time it takes for a variety of organisms to die across a range of temperatures. These rate effects (also called thermal sensitivity of trait responses) are typically reported as Q_{10} values, which reflect the change in the trait value when the temperature varies by 10 °C. Alternatively, rate effects can be expressed more formally as average activation-energy values (E_a). Reporting E_a values for thermal sensitivities of traits is often, but not always[5], preferred to reporting Q_{10} values, because of the underlying modelling connection between E_a and the kinetics of biochemical reactions[5,6].

The authors found that the temperature sensitivities (Q_{10} and E_a values) of biological processes in the permissible temperature range, taken from assessments of more than 300 species, were consistent with those previously reported for traits such as movement, feeding and metabolism. By contrast, the temperature sensitivity of heat failure, calculated using data from more than 100 species, was extremely high and well above the rates of temperature sensitivity typically reported in the permissible range.

These high sensitivities for heat failure suggest that when there are no options to escape hot events, species can, on average, encounter strikingly high heat-failure rates, estimated as a doubling of this rate per 1 °C increase in temperature. Furthermore, the thermal sensitivities of heat failure for vertebrates, such as fishes and amphibians, were higher than those for insects and marine invertebrates, suggesting that vertebrates are particularly vulnerable when facing extreme warm conditions near their survival limit.

By combining the estimates of thermal sensitivity for terrestrial and aquatic species with average and maximum temperatures predicted to occur because of climate change, Jørgensen *et al*. find that both aquatic and terrestrial ecosystems will experience rates of heat failure approximately two to eight times higher than under current conditions. Using two species as examples — an ant and a fish — the authors reveal that terrestrial and aquatic species are already experiencing maximum temperatures that fall in the stressful-temperature region, and that future scenarios of rising temperatures will only exacerbate these patterns.

These predicted increases in heat-failure rates could be catastrophic for local populations, despite potential buffering mechanisms such as variations in temperature over time or the use of sites that provide thermal refuges. The authors recognize that the value of these warming-risk estimates also requires a good understanding of temperatures experienced by ectotherms in the wild, including the timing, severity and duration of maximum temperatures[7]. Moreover, other processes might also protect organisms from the potentially lethal effects of heatwaves, including behavioural and physiological strategies to combat heat, or having the ability to mount a response to extreme temperatures after an acute pre-exposure to a high temperature[8].

A deeper understanding of the responses underlying heat failure is urgently needed. For example, what is the relative order in which various cellular and system processes break down at extreme temperatures? And is this order maintained across different groups of related species? Key repair mechanisms might have a role in combating exposures to lethal temperatures, up to a point. However, determining the transition temperature at the boundary between permissive and stressful temperatures (which Jørgensen *et al*. term the critical temperature, T_c) can be highly challenging. Indeed, lethal tests of the kind needed to investigate these processes are often not possible in vertebrates for ethical reasons, or might be less appropriate for immobile life stages (such as eggs), which have no or limited behavioural responses.

There is clearly a need to develop innovative methods to examine the progression of cellular stress responses leading to heat death[9] in both controlled and wild populations. Moreover, the use of multiple-inference approaches to model temperature-sensitivity data for both biochemical and trait responses will be essential to advance the field[10]. Jørgensen and colleagues' study highlights the complexity underlying lethal limits, and the urgent need to scrutinize these limits to better predict the vulnerability of species to climate warming.

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Cell biology

Assembly surprise for membrane proteins

Ben C. Berks

Membrane-spanning proteins have many crucial roles in the cell. New findings challenge our current understanding of the route by which such proteins are inserted into the membranes of animal cells. **See p.161 & p.167**

Proteins in cellular membranes contain one or more segments known as transmembrane domains (TMDs). Insertion of these TMDs into the membrane is a key step during the production of new membrane proteins. In eukaryotic cells (those with a nucleus), this insertion process occurs mainly at one organelle — the endoplasmic reticulum (ER). Here, TMDs are integrated into the membrane as they emerge from the protein-producing ribosome. For many decades, the accepted view has been that TMD insertion occurs through the Sec61 protein complex — the same machinery that transports proteins into the interior of the ER[1,2]. However, Smalinskaitė *et al*.[3] (page 161) and Sundaram *et al*.[4] (page 167) now challenge this understanding.

These two papers show that when ribosomes insert membrane proteins with multiple TMDs (termed multipass proteins), they recruit not only the Sec61 complex, but also other membrane proteins to form an ensemble called the multipass translocon (MPT). Moreover, Smalinskaitė *et al*. report the remarkable finding that some multipass proteins that trigger assembly of the MPT are inserted into the membrane without passing through the classic Sec61 complex at all. These studies overturn current thinking that TMDs are usually inserted into the membrane exclusively by the Sec61 complex. The findings suggest that, in fact, responsibility for TMD integration passes to the MPT as protein synthesis proceeds.

It is well established that the Sec61 complex has a role in the insertion of membrane proteins. However, previous work by some

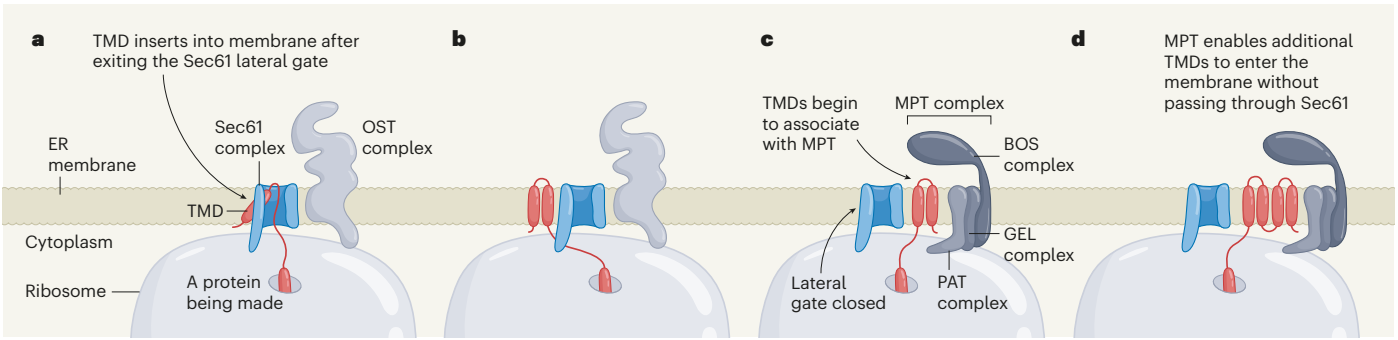

Figure 1 | Protein insertion into the membranes of an organelle in animal cells. a, **b**, As the ribosome generates membrane proteins, the insertion of a segment called a transmembrane domain (TMD) into the membrane of the endoplasmic reticulum (ER) can occur by a process mediated by the protein complex Sec61 (shown here in cross-section). Sec61 associates with complexes called OST and TRAP (not shown). In this well-established process, the TMD enters the membrane through a side opening of the central pore of Sec61 called a lateral gate. **c**, **d**, Smalinskaitė *et al.*[3] and Sundaram *et al.*[4] provide evidence indicating that some TMDs, although typically not the first few TMDs of a protein, are inserted through an alternative process. This requires a complex called MPT, composed of three complexes called BOS, GEL and PAT. MPT occupies the position normally held by OST, and when MPT associates with the Sec61 complex, the lateral gate is closed. (Adapted from Fig. 2 of ref. 4.)

of the current authors identified other membrane proteins of the ER that also seem to be associated with the generation of multipass membrane proteins[5,6]. Smalinskaitė *et al.* and Sundaram *et al.* now confirm that these other proteins are part of a linked network of three ribosome-bound complexes that are termed GEL, PAT and BOS. These complexes are positioned in the membrane next to the Sec61 complex, and together form the MPT (Fig. 1). Deleting components of the MPT impairs the correct insertion of multipass membrane proteins, but not of membrane proteins with a single TMD, confirming that these MPT constituents are involved in the formation of multipass proteins.

Intriguingly, the MPT is not the only newly discovered player in the assembly of multipass membrane proteins. A complex in the ER called the EMC inserts the first TMD of certain proteins into the membrane before ribosomes dock at the Sec61 complex[7].

Smalinskaitė *et al.* and Sundaram *et al.* investigated the progression of multipass-protein insertion by characterizing intermediates stalled at various stages during the integration process. The authors found that ribosomes that synthesize multipass membrane proteins interacted at first with the established insertion machinery. This machinery comprises not only the Sec61 complex but also a complex of poorly defined function called TRAP, and the oligosaccharyl transferase complex (OST), which adds sugar groups to most inserted proteins.

However, once an initial segment of the protein containing between one and three TMDs had been integrated into the membrane, the MPT was recruited to the ribosome, and this association was retained during the insertion of further TMDs. The MPT occupied the same location relative to the ribosome as the OST, and recruitment of the MPT therefore requires the OST to be displaced from the insertion apparatus. The remodelling of the ribosome-associated insertion apparatus seems to be triggered by as-yet-unknown features of the multipass protein that is being synthesized. An obvious challenge for future research is to elucidate exactly how the protein being inserted into the membrane drives these changes.

Smalinskaitė *et al.* report that once the MPT has assembled, the Sec61 complex is no longer involved in integrating TMDs into the membrane. The first evidence for this conclusion is structural. The Sec61 complex forms a transmembrane channel that enables it to carry out the dual functions of transporting the protein into the ER and integrating the TMDs. Water-soluble proteins cross the membrane through the channel, whereas TMDs exit the channel into the membrane by opening a side 'seam' of the complex termed the lateral gate[8] (Fig. 1). Smalinskaitė *et al.* show that recruiting the MPT locks this lateral gate closed, and thus blocks the insertion of TMDs through Sec61.

The second piece of evidence comes from experiments examining the membrane insertion of MPT-recruiting multipass proteins in which the first TMD is inserted by the EMC rather than by Sec61. Smalinskaitė *et al.* report that insertion of these proteins into the membrane was unaffected by Sec61 inhibitors. Thus, there are MPT-recruiting multipass proteins that can be routed into the membrane without passing through the Sec61 complex at any stage. Together, these observations suggest that the MPT takes over the role of TMD insertion from Sec61 (or, in some cases, from the EMC) in the later stages of the formation of multipass membrane proteins.

The observations in both papers suggest a revised model for the production of multipass proteins. In this scenario, the ribosome is initially associated with a complex that contains Sec61, OST and TRAP — consistent with the current model of membrane-protein formation. However, only the first few TMDs of the substrate protein are integrated into the membrane by the Sec61 complex. At this point, the OST disengages from the ribosome and is replaced by the MPT, which takes over the insertion of the membrane protein from Sec61. For multipass proteins in which the first TMD is integrated by the EMC, the Sec61 complex might not be involved in TMD integration at all, but instead serve to dock the ribosome to the membrane to allow the subsequent assembly of the MPT.

The roles of the various components of the MPT in membrane-protein insertion, and how their interactions with the inserting protein are organized, have yet to be determined. The PAT complex probably sequesters any highly polar regions in the TMDs away from the non-polar membrane environment until they can be buried in the interior of the fully assembled protein[6]. One subunit of the GEL complex is similar to a bacterial protein that has TMD-inserting activity[5], and could therefore be a good candidate for 'feeding' TMDs into the membrane.

Not all components of the MPT have obvious equivalents in non-animal eukaryotes. This raises the question of why animal multipass proteins require a biosynthetic apparatus that is more complex than that of other eukaryotes.

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