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CELL SCIENCE AT A GLANCE

Targeting and translocation of proteins to the endoplasmic reticulum at a glance

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ABSTRACT

The evolutionary emergence of organelles was a defining process in diversifying biochemical reactions within the cell and enabling multicellularity. However, compartmentalization also imposed a great challenge–the need to import proteins synthesized in the cytosol into their respective sites of function. For example, one-third of all genes encode for proteins that must be targeted and translocated into the endoplasmic reticulum (ER), which serves as the entry site to the majority of endomembrane compartments. Decades of research have set down the fundamental principles of how proteins get from the cytosol into the ER, and recent studies have brought forward new pathways and additional regulators enabling better definition of the rules governing substrate recognition. In this Cell Science at a Glance article and the accompanying poster, we give an overview of our current understanding of the multifaceted and regulated processes of protein targeting and translocation to the ER.

KEY WORDS: GET, Organelles, SND, SRP, Protein targeting, Endoplasmic reticulum

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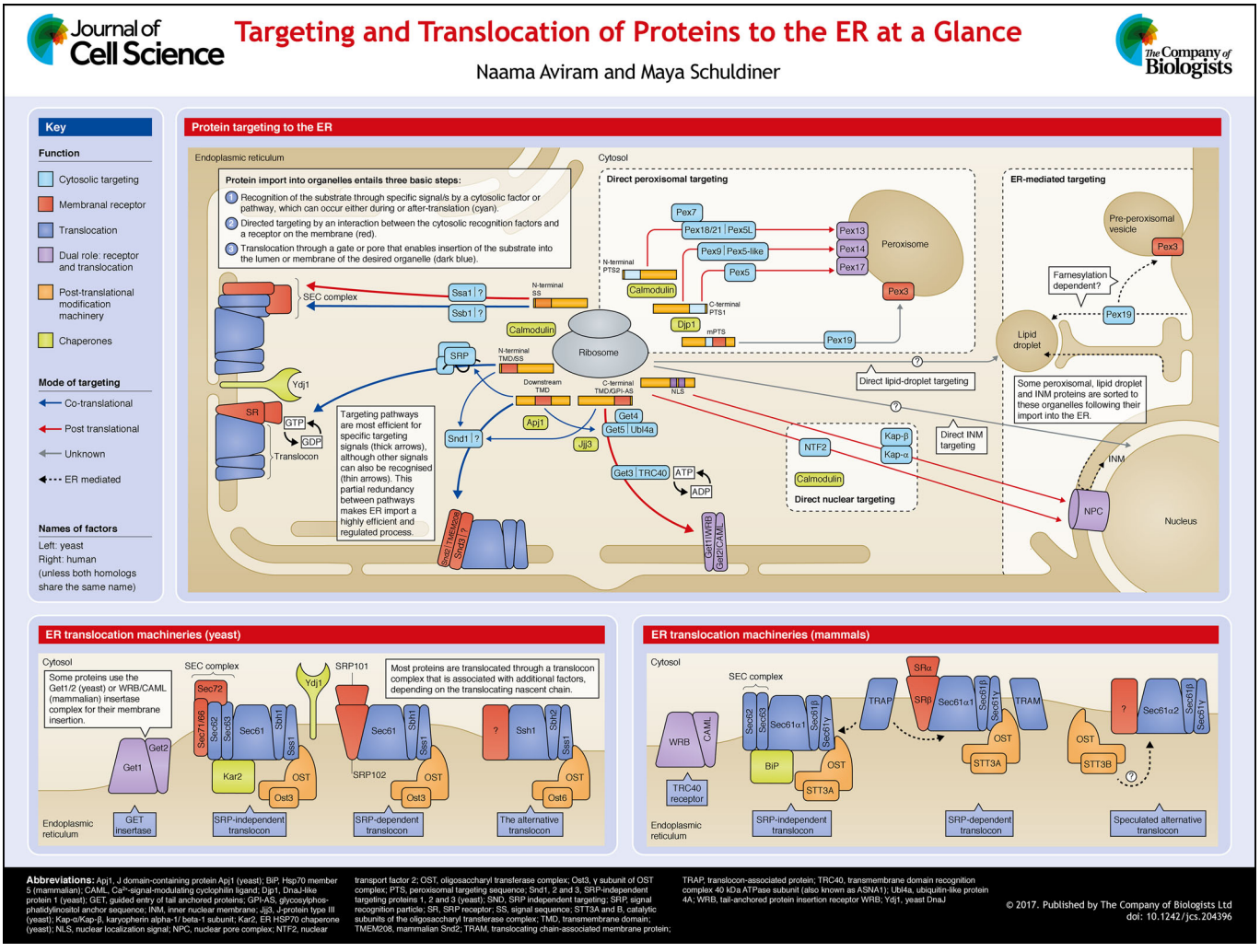

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Introduction

Organelles constitute distinct biochemical niches within the cell, allowing diversification of complex life processes. However, a consequence of creating compartmentalization is the need to correctly sort, and often import, the proteins that inhabit each organelle and function within it. Protein import into a specific organelle entails three basic steps: (1) Recognition of the substrate through specific signal/s by a cytosolic factor or pathway; this recognition prevents protein mistargeting, premature protein folding or protein aggregation in the cytosol, and can occur either during or after translation. (2) Directed targeting by an interaction between the cytosolic recognition factor(s) and a receptor on the target membrane. (3) Translocation through a gate or pore that enables insertion of the substrate into the lumen or membrane of the desired organelle.

In eukaryotic cells, the three largest targeting destinations are mitochondria (reviewed in Harbauer et al., 2014; Wasilewski et al., 2017), chloroplasts (reviewed in Bölter and Soll, 2016; Lee et al., 2017) and the endoplasmic reticulum (ER). The ER is the entry site to the secretory/endomembrane system as well as to peroxisomes, lipid droplets (LD) and the inner nuclear membrane (INM). Hence, it is not surprising that ∼30% of all eukaryotic genes encode for proteins that must target and translocate to the ER (Chen et al., 2005; Choi et al., 2010; Wallin and von Heijne, 1998). Protein targeting to the ER has been the subject of intensive research for decades and is the focus of this article. We try to provide a broad overview of the factors and pathways that facilitate ER import, highlight the diversity of signal motifs that mediate recognition by different targeting and translocation pathways, and discuss the intricate interactions between the pathways that make the process of protein import to the ER so robust.

Recognition and targeting of ER substrates

The first ER-targeting pathway described is dependent on recognition of substrates by a large ribonucleoprotein complex, the signal recognition particle (SRP), which is conserved from bacteria to mammals (Walter and Blobel, 1981a,b; Walter et al., 1981). The SRP associates tightly with the ribosome and, through a hydrophobic cleft, recognizes secretory proteins with hydrophobic motifs as they are being translated (Keenan et al., 1998). Studies in bacteria and yeast suggest that SRP prefers to cater for highly hydrophobic transmembrane domains (TMDs) or cleavable signal sequences (Ast et al., 2013; Ng et al., 1996; Schibich et al., 2016) (see poster).

It was long thought that SRP binds the ribosome-nascent chain complex only after the emergence of the nascent hydrophobic motif from the ribosome exit tunnel; however, recent studies suggest that, for certain substrates, SRP already binds to the ribosome at earlier stages during translation (Berndt et al., 2009; Chartron et al., 2016; Voorhees and Hegde, 2015). Notably, SRP molecules do not exist in equimolar amounts to ribosomes. Hence, in order for SRP to find its substrates, it quickly scans many nascent polypeptides and dissociates from translating ribosomes if it does not encounter a targeting signal (Holtkamp et al., 2012). Consequently, endomembrane proteins that harbor targeting motifs positioned further away from their N-terminus might be less efficiently captured by such a scanning system and, hence, also use alternative, SRP-independent, pathways.

SRP targets ribosome-nascent chain complexes to the translocon by binding to its ER-localized SRP receptor (SR) (Gilmore et al., 1982a,b; Meyer et al., 1982) (see poster). The SR is composed of the soluble SR α-subunit, which is anchored to the membrane-embedded SR β-subunit. Both SRP and SR comprise GTPase activity; when both are bound by GTP, they form a complex on the ER membrane. Following GTP hydrolysis, SRP and SR dissociate, and are recycled for a new round of targeting (Halic and Beckmann, 2005; Shan et al., 2009). Therefore, targeting by the SRP/SR pathway is co-translational and GTP dependent (see poster).

Regardless of a particular targeting pathway, it appears that the majority of proteins can be targeted to the ER co-translationally (Jan et al., 2014). An exception to this is the group of tail-anchored (TA) proteins, which harbor a single TMD at their very C-terminus. Since the targeting TMD is only exposed after translation has terminated, this group of proteins must have the capacity to be targeted to the ER post-translationally and, hence, in an SRP-independent manner (Borgese and Gaetani, 1983). The machinery that targets TA proteins was discovered recently, and comprises the transmembrane recognition complex of 40 kDa (TRC40, officially known as ASNA1) pathway in mammals (Favaloro et al., 2008, 2010; Stefanovic and Hegde, 2007) and the homologous yeast guided entry of tail-anchored proteins (GET) pathway (Jonikas et al., 2009; Schuldiner et al., 2008).

Recognition of substrates by the GET/TRC40 pathway occurs on the ribosome through the ribosome-associated chaperone Sgt2 (yeast)/SGTA (mammals) (Shao et al., 2017), which hands over the TA protein to the Get4-Get5 pre-targeting complex in yeast (Denic et al., 2013), or the Bat3-Get4-Ubl4a complex in mammals (Mariappan et al., 2010; Shao et al., 2017). After their initial recognition, TA proteins are passed to the ATPase chaperone Get3/TRC40, which forms a hydrophobic binding cleft upon its dimerization (Mateja et al., 2015). The interaction with TA protein induces ATP hydrolysis through the ATPase activity of Get3/TRC40 (Rome et al., 2013). Targeting to the ER occurs through binding of Get3/TRC40 to an ER-localized membrane receptor that comprises a heterodimer of the membrane proteins Get1 and Get2 (Get1-Get2) in yeast (Schuldiner et al., 2008) or CAML and WRB (CAML-WRB) in mammals (Yamamoto and Sakisaka, 2012). Receptor binding releases the TA protein from Get3/TRC40 and subsequent binding of a new ATP molecule is required for its complete dissociation, freeing Get3/TRC40 for another round of targeting (Mariappan et al., 2011) (see poster).

Although the GET/TRC40 pathways were initially discovered in the context of TA protein targeting, the GET pathway was later shown to also recognize and mediate the targeting of another group of SRP-independent proteins, the glycosylphosphatidylinositol (GPI)-anchored proteins in yeast. In the GET pathway, GPI-anchored proteins are recognized through their hydrophobic C-terminal GPI-anchor motif, whose hydrophobicity resembles that of TA proteins (Ast et al., 2013). TRC40 has also been suggested to target short secretory proteins in mammalian cells (Johnson et al., 2012).

Despite its broad substrate range, the TRC40/GET pathway does not appear to be able to cater for all SRP-independent substrates. Indeed, we have recently described a new SRP-independent targeting (SND) pathway (Aviram et al., 2016), which can recognize and target proteins with ER-targeting motifs that are not recognized efficiently by either SRP or TRC40/GET (see poster).

It is still not entirely clear how substrate recognition and specificity are mediated via the SND pathway; however, the ribosome-associated SND protein 1 (Snd1) might play a role. Targeting, most likely, involves the ER-localized receptor, which comprises the membrane proteins Snd2 and Snd3 (officially known as ENV10 and PHO88, respectively). The SND pathway is conserved in mammals; the human homolog of Snd2 is TMEM208 (Aviram et al., 2016), which has recently been shown to function in the targeting of substrates with downstream TMDs and shares conserved functional interactions with the human TRC40 pathway (Casson et al., 2017; Haßdenteufel et al.,

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2017). The mammalian equivalent of Snd1 and Snd3 still await identification.

Interestingly, some proteins can be recognized by more than one of the above pathways, presumably allowing for a greater level of regulation and efficiency of protein targeting (Aviram et al., 2016; Zhang et al., 2016); this would also allow buffering of functional loss of one pathway by another (see poster). For example, we have shown that overexpression of the SND pathway rescues the lethality caused by loss of SRP targeting in yeast (Aviram et al., 2016).

In addition to the targeting pathways mentioned above, other cytosolic factors, mainly chaperones, recognize SRP-independent substrates; among these are calmodulins (CALM1, CALM2, CALM3) in mammals (Shao and Hegde, 2011), the yeast Hsp40 proteins Apj1, Jjj3 and Ydj1 (Ast et al., 2013), and the yeast Hsp70 proteins Ssa1 and ribosome-associated molecular chaperone Ssb1 (Tripathi et al., 2017). Additionally, the nascent-chain-associated complex (NAC) has been suggested to modulate SRP activity in yeast (del Alamo et al., 2011). Notably, for the majority of these chaperones it is still unclear whether their sole role is preservation of nascent polypeptides in a translocation-competent misfolded state, or whether they take part in active targeting. The only clear example for a targeting role is that Ssa1 or Ssb1 function in a post- or co-translational manner, respectively; i.e. both bind directly to the SEC membrane complex in the ER (see below) to enable the targeting of proteins containing signal sequences that are too weak to fully engage SRP (Tripathi et al., 2017).

The substrate diversity of ER-designated proteins has encouraged the evolutionary development of multiple targeting pathways, each catering most efficiently for proteins with different characteristics, i.e. different positions of the TMD, different hydrophobicity of the signal sequence etc. (see poster). Regardless of the exact pathway a particular protein takes to reach the ER, the vast array of factors involved in ER-targeting – in addition to a degree of redundancy between them – solve this biological challenge and allow for robust, efficient and, most probably, regulated (albeit this is little studied) recognition of a variety of substrates and their efficient ER targeting.

Translocation into the ER

Once a protein has reached its target membrane, the work is not done – it now has to either pass through or become integrated into its target membrane in a process called translocation (see poster). The best-studied ER translocation route is through the translocon channel, a heterotrimeric complex consisting of the Sec61α pore-forming subunit and the two accessory proteins Sec61 beta homolog 1 (Sbh1) and Ssh suppressor (Sss1) (in yeast) or Sec61β and Sec61γ (in mammals) (Görlich and Rapoport, 1993). Activation of the channel requires the engagement of hydrophobic signals within the translocating nascent chain, which weakens its gating (Voorhees and Hegde, 2016; Voorhees et al., 2014). The aqueous environment within the translocon channel enables the hydrophilic nascent chains to pass through it, while allowing the hydrophobic TMDs to integrate into the membrane through a lateral gate that exists in the Sec61α pore and can open towards the lipid bilayer (Gogala et al., 2014). Ribosomal docking on the translocon has been suggested to induce conformational changes to the translocon needed for substrate translocation (Voorhees et al., 2014). It was shown to shield membrane proteins from aggregation throughout their co-translational translocation, and to allow their correct co-translocational folding (Conti et al., 2014; Patterson et al., 2015). However, ribosomal docking is not necessary for translocation, as post-translational translocation occurs as well (Rothblatt et al., 1989).

The Sec61 translocon can be found in different complexes, each associated with different accessory proteins. Therefore, each translocon complex handles translocating nascent chains with different characteristics. For SRP-dependent substrates, the translocon associates with the SR, whereas for SRP-independent substrates, the translocon is associated with the SEC auxiliary complex. SEC is composed of the evolutionary conserved essential proteins Sec62 and Sec63 (Meyer et al., 2000; Tyedmers et al., 2000) and, in yeast, also contains the non-essential proteins Sec71 (also named Sec66) and Sec72 (Harada et al., 2011). Sec63 has a J-domain located in the ER lumen, which recruits the ER HSP70 chaperone Kar2 (yeast)/BiP (mammals) that utilizes its ATPase activity to facilitate threading of the nascent chain into the ER lumen (Brodsky et al., 1995; Matlack et al., 1997; Tyedmers et al., 2003). Recently, it has been shown that the Sec71 and Sec72 subunits of SEC support the initial steps of SRP-independent translocation, both during and after translation, depending on the chaperones they interact with (Tripathi et al., 2017). The SEC complex appears to be dynamically recruited to the Sec61 translocon, depending on the nascent chain being translocated (Conti et al., 2015). It can be displaced by the SR (Jadhav et al., 2015), presumably, when physiological conditions favor SRP-dependent translocation, such as in the presence of high glucose levels (Webb et al., 2000).

In mammals, additional factors were shown to facilitate the translocation process (reviewed in Aviram and Schuldiner, 2014). These include the translocating-chain-associated membrane (TRAM) protein, which is a part of the minimal apparatus that allows reconstitution of translocation *in vitro*, and the translocon-associated protein (TRAP) that assists in translocation initiation (Fons et al., 2003).

Interestingly, not all proteins are translocated through the Sec61 translocon. TA proteins that are targeted by the GET/TRC40 pathway can be inserted into the membrane post translationally in a translocon-independent manner, by using the insertase function of the Get1-Get2/CAML-WRB complex (Wang et al., 2014). An intriguing open question is how the GET/TRC40 pathway mediates the translocation process of non-TA proteins, as these proteins need to be targeted to the translocon for complete translocation (Ast et al., 2013; Johnson et al., 2012). Here, a yet-unidentified factor might transfer substrates from the targeting machinery to the translocon.

Another intriguing aspect in this context is the existence of a second, alternative translocon, which is far less studied. Similarly to the canonical Sec61 translocon, the yeast alternative translocon comprises three subunits, the pore-forming Sec sixty-one protein homolog 1 (Ssh1), Sec61 beta homolog 2 (Sbh2) that is homologous to Sbh1, and Sss1 that is shared with the canonical translocon complexes (Finke et al., 1996). It appears that the presence of both Sec61 and Ssh1 translocons is required for optimal ER-translocation capacities because, in the absence of either, translocation of some substrates is hampered (Finke et al., 1996; Jiang et al., 2008; Wilkinson et al., 2001; Wittke et al., 2002). Interestingly, loss of Ssh1 in yeast yields phenotypes that are distinct from those owing to mutation of Sec61 (Wilkinson et al., 2001). However, only Sec71 has so far been shown to preferentially translocate through the Ssh1 translocon (Spiller and Stirling, 2011).

Genome sequencing has revealed that genomes of other species, including humans, encode for a homolog of Sec61α, the pore-forming subunit (Görlich et al., 1992). In humans this homolog, Sec61α2 (SEC61A2), has 95% sequence similarity to Sec61α1 and

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might only be a recent duplication with no functional diversification. However, because this subunit has not been studied, it remains to be determined why a second copy is encoded in the genome. A fascinating quest for the future is, thus, to uncover the divergent functions or substrate specificity of such paralogous complexes.

Now what? Post-translocational sorting steps

Once a protein is translocated into the ER it can either stay in the ER as a resident protein, continue through the Golgi complex to its site of function in the secretory and/or endocytic pathway (Geva and Schuldiner, 2014), or be targeted directly to several other non-secretory organelles (see poster)

Proteins destined for peroxisomes

Unlike peroxisomal matrix proteins that are targeted directly to peroxisomes (see Box 1), some peroxisomal membrane proteins (PMPs) have been shown to be inserted into the ER prior to their delivery to peroxisomes (Tam et al., 2005; van der Zand et al., 2010) by Pex3 and Pex19 (Smith and Aitchison, 2013).

Proteins destined for lipid-droplets

Some lipid droplet (LD) proteins with a TMD have been shown to first translocate to the ER and then sort to LDs by accumulating on the outer leaflet of the ER membrane at sites of LD biogenesis, or by diffusing through membrane bridges (reviewed in Kory et al., 2016). Interestingly, Pex19 in mammalian cells may also target LD proteins directly when it is farnesylated (Schrul and Kopito, 2016).

Proteins destined for the inner nuclear membrane

Proteins with a TMD can be directed from the ER to the continuous inner nuclear membrane (INM) (Burns and Wente, 2012). Such sorting can occur by a diffusion-retention mechanism (Ungricht et al., 2015). Another method of sorting to nuclear membranes is by direct binding to importins, which also target soluble nuclear proteins (see Box 2) (King et al., 2006). As translocon complexes are also present in the INM (Smoyer et al., 2016), some proteins might also directly translocate (post translationally) into the INM.

The combinatorial complexity of ER import–Are all translocation routes the same?

Although it is clear that there are four different translocation routes into the ER (see poster) it is not always clear why translocation can occur in different 'flavors'. One difference is the coupling to the cytosolic targeting routes: receptors of different targeting pathways can be found associated with distinct subpopulations of the translocon complex. For instance, although both the SR (Halic et al., 2006) and the membrane complex participating in the SND pathway (Aviram et al., 2016) interact with the translocon, either interaction seems to be mutually exclusive (Aviram et al., 2016), suggesting that there is a physical separation between translocons bound to either one of the two targeting receptors.

However, another aspect of having different translocons is their capacity to bind different luminal machineries for post-translocational modifications, such as N-linked glycosylation, which is facilitated by the translocon-associated oligosaccharyl transferase (OST) complex (Pfeffer et al., 2014). In yeast, two isoforms of the OST complex exist that differ from each other by a single (Ost3 or Ost6) subunit (Schwarz et al., 2005). Each OST complex isoform specifically interacts with one translocon: the Ost3-containing isoform interacts with the Sec61-containing translocon, and the Ost6 isoform with that comprising Ssh1 (Yan and Lennarz, 2005). Moreover, Ost3 and Ost6 bind to different peptides (Jamaluddin et al., 2011) and modify different substrates (Schulz and Aebi, 2009). The presence of two OST complex isoforms is also conserved in mammals, and comprise subunits STT3A and STT3B (Ruiz-Canada et al., 2009). It will be of interest to investigate further whether interaction of these subunits with the two different mammalian translocons is also conserved.

Another such modification is GPI-anchor protein addition. Interestingly, GPI biosynthesis enzymes are concentrated in a specific ER subcompartment (Ilgoutz et al., 1999) that harbors a

Box 1. Peroxisomal targeting

Different targeting machineries support the targeting of peroxisomal matrix proteins, which are recognized by one of the two peroxisomal targeting sequences, PTS1 and PTS2. PTS1 is a C-terminal Ser-Lys-Leu (SKL) motif that is recognized by Pex5, a targeting factor that is conserved from yeast (Van der Leij et al., 1993) to humans (Wiemer et al., 1995). A subset of PTS1-containing proteins is also specifically recognized by the Pex5-like protein Pex9 (Effelsberg et al., 2016; Yifrach et al., 2016) that, under specific growth conditions, targets these proteins into peroxisomes. PTS2, a nine-residue motif that is located in the N-terminus of the protein, and is recognized by Pex7 (Marzioch et al., 1994) – another highly conserved protein – and, in yeast, one of its two cofactors Pex18 or Pex21 (Grunau et al., 2009) or, in humans, a long form of Pex5 (Pex5L) (Emmanouilidis et al., 2016). Both Pex5 and Pex7 function by interacting with the peroxisomal docking complex Pex13 –Pex14 (and also Pex17 in yeast) (Emmanouilidis et al., 2016).

Peroxisomal membrane proteins that have a membrane peroxisomal targeting signal (mPTS) are targeted directly to peroxisomes by Pex19 that, for these targeting events, binds directly to Pex3 in the membranes of mature peroxisomes (Yagita et al., 2013).

Interestingly, some peroxisomal proteins do not have a PTS, and these proteins have been shown to arrive at the peroxisome by 'piggybacking' other proteins that comprise either a PTS1 (Islinger et al., 2009) or PTS2 (Effelsberg et al., 2015; Kumar et al., 2016). Additionally, Pex5 has been suggested to act in a PTS1-independent manner, which may explain the peroxisomal import of some proteins that lack a PTS (van der Klei and Veenhuis, 2006). Additional cytosolic factors are emerging as facilitators of protein import into peroxisomes, with the HSP40 Djp1 (Dobriyal et al., 2017; Hettema et al., 1998) and calmodulin (Corpas and Barroso, 2017) among them.

Box 2. Nuclear targeting

One of the most studied organelles in the context of protein import is the nucleus. Soluble proteins are imported through the nuclear pore complex (NPC), the gate between the cytoplasm and the nucleoplasm. The NPC is permeable to passive diffusion of small molecules with a molecular mass of less than ~40 kDa (Christie et al., 2016).

For larger proteins, active targeting is required and this is mediated by cargo receptors called karyopherins (Bauer et al., 2015). Cargo receptors that can directly bind the substrate and the NPC are referred to as karyopherin-β (Kap-β). However, most of the nuclear import is mediated through an additional adaptor, karyopherin-α (Kap-α), that facilitates binding of the substrate to the NPC (Christie et al., 2016). Karyopherins cater for proteins that contain a nuclear localization signal (NLS); they can function in either import to or export from the nucleus and, depending on the directionallity of their function, are then called importins or exportins, respectively. The directionality of their function is dictated by the small GTPase Ran and its accessory proteins (Künzler and Hurt, 2001). Interestingly, Ran itself is targeted to the nucleus by a non-karyopherin transport factor (NTF2) (Ribbeck et al., 1998). This is not the sole karyopherin-independent import route as other machineries have also been described, including calmodulin-mediated import (Hanover and Sweitzer, 1996), as well as spontaneous import of some proteins that harbor internal NPC-interacting domains (Kumeta et al., 2012).

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unique membrane composition, enriched in ceramides and sterols (Bagnat et al., 2000). It is, therefore, tempting to speculate that GPI-anchored proteins are targeted and translocated directly into these ER subdomains. If this is the case, and because GPI-anchored proteins are sorted into subpopulations of ER-derived vesicles (Muñiz et al., 2001) that are distinct from other vesicles (Watanabe and Riezman, 2004), GPI-anchored proteins might follow a unique biogenesis route, from their targeting and translocation until their export and maturation.

Several types of translocation hub might easily be created in the ER membrane (see poster) – on the basis of 'platforms' that contain Sec61 or Ssh1, either with or without the auxiliary complexes and by encompassing the various protein-modification machineries required and proximal to different exit sites. Future research in the area of translocation should focus on characterizing the subgroups of the translocating clientele, in order to better understand how proteins with different biophysical properties choose their optimal translocation machinery and whether this streamlines protein production.

A bird's eye view of protein targeting and translocation

Here, we have focused mainly on protein targeting and translocation into the ER; however, many of the outlined basic principles also apply to other organelles. Taking a step back, it is important to appreciate the three fundamental concepts that have been described for targeting and translocation: (1) Each and every protein that is designated for a particular organelle has evolved to contain signals that make it recognizable by dedicated machineries, which then transfer it from the site of its synthesis to its final functional cellular localization. Interestingly, in most cases the same signals mediate both targeting and translocation, and are often also used for later sorting; but this is not a necessity. (2) Many targeted substrates can be recognized by members of more than one pathway (Aviram et al., 2016; Yifrach et al., 2016; Zhang et al., 2016). Such functional redundancy might assure robust and adaptable targeting when the pathways target the same organelle, and dual targeting when the pathways are destined to different organelles. However, this might create the problem of potential mistargeting, which must also be dealt with. More generally, it would be interesting to decipher the physiological or environmental contexts that make specific pathways more dominant. (3) It is becoming increasingly clear that, for each organelle, more than a single targeting and translocation pathway exists. One future aim is, therefore, to understand the interplay between these pathways, and how they compete for upstream and downstream factors.

It is also becoming apparent that some factors (e.g. Pex19, calmodulin or HSP40s) appear to aid protein targeting to more than one organelle. Such factors might only have one set of substrates that they target to several organelles, thereby creating dual or multiple localization (Karniely and Pines, 2005). However, these factors could also interact with other proteins in order to generate targeting specificity, and any inter-organelle interplay remains to be uncovered.

Perspectives and conclusions

Looking into the future, many important and interesting questions are still open in the field of targeting and translocation. For many of the chaperones and protein-targeting factors, a detailed mechanistic understanding is still missing. Additionally, the dedicated targeting machinery is still unknown for many proteins. Although autonomous targeting could occur, the capacity of a protein to target and translocate into an isolated membrane *in vitro* does not mean that this occurs in the dense milieu of the cytosol *in vivo*. To enable robust regulation, the majority of proteins is most likely to be associated with some targeting factors. Hence, any potential targeting pathways for such 'orphan proteins' are yet to be discovered.

More broadly, one must appreciate the vast array of factors involved in protein targeting and their import into organelles. More than half of the eukaryotic proteome has to arrive at the various cellular organelles in order to sustain life; and, while some of the rules that dictate correct sorting and import of proteins are generally understood, the specific features and crosstalk between pathways are still a mystery. Hopefully, future work will help gaining a deeper understanding of this beautiful and intricate aspect of cell biology.

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Cell science at a glance

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