NEWS & VIEWS

STRUCTURAL BIOLOGY

Clamour for a kiss

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Precisely how proteins snake their way through channels in cell membranes is unclear. Complexes between the SecY channel and its motor protein, and the use of a 'molecular endoscope', provide fascinating clues.

To cross cellular membranes, secretory proteins use specialized channels such as that known as SecY. When ribosomes start synthesizing secretory proteins, the ribosome–protein complex is hijacked to the mouth of SecY. There the nascent 'pre-protein' chains, which carry specific recognition tags known as signal peptides, are threaded through the endoplasmic reticulum — a membranous mesh inside the cells of eukaryotic organisms — or through the plasma membrane that surrounds prokaryotic cells such as bacteria. In bacteria, a 'push' motor called SecA can also mediate ribosome-independent protein secretion through SecY channels, using the cell's energy currency ATP in the process. The structure of such cellular machines dictates their function and so can be highly informative. But despite having known for some time[1,2] what the separated parts — SecY and SecA — look like, we are no wiser about what they look like when united. In this issue, three studies[3–5] provide eagerly awaited snapshots of how the SecY channel and the SecA motor come together in a 'kissing' complex, and how they might move to mediate protein translocation.

SecY channels come in pairs in which each has its own specific functions — one half seems to carry out the actual translocation of proteins, the other holds the SecA motor in place[6]. Near the cytoplasmic entrance of both channels are several docking sites for ribosomes and motors, and at their exits a plug structure[1,7]. Motors also come in pairs and each copy comprises four domains: two make up the 'engine' and sandwich ATP molecules between them[2]; the other two form the 'business end' for translocation and can be thought of as 'hands' (Fig. 1a).

Zimmer et al.[3] (page 936) share the first glimpse of a single bacterial protein-conducting channel in complex with a single SecA motor (Fig. 1b) — a technical feat because detergent extraction of proteins from membranes often dissociates them from their binding partners. The channel seems to have undergone conformational changes compared with its closed state[1], including loosening of its plug. Moreover, one of the motor hands has swivelled around its slender stem in such a way as to delimit a tubular enclave, forming a continuum with the channel. Pre-protein
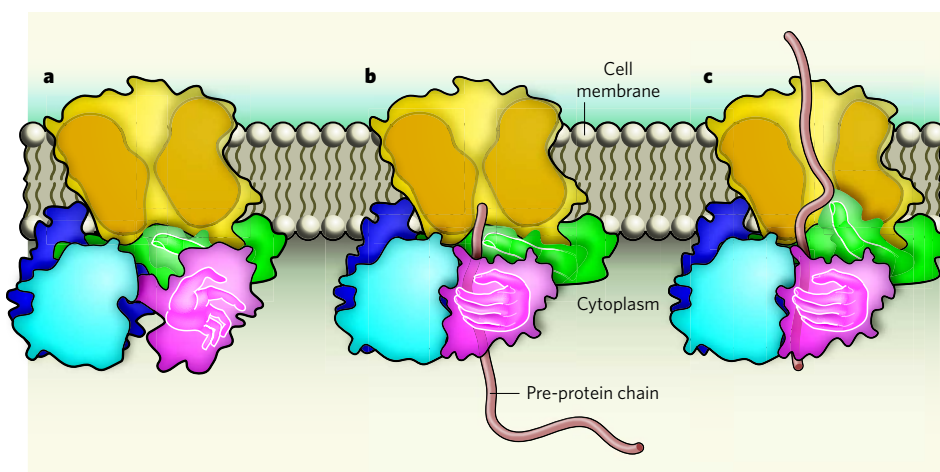

Figure 1 | Protein translocation in bacterial cells. This simplified representation is based on both earlier studies and the new findings[3–5]. In this cut-away view of the membrane, the SecA motor lies flat against the cytoplasmic side of the SecY channel (yellow), and consists of a two-domain ATP-powered engine (light and dark blue) and two 'business-end' domains (green and magenta; depicted as hands). **a**, Initially, the channel pore is sealed by both a constriction halfway through it and a mobile plug domain (not shown) near its exit. The pre-protein-binding domain of the motor (magenta) is in the open state, exposing an elongated corridor that connects to the entrance of the channel. This open state is seen in structures of the isolated motor. **b**, Swivelling this domain around its stem would allow it to embrace a secretory protein chain. At this stage, a finger (green) from the second hand of SecA might be in close contact with the chain. **c**, When ATP (not shown) is present, the engine conformation changes and the finger could move upwards, pushing or dragging the protein chain into the pore. This motion, or other conformational changes, leads to the opening of the pore.

chains interact with this region of the motor[8] and — although not present in this structure[3] — can be imagined to occupy it. For this to occur, the enclave, which is tightly packed and extremely narrow in the present structure, would have to open up. The authors[3] find that a two-helix finger from the motor's other hand dips near the cytoplasmic mouth of the channel, next to where the pre-protein chain would be. This finger is an essential switch that couples ATP expenditure to protein movement[9]. Could it somehow drive the chain through the channel?

To test this possibility, Erlandson et al.[4] (page 984) used the pre-protein as an endoscope, trapping it within the channel to study what it 'sees' around it. They constructed mutated derivatives of the pre-protein, the channel and the motor that contained strategically placed cysteine residues. When two copies of this amino acid are juxtaposed within roughly

5 angstroms of each other, even if they are on different proteins, they can form covalent bonds and so reveal proximity. In this case, detergent extraction is unnecessary, and the complex remains fully functional.

These analyses show that, at first — when the motor is idle — specific residues from the pre-protein are in proximity to the 'fingertip'. In a subsequent ATP-driven step, these residues are carried well into the channel (Fig. 1c). On the basis of the chain lengths these authors[4] used, it would be expected that only short segments of the pre-protein — which would have to be stretched — can travel forwards in each cycle. Several repeats of this motion would be required for a complete pre-protein chain of a hundred to hundreds of residues to be pushed across to the other side. Thus a flick of the fingertip could provide a mechanical motion that drags or pulls the trapped pre-protein forwards. Alternatively, the finger could prise the channel

open and/or prevent back-slippage, allowing forward movement of pre-protein segments by Brownian motion. Detailed kinetic assays will be necessary to discriminate between these possibilities.

To visualize the channel–motor complex, Zimmer et al.[3] had to immobilize it in a crystal lattice. Consequently, they could capture the structure of only one of its possible states — much like a party snapshot freezes a single move on the dance floor. Synthesis of the full spectrum of channel and/or motor motions will necessitate more snapshots. Tsukazaki et al.[5] present one such additional picture on page 988, revealing a different conformational state of the SecY channel.

In these authors' structure, the motor is absent. Instead, they used an antibody to hold a single SecY channel in a particular state. The antibody binds specifically to a motor-binding site and freezes the channel in what is proposed to be a 'pre-open' state. This conformation exposes a cleft with access to the cytoplasm, which Tsukazaki and colleagues hypothesize[5] could attract signal peptides. Whether these antibody-driven effects faithfully mimic those driven by the binding of the motor is unknown. Nevertheless, they reveal the inherent repertoire of potential channel motions.

Like Erlandson and colleagues[4], Tsukazaki et al. also used cysteine cross-links to study a functional complex of the channel with the motor. These experiments revealed that a region of the motor undergoes significant conformational changes when it binds to the channel. These interactions probably involve the second copy of the channel, which is absent from Zimmer and colleagues' channel–motor structure. This second copy is thought[1] to act as a docking station for the engine part of the motor.

The implications of these channel–motor 'kiss-and-tell' structures for understanding the mechanics of pre-protein translocation are important. A possible pathway for the pre-protein to follow has now been coarsely charted. The range of possible channel conformations and the motions of the motor's domains can now be predicted and tested. A second copy of the motor is apparently not required for interaction with a single translocating channel. Similarly, the remarkable ATP-driven motions of the motor's hands that accompany protein secretion[10] need not lead to profound membrane penetration of the motor. But to confirm these and other conclusions, experimentally less disruptive isolation of the complexes in a functional form and freezing of other conformational states will be necessary.

Solving structures of membrane proteins is not a trivial pursuit. As we contemplate in awe the reported achievements[3–5], a wish list of future findings comes to mind: higher resolution structures of the twin channel–motor complex, accompanied by a trapped pre-protein chain; understanding how the motor recognizes pre-proteins and how it converts the chemical energy of ATP into mechanical

work; and determining the dynamics of this astonishing cellular nanomachine.   ∎

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CONDENSED-MATTER PHYSICS

Surviving the transition

Kristian Helmerson

Observations of the birth of a superfluid have uncovered details of the microphysics of phase transitions. Whether these results can be used to model such transitions in the early Universe is an open question.

The spontaneous formation of topological defects — stable configurations of matter — is thought to accompany most continuous, non-equilibrium phase transitions in condensed-matter physics. But the study of the microscopic dynamics underlying the formation of such defects is a challenge, especially in superfluid transitions. On page 948 of this issue, Weiler et al.[1] report observations of topological defect formation in a particularly helpful superfluid phase transition — the Bose–Einstein condensate (BEC) transition— in a trapped gas of rubidium atoms. These observations offer unique insights into the microscopic physics of phase transitions.

Approximately 10^{-35} seconds after the Big Bang, the Universe underwent a phase transition resulting from the cooling associated with its rapid expansion. We generally think of a phase transition driven by cooling as one that takes a system from a disordered state to an ordered one — for example, water turning into ice. Yet the Universe is not an ordered state, but is filled with galaxies, stars and other celestial bodies. A way out of this dilemma was proposed by Thomas Kibble[2,3] in 1976. He argued that, as the Universe cooled and approached the phase transition, large fluctuations in the vacuum in the form of topological defects, such as cosmic strings, were formed. These defects, which persisted even after the transition, eventually led to the formation of larger structures in the Universe.

The key to the survival of these topological defects is that they are formed locally on a timescale that is shorter than the time it would take light to propagate from the location of one defect to another; hence the defects would not be able to know about one another. In the parlance of physics, this means that the formation of the defects is not causally connected. Using similar reasoning, Wojciech Zurek suggested[4,5] that in a

condensed-matter system, a second-order phase transition — in which the transition between the old and the new phase is continuous — could result in defect formation if the transition is crossed rapidly enough.

Zurek went on to propose a specific experiment involving the phase transition of liquid helium ^{4}He, at a temperature of 2.17 Kelvin, from a normal fluid to a superfluid; that is, a fluid with no viscosity. If the helium could be cooled rapidly enough, then topological defects in the form of quantized vortices would be produced. In this case, however, the timescale for the causal connection of the defects is not determined by the speed of light but by the speed of sound in the bulk superfluid.

Since Zurek's suggestion, the mode of formation of topological defects, which is known as the Kibble–Zurek mechanism, has been observed in liquid crystals, arrays of superconducting Josephson junctions, nonlinear optical systems, fluid convection systems and superfluid ^{3}He. But, surprisingly, it has not been observed in ^{4}He, the quintessential superfluid and the system referred to in Zurek's original proposal. Weiler et al.[1] now report observing such defects in a BEC transition of a trapped atomic gas.

In their experiments, Weiler et al. evaporatively cooled a gas of weakly interacting rubidium (^{87}Rb) atoms confined in a magnetic trap. Evaporative cooling normally occurs slowly, such that the system being cooled is close to reaching thermal equilibrium. This is similar to what occurs when the hottest (highest-energy) molecules evaporate from a cup of coffee and the rest of the coffee 're-thermalizes' — that is, comes back to thermal equilibrium — at a lower temperature. The system can, however, be thrown out of equilibrium by rapidly modifying the energy distribution. Weiler et al. achieved this by using radio-frequency transitions that