61 complex did not appreciably impair insertion of several different N_{exo} signal anchors, including that from β1AR (86). As expected based on earlier work (92), N_{cyt} signal anchors and cleavable signal sequences were completely dependent on Sec61. Conversely, purified EMC in synthetic liposomes was sufficient to mediate N_{exo} signal

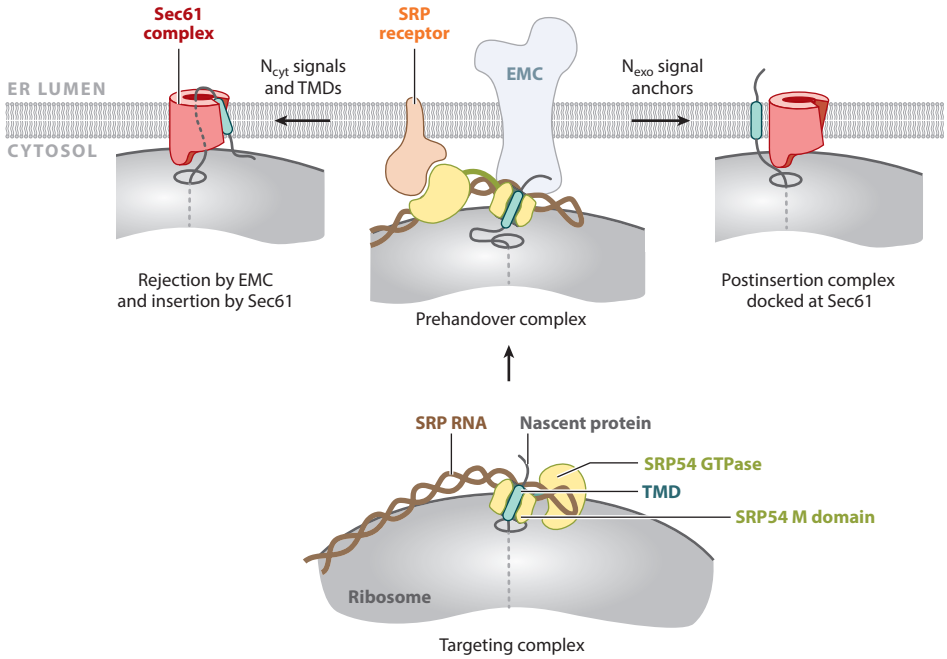

Figure 5

Model for cotranslational insertion of N_{exo} signal anchors by EMC. The prehandover complex (114, 115) is proposed to be sampled by EMC before the ribosome docks onto the Sec61 complex. Substrates suitable for EMC are inserted, whereas other proteins are rejected and instead engage the Sec61 complex. Abbreviations: EMC, ER membrane protein complex; ER, endoplasmic reticulum; M domain, methionine-rich domain; N_{cyt}, N terminus facing the cytosol; N_{exo}, N terminus facing the exoplasmic side of the membrane; SRP, signal recognition particle; TMD, transmembrane domain.

anchor insertion in vitro. After EMC-mediated N_{exo} signal anchor insertion, the ribosome tightly docks onto Sec61 where the remainder of the protein is synthesized and inserted. The transient role of EMC followed by stable docking on Sec61 (**Figure 5**) probably explains earlier findings that an N_{exo} signal anchor is close to Sec61 shortly after insertion (93, 94).

Insertion of the signal anchor in the correct topology seems to be the main EMC-dependent step for GPCR biogenesis. Support for this conclusion comes from two observations. First, the EMC step can be bypassed by appending a signal sequence and soluble translocated domain to the N terminus of an N_{exo} signal anchor (86). Here, the substrate has already initiated translocation through Sec61 when the TMD emerges, allowing its insertion via Sec61's lateral gate. GPCRs engineered in this manner are no longer EMC-dependent in vitro or in cells, indicating that EMC does not participate in its biogenesis. Second, EMC's cytosolic subunits are large, so it cannot fit between the ribosome and membrane when the ribosome is bound to Sec61 (84). This suggests that EMC acts before ribosome docking onto Sec61, after which EMC cannot reach newly emerging downstream TMDs.

Although N_{exo} EMC substrates would seem very different from TA protein substrates, they in fact require the same biochemical reaction: translocation of a relatively short hydrophilic segment of polypeptide across the membrane concomitant with membrane insertion of a hydrophobic TMD (95). In both cases, the translocated segment typically does not have positive charges near

the TMD. Although Sec61-independent insertion of N_{exo} signal anchors might seem surprising, it should not have been in hindsight: N-tail translocation was long known to be Sec-independent in both *Escherichia coli* and the mitochondrial inner membrane (96–99). In both of these cases, N-tail translocation is mediated by an Oxa1 superfamily member (YidC in *E. coli*, Oxa1 in mitochondria). EMC's ability to perform this task is consistent with EMC3 being a member of this superfamily.

As with the TMDs of TA proteins, different N_{exo} signal anchors show different degrees of EMC dependence, with shorter and more hydrophobic TMDs being capable of EMC-independent insertion (86). Whether this EMC-independent insertion is explained entirely by Sec61 (94) or involves another factor, such as the other Oxa1 superfamily member TMCO1 (17), remains to be investigated. The role of EMC in N_{exo} signal anchors seems to explain why this class of proteins (sometimes called Type III membrane proteins) is refractory to Sec61 inhibitors (100–102). Thus, one role for EMC is in the insertion of terminal TMDs that are close to either the N or C terminus.

4. STRUCTURES OF THE YEAST AND HUMAN EMC

The assignment of at least some functions to EMC (73, 86) combined with the wide range of physiologically important phenotypes (41) motivated several groups to determine the structure of EMC. The series of structures and accompanying mutagenesis experiments that emerged in the past two years are noteworthy for two reasons. First, they provide an independent line of evidence that EMC3 is an Oxa1 superfamily member with several features that define this family. Second, surprisingly little new biological insight was immediately evident from simply seeing the structure, leaving one to speculate about EMC's proposed functions and mechanisms. This section describes the EMC structures and speculates about their implications for EMC's known function in terminal TMD insertion.

4.1. The Conserved Architecture of EMC

Multiple cryo–electron microscopy structures of yeast and human EMC, in detergent or in lipid nanodiscs, have been reported (83, 84, 103, 104). They show that EMC has a tripartite architecture with large domains in the ER lumen, membrane, and cytosol (**Figure 6*a***). Most of the lumenal domain is contributed by EMC1, whose most prominent feature in both yeast and humans is an eight-bladed β-propeller. A single β-strand of one blade in this propeller is contributed by the otherwise small C-terminal lumenal domain of EMC4, providing a stable anchor for EMC4 in the lumen. Human EMC1 contains a second eight-bladed β-propeller associated with the first one in a perpendicular orientation. Yeast seems to have lost more than half of this second propeller, retaining approximately three and a half blades. The periphery of EMC1's conserved β-propeller is associated with β-sandwich domains from the lumenal domains of EMC7 and EMC10. Although β-propellers and β-sandwiches are typically protein interaction modules, partners for EMC's lumenal domain have not been established.

The cytosolic domain is composed primarily of EMC2, a tetratricopeptide (TPR)-repeat protein whose α-helices are arranged as a semicircle that is tilted ∼30° relative to the membrane plane. The cytosol-facing side of human EMC2 is covered by EMC8 (or its paralog EMC9) and short segments of the cytosolic tails of EMC4 and EMC5. In yeast, this region is more exposed, containing only the cytosolic tails of EMC4 and EMC5. The cytosolic domains of EMC3, contributed by its C-terminal tail and a long loop between its first and second TMDs, form a three-helix bundle that wraps around the outside of EMC2's semicircular TPR repeats. The cradle formed by the inner aspect of EMC2 contains the cytosolic tail of EMC5, which in yeast is expanded and more completely fills the EMC2 cradle.

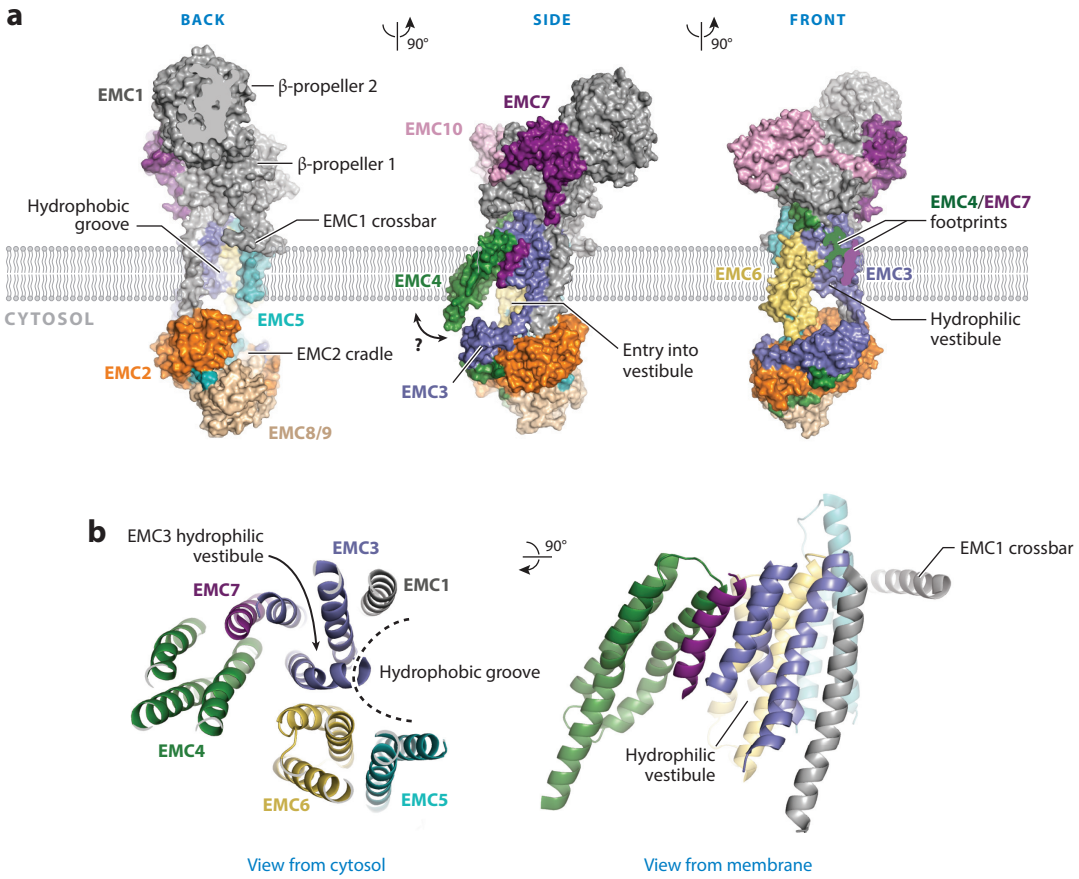

Figure 6

(*a*) Overview of mammalian EMC. The model is a composite based mostly on human EMC in lipid nanodiscs (PDB ID: 7ADO) but with the AlphaFold2 model for EMC4 (137) placed in the position established by O'Donnell et al. (84) and Bai et al. (103). The TMD of EMC7 was placed as a poly-alanine helix in the position proposed by O'Donnell et al. based on site-specific photocrosslinking to EMC3 (84). (*b*) Close-up of the EMC membrane domain using the composite model shown in panel *a*. Abbreviations: EMC, endoplasmic reticulum membrane protein complex; PDB ID, Protein Data Bank identifier; TMD, transmembrane domain.

The membrane domain contains a stable, well-resolved nine-TMD subdomain adjacent to an evidently dynamic, poorly resolved subdomain with five TMDs (**Figure 6*b***). The stable subdomain contains six TMDs from the EMC3–EMC6 subcomplex (discussed in Section 4.2) with the two TMDs of EMC5 associated with EMC6 and the single TMD of EMC1 associated with EMC3. Together, they form a large horseshoe-shaped hydrophobic groove that spans the membrane on the back side of EMC. A peripheral amphipathic helix from EMC1, termed the crossbar or horizontal helix, runs across the open side of this groove on the lumenal end. The crossbar's position is somewhat variable among the different structures. The cytosolic end of the groove leads into the EMC2 cradle in human EMC but is mostly occluded by the expanded cytosolic domain of EMC5 in yeast.

The dynamic subdomain contains four, or possibly five, TMDs that sit on the front side of EMC, covering EMC3 and part of EMC6. Three TMDs are contributed by EMC4, one by EMC7, and possibly one by EMC10 (although its position has not been visualized). Because

this region of the EM maps is not well resolved, their placements based solely on structural data are somewhat tenuous. However, site-specific diazirine probes located in TMD2 and TMD3 of EMC3 crosslink to EMC7 and EMC4, respectively (84). Furthermore, structure predictions based on coevolutionary constraints and protease-protection assays of topology show that EMC4 is a three-TMD protein (84). These constraints, together with another crosslink between the cytosolic domain of EMC3 and EMC4, allowed reasonably confident placements of EMC4 and EMC7 into an otherwise moderate-resolution mammalian EMC map. EMC4 was placed similarly in a yeast structure that was well resolved in this region (103). The single TMD of EMC10 has not been visualized in the structures but is likely to be peripherally located, given that its deletion has no impact on the remainder of EMC.

Thus, the EMC3–EMC6 subcomplex is centrally located within the membrane domain of EMC. The back side of this central subcomplex contains EMC5 and EMC1, whereas the front side contains EMC4, EMC7, and possibly EMC10. The back side contains the membrane-spanning hydrophobic groove, whereas the front side contains the dynamic subdomain with a large gap between the cytosolic regions of the EMC3–EMC6 subcomplex and the EMC4–EMC7 subcomplex. On the EMC7 side, this gap would contain the cytosolic loops of EMC3, EMC7, and perhaps EMC10, so it may not be as open as depicted in the structural models. Nonetheless, this large cytosol-accessible vestibule is a striking feature of EMC's structure that probably facilitates TMD insertion as discussed next.

4.2. The Ancient EMC3–EMC6 Core

As anticipated from sequence analysis and structure prediction (17), the three-helix bundle of EMC3 is structurally similar to the core of bacterial YidC (105) and archaeal YidC-like protein (Ylp1) (5). Structures of TMCO1 and human GET1, reported shortly thereafter (106, 107), have the same membrane domain arrangement common to all Oxa1 superfamily members. Notably, GET2 is structurally similar to EMC6 (68), and both were recently appreciated to be part of a widely conserved family of binding partners for many Oxa1 superfamily members, including Ylp1 and TMCO1 (4). The family members that do not have this binding partner, such as YidC, instead have an intramolecular domain that occupies the same binding surface (105). Hence, YidC superimposed onto EMC3 causes one of its additional TMDs to (roughly) align with the EMC3-interacting TMD of EMC6 (103). Thus, the EMC3–EMC6 subcomplex is an exceptionally widely conserved module that seems to have originated in archaea prior to the emergence of eukaryotes (**Figure 7**).

The Oxa1 superfamily fold is characterized by three TMDs arranged to form a hydrophilic vestibule that penetrates roughly halfway through the membrane. The interior of this vestibule typically contains a highly conserved basic amino acid, most often an arginine. The vestibule is thought to thin and distort the lipid bilayer locally, which might facilitate polypeptide translocation (108). Characteristic of this family, EMC3 similarly contains a hydrophilic vestibule with conserved basic residues. As with other Oxa1 superfamily members, mutation of the vestibule's basic amino acid impairs TMD insertion (discussed in detail in Section 4.3), supporting the idea of a shared mechanism (83, 103–105). Furthermore, the membrane was seen to be thinner adjacent to EMC3's vestibule in one structure determined in lipid nanodiscs (83). Some caution is warranted with this conclusion because EMC7 and EMC4 would normally reside where the membrane was seen to be thinned.

The first cytosolic loop (typically between TMD1 and TMD2) of the Oxa1 superfamily almost always contains a two-helix hairpin (**Figure 7**). Although the length and characteristics of this hairpin vary widely, it is often involved in recruiting substrate either directly or indirectly.

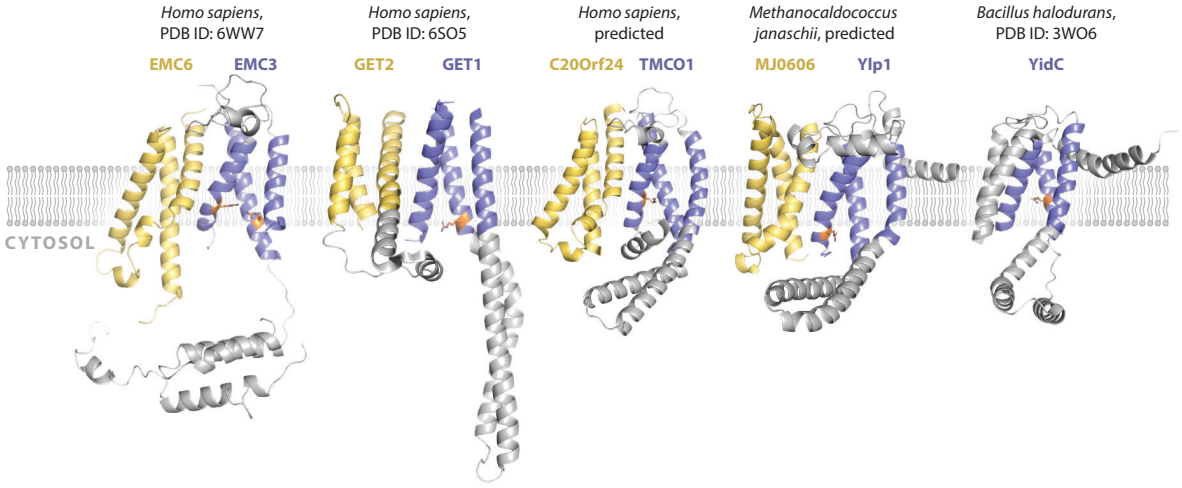

Figure 7

The conserved EMC3–EMC6 core membrane domain compared to homologous heterodimers. Ylp1 is the archaeal YidC-like protein (MJ0480) and is predicted to form a complex with an archaeal EMC6-like protein termed MJ0606. Similarly, the EMC3-related protein TMCO1 is predicted to form a complex with the mammalian EMC6-like protein C20Orf24. YidC is shown for comparison with the colored region homologous to EMC3's membrane domain. Lysine or arginine residues in the hydrophilic vestibule are shown as orange sticks. Abbreviations: EMC, endoplasmic reticulum membrane protein complex; GET, guided entry of TA protein; Ylp1, YidC-like protein.

For example, the hairpin in GET1 directly engages GET3 (57, 65), the hairpin in YidC may interact with SRP and the SRP receptor (SR) (109), and the hairpin in TMCO1 interacts with the ribosome (106). EMC3's hairpin is unusually long, with its two helices interacting with a helix from EMC3's C-terminal tail. This three-helix domain interacts with EMC2. Charge reversal and hydrophobicity-altering mutations in this region impair substrate TMD insertion consistent with a role in substrate recruitment (**Figure 8**) (83, 104), although this has not been investigated in biochemical studies. Taken together, the key features of Oxa1 family members required for their function as transporters seem to be conserved in EMC3. Although EMC6 seems to be structurally well conserved with other family members, the lack of functional information on any family member [beyond a targeting role for the GET2-specific cytosolic domain (56, 57, 65)] limits insights into EMC function.

4.3. The Translocation Paths Within EMC

EMC-mediated TA protein insertion presumably involves at least transient interactions between EMC and the substrate's TMD or its flanking regions. In one model based on current views of YidC function (15, 105, 110, 111), the hydrophilic TMD-flanking domain of the substrate would access the hydrophilic vestibule contributed by the front face of EMC3. This would allow this region of the substrate to move part of the way across the membrane, facilitating its eventual translocation to the lumen. The TMD, which for EMC substrates is moderately hydrophobic, might initially engage EMC3 at its interface with EMC4, analogously to how signal sequences (which are also moderately hydrophobic) bind at the lateral gate of Sec61 (112). From this waystation at the interface of protein and lipid, the TMD would egress laterally into the membrane. This energetically favored reaction would drive concomitant translocation of the flanking domain.

This model for TA protein insertion is supported by the clustering of mutations that impede TA protein insertion on the front side of EMC (**Figure 8**). This includes point mutations of key

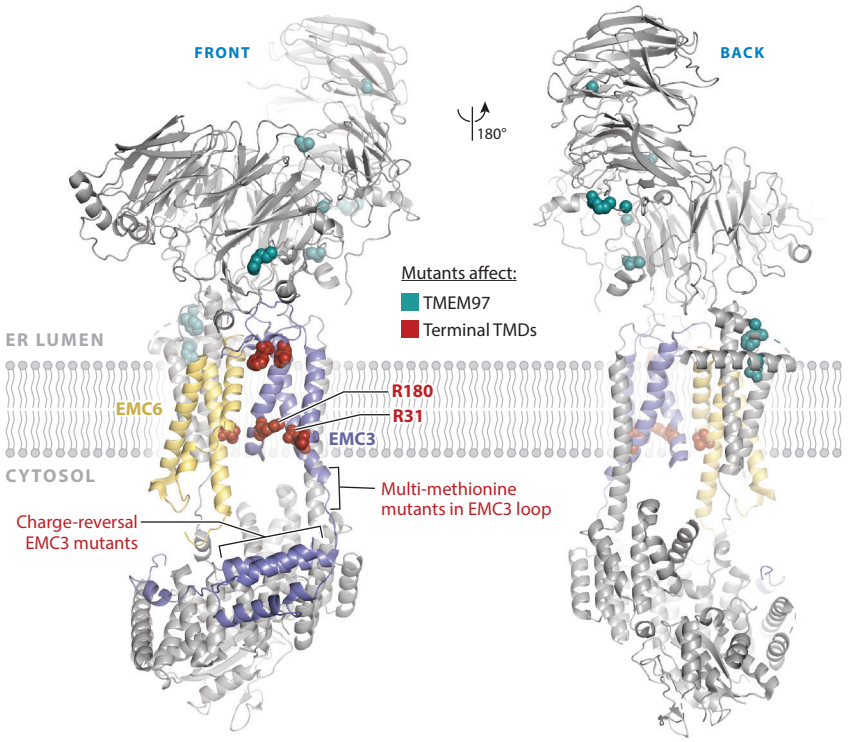

Figure 8

Two views of human EMC (PDB ID: 6WW7) in which point mutations that affect terminal TMD substrates (*red*) and TMEM97 (*teal*) are indicated. The point mutations that affect terminal TMD insertion are located on the front side and involve the EMC3–EMC6 subcomplex. By contrast, mutations that affect TMEM97 are more distributed, with most of them in the lumenal domain of EMC1. This structure does not show the membrane domains of EMC4 or EMC7, which would be located in front of EMC3 in the front view (see **Figure 6**). Abbreviations: EMC, ER membrane protein complex; ER, endoplasmic reticulum; PDB ID, Protein Data Bank identifier; TMD, transmembrane domain.

residues in the hydrophilic vestibule and combinations of mutations in the cytosolic domains of EMC2 and EMC3 (83, 103, 104). Of these, the most straightforward to rationalize are the EMC3 vestibule mutations that might impede substrate entry. Mutation of a subset of methionines in a methionine-rich cytosolic loop of EMC3 impairs TA protein insertion (83), perhaps by reducing the efficacy of TMD recruitment through hydrophobic interactions. Charge-reversal mutations in the cytosolic region also affect TA protein insertion (104), but the reason is currently unclear. In each of these cases, biochemical analysis is required to determine the precise step(s) at which TA protein interaction or insertion is blocked.

As discussed in Section 4.1, the front face of EMC3 might be partially or even completely occluded in the inactive state by EMC4 and EMC7. Consistent with this idea, both EMC4 and EMC7 can be photocrosslinked to probes on the front-facing TMDs of EMC3 in native ER membranes (84). Thus, substrate access to the EMC3 hydrophilic vestibule or TMD access to the EMC3–EMC4 interface might require EMC4 to partially move away from EMC3. An attractive model is one where an initial interaction of substrate with the cytosolic domain of EMC facilitates this movement to allow substrate access to the EMC3 transporter (**Figure 9**). Such a gating mechanism would avoid constitutive exposure of a hydrophilic half-channel to the membrane.

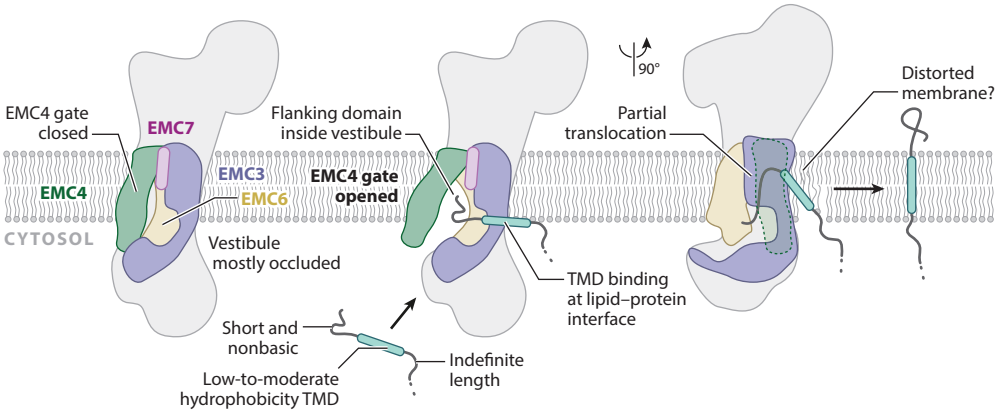

Figure 9

One model for EMC-mediated insertion of moderately hydrophobic terminal TMDs. A gate containing EMC4 is proposed to open in response to substrate binding, allowing the substrate's hydrophilic flanking domain to enter EMC's hydrophilic vestibule. TMD entry into the membrane is aided by partial transport of the hydrophilic flanking domain through the vestibule. Complete TMD entry into the membrane drives the completion of flanking domain transport through the locally distorted membrane. The third diagram is turned by 90° relative to the first two to show the front of the EMC3–EMC6 subcomplex with a transparent EMC4. Abbreviations: EMC, endoplasmic reticulum membrane protein complex; TMD, transmembrane domain.

In the context of this model, it is noteworthy that EMC4, EMC7, and EMC10 are each anchored to the lumenal domain of EMC1, with EMC4 also anchored to EMC2 in the cytosol. This might allow these proteins to tolerate only weak interactions with EMC3 in the membrane, thereby facilitating their movement in response to substrate translocation. Interestingly, slightly shortening the linker between EMC4's membrane domain and its cytosolic EMC2-interacting domain impaired EMC function in yeast (103). As with the other mutations, the biochemical basis of this impairment needs to be investigated, but it is consistent with the need for EMC4 flexibility as part of EMC's insertion reaction.

An alternative but very similar model is one where substrates access EMC3's frontside vestibule from the side near EMC6. The only evidence against this route of access is that the methionine-rich cytosolic loops of EMC3, proposed to initially engage substrate, are on the EMC7 side. A third proposed route via the hydrophobic groove on the back side of EMC now seems relatively unlikely (84). This was based on the observation that the cradle formed by EMC2 (or the EMC2–EMC8 or EMC2–EMC9 complexes) can crosslink to a substrate TMD in isolation. Because this cradle is continuous with the hydrophobic groove (whose size is large enough to fit a TMD), insertion via this path was suggested. However, the cradle is partially or completely occluded by EMC5's cytosolic domain in native mammalian and yeast EMC, arguing against this insertion path. Indeed, recent crosslinking experiments indicate that EMC5's cytosolic domain reduces TMD interaction with the EMC2–EMC8 subcomplex (113).

4.4. Can EMC Access ER-Targeted Ribosomes?

Although the insertion of TA proteins and N_{exo} signal anchors are conceptually similar in many ways, only the latter occurs cotranslationally. This is an important difference for two reasons. First, the time available for cotranslational insertion is likely to be very short because the ribosome is thought to dock onto Sec61 almost immediately after SRP and its receptor have dissociated (14, 22). After Sec61 docking, EMC cannot access the nascent chain unless the TMD can reach at least ~110Å away from the ribosome exit tunnel (84). Second, the sizes of EMC's cytosolic domain and

the ribosome impose steric constraints on how the substrate can approach and access different regions of EMC.

As noted in Section 3.3 (**Figure 5**), the only point at which EMC can insert N_{exo} signal anchors is between SRP-mediated targeting and ribosome handover to Sec61. Structures of the prehandover complex between the ribosome, SRP, and SR show that the exit tunnel of the ribosome cannot come close to the membrane without severely clashing with the SRP–SR complex (114, 115). Hence, the prehandover complex cannot engage Sec61, even though the region around the exit tunnel is mostly vacated by the movement of SRP54's GTPase domain to a distal site on SRP RNA. Notably, the GTPase activities of both SRP54 and its associated SRα are inhibited, and this inhibition seems to be a eukaryotic-specific step. Thus, this prehandover complex is poised to be sampled by EMC, whose cytosolic domain can approach the exit tunnel without competition.

An attractive model is that EMC can specifically sample this complex, and perhaps even stimulate GTP hydrolysis by the SRP and SR. In this way, the signal sequence would be released from SRP in the vicinity of EMC. If the properties of the substrate match EMC's preference, insertion occurs in the N_{exo} orientation. Otherwise, the ribosome docks onto Sec61, which can then mediate N_{cyt} insertion of the signal sequence or TMD. Because EMC3's cytosolic vestibule contains conserved positive charges, signal sequences and N_{cyt} signal anchors [whose N-terminal flanking domains typically contain positive charges (88, 116, 117)] are rejected, leaving them to be inserted by Sec61. The limited time available to EMC may help impose this positive inside rule because TMDs preceded by nearby positive charges would be inserted relatively slowly.

Consistent with this stepwise model, N_{exo} signal anchors are inserted in the N_{cyt} orientation in the absence of EMC (86). Furthermore, a model in which EMC samples all SRP-targeted substrates before delivery to Sec61 predicts that some frequency of inappropriate insertion occurs. Hence, signal sequences and N_{cyt} signal anchors with short N-terminal domains might be inserted in the N_{exo} topology. Recently, the P-type ATPase ATP13A1 (Spf1 in yeast) was shown to dislocate precisely these types of errors, which accumulate when ATP13A1 is depleted (118). The copurification of Spf1 with EMC in one study (119) suggests the intriguing hypothesis that they work together to ensure accurate topogenesis of signal peptides and signal anchors. One prediction of such a model is that a subset of topology errors observed in ATP13A1 knockouts will be reduced by also eliminating EMC.

It has been suggested that EMC might also engage TMDs inserted through the lateral gate of Sec61 (119). This idea comes from indirect evidence examining the mRNA footprints of ribosomes in proximity to EMC (discussed in more detail in Section 5.2). Such an interaction is feasible only if the ribosome detaches from Sec61 or if the TMD diffuses at least ∼110 Å away from the lateral gate. Both are feasible, but represent relatively special circumstances. Thus, a general role in chaperoning poorly hydrophobic TMDs shortly after their insertion seems unlikely. Such a function might instead be carried out by the abundant PAT complex, which has been shown to directly interact with partially hydrophilic TMDs (29) and is probably recruited to ribosomes synthesizing multipass membrane proteins along with other components, including TMCO1 (106).

5. THE SUBSTRATE RANGE OF EMC

TA proteins and N_{exo} signal anchors represent two reasonably well-established classes of direct EMC substrates supported by a combination of in vitro reconstitution experiments and endpoint assays in cells. Satisfyingly, the homology between the EMC3–EMC6 subcomplex and the GET1–GET2 complex matches with EMC's TA protein substrates, whereas EMC3's membership in the Oxa1 superfamily matches with the cotranslational N_{exo} translocation activity assigned to both YidC and Oxa1. Yet, EMC is far more elaborate than these other systems, mutations in regions

far from the EMC3–EMC6 core affect the biogenesis of certain membrane proteins, and complete EMC deletion affects non-TA and non-N_{exo} membrane proteins. These observations imply a broader but unknown role for EMC.

5.1. Proteomic Analysis of EMC–Deficient Cells

Three studies have analyzed the proteome of mammalian cells acutely or stably depleted of EMC in an attempt to determine the full complement of EMC substrates (71, 119, 120). Such an approach benefits from being unbiased but also has a number of caveats. First, only substrates that cannot be made by another pathway are sufficiently depleted to detect reliably. Indeed, it is noteworthy that although TA proteins of moderate hydrophobicity can be inserted by EMC, only those very-low-hydrophobicity substrates that cannot use the GET pathway were found. Second, cells compensate quickly by the induction of stress responses, potentially blunting the impact of EMC loss. Third, components of other biogenesis pathways might depend on EMC, thereby leading to indirect effects that can be challenging to deduce.

These caveats notwithstanding, the proteomics studies so far do show some patterns. First, although some TA and N_{exo} membrane proteins are reduced, they do not emerge as a major set of hits. This could be due to alternative insertion pathways, minimal numbers of GPCRs expressed in the cell types analyzed, and low abundance of many such proteins. Second, two of the studies found an enrichment for multipass membrane proteins containing more than four TMDs (119, 120). The third study found only two membrane proteins: SQS (the only protein found in all three studies) and SGPL1 (an N_{exo} substrate) (71). Third, the two studies that used the same cell type (HeLa cells) had modest overlap at a high stringency cutoff but reasonably good overlap when the stringency was relaxed. Finally, a study performing proteomics on EMC1-knockdown *Xenopus* embryos identified 21 statistically significant proteins with at least 50% reduction (121). Although this list did include several membrane proteins, the overlap with the other studies is modest. Overall, the number of validated membrane proteins dependent on EMC for normal expression is approximately a dozen.

Although this analysis provides important leads for in-depth analysis (as was productive for both SQS and GPCRs), a different approach is probably needed to curate a more complete list. Reduced steady-state expression depends on the failed biogenesis product being effectively degraded, so many substrates may not be detected using overall levels of protein alone. Because defects in insertion, topology, and ER retention all lead to altered glycosylation and altered cell surface exposure, an enrichment strategy focused on one or both of these parameters might allow a deeper proteomic analysis. For example, selective surface labeling, fractionation, or lectin binding could provide greater discriminatory power. It could be argued that at this stage, finding more candidate substrates is of lower importance than determining why EMC is needed for putative substrates that do not have a terminal TMD in need of insertion.

5.2. Profiling of EMC–Proximal Ribosomes

A second global approach to identifying EMC substrates was based on determining which translating mRNAs in yeast are in proximity to EMC (119). In this approach (122), the biotin ligase BirA was fused to the cytosolic domain of EMC (the C terminus of EMC5) in a yeast strain where the BirA target peptide is located at the C terminus of the large ribosomal subunit of RPL10a. Ribosomes that are close to EMC are selectively biotinylated, allowing their purification and sequencing of the mRNA footprints protected by the small subunit. In principle, this provides a readout of both which mRNAs are translated in the vicinity of EMC and when during that mRNA's translation EMC is nearby.

Similar to other ER-localized BirA fusions (to Ubc6, Ssh1, and Sec63), EMC-labeled ribosomes were strongly enriched for footprints from mRNAs encoding all cotranslationally translocated secretory and membrane proteins. Conversely, mRNAs coding for cytosolic proteins, mitochondrial proteins, and posttranslationally targeted ER proteins (e.g., TA proteins) were relatively depleted. Beyond this, the EMC-BirA fusion did not enrich any obvious subset of mRNAs appreciably more than other ER-localized BirA fusions.

Importantly, the data set contained a positive control for the degree of enrichment one might expect if BirA was genuinely adjacent to an mRNA during its translation: BirA fused to the N terminus of Ssh1, a ten-TMD protein. Here, BirA would be translated and folded just as the first TMD of Ssh1 targets to the ER membrane for insertion. BirA would then be located precisely where a putative chaperone that interacts with Ssh1 should be during the remainder of Ssh1 biogenesis, then move away from Ssh1-translating ribosomes upon termination. The degree of selective enrichment of Ssh1 mRNA footprints in the BirA-Ssh1 cells is both striking and unique. Because no mRNA showed this behavior in the EMC–BirA strain, it seems that EMC does not robustly engage any protein during the majority of its synthesis.

A finer analysis to search for transient EMC proximity identified local enrichments in 53 mRNAs, most of which code for multipass membrane proteins (119). In the most enriched example (Fks1, and the highly homologous Gsc2), the enrichment mapped to regions of the mRNA that code for its six-TMD and nine-TMD bundles. The very similar pattern in both of these mRNAs, and the coimmunoprecipitation of both full-length proteins with EMC, suggest a direct role in their biogenesis that begins cotranslationally and ends posttranslationally. EMC has also been linked to the biogenesis of Yor1, an ABC transporter that similarly has two bundles of TMDs separated by a large cytosolic domain (37, 123). Loss of EMC seems to cause ribosome collisions along the Yor1 mRNA (123), providing indirect evidence that EMC might interact cotranslationally with nascent Yor1 protein.

These analyses suggest that EMC is near ribosomes that have begun inserting TMDs of a multipass membrane protein. Given the limited space around the ribosome–Sec61 complex, this interaction would need either the ribosome to detach from Sec61 or substrate TMDs to diffuse sufficiently far away from Sec61. It is therefore noteworthy that Fks1, Gsc2, and Yor1 each have very long cytosolic domains between their two bundles of TMDs. Engagement of the first bundle by EMC might chaperone it until the second bundle is synthesized and successfully assembles with the first. This could explain the observed ribosome biotinylation (especially if trailing ribosomes in a polysome are biotinylated by EMC–BirA) and the persistent association between EMC and these proteins.

5.3. Is EMC a Membrane Chaperone?

A chaperone function for EMC during membrane protein folding or protein complex assembly is attractive for a number of reasons. First, both YidC and Oxa1 have been proposed to function as chaperones by using their membrane-embedded hydrophilic grooves to engage membrane protein substrates (15, 19). Second, a variety of membrane proteins whose steady state levels are impacted by EMC are neither TA proteins nor begin with an N_{exo} signal anchor (**Table 1**). Third, EMC coimmunoprecipitates with at least some putative substrates and their presumed substrate-specific chaperones (119).

Although this model is attractive, it remains relatively poorly explored. If EMC functions analogously to YidC, the interaction with its substrate might be expected to involve the same region of EMC as for TMD insertion. However, EMC mutagenesis studies suggest this is not the case for at least some substrates (104). Here, TMEM97 was unaffected by EMC3 mutations that strongly

Table 1 A subset of multipass membrane proteins established to be strongly affected by EMC for unclear mechanistic reasons

Protein(s)	Function	Topology	Reference(s)
Acetylcholine receptor & $GABA_A$ receptor	Ligand-gated ion channels	Pentameric complex; each subunit has a signal sequence with four TMDs & large lumenal domain	38
Pma1 (*Saccharomyces cerevisiae*)	Ion transport	Ten TMDs N_{cyt} signal anchor Short lumenal loops Large cytosolic domain	138
Na^+/K^+ ATPase	Ion transport	Ten TMDs N_{cyt} signal anchor Short lumenal loops Large cytosolic domain	39
TRP channels	Ion transport	Six TMDs Begins with N_{cyt} signal anchor	39, 139
Frizzled receptors FZD2, FZD4, FZD7	Signaling GPCR	Seven TMDs Signal sequence Large lumenal domain	120, 121
Yor1ΔF (*S. cerevisiae*)	Ion transport; ΔF is a partially destabilizing mutant of Yor1	Two bundles of six TMDs N_{cyt} signal anchor Large cytosolic ATPase domains Short lumenal domains	37, 123
ABCA3	Phospholipid transport	Two bundles of six TMDs N_{cyt} signal anchor Large cytosolic ATPase domains Large lumenal domains	140
TMEM97	Sigma-2 receptor, function unknown	Four TMDs N_{cyt} signal anchor Small lumenal domains	104, 119
SOAT1	Sterol O-acyltransferase	Nine TMDs N_{cyt} signal anchor Short lumenal loops	71

Abbreviations: EMC, endoplasmic reticulum membrane protein complex; GPCR, G protein–coupled receptor; N_{cyt}, N terminus facing the cytosol; TMD, transmembrane domain; TRP, transient receptor potential.

affect SQS or β1AR biogenesis, whereas various mutations in the lumenal domain of EMC1 impact TMEM97 without impairing the other two. These findings provide compelling evidence that EMC can affect protein biogenesis by mechanisms unrelated to its function in TMD insertion.

The nature of this additional activity is currently unclear. TMEM97 interaction with EMC has not been examined, and it is mysterious how mutations in the lumenal domain of EMC that are relatively far from the membrane could affect membrane proteins (such as TMEM97) that protrude only modestly into the lumen. Although the back side hydrophobic groove is large enough to engage a substrate TMD, its highly hydrophobic surface would seem incompatible with EMC's proposed preference for hydrophilic TMDs. Additionally, it is not clear what might go wrong for the clients of EMC's proposed chaperone activity. Possibilities include failed folding, the wrong topology, aggregation or inappropriate interactions. Each of these would lead to promiscuous degradation and reduced overall expression levels.

It is worth noting that although much has been written about YidC acting as a chaperone, disentangling this proposed function from its role in TMD insertion is nontrivial. The strongest evidence comes from the study of LacY, a lactose transporter. In this model, LacY has been shown to physically interact with YidC, and in the absence of YidC, it has been shown to misfold as monitored by conformation-specific antibodies (124–126). Importantly, separate probes of topology provided evidence of correct membrane insertion. Analogous studies for an EMC substrate would be valuable in establishing a chaperone role.

6. FUTURE DIRECTIONS

A combination of evolutionary, biochemical, structural, and functional analyses have now converged on a functional role for EMC in TMD insertion of N_{exo} and TA proteins. An important goal is to determine the mechanistic basis and substrate range of TMD insertion by EMC. This is a tractable problem given the availability of model substrates, in vitro assays, EMC structures, and insights from other Oxa1 superfamily members. In addition to N_{exo} and TA proteins, a role for EMC in inserting internal TMDs of multipass proteins should be investigated. Both YidC and Oxa1 insert multipass proteins using their transporter activity to translocate the loops between adjacent TMDs concomitant with their insertion (127–129). Although EMC is not likely to perform this function cotranslationally, it might insert pairs of TMDs posttranslationally. Several studies indicate that marginally hydrophobic TMDs, which are particularly common in membrane transporters, may not insert effectively when they are first synthesized (130–136). If so, an initially misinserted or partially inserted membrane protein might need posttranslational insertion steps, a function suitable for EMC.

A second broad goal is to determine whether and how EMC functions as a membrane protein chaperone beyond its TMD-insertion function. This would be aided by first identifying a model substrate that interacts with EMC, has a clear phenotype in EMC-deficient cells, is relatively small, and for which assays of folding and topology are tractable. With such a candidate substrate in hand, one can determine which region(s) of EMC engage which parts of the substrate and define the specific consequences of not having EMC. This should provide a starting point to understand whether EMC's chaperone function is to protect interaction surfaces, maintain a protein's appropriate topology, help reorient TMDs to the correct topology, or something else. Having a full picture of EMC functions and mechanisms will provide insight into the fundamental process of membrane protein biogenesis and help explain the basis for the severe consequences of its dysfunction.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I thank Aaron Lewis, Patrick Chitwood, Luka Smalinskaitė, and Haoxi Wu for productive discussions and Aaron Lewis for help rendering figures and insightful comments on this manuscript. Research in the author's lab is supported by the Medical Research Council of the UK (MC_UP_A022_1007).

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