The quest for a better resolution of protein-translocation processes

Conference on the Control, Co-ordination and Regulation of Protein Targeting and Translocation

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The EMBO Conference on the Control, Co-ordination and Regulation of Protein Targeting and Translocation took place between 25 and 28 October 2008, in Sainte-Maxime, France, and was organized by T. Pugsley & R. Zimmermann. Credit: Hôtel les Jardins de Sainte-Maxime, France.

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See Glossary for abbreviations used in this article.

Introduction

The 2008 European Molecular Biology Organization Conference on the Control, Co-ordination and Regulation of Protein Targeting and Translocation was held in sunny Sainte-Maxime, on the Côte d'Azur in Southern France. It brought together almost 200 scientists who work on protein translocation in different organelles, as well as in different kingdoms and domains of life, thereby providing a unique opportunity to discuss commonalities and differences between the operational mechanisms.

The conference took place during an interesting time for this field of research. The biological question is as important as ever: how are huge macromolecules transported across membranes that are designed, in many cases, to be impermeable even to protons? Protein translocases have been studied for several decades, and the most famous examples have been subjected to exhaustive biochemical, structural and genetic analyses. Although it is undeniable that these 'traditional' approaches are continuing to bear fruit and elucidate the basic translocation processes, it is becoming increasingly evident that, on the whole, protein translocases are not simply machines that grab proteins on one side of the membrane and send them to the other by a standard—but impressive—mechanism. Instead, and as the conference title implies, they are sophisticated systems that can be controlled and adapted to the types of protein substrate being transported, and to the prevailing physiological status of the organism or organelle. Here, we give an account of some of these exciting new areas. For background, Fig 1 shows the key protein translocases under discussion, as well as their evolutionary relationships.

The dynamics of the translocation apparatus

Protein-translocation machineries are multisubunit complexes that undergo dynamic changes both in their subunit composition

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Glossary

BAM	β -barrel assembly machinery
ER	endoplasmic reticulum
ERAD	ER-associated degradation
Erv1	essential for respiration and viability 1
ESR	electron paramagnetic-resonance spectroscopy
FeS	iron sulphur
Hot13	Helper of Tim 13
LacY	lactose permease
Mas37	mitochondrial assembly 37
MIA	mitochondrial intermembrane-space import and assembly
OMP	outer membrane protein
OXA	oxidase assembly
Pam17	presequence translocase-associated motor 17
PE	phosphatidylethanolamine
pK _a	acid dissociation constant
SAM	sorting and assembly machinery
Sec	secretory
Tam41	translocator assembly and maintenance 41
Tat	twin arginine translocase
TIC	translocase of the inner chloroplast envelope
TIM	translocase of the inner membrane
TOB	topogenesis of mitochondrial outer membrane β -barrel proteins
TOC	translocase of the outer chloroplast envelope
TOM	translocase of the outer membrane
UPR	unfolded protein response
VCAM1	vascular cell-adhesion molecule 1

and in their conformational states. The *Escherichia coli* general Sec system has been a favoured model system among protein translocases for many years, and it is becoming increasingly clear that substrate recognition and translocation involve a complex—and adaptable—series of dynamic changes among the different Sec subunits. T. Economou (Heraklion, Greece) discussed the multitude of conformational changes involved in substrate recognition by SecA, which is a molecular motor that promotes the ATP-driven translocation of unfolded polypeptides across the SecYEG pore in bacteria. In particular, the question was addressed of how SecA is activated for ATP hydrolysis upon binding of the signal sequence of preproteins. L. Randall (Columbia, MO, USA) reported on a series of site-directed spin-labelling and ESR experiments aimed at identifying the surface of SecA that interacts with each of the binding partners that it encounters during the dynamic cycle of export. This analysis revealed not only that there are overlapping binding sites for the molecular chaperone SecB, the precursor polypeptides and the major subunit of the translocation pore SecY, but also sites of interaction that are unique for each partner. Therefore, Randall proposed a model that links SecA conformational changes at the SecYEG pore with the ATPase cycle.

The translocases that are present in the outer and inner membrane of the mitochondria have also been the focus of intense research because the vast majority of mitochondrial proteins are synthesized as precursor proteins in the cytoplasm and subsequently targeted to the organelle, where they have to be translocated and sorted to the correct submitochondrial destination. The meeting effectively marked an end to a decade of research, which was characterized by a search for new mitochondrial translocation complexes and components. In 1997 only two complexes—the outer membrane translocase, known

as TOM, and the inner membrane translocase TIM23—and about a dozen subunits of these complexes were known. Eleven years later, we count five new machineries—TOB/SAM, TIM22, MIA, OXA and the small TIMs—and a total of 37 proteins as components of the mitochondrial translocation complexes (Bolender *et al*, 2008; Neupert & Herrmann, 2007). Recent meetings have been characterized by reports of newly identified components; however, this ‘gold rush’ seems to be over, as not a single new component was reported at the current conference (Fig 2 summarizes the components that are now known). In the years to come, the mitochondrial import field is probably going to develop in very different ways, with a greater emphasis on the roles of these multiple components and by providing insight into how they interact. Protein dynamics seem to be a core feature of the mitochondrial import systems. W. Neupert (Munich, Germany) and T. Endo (Nagoya, Japan) discussed the active remodelling of the TIM23 complex during the translocation of precursor proteins across the mitochondrial inner membrane. Neupert considered that the membrane-embedded parts of the complex and the subunits that form the import motor act as a single structural entity that can alternate among three main conformations: first, with no substrate in the translocase; second, translocating preprotein into the matrix; or third, sorting a substrate to the inner membrane. He proposed that Tim21 and Pam17 function as two antagonistic regulatory proteins in this dynamic organization. Endo concentrated on the transfer of preproteins through the intermembrane space. He presented data indicating that the TOM and TIM23 complexes are in permanent contact through dynamic interactions between the carboxy-terminal domain of Tom22 and Tim50, and further proposed that interactions between the coiled-coil domains of Tim23 and Tim50 promote the transfer of the precursor protein from the TOM complex to the TIM23 machinery.

Another mitochondrial import process that attracted considerable attention is that mediated by the MIA machinery, which catalyses the formation of disulphide bonds in precursor proteins, thereby promoting their import into the mitochondrial intermembrane space (Tokatlidis, 2005). N. Pfanner (Freiburg, Germany) and J. Herrmann (Kaiserslautern, Germany) addressed the dynamic composition of this machinery. The known components of the machinery are a sulphhydryl oxidase, Erv1, which oxidizes a disulphide carrier protein, Mia40, which subsequently transfers the disulphide to the substrate protein. A popular model has suggested that Mia40 alternates between the association with the substrate or with Erv1. By contrast, Pfanner reported on the characterization of a ternary complex in which both the substrate protein and Erv1 interact with Mia40. Therefore, he proposed that MIA-mediated import could be considered to be a disulphide-channelling process instead of a disulphide-relay system. Herrmann described Hot13—an intermembrane space protein—as a zinc-binding protein that maintains Mia40 in a zinc-free state, thereby improving the oxidation of Mia40 by Erv1.

The overall message seems to be that, collectively, translocation machineries are dynamic entities that can have several structural flavours. Unfortunately, our current biochemical assays are programmed to provide only a snapshot of this rich repertoire or even to influence it by altering the environment of these complexes.

The problem of lateral gating of translocons

Lateral gating is another area of protein dynamics that shows how flexible protein translocases need to be; for example, in contrast to ion channels, protein-conducting channels must be capable of both directing hydrophilic protein substrates directly across the

Fig 1 | The major protein-translocation machineries in eukaryotic and prokaryotic cells. The transport systems of the bacterial cytoplasmic membrane are conserved in eukaryotes; homologous systems are colour coded. Homologues of bacterial Sec and Tat systems are found in the chloroplast thylakoid, and a Sec-type system is also present in the ER. Homologues of the bacterial YidC integrase are present in both chloroplasts and mitochondria. The core component of the bacterial outer membrane BAM complex, which is involved in β -barrel protein insertion, also has homologues in the mitochondrial and chloroplast outer membranes (SAM/TOB and Toc75-V, respectively). However, eukaryotic organelles have also evolved new systems to import nuclear-encoded proteins: the TOM and TIM import systems in the mitochondrial outer/inner membranes, the TOC and TIC import systems in chloroplast envelopes, and the peroxins in the peroxisomal membrane. Specialized secretion systems, which are unique to bacteria, are not depicted in this illustration. Archaea, which are not depicted in the illustration, often have both Sec and Tat systems. Alb, albino 3; BAM, β -barrel assembly machinery; ER, endoplasmic reticulum; Oxa, oxidase assembly; SAM/TOB, sorting and assembly machinery/topogenesis of mitochondrial outer membrane β -barrel proteins; Sec, secretory; Tat, twin arginine translocase; TIC, translocase of the inner chloroplast envelope; TIM, translocase of the inner membrane; TOC, translocase of the outer chloroplast envelope; Toc75-V, chloroplast outer membrane homologue of Omp85/YaeT/BamA; TOM, translocase of the outer membrane; YidC, membrane protein integrase of the bacterial inner membrane (*E. coli* homologue of mitochondrial Oxa1p).

membrane and opening up (gating) laterally to allow hydrophobic segments of membrane-spanning proteins to become integrated into the bilayer. Several speakers addressed this complex issue. A. Driessen (Groningen, The Netherlands) presented site-directed cysteine crosslinking data on the translocation and membrane protein-insertion activities of the bacterial SecY pore, in which the presumed lateral gate is artificially constrained by a disulphide bond or by bifunctional crosslinkers with different spacer lengths. Notably, the co-translational insertion of transmembrane segments into the membrane is unaffected by the immobilization of the lateral gate. This suggests that hydrophobic transmembrane segments slide into the membrane through a different path on SecY, rather than inserting vectorially into the aqueous pore region followed by subsequent lateral diffusion into the lipid bilayer.

R. Gilmore (Worcester, MA, USA) discussed experiments on Sec61p, which is the ER equivalent of the bacterial SecY pore. He used a ubiquitin-translocation assay to estimate the length of a nascent membrane protein that needs to be exposed to the cytoplasm before the ribosome–nascent chain complex binds to Sec61p, thereby opening (activating) the translocon. Considering the rates of protein synthesis *in vivo*—which are 6–8 aminoacyl residues per second—the experiments with nascent membrane proteins suggest that there

is a 15–20 s delay between the emergence of the transmembrane domain of a protein from the large ribosomal subunit and the opening of the channel of the translocon. Additional time is required for translocon gating by multispans membrane proteins, suggesting that the movement of the transmembrane domains past the lateral gates of the translocon is the rate-limiting step during membrane integration. S. High (Manchester, UK) discussed the insertion mechanism of a membrane protein with two transmembrane domains at the Sec61 complex in the ER. By using site-specific crosslinking experiments, High showed that the first transmembrane domain remains stalled at the translocon for the entire duration of the synthesis of the second transmembrane segment and the terminal loop, pointing to a functional link between protein synthesis and release at the translocon. In summary, membrane protein insertion through the translocon seems to be a highly coordinated event in order to ensure correct folding and assembly.

Quality control of protein transport

The most thoroughly characterized quality-control system is the one operating in the ER, which is a compartment specialized in the folding and subsequent export of translocated proteins. Unfolded proteins are typically denied access to the export pathway until they

Fig 2 | Components of the mitochondrial protein-import machineries. The mitochondrial proteins that are synthesized in the cytoplasm are initially recognized by the TOM complex, which mediates their translocation across the outer membrane. The subsequent sorting of proteins to their final destination requires the action of additional import complexes. Proteins containing a presequence are transferred from the TOM complex to a specialized translocase of the inner membrane—the TIM23 machinery—that can translocate proteins into the matrix or the inner membrane. Precursors of inner membrane polytopic proteins—such as those of the mitochondrial carrier family—are relayed to the TIM22 complex, which mediates their integration into the inner membrane. The small proteins that reside in the intermembrane space are recognized by the MIA machinery on their exit from the TOM complex. Finally, β -barrel precursors are relayed from the TOM complex to a dedicated complex in the outer membrane, the TOB/SAM complex, which facilitates their assembly into the outer membrane. For clarity, each type of import machinery is presented in a different colour. Erv1, essential for respiration and viability 1; Hot13, Helper of Tim 13; Hsp70, heat-shock protein 70; IM, inner membrane; IMS, intermembrane space; Mas37, mitochondrial assembly 37; Mge1, mitochondrial heat shock protein related to GrpE1; MIA, machinery for import and assembly of intermembrane space proteins; OM, outer membrane; Oxa1, oxidase assembly 1; Pam17, presequence translocase-associated motor 17; TIM, translocase of the inner membrane; TOB/SAM, topogenesis of mitochondrial outer membrane β -barrel proteins/sorting and assembly machinery; TOM, translocase of the outer membrane.

fold correctly, and those that fail to do so are disposed of by a process known as ERAD. This involves the retrotranslocation (dislocation) of misfolded substrates from the lumen of the ER, across the ER membrane back into the cytosol, followed by their ubiquitination and degradation by the proteasome. Under stress conditions, in which the workload imposed on the ER exceeds its capacity, cells respond by increasing the transcription of genes coding for ER chaperones as well as for ERAD components, and by attenuating protein synthesis—the so-called unfolded protein response. R.S. Hegde (Bethesda, MD, USA) and D. T. Ng (Singapore) reported on additional ways in which the secretory pathway can respond to stress.

Hegde discussed an immediate response—which precedes transcriptional control—of the acutely stressed ER, consisting of the rejection of several substrates by the translocon, with the consequent reduction of the workload on the ER lumen. This pre-emptive

quality-control mechanism is substrate specific, and the Hegde group has previously shown that the basis for the specificity lies in the unique amino-terminal ER-targeting signal sequence that each substrate carries (Kang *et al*, 2006). Constitutive signals (such as that of preprolactin) that allow translocation under all conditions can be distinguished from regulated ones (such as that of prion protein) that are not translocated under stress. At the meeting, Hegde reported on the different protein–protein interactions that constitutive and regulated signal sequences engage in at the translocon, which suggest that regulated signal sequences interact in a more dynamic manner than constitutive ones and require additional factors to initiate translocation successfully.

At the other extreme, Ng reported that some misfolded proteins in the ER lumen can escape ERAD, exit the ER and reach the Golgi complex. However, yeast cells have a back-up system to handle this

situation and counteract the potential damage that could occur if the misfolded substrates were to be secreted. Ng showed that the misfolded proteins are recognized in the Golgi complex and sorted to the vacuole for degradation. Understanding the molecular basis for this recognition and for the subsequent sorting events is an important future goal.

Quality-control mechanisms clearly operate in all domains of life, and the conference featured talks that show such processes in several other protein-transport systems. C. Robinson (Warwick, UK) discussed the Tat system in *E. coli*. This system is unusual in that it transports fully folded proteins, including periplasmic proteins that bind to complex cofactors—such as FeS centres—in the cytoplasm. This raises the question of how the system ‘knows’ whether the cofactors are inserted correctly and the proteins are properly folded. Robinson reported that the mutation of the FeS ligands in two Tat substrates blocked their export, showing that the system could indeed ‘proofread’ these proteins. Unexpectedly, the Tat translocase then directly initiates the disposal of the unwanted, mutated proteins—which is an unusual type of quality-control mechanism as the translocase is usually bypassed in these situations. The work of R. Erdmann (Bochum, Germany) on peroxisome biogenesis is also related to quality control. Erdmann reported on the ubiquitination cycle of the peroxisomal import receptor Pex5. The peroxisomal import system—like bacterial and chloroplast Tat systems—translocates fully folded proteins across the peroxisomal membrane. The peroxisomal precursors are bound in the cytosol by recycling peroxins—also known as Pex proteins—that deliver them to the peroxisomal membrane and initiate their translocation by mechanisms that are not yet fully understood. The receptors themselves must subsequently recycle back to the cytosol to pick up additional substrate, which is a process that requires their dislocation from the peroxisomal membrane. In the case of Pex5, the Erdmann group has shown that ubiquitination is required for its dislocation and that, depending on whether it is monoubiquitinated or polyubiquitinated, it is either reused or sent to the proteasome for degradation (Platta *et al.*, 2007). At the meeting, he reported the identification of all the components involved in this ubiquitination cycle, including the E3 ligases, which were previously unknown. Moreover, he showed that two different E2–E3 complexes are involved in Pex5 ubiquitination and determine whether the protein becomes monoubiquitinated or polyubiquitinated.

On a different note, J. Soll (Munich, Germany) brought up some interesting ideas regarding the chloroplast import machinery. In this case, it also seems as if the translocase is subject to control systems, which involve redox regulation. Soll presented evidence that the TOC–TIC machinery in the outer and inner envelopes of the chloroplast might include components that contain redox cofactors. In support of this hypothesis, reagents that are known to perturb the redox state of the chloroplast stroma were shown to affect the import of proteins *in vitro*. Therefore, protein import might be fine-tuned in response to the metabolic and/or physiological state of the chloroplast interior.

Evolution of translocation/insertion machinery

The evolution of protein-transport systems has long been an area of interest, in part because two major organelles—the chloroplast and the mitochondrion—evolved from endosymbiotic bacteria. The membranes of Gram-negative bacteria, as well as those of chloroplasts and mitochondria, are the only cellular membranes that contain β -barrel

proteins. The β -barrel precursors are assembled into their target membranes by specialized machinery in the corresponding membrane, namely, the BAM system in bacteria and the TOB/SAM complex in mitochondria. The evolutionary relationships among these systems are further highlighted by the similarity of their main components. Homologues of Omp85/YaeT/BamA—which mediate the integration of β -barrel precursors in bacteria—are found in the outer membrane of both chloroplasts and mitochondria, and are known as Toc75-V and Tob55/Sam50, respectively (Paschen *et al.*, 2005). The mitochondrial Tob55 was also found to promote membrane integration of mitochondrial β -barrel proteins, whereas a similar function of Toc75-V has not yet been shown. T. Lithgow (Melbourne, Australia) discussed the degree of similarity between β -barrel assembly in bacteria and in mitochondria using the α -proteobacterium *Caulobacter crescentus* as a model organism. He reported that, although the central mitochondrial component is derived from the bacterial BamA, no mitochondrial homologues were found for the other bacterial Bam proteins. Similarly, the two interacting partners of Tob55 in mitochondria—which are Tob38 (also known as Sam35 or Tom38) and Mas37 (also known as Sam37)—do not seem to have homologues in bacteria. Overall, it seems that although the mitochondrial machinery for inserting β -barrel proteins was derived from the bacterial system, some modifications occurred during evolution to meet the requirements of the organelle. D. Rapoport (Tübingen, Germany) discussed the evolution of the β -barrel proteins, and showed that bacterial β -barrel proteins expressed in yeast cells were imported into mitochondria and integrated into the outer membrane as native-like oligomers. Rapoport and J. Tommassen (Utrecht, The Netherlands) showed that the reciprocal approach was also successful and that a mitochondrial β -barrel protein expressed in *E. coli* was correctly integrated into the outer membrane of the bacteria, suggesting that although the machinery that sorts β -barrel proteins was modified during the evolution of mitochondria from bacteria, its substrate proteins were not subject to such divergent evolution.

New drugs that target protein translocation

The development of drugs that can specifically interfere with defined steps of protein translocation is an exciting prospect for both basic and translational research. C. Koehler (Los Angeles, CA, USA) introduced a promising chemical genetic approach for studying protein translocation in mitochondria. She described *in vivo* and *in vitro* screens that were used to test a collection of 50,000 drug-like small molecules for their potential activity in modulating protein translocation. The *in vivo* screen led to the identification of three specific inhibitors, of which two interfere with the function of the small Tim proteins in the intermembrane space, whereas one inhibits protein translocation through the TOM complex in the outer membrane. The identities of the molecular targets of these inhibitors remain unknown, although their effects are quite specific. In addition, Koehler discussed the results of an *in vitro* screen, which identified a fourth molecule as an inhibitor of recombinant Erv1. J. Taunton (San Francisco, CA, USA) reported on the cotransins, which are drugs derived from the cyclodepsipeptide compound HUN-7293 that was initially discovered owing to its remarkably specific effect on the expression of VCAM1 and later shown to exert this effect by signal sequence-specific inhibition of pre-VCAM1 translocation across the Sec61 translocon (Garrison *et al.*, 2005). Taunton reported on the development of new cotransin variants and showed that the spectrum of inhibited secretory proteins is sensitive to structural alterations in the cyclodepsipeptide side chains. These

results indicate that it might be possible to develop 'magic bullets' that could specifically inhibit the secretion of disease-related molecules.

What about lipids?

A traditionally neglected aspect in the field of protein translocation is the involvement of membrane lipids in this process. The results presented by Pfanner and M. Bogdanov (Houston, TX, USA) addressed this issue. Pfanner reported on the function of Tam41, which was originally identified as a protein involved in the translocation of preproteins by the TIM23 complex. He showed that the deletion of this protein leads to various pleiotropic effects and suggested that all these observations can be explained by the role of Tam41 in the biosynthesis of cardiolipin.

Bogdanov reported on his studies of the effect of lipid composition on the topology of a multispanning membrane protein, LacY of *E. coli*. LacY has 12 transmembrane segments, which are organized in two six-transmembrane bundles, with both the N and the C terminus normally facing the cytoplasm. The cytoplasmic loops have a net positive charge, as predicted by the positive inside rule, but also carry negatively charged residues. Previous studies revealed that PE is required for the native topology of the protein. In cells incapable of synthesizing PE, the six N-terminal transmembrane segments are inserted with inverted topology, so that the positively charged loops face outside. Furthermore, if these cells are induced to synthesize PE, LacY undergoes post-translational inversion of the N-terminal transmembrane bundle, presumably without assistance from the proteinaceous translocation machinery (Bogdanov *et al.*, 2008). At the meeting, Bogdanov reported extensive mutagenesis analyses that led him to propose that PE, possibly by increasing the pK_a of ionizable amino-acid residues in the microenvironment close to the bilayer, is required to allow positive charges in the cytoplasmic loops, in order to exert their full retention potential and to dampen the translocation potential of acidic residues.

Concluding remarks

The conference provided an up-to-date panorama of the protein-translocation field by bringing together a broad spectrum of topics that essentially covered all known translocation systems, and addressed their structure-function relations, regulation and evolution. The meeting highlighted how a combination of structural and biochemical methods can generate a detailed picture of the dynamics of protein translocation. We anticipate that the combination of these two methodologies will provide the field with new high-resolution models of the translocation process in the near future, which will hopefully be presented at the next meeting on this exciting topic.

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REFERENCES

- Bogdanov M, Jun Xie J, Heacock P, Dowhan W (2008) To flip or not to flip: protein-lipid charge interactions are a determinant of final membrane protein topology. *J Cell Biol* **182**: 925–935
- Bolender N, Sickmann A, Wagner R, Meisinger C, Pfanner N (2008) Multiple pathways for sorting mitochondrial precursor proteins. *EMBO Rep* **9**: 42–49
- Garrison JL, Kunkel EJ, Hegde RS, Taunton J (2005) A substrate-specific inhibitor of protein translocation into the endoplasmic reticulum. *Nature* **436**: 285–289
- Kang SW, Rane NS, Kim SJ, Garrison JL, Taunton J, Hegde RS (2006) Substrate-specific translocational attenuation during ER stress defines a pre-emptive quality control pathway. *Cell* **127**: 999–1013
- Neupert W, Herrmann JM (2007) Translocation of proteins into mitochondria. *Annu Rev Biochem* **76**: 723–749
- Paschen SA, Neupert W, Rapaport D (2005) Biogenesis of beta-barrel membrane proteins of mitochondria. *Trends Biochem Sci* **30**: 575–582
- Platta HW, El Magraoui F, Schlee D, Grunau S, Girzalsky W, Erdmann R (2007) Ubiquitination of the peroxisomal import receptor Pex5p is required for its recycling. *J Cell Biol* **177**: 197–204
- Tokatlidis K (2005) A disulfide relay system in mitochondria. *Cell* **121**: 965–967

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