# The yeast voltage-dependent anion channel Porin: more IMPORTant than just metabolite transport

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## Abstract

Porin is is crucial for metabolite flux in mitochondria. In this issue, Sakaue et al. and Ellenrieder et al. describe an unexpected role for Porin in mitochondrial protein import by regulating the oligomeric state of the major protein import gate, the TOM complex. and the inner membrane insertion of metabolite carriers.

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Protein import into mitochondria is critical for mitochondrial function and cell fitness. 99% of mitochondrial proteins are translated in the cytosol and imported through one essential mitochondrial import gate, the translocase of the outer membrane (the TOM complex). TOM is a multi-subunit complex made up of three main receptor proteins, Tom20, Tom22 and Tom70; three small channel-modulating Tom5,Tom and Tom7 subunits; and the key import channel Tom40 (Pfanner et al 2019). Previous studies have suggested that the TOM complex exists in many different states that alter the channel components (Shiota et al 2015 and Gornicka et al 2014). A combination of the detailed mapping of the architecture of the TOM complex by site-specific crosslinking (Shiota et al 2015) and the determination of the structure of the *Neurospora Crassa* TOM complex (Bausewein et al 2017) indicated that the TOM complex adopts both a trimeric and dimeric oligomeric structure. The abundant outer membrane channel, Porin, is a voltage-dependent anion channel whose role in transporting metabolites and ions is well established but a role in protein import into mitochondria was hitherto unknown. Now, two papers by the Endo and Pfanner groups, respectively, elegantly describe a novel role for Porin whereby this protein plays a critical role in the transition of the TOM complex between the trimer and dimer state and directly impacts on the pathway that inserts the metabolite carrier proteins in the inner mitochondrial membrane (**Figure 1**)

Sakaue et al. provide a comprehensive analysis of a novel role for yeast Porin in regulating the dynamic transition of the TOM complex from a trimeric to a dimeric state. Although such a dynamic behaviour of the TOM complex has been previously suggested (Model et al 2002), Sakaue et al. describe a molecular handle and mechanistic framework to this process. Porin regulates this dynamic transition through an interaction with the major Tom receptor, Tom22, which is normally present in the trimeric form of the TOM channel. Sequestration of Tom22 off the trimer favours the dimeric form of the TOM channel, which favours the import of a subset of mitochondrial proteins including substrates of the Mia40 import pathway. A strain lacking Porin is severely depleted in the small Tim chaperone proteins, Tim9 and Tim10, which are dependent on Mia40 for their import. Gornicka et al. (2014) previously showed that Mia40 substrates do not require Tom22 for import reinforcing the work of Sakaue et al. (2019). The TOM receptor Tom6 stabilises the Tom22

receptor, preventing its dissociation from the trimeric TOM complex by inserting itself between two Tom40 channels.

The authors exemplify this dynamic process during the cell cycle as interactions between Tom6 and Tom40 as well as Porin and Tom22 change depending on the cell cycle stage. During M phase Tom6 interacts with Tom40 to stabilise the trimeric Tom complex, thus retaining Tom22. During S phase, Porin interacts with Tom22 to favour the dimeric complex. The results of Sakaue et al. highlight a novel role for Porin as a key controller of the dynamic transition of the TOM complex that is critical for this machinery to recognise and transport different preproteins efficiently. Additionally, they provide new evidence that the dynamic transitions of the TOM channel must be seen in the context of a cell-cycle regulated process both through the Tom6-Tom40 association and through the Porin-Tom22 association.

Ellenrieder et al. elucidate a novel role for yeast Porin as a coupling factor assisting the transport of metabolite carrier proteins from the outer membrane to the Tim22 insertion machinery of the inner membrane. The authors show that loss of Porin results in a reduction of several metabolite carriers. Interestingly, the ion flux activity of Porin is not required for the import of the metabolite carriers. The carrier import machinery comprising the Tim22 channel and small Tim chaperone complex Tim9/10 both interact with Porin. The combined results strongly support a role for Porin in the import of the metabolite carriers. The authors go on to dissect the exact stage at which Porin is involved in the well-characterised metabolite carrier import pathway. The two early stages of metabolite carrier import involving ATP-dependent cytosolic chaperones (Stage I) and accumulation on the Tom70 receptor (Stage II) are unaffected by Porin. By contrast, the later stages of translocation across the intermembrane space (Stage III), accumulation at the Tim22 channel (Stage IV) and insertion into the inner membrane (Stage V) are clearly affected when Porin is deleted. The defect is particularly evident at stage III suggesting that Porin is involved in the transfer of the metabolite carriers to the inner membrane. This is an intriguing finding as it has been previously shown that the interaction of the carrier preprotein at stage III with the soluble small Tim chaperones in the IMS is sufficient to drive the complete insertion of the carriers in in vitro reconstitution and in vivo experiments (Luciano et al 2001, Weinhäupl et al 2018).

The involvement of Porin in this process indicates a more elaborate mechanism that originally thought.

Both articles shed new light in the functions of Porin in mitochondria. They demonstrate that Porin is intimately linked to the mitochondrial protein import machineries, in a manner which independent of its metabolite transport function. Yet, why this intimate link is required for the import of certain proteins is still unclear. Sakaue et al. show that the intermembrane space (IMS) proteins dependent on Mia40 favour the dimeric TOM complex over the trimeric. They suggest that loss of the Tom22 receptor makes the Tom40 channel more accessible to substrates that depend on disulphide bond formation for their folding. One key question that arises from this study is what sequence or surface determinants are specific to (or more accessible in) the dimeric TOM complex that the IMS proteins recognise to facilitate their import. The physiological significance of the cell cycle stage-dependent regulation of the TOM complex is also intriguing as this could act as a quality control mechanism to orchestrate the efficient import of all mitochondrial proteins via their preferred TOM complex. Even more surprisingly, the effect of Porin on protein import goes beyond the level of the outer membrane channel dynamics to optimise transfer of carrier precursors across the IMS. Both papers provide exciting data that pave the way for future studies to investigate the nuances that dictate how Porin regulates the oligomeric state of the TOM channel and how it physically aids the import of the metabolite carriers.
In broader terms, it will be interesting to investigate whether these unexpected roles of Porin are conserved in mammals as the TOM complex and protein import pathways are linked to human diseases.

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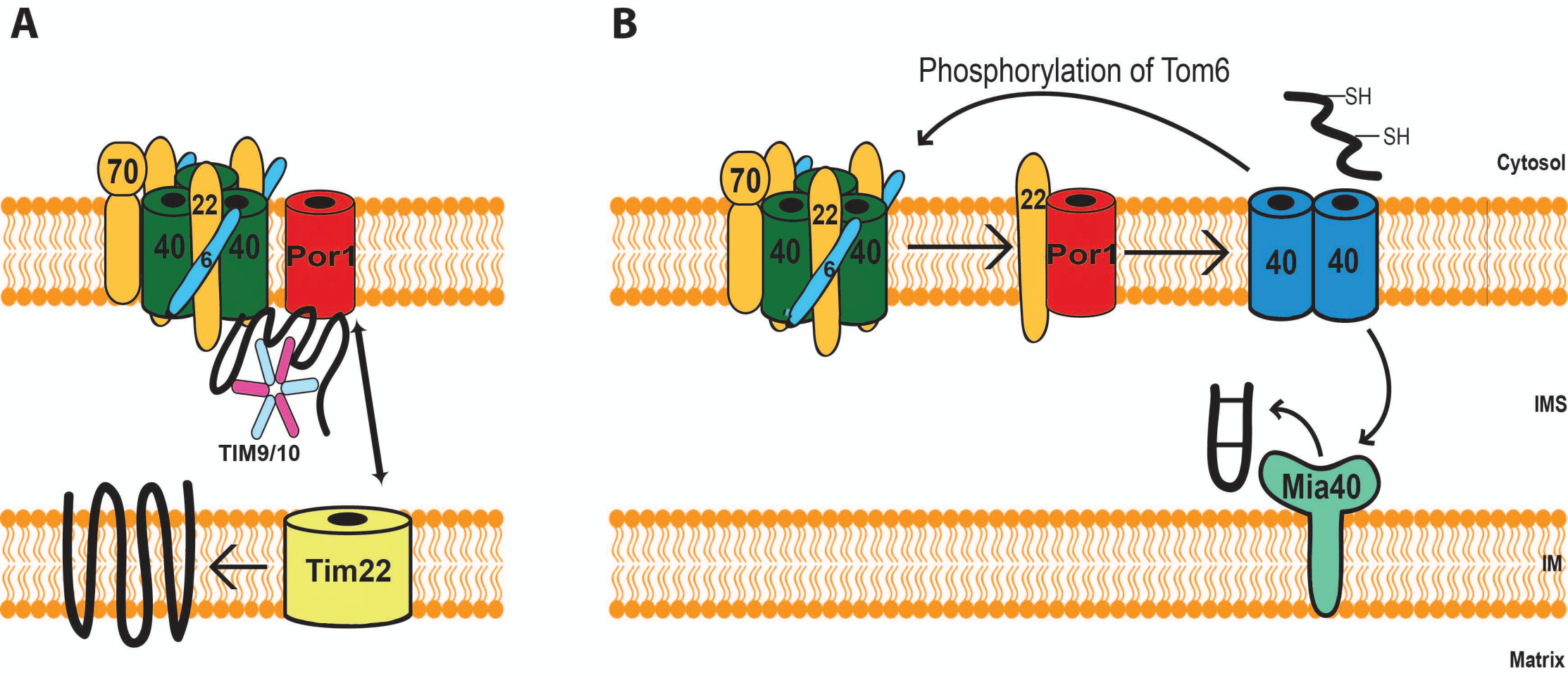
**Figure Legend: The Voltage dependent anion channel Porin plays a critical role in protein import in the yeast S. cerevisiae.** A: Porin facilitates the transfer of metabolite carriers across the intermembrane space (IMS) in the carrier import pathway. The effect of Porin is at the same stage of import as the small Tim chaperones (the Tim9 and Tim10 complex, shown in the IMS). The earlier stages of import of the carrier precursors, which occurs in the cytosol and in association with the TOM complex and in which Porin does not participate, are not shown for simplicity. B: Porin modulates the trimer-dimer transition of the TOM complex, by segregating unassembled Tom22 off the TIM trimer. This is a cell-cycle controlled process in which phosphorylation of Tom6 (light blue) also plays a role. The trimeric form of the

TOM complex is shown in green and the dimeric form in blue. Porin is depicted in red, Mia40 in green, Tom22, Tom70 in orange and Tim22 in yellow.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6
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