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CELL SCIENCE AT A GLANCE

Membrane insertases at a glance

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ABSTRACT

Protein translocases, such as the bacterial SecY complex, the Sec61 complex of the endoplasmic reticulum (ER) and the mitochondrial translocases, facilitate the transport of proteins across membranes. In addition, they catalyze the insertion of integral membrane proteins into the lipid bilayer. Several membrane insertases cooperate with these translocases, thereby promoting the topogenesis, folding and assembly of membrane proteins. Oxa1 and BamA family members serve as core components in the two major classes of membrane insertases. They facilitate the integration of proteins with α-helical transmembrane domains and of β-barrel proteins into lipid bilayers, respectively. Members of the Oxa1 family were initially found in the internal membranes of bacteria, mitochondria and chloroplasts. Recent studies, however, also identified several Oxa1-type insertases in the ER, where they serve as catalytically active core subunits in the ER membrane protein complex (EMC), the guided entry of tail-anchored (GET) and the GET- and EMC-like (GEL) complex. The outer membrane of bacteria, mitochondria and chloroplasts contain β-barrel proteins, which are inserted by members of the BamA family. In this Cell Science at a Glance article and the accompanying poster, we provide an overview of these different types of membrane insertases and discuss their function.

KEY WORDS: BAM complex, β-barrel proteins, EMC complex, GET complex, Membrane insertases, Oxa1, Protein biogenesis, Translocases, YidC

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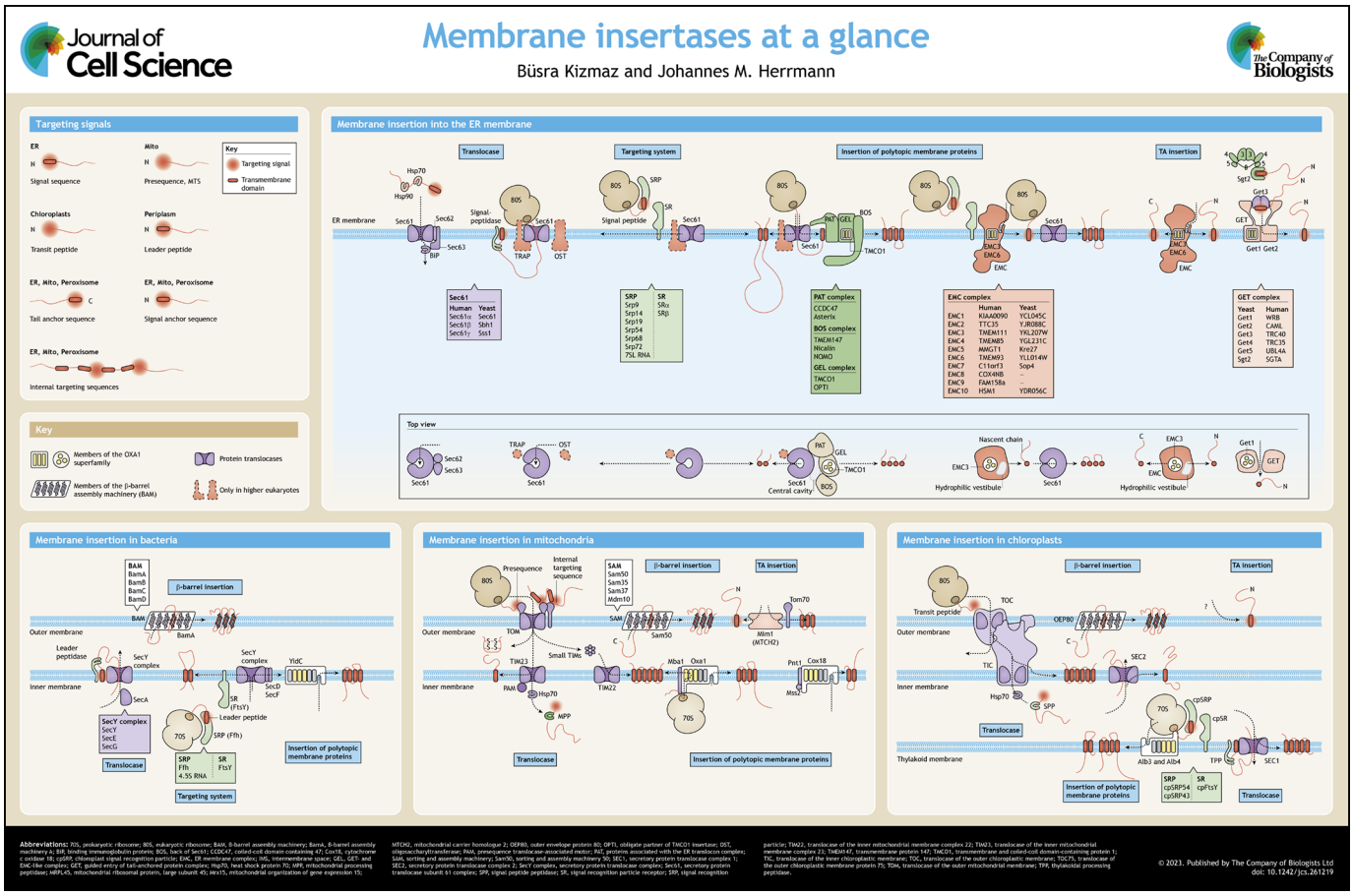
Introduction

Integral membrane proteins make up about one third of all cellular proteins. They can span the membrane with one α-helical transmembrane domain close to the N terminus of a protein (referred to as a signal anchor), on the C-terminus (referred to as a tail anchor) or somewhere in the middle of a protein. Polytopic membrane proteins have either several α-helical transmembrane domains or a series of membrane-embedded β-strands. The outer



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membranes of bacteria, mitochondria and plastids contain this second type of integral membrane protein, also called β-barrel proteins. These pore-forming proteins comprise eight (such as in the abundant OmpA porin of *Escherichia coli*) to 26 β-strands, in which the most N-terminal strand is bound to the most C-terminal one to form a cylindrical structure around a central hydrophilic pore (Doyle and Bernstein, 2022). The residues that form these sheets alternatingly consist of hydrophobic residues (facing the lipid bilayer of the membrane) and polar residues (facing the interior of the cylinder). The machineries which promote the insertion of integral membrane proteins with α-helical transmembrane domains as well as of β-barrel proteins are introduced in this short review and illustrated on the accompanying poster.

Translocases and insertases facilitate membrane protein biogenesis

Translocases and insertases are proteins that assist in the membrane insertion of integral membrane proteins. Translocases allow the complete translocation (secretion) of proteins across the lipid bilayer whereas insertases mediate the insertion of hydrophobic protein segments into the membrane. Some translocases form water-filled protein-conducting channels, whereas others allow protein translocation through protein–lipid interfaces in destabilized membranes (Wu and Rapoport, 2021). Given that hydrophilic proteins are unable to penetrate membranes on their own, translocases, such as the SecY complex, the Sec61 complex and the translocases of the outer and inner membrane of mitochondria are essential for protein translocation and, thus, for the viability of organisms.

Insertion into the membrane often occurs spontaneously *in vitro* even when no helping insertases are present (Kiefer and Kuhn, 1999), but *in vivo*, such factors are important to increase insertion rates and to provide target membrane specificity. Thus, insertases are comparable to enzymes that catalyze an exergonic reaction. Most insertases for α-helical transmembrane domains are not essential, and their deletion causes rather moderate defects. This might explain why many insertases, such as the proteins of the guided entry of tail-anchored proteins (GET complex), the ER membrane complex (EMC) or the GET- and EMC-like (GEL) complex have been identified only recently (Schuldiner et al., 2008; Guna et al. 2018; McGilvray et al., 2020).

Membrane insertion as well as membrane translocation often occurs during protein synthesis. This co-translational mode is typically facilitated by a signal recognition particle (SRP), which recognizes targeting sequences in nascent chains and directs ribosome–nascent chain complexes to insertases or translocases in target membranes (Akopian et al., 2013). Post-translational targeting does not make use of SRPs.

Protein insertion into the inner membrane of bacteria

The bacteria SecY complex serves as a protein translocase for the secretion of soluble proteins, as well as for the lateral integration of integral membrane proteins (see poster). The SecY complex is a trimeric complex (consisting of SecY, SecE and SecG), which forms an hourglass-shaped translocation pore with a lateral gate on one side of its wall (Van den Berg et al., 2004), which dynamically flickers between its closed and open state to allow the release of transmembrane segments into the lipid bilayer (Mercier et al., 2021). Opening of the lateral gate is stimulated by the hydrophobicity of the membrane segment in a penetrating substrate, as well as by protein ligands of the SecY complex, such as the integral membrane protein YidC (Mercier et al., 2021; Sachelaru et al., 2013). Post-translational translocation through the SecY complex is driven by SecA, which pushes proteins across the membrane in an ATP-consuming reaction (Kedrov et al., 2011) (see poster). Co-translational translocation is facilitated by the bacterial SRP (consisting of the protein Ffh and its associated 4.5S RNA co-factor) and its cognate receptor FtsY (Miller et al., 1994).

YidC serves as insertase, which receives substrates from the SecY complex to promote their further topogenesis and folding. The SecY-associated membrane proteins SecD and SecF, together with YidC, form a lipid-filled chamber that has been suggested to serve as folding compartment for nascent membrane proteins (Botte et al., 2016). The SecY complex and YidC are ubiquitously found in prokaryotes, whereas SecD and SecF, are not strictly conserved. Membrane proteins lacking larger periplasmic domains can also be inserted by YidC in a SecY-independent fashion (Serek et al., 2004). The YidC protein of *E. coli* comprises six transmembrane domains, five of which form a positively charged hydrophilic groove within the membrane plane (Kumazaki et al., 2014; Wickles et al., 2014; Chen et al., 2017). Membrane thinning, the disturbance of tightly packed membrane lipids and negative charges in the translocated segments promote the insertion and intramitochondrial folding of inner membrane proteins; this allows proteins to reach their correct topology in the lipid bilayer, a process referred to as topogenesis (Endo et al., 2022; McDowell et al., 2021; Komar et al., 2016). Given that YidC facilitates protein folding and the assembly of oligomeric proteins (e.g. of the *c* ring of the F_o part of the ATPase), its role is comparable to that of chaperones for water-soluble proteins.

Membrane insertion into the inner membrane of mitochondria

Mitochondria and chloroplasts were derived from bacteria and still contain genomes for the synthesis of a small number of proteins. Mitochondria reduced the coding information of their genome to a minimum, and mitochondrial ribosomes of animals and fungi only synthesize a small number of highly hydrophobic membrane proteins. Membrane insertion of mitochondrial translation products is facilitated by the mitochondrial YidC homolog Oxa1 (called OXA1L in animals; for a review, see Homberg et al., 2023). Oxa1 was the first representative of the Oxa1 family discovered (therefore coining the family name) and received its name for its critical relevance for cytochrome oxidase assembly (Bonnefoy et al., 1994; Bauer et al., 1994). Oxa1 shares the five groove-forming transmembrane domains with YidC and presumably promotes the insertion and assembly of its substrates in a YidC-like fashion. Mitochondria of most eukaryotes do not contain an SRP system but rather directly connect the ribosome to the inner membrane (Szyrach et al., 2003; Jia et al., 2003). A C-terminal coiled-coil domain of Oxa1 serves as a ribosome anchor, which binds close to the tunnel exit of the ribosomal large subunit and tethers it to the site of membrane insertion (Pfeffer et al., 2015; Itoh et al., 2021). The membrane-associated protein Mba1 (called MRPL45 in animals) further bridges the gap from the exit tunnel to the membrane-embedded region of Oxa1 and supports protein insertion into the inner membrane together with Mrx15 and Mdm38 (Ott et al., 2006; Moller-Hergt et al., 2018; Wenger et al., 2023; Lorenzi et al., 2016) (see poster).

Mitochondrially encoded inner membrane proteins of animals and fungi have lost any larger domains exposed to the intramembrane space (IMS), making a SecY-analog dispensable, with one exception – the C-terminus of Cox2 forms a large, highly negatively charged

Journal of Cell Science (2023) 136, jcs261219. doi:10.1242/jcs.261219

domain essential for cytochrome oxidase function. Translocation of this domain depends on a specific Oxa1 paralog, called Cox18 and its complex partners Pnt1 and Mss2 (Bourens and Barrientos, 2017; Saracco and Fox, 2002). The mechanism, by which the Cox18 complex mediates the export of the Cox2 C-terminal tail is still unresolved.

Some nuclear encoded proteins also use Oxa1 for their insertion into the inner membrane. However, most of these proteins are inserted following a stop-transfer mechanism by the translocases of the inner membrane, the TIM23 and TIM22 complexes. The composition and function of the mitochondrial import machinery have been described in depth in excellent previous reviews, to which we refer the reader to for further details (Chacinska et al., 2009; Horten et al., 2020).

Membrane insertion of mitochondrial tail-anchored proteins

Many proteins of the mitochondrial outer membrane are tethered to the mitochondrial surface by a C-terminal tail-anchor. In yeast, these proteins are inserted into the membrane by an oligomer of the single-spanning protein Mim1 (Becker et al., 2011; Papic et al., 2011). In human mitochondria, the polytopic outer membrane protein MTCH2 plays an analogous role as tail anchor insertase (Guna et al., 2022). However, the mechanisms by which the Mim1 complex and MTCH2 facilitate membrane insertion are not known. Comparable insertases for tail-anchored proteins presumably also exist on chloroplasts but have not been described to date.

Membrane insertion into the thylakoid membrane of chloroplasts

The thylakoid membrane of chloroplasts is characterized by an extremely high protein content. The chloroplast genome still encodes several proteins that are secreted into the thylakoid lumen, as well as to the IMS. Their translocation closely resembles the translocation process at the bacterial inner membrane as these proteins can carry N-terminal leader peptides, employ an SRP system for targeting and are transported through SecY-like translocases. However, chloroplasts show several deviations from the bacterial system (see poster).

There are two distinct Sec complexes (SEC1 and SEC2) to direct proteins to the thylakoid lumen or the IMS, respectively (Li et al., 2017), consisting of distinct SecY, SecE and SecA subunits; there is no SecG in plastids of higher plants.

The SRP protein (cpSRP54) in plastids lacks an RNA component but is supported by the additional plant-specific co-factor cpSRP43; cpSRP43 promotes protein targeting in conjunction with cpSRP54, but it also acts independently as a chaperone to protect chlorophyll biosynthesis proteins against high temperature (Jaru-Ampornpan et al., 2010; Ji et al., 2021; Horn et al., 2015). The SRP system in plants cooperates with the YidC homologs of plastids, Alb3 and Alb4, which serve as membrane insertases for a large spectrum of thylakoid proteins. The nuclear-encoded light-harvesting-complex proteins (LHCPs) represent the predominant substrates of Alb3 (Bals et al., 2010; Rathod et al., 2022).

Membrane insertion into the ER membrane

The ER is the professional compartment for the synthesis and sorting of secretory and membrane proteins. Owing to the predominantly co-translational mode of protein biogenesis on the ER membrane, the ER surface is covered with cytosolic ribosomes. N-terminal signal sequences and internal transmembrane domains in nascent chains serve as targeting information, which is decoded by the eukaryotic SRP (Akopian et al., 2013), a large ribonucleoprotein complex consisting of six proteins, Srp9, Srp14, Srp19, Srp54, Srp68 and Srp72, and the 7SL RNA. The SRP recruits the ribosome–nascent chain complex to the ER-tethered heterodimeric SRP receptor. Srp54 and the α-subunit of the SRP receptor (SRPRA) are GTPases; in a nucleotide-controlled, timer-like mechanism, they control the release of the ribosome from the SRP and the transfer of the ribosome to the Sec61 complex (Wild et al., 2019; Miller et al., 1993; Wild et al., 2001) (see poster). Additional targeting components, including the yeast proteins Snd1, Snd2 and Snd3 (for SRP-independent targeting), help to recruit nascent chains to the Sec61 complex, particularly if the hydrophobicity of their signal sequence is too low for efficient SRP binding (Aviram et al., 2016; Tirincsi et al., 2022).

The Sec61 complex is a heterotrimeric protein that shows high similarity to the bacterial SecY complex. This Sec61 complex represents the entry gate for proteins that are translocated into the ER lumen and inserts membrane proteins via its lateral gate into the ER membrane. In mammalian cells, the translocation into the ER occurs predominantly co-translationally and the SRP promotes a direct binding of the ribosome to the Sec61 complex (Becker et al., 2009). Most secretory proteins in yeast cells and some in mammalian cells are translocated into the ER post-translationally. In that case, Sec62 and Sec63 associate with the Sec61 translocon and drive the translocation reaction in conjunction with the luminal Hsp70 protein BiP (also known as HSPA5; Kar2 in yeast) (Schlenstedt et al., 1990; Brodsky et al., 1993).

The Sec61-mediated protein translocation and the role of associated enzymes such as the oligosaccharide transferase (OST) complex and the signal peptidase are already well described in excellent reviews (Rapoport et al., 2017; Voorhees and Hegde, 2016). However, more recent studies have identified several other insertases in the ER membrane that collaborate with or complement the function of the Sec61 complex – the GET complex, the EMC complex and the TMCO1-containing GEL complex, each containing a specialized member of the Oxa1 family as one of their core components (Anghel et al., 2017) (see poster).

The GET complex was initially identified in yeast (Schuldiner et al., 2008) but is ubiquitously present in eukaryotes. A heterodimer of Get1 (also known as WRB in humans) together with Get2 (CAML in humans, and also known as CAMLG) facilitates the insertion of tail-anchor proteins into the ER (McDowell et al., 2020; Wang et al., 2014; Schuldiner et al., 2008) (see poster). Protein targeting (which for tail-anchor proteins is always a post-translational event) is mediated by several cytosolic proteins, such as Sgt2 (SGTA), Get5 (UBL4A), Get4 (also known as TRC35) and Get3 (also known as TRC40) (Zhang et al., 2021). Get3 is an ATP-binding protein that delivers tail-anchored proteins to the Get1–Get2 insertase module to support their membrane insertion (Mariappan et al., 2011; Stefer et al., 2011; Fry et al., 2022). Get3 also serves as chaperone independently of its role in tail-anchor insertion (Ulrich et al., 2022; Powis et al., 2013).

The EMC was likewise first identified in yeast (Jonikas et al., 2009) but also found in other eukaryotes. It consists of 10 subunits in humans, seven of which are embedded into the ER membrane (Pleiner et al., 2020) (see poster). The EMC of yeast is highly similar but comprises only eight subunits (Bai et al., 2020; Miller-Vedam et al., 2020). The Oxa1 homolog Emc3, together with Emc6, comprises the catalytic core of the EMC by forming a hydrophilic vestibule with critical positively charged residues on its surface. These were proposed to facilitate protein insertion into a locally thinned and disorganized membrane, and thus appear to apply the same catalytic mechanism used by the much simpler monomeric Oxa1 family members (Pleiner et al., 2020). A fusion of Emc3 and Emc6 that was artificially targeted to the mitochondrial inner

membrane even suppresses the defects of Oxa1-deficient yeast mutants, supporting the generally conserved mode of function of the different Oxa1 members in membrane protein insertion (Güngör et al., 2022). The EMC promotes the insertion of two types of membrane proteins (O'Keefe et al., 2021; Tian et al., 2019; Chitwood et al., 2018; Guna et al., 2018). First, it inserts the first transmembrane domains of some multi-pass membrane proteins; and second, many tail or signal-anchored ER proteins are inserted by the EMC complex, often in cooperation with the Sec61 translocon (Wu and Hegde, 2023).

A third ER membrane insertion complex was described only recently and is absent from yeast and only found in animals – the GEL complex contains the Oxa1 homolog TMCO1, which was proposed to serve as an insertase for multi-pass proteins (McGilvray et al., 2020) and a further subunit called OPTI (also known as RAB5IF). This GEL complex was found to bind two further protein complexes, BOS (for back of the translocase; consisting of nicalin, TMEM147 and NOMO) (Dettmer et al., 2010; McGilvray et al., 2020) and PAT [formed by CCDC47 and asterix (also known as WDR83OS)] (Sundaram et al., 2022; Chitwood and Hegde, 2020). This Sec61–BOS–GEL–PAT supercomplex was proposed to serve as a specialized translocon for multi-pass proteins, utilizing a lipid-filled central cavity for intra-membrane folding of its substrates, reminiscent to the SecY–YidC–SecD–SecF complex of the bacterial inner membrane (Botte et al., 2016; Smalinskaite et al., 2022). However, the mechanistic properties of this supercomplex are still poorly understood.

In higher eukaryotes, secretory proteins make use of another isoform of the Sec61 translocon, in which Sec61 is bound to the OST complex that also occupies the 'back' of Sec61 and thus competes with the insertion modules for its position on the translocase (Sundaram et al., 2022; Gemmer et al., 2023). The translocon-associated protein (TRAP) complex represents another Sec61-bound subcomplex of the ER translocon.

In summary, the ER membrane contains at least three functionally distinct membrane insertases, which all employ Oxa1 homologs as catalytically active core subunits. These three complexes evolved presumably from an archaeal Oxa1 homolog, which, like Get1, Emc3 and TMCO1, contain three transmembrane domains (Borowska et al., 2015). Moreover, unlike the bacterial and organellar Oxa1 family members, the ER isoforms function as part of multi-subunit complexes, which apart from acting as insertases have further roles in membrane protein biogenesis.

Membrane insertion of β-barrel proteins

β-barrel proteins are exclusively found in outer membranes of bacteria and bacteria-derived organelles. The membrane integration of β-barrel proteins is conceptually very different from that of proteins with α-helical transmembrane domains. For β-barrel proteins, a mature cylindrical architecture is a prerequisite for their membrane incorporation. In bacteria, their insertion is mediated by a specialized β-barrel protein that is part of the so-called β-barrel assembly machine (BAM) (see poster). BamA is a crucial central player of the BAM complex (Bakelar et al., 2016; Noinaj et al., 2013). BamA itself is a β-barrel protein and has the ability to open its cylindrical structure between its first and last β-strand (Doyle and Bernstein, 2021; Hohr et al., 2018). This allows the accommodation of β-hairpins of its substrates, which are integrated into the lipid bilayer in a stepwise manner (Tomasek et al., 2020).

The sorting and assembly machinery (SAM) of mitochondria works in a comparable fashion. Here, its central β-barrel protein Sam50 (SAMM50 in animals) integrates its substrates into the membrane and facilitates their assembly with assistance of several other subunits of the SAM complex (Takeda et al., 2021, 2023). Substrate release from SAM is triggered by another β-barrel protein, Mdm10, which replaces the substrate from Sam50 once it is fully integrated into the inner membrane (Meisinger et al., 2004).

Chloroplasts insert β-barrel proteins via their general protein translocase of the outer membrane of the TOC complex, and OEP805, which is related to BamA and Sam50 (Day et al., 2019). Molecular details of the β-barrel integration process in plastids are not known. However, the architecture of the chloroplast import machinery has been recently revealed by cryo-electron microscopy, showing the presence of several embedded β-barrel proteins (Jin et al., 2022; Liu et al., 2023). These structural insights will be a good basis to study the mechanistic details of β-barrel protein insertion in chloroplasts in the future.

Final remarks

The Sec complex, as well as the translocases of mitochondria and chloroplasts, had already been discovered by the 80s and 90s. In contrast, membrane insertases, such as the GET, EMC and GEL complexes, have only been recently identified. Given that hundreds of membrane proteins are substrates of these complexes, including many of high clinical relevance, their molecular mode of action is a hot topic in the field.

In addition to these insertases, intracellular membranes also have extractors, complexes that can revert the insertion of proteins. These factors are crucial for proof-reading and for the removal of mis-localized proteins. Examples for such extractors are the Msp1 (ATAD1) AAA protein of the mitochondrial outer membrane (Wohlever et al., 2017; Okreglak and Walter, 2014; Chen et al., 2014; Wang et al., 2022), Spf1 (P5A-ATPase) of the ER (McKenna et al., 2022; McKenna et al., 2020) and the SP1 and SP2 proteins of the outer membrane of chloroplasts (Ling et al., 2021). It will be exciting to study the interplay of insertases with these extractor systems on a mechanistic level in the future. It appears likely that cycles of insertion and extraction reactions ensure the correct targeting of each given membrane protein to its specific intracellular membrane.

Competing interests

The authors declare no competing of financial interests.

Funding

The work in our groups is funded by the European Research Council (ERC 101052639 MitoCyto to J.M.H.), the Deutsche Forschungsgemeinschaft (HE2803/10-1 and GRK2737-STRESSistance to J.M.H.), and the Landesforschungsinitiative Rheinland-Pfalz BioComp (to J.M.H.).

High-resolution poster

A high-resolution version of the poster is available for downloading at https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.261219#supplementary-data.

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