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SPOTLIGHT

Chasing the right tail: How the ER membrane complex recognizes its substrates

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Tail-anchored proteins are tethered to membranes of the ER, mitochondria, and peroxisomes. In this issue, Pleiner and colleagues (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202212007) show that the ER membrane complex (EMC) uses an inbuilt charge-dependent selectivity filter to specifically insert ER tail-anchored proteins according to their topology signals and to prevent the misincorporation of mitochondrial proteins.

In eukaryotic cells, most of the proteins that are synthesized on cytosolic ribosomes have to be transported across or into internal membranes to reach their final destination in one of the different cellular compartments. Targeting signals in the nascent polypeptides serve as address labels that ensure the correct delivery of the newly synthesized proteins. The signal sequences of proteins of the endoplasmic reticulum (ER) and the matrix-targeting sequences of mitochondrial proteins, being the archetypes of such targeting signals, were discovered already three decades ago (1). These sequences are recognized initially by either targeting factors in the cytosol (such as the signal recognition particle in the case of ER proteins) or on the target membranes (such as the receptors of the translocase of the outer mitochondrial membrane). Signal sequences and matrix-targeting sequences are positioned at the N-terminus of the corresponding proteins so that the targeting information is presented as soon as the first portions of newly synthesized proteins emerge from the exit tunnel of the ribosome. This positioning on the N-terminus allows the co-translational translocation of proteins into their target compartment and reduces the likelihood for the nascent protein to be folded in the cytosol prior to the translocation process.

In contrast, a large class of cellular proteins displays their targeting information on the C-terminus: Tail-anchored (TA) proteins are characterized by C-terminal transmembrane domains followed by only a very short hydrophilic stretch of about 5–40 residues. These C-terminal membrane anchors tether proteins to the cytosolic surface of the cellular compartments that face the cytosol. Since the tail anchor domain is only presented after the termination of translation, the targeting of TA proteins occurs generally post-translationally. Thereby, TA proteins have to embark on targeting routes to their destination membranes that are distinct from those used by proteins with classical N-terminal targeting sequences.

The "code" by which tail anchor segments specify their targeting membrane was only recently discovered (Fig. 1 A; 2, 3): TA proteins with very hydrophobic transmembrane domains are bound by Sgt3/SGTA and Get3, cytosolic proteins of the guided entry of TA proteins (GET) pathway, and inserted into the ER by the Get1/Get2 complex (4, 5). For proteins with moderately hydrophobic membrane anchors, the short C-terminal stretch is relevant: if neutral or negatively charged residues follow their membrane anchor, proteins are inserted into the ER membrane by the ER membrane complex (EMC), whereas proteins with positive residues at their C-terminus are excluded from the ER and typically found in mitochondria and peroxisomes (Fig. 1 A). A study in this issue of *JCB* (6) now reveals for the first time how these charge signals are deciphered.

The human EMC is a large membrane-embedded structure consisting of 10 subunits of which the molecular architecture has recently been resolved by cryo-electron microscopy (9, 10). The catalytic core of the EMC consists of EMC3 and EMC6, two membrane-embedded subunits that form the catalytic center for TA protein insertion (Fig. 1 B). EMC3 belongs to the OXA1 family of protein insertases, the members of which integrate proteins into the inner membranes of bacteria and mitochondria as well as the thylakoid membrane of chloroplasts. These insertases are postulated to disorganize the lipid packaging in their surroundings to mediate protein insertion into locally thinned and distorted bilayers (11). The different members of the OXA1 family are universal insertases that can even be functionally exchanged between different cellular membranes (12).

The detailed analysis of the molecular architecture of the EMC (9) allowed insights into the mechanism by which it recognizes its substrate proteins (6): (i) On one side of its cytosol-exposed surface, a hydrophobic crevice formed by EMC3 and a hydrophobic helix of EMC7 serve as binding sites for the

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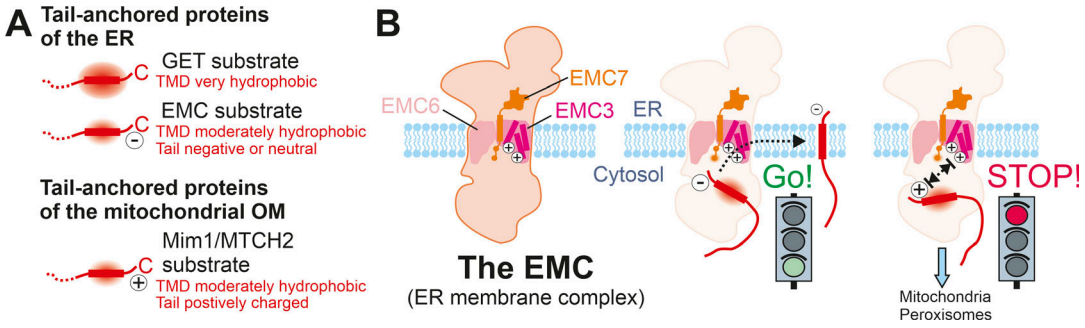

Figure 1. **The features of C-terminal sequences direct TA proteins to their correct intracellular target membrane using a selectivity filter of the EMC in the ER membrane. (A)** The properties of the C-terminal membrane anchors and the short subsequent sequences serve as targeting signals for TA proteins. The membrane integration of TA proteins of the yeast and human mitochondrial outer membrane is facilitated by Mim1 and MTCH2, respectively (7, 8). **(B)** The EMC complex promotes the translocation of negatively charged protein segments across the ER membrane, using their affinity to two arginine residues in EMC3. These positive charges serve as a selectivity filter to repel TA proteins with positive charges in their tail, preventing the mis-insertion of TA proteins destined for mitochondria and peroxisomes.

transmembrane domain of newly synthesized TA proteins. The interaction of these hydrophobic regions with the transmembrane domains of TA proteins was demonstrated by crosslinking experiments with a zero-length disulfide crosslinker and by site-specific mutants that resulted in compromised protein insertion. (ii) On the other side of its cytosol-exposed surface, EMC3 forms the hydrophilic vestibule which exposes two positively charged arginine residues (R31 and R180) to the cytosol serving as charge-driven selectivity filter (Fig. 1 B): negatively charged or neutral tails in TA proteins are stimulated by these charges to engage with the EMC and to insert into the ER membrane. On the contrary, proteins with positive charges following their transmembrane segment are repelled by the charges in EMC3, preventing the mis-insertion of mitochondrial and peroxisomal proteins into the ER membrane. The authors proved their selectivity filter model through an elegant experiment; they generated a mutated EMC complex in which they had replaced the positive charges in EMC3 by negative ones. These changes compromised the insertion of an ER TA protein. However, if the negative charges in that model protein were simultaneously replaced by positive charges, protein insertion was restored.

The charge-driven selectivity filter in the EMC seems to be of relevance not only in the context of TA proteins. The EMC complex facilitates the insertion and topogenesis also of many multispan ER proteins, in particular of those that lack a signal sequence. G protein–coupled receptors (GPCRs), a family with hundreds of members in the human proteome, belong to this group of proteins. Pleiner et al. (6) show that the charges in EMC3 are also critical for the correct orientation of such GPCRs, which almost consistently expose negative charges in their N-terminus that transfers to the ER lumen in an EMC-driven reaction (9). Thus, the selectivity filter in the EMC complex determines the topology of a multitude of cellular membrane proteins and confers an orientation in which positively charged residues flanking a transmembrane domain are retained in the cytosol and negatively charged residues are translocated. This "positive-inside rule" in protein topogenesis according to which positive charges remain in the cytosol was already discovered by Gunnar von Heijne three decades ago (13). The detailed analysis of the structure of the membrane core of the EMC complex finally elucidates the molecular mechanisms underlying this topogenesis code of eukaryotic membrane proteins.

In the future, it will be interesting to learn how the reciprocal selection works, namely, how the mitochondrial insertion machinery avoids the misincorporation of ER-destined TA proteins.

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