Seaside transportation—from structure to function of translocation machines

Trevor Lithgow & Gabriel Waksman

The EMBO conference 'From Structure to Function of Translocation Machines' took place in April 2013 in Dubrovnik, Croatia. The meeting brought together a mix of established and aspiring researchers to discuss a wealth of unpublished data and ideas in a lively scientific programme of talks designed to shatter the established dogma. From new ways to envisage known protein transport pathways to a brand new and totally unconventional protein transport system, surprises were part and parcel of this excellent EMBO conference.

Not unlike the walled city of Dubrovnik, trying to keep out centuries of potential invaders, the membrane-bound compartments of cells are selective about protein transport—they ensure that only select and appropriate proteins are allowed entry. Continuing the 'science by the sea' theme from the previous meeting in Sardinia, the 2013 EMBO conference on 'From Structure to Function of Translocation Machines' was set in an idyllic location on Croatia's Dalmatian Coast, just a stone's throw from the old town of Dubrovnik.

A main theme of the meeting this year was the use of protein structure to inspire functional understanding of protein transport pathways. With participants from 20 countries from across Europe, the Americas, Asia and Australia, this vibrant meeting showcased the best that science can offer: unashamedly unpublished observations and ideas, lively discussions, critical appraisal of data, and speculation that will drive the next few years of investigation in this expanding and highly topical area of research. The conference organizers, Nica Borgese (Institute of Neuroscience, Milan, Italy) and Peter Rehling (Göttingen U., Germany), welcomed established investigators, new researchers entering the field and postgraduate students—one-third of the attendees—all learning about the latest developments in their field.

Dynamic and modular: the translocon
Perhaps the key theme running through the meeting was that protein translocation machines are modular in design and dynamic by nature. Many of the breakthroughs

Dubrovnik, Croatia. Impenetrable walls, with entry portals designed to allow only highly selective transport into the old city. Photo credit: Dr Chaille Webb (Monash University, Australia).

announced in Dubrovnik focused on protein transport across the cytoplasmic membrane of bacteria. The translocon comprises three proteins contributing 13 transmembrane segments to a clam-shaped molecule that forms a tightly regulated pore for proteins to be threaded across the bacterial membrane. An equivalent translocon (the Sec61 complex) is found in the endoplasmic reticulum of eukaryotic cells.

In the course of co-translational protein targeting, ribosomes dock onto the translocon. Roland Beckman (U. Munich,

Germany) detailed the engagement of a bacterial ribosome captured with a client protein, by using high-resolution electron microscopy to visualize the structural changes required and the precise route taken during polypeptide transfer through the translocon. Tom Rapoport (Harvard U., USA) reported work done in collaboration with Chris Akey (Boston U., USA) to visualize the structure of the translocon in action. Rapoport and Akey used cryo-electron microscopy to analyse ribosome-channel structures in both 'resting' and 'active'

conformations. Non-translating ribosome–translocon complexes derived from bacteria showed the channel in its closed state, indicating that ribosome binding causes only minor changes. Conversely, the structure of a translocating ribosome–translocon complex showed that the nascent chain opens the channel, causing mostly rigid body movements of the amino- and carboxy-terminal halves of the translocon. The polypeptide inserts as a loop into the translocon channel, with the hydrophobic region of the signal sequence intercalated into the open lateral gate.

The mechanics of the docking reactions between various ‘modules’ with the translocon were revealed through a series of biochemical stories told by Ian Collinson (Bristol U., UK) and Hans-Georg Koch (U. Freiburg, Germany). Collinson described attempts to determine the functional significance of different structural states of the bacterial protein secretion machinery. A conformational change within SecA, driven by the association of the translocon, corresponds to the activation of the ATPase and the relocation of elements within the translocon. This was observed on signal-sequence binding and seems to activate the channel for protein secretion but not for membrane insertion. By using a distinct experimental system, Koch explained the interplay between the ribosome and the translocon during the insertion of membrane proteins, and how this is modulated by contrasting engagements with either an ‘FtsY receptor module’ or a membrane integration machine called YidC.

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The attention of the meeting was also drawn to the homologous translocon found in the endoplasmic reticulum of mammalian cells. Bill Skach (Oregon Health & Science U., USA) presented studies that used a finely tuned translocation assay system to measure the specific recruitment of select modules mediating glycosylation, proteolytic processing and protein folding into the translocon machine. Taken together, these and the other studies presented in Dubrovnik could serve as a blueprint for

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how to tackle structural studies on any piece of cellular machinery, and have revealed once again the intimate degree to which the function of the translocon is understood.

Mitochondrial translocation machines

Mitochondria evolved from intracellular bacteria, and elements of the ancestral bacterial protein transport pathways are evident today, sitting and working alongside protein translocases that are uniquely eukaryotic. In this context, exciting revelations were delivered at the meeting. Nikolaus Pfanner (U. Freiburg, Germany) presented the first evidence of a SAM–TOM (sorting and assembly machinery–translocase of the outer mitochondrial membrane) interaction. Mitochondrial proteins are encoded on genes in the nucleus, translated on ribosomes in the cytosol and recognized and imported into mitochondria by the TOM complex. Once imported, outer membrane proteins are transferred to the SAM for insertion into the outer mitochondrial membrane. Optimal protein assembly, it seems, is managed through the formation of direct contact between the translocases. Remarkably, both the TOM and SAM complexes are themselves formed of protein modules, so that the TOM–SAM assembly becomes one of the most complicated—and beautiful—protein transport machines yet described.

Mitochondria are also the site of several crucial steps in lipid biosynthesis. Toshiya Endo (Nagoya U., Japan) reported new details on the stepwise transfer of lipids through the mitochondrial membranes and their transport out into the membranes of the endoplasmic reticulum—a process orchestrated by the endoplasmic reticulum–mitochondria encounter structure (ERMES). Structural studies on the ERMES are in progress, seeking to capture lipid occupancy within this fascinating molecular machine. Detailed electron tomography and fluorescence microscopy images of the membrane contacts between the endoplasmic reticulum and the mitochondrial outer membrane were showcased by Gia Voeltz (U. Colorado, Boulder, USA). These junctions also have an important role in

decision-making to determine the effects of extracellular stimuli, as illustrated by Gisou van der Goot (EPFL, Lausanne, Switzerland).

Structures reveal function

Several surprises and not a small amount of controversy came from several beautiful structures revealed at the meeting, including those of the ribosome–SecYEG complex engaged in translocation of a nascent chain (see above by Roland Beckman and Tom Rapoport), the C-ring of bacterial type III secretion systems (Melanie McDowell, U. Oxford, UK), TatC from the TatABC transport machinery (Sarah Rollauer, U. Oxford, UK), the GET complex involved in the delivery of tail-anchored proteins to their target membranes (Bil Clemons, Caltech, USA), and new insights in pilus biogenesis and assembly by usher proteins (Gabriel Waksman, UCL and Birkbeck, London, UK).

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TatABC is an important export system in bacteria, as it is a main pathway for the transport of large folded proteins out of bacteria. The stoichiometry of the various components in this truly dynamic machine is still unclear, and, as Matthias Muller (U. Freiburg, Germany) noted in his presentation, might be varied in the course of its action. The crystallography of large complexes was also brought to life by the structure of the FimD usher transporter with a three-subunit protein polymer inserted, with the last subunit still bound to its cognate chaperone (Gabriel Waksman). This structure was subsequently used to unravel energy tracks that guide the polymer during translocation within the FimD pore lumen.

Just as exciting as the revelations from structural biology presented at this meeting is the prospect of future, groundbreaking dissections of protein transport, as promised by recent successes in purifying large complexes that can be submitted to cryo-electron microscopy. Collison and co-workers, for example, have purified an intact translocon complex bound to modules of YajD, SecD, SecE and YidC—a spectacular arrangement that will make

it possible to elucidate intimate relations between the translocon and YidC. YidC function itself was addressed in detail at this meeting by Andreas Kuhn (U. Hohenheim, Stuttgart, Germany), who presented the structure of this essential protein insertase bound to the ribosome, achieved in collaboration with Roland Beckman.

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Size might not be everything but it does matter, and the field is moving into areas of *in cellulo* analysis of molecular machines by cryo-electron tomography. This medium-resolution technique allows the visualization of large macromolecular complexes, and Vicki Gold (MPI of Biophysics, Frankfurt, Germany) illustrated the potential of this technique. The future use of structural biology to analyse transport systems is bright and will no doubt continue to provide insights and excitement at future EMBO meetings.

Transport assembly and function

Another exciting aspect of the meeting was a renewed emphasis on the regulatory mechanisms underpinning translocation system assembly and function. Links between transport systems and ubiquitination and deubiquitination pathways were the subject of extensive studies and discussions. A fascinating talk by Manu Hegde (MRC Laboratory of Molecular Biology, Cambridge, UK) demonstrated that discriminating

'right' from 'wrong' during membrane protein quality control depends crucially on deubiquitinases working to oppose ubiquitin ligases. Ubiquitination and deubiquitination proteins were shown to be integral parts of some important transport systems, such as endoplasmic reticulum-associated protein degradation (ERAD), which is responsible for cleaning up the endoplasmic reticulum from misfolded proteins, and the protein import machinery in peroxisomes. Indeed, these systems also highlight our growing awareness of how and why regulated protein transport pathways have evolved.

An exciting thread of talks covered the ERAD pathway, which targets misfolded proteins of the endoplasmic reticulum for degradation in the cytoplasm (Pedro Carvalho, Centre for Genomic Regulation, Barcelona, Spain); the symbiont-specific ERAD-like machinery (SELMA) protein translocase, found in diatoms and related organisms, in which it serves to assist a new protein translocation event required only in these organisms that have undergone secondary endosymbiosis (Kathrin Bolte, Philipps-U. Marburg, Germany); and the peroxisomal protein translocase (Ralf Erdmann, Ruhr-U. Bochum, Germany). These transport systems have different cellular functions but they all contain homologous components at their core and probably all derive from an ancestral protein transport system, presumably—although debate might continue on this topic—situated in the endoplasmic reticulum of ancient eukaryotes.

Come to the next meeting...

We hope that this report makes clear the breadth and excitement of the meeting, although we have only scratched the surface of what was presented and discussed. The enormous complexity of some of the systems

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under investigation is mind-blowing, and the coming years promise to yield exciting developments in our understanding of the molecular basis for transport by these systems. Even those systems for which the molecular basis of substrate transport was elucidated early on—because they are remarkably small and self-contained—are still providing new glimpses of complex molecular machines caught in the act of translocating proteins across membranes. With the constant improvement of visualization techniques, notably in electron microscopy—including the breakthrough in direct electron detection—our molecular understanding of these beautiful machines is expected to increase markedly. It is our view that future EMBO meetings will be just as exciting as the one we have just experienced. So please, join us at the next one.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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