1 Review

Q3 The mitochondrial unselective channel in *Saccharomyces cerevisiae*

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6 **A R T I C L E   I N F O**

A B S T R A C T

Opening of the mitochondrial permeability transition (MPT) pore mediates the increase in the unselective permeability to ions and small molecules across the inner mitochondrial membrane. MPT results from the opening of channels of unknown identity in mitochondria from plants, animals and yeast. However, the effectors and conditions required for MPT to occur in different species are remarkably disparate. Here we critically review previous and recent findings concerning the mitochondrial unselective channel of the yeast *Saccharomyces cerevisiae* to determine if it can be considered a counterpart of the mammalian MPT pore.

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Q6 ## 1. Introduction

The endosymbiont model proposes that mitochondria originated from an α-proteobacteria that learned to live inside an eukaryotic ancestor (Gray et al., 1999). These endosymbionts became mitochondria once protein and metabolite carrier proteins were inserted in their outer and inner membranes so the host cell could manage mitochondrial protein content and exchange ions and molecules thus controlling aerobic metabolism (Cavalier-Smith, 2006). In mitochondria, oxidative phosphorylation can behave as a double edge sword since the highly efficient oxidative metabolism can produce toxic reactive oxygen species (ROS) at a high rate (Korshunov et al., 1997). ROS and calcium ions can alter molecules in the mitochondrial inner membrane thus affecting the permeability status of the mitochondrion (Lindsay et al., 2015). This permeability shift, also known as the mitochondrial permeability transition (MPT) can lead to organelle swelling, ATP depletion and cell death (for a recent perspective see Kwong and Molkentin, 2015).

In mammalian mitochondria, a pore allowing unselective traffic of solutes with a molecular exclusion cutoff around 1.5 kDa was reported (Haworth and Hunter, 1979) and termed the mitochondrial permeability transition (MPT) pore (for a review see Bernardi, 2013). In

Saccharomyces cerevisiae, three different groups detected a MPT albeit with differing characteristics (Guérin et al., 1994; Jung et al., 1997; Prieto et al., 1995). These groups hypothesized that this transition could be triggered by the opening of a channel termed either the yeast mitochondrial unselective channel ($_{sc}$MUC) or the yeast mitochondrial permeability transition pore (Manon et al., 1998). The pores in mammalian and *S. cerevisiae* mitochondria exhibit similar dimensions (Jung et al., 1997) and are regulated by pH and Mg^{2+} in a similar fashion (Guérin et al., 1994). However, key effectors such as cyclosporine A (CsA) and Ca^{2+} apparently lack effects on the $_{sc}$MUC (Jung et al., 1997). This has led to propose that the $_{sc}$MUC could be considered an inaccurate model for understanding MPT (Manon et al., 1998). Later hypotheses however propose that the MPT pore and the $_{sc}$MUC may be more similar than previously thought (Azzolin et al., 2010; Vianello et al., 2012). Evidence also shows that matrix Ca^{2+} can open a Ca^{2+}-release pore under appropriate experimental conditions (Carraro et al., 2014; Yamada et al., 2009). Furthermore, we recently found that $_{sc}$MUC is modulated by ubiquinone derivatives (Gutiérrez-Aguilar et al., 2014b). This family of compounds affects the MPT pore status downstream of cyclophilin D (CypD) regulatory site (Basso et al., 2005; Fontaine et al., 1998a,b). Thus one main difference between the MPT pore and the $_{sc}$MUC resides on CsA sensitivity. Although CsA potently desensitizes the MPT pore, $_{sc}$MUC is apparently not modulated by this undecapeptide. Here we examine known structural, regulatory and physiological features of the $_{sc}$MUC in order to determine whether it shares identity with the MPT pore.

2. Regulatory features of the $_{sc}$MUC

During the 90's, different groups studied channel-dependent mitochondrial unselective transport of molecules triggered by phosphate depletion, high respiratory rates and ATP/GDP addition in the baker's yeast (Guérin et al., 1994; Prieto et al., 1995). Although strain-dependent differences were found on the transported molecules, the consensus was that these permeabilities were unselective. More recently, Bradshaw and Pfeiffer (2013) have shown that the ATP synthase inhibitor oligomycin abolishes strain-dependent differences on $_{sc}$MUC activity. While the reasons underlying such result remain to be understood, the authors proposed that oligomycin could bind ATP synthase to induce opening of the $_{sc}$MUC mediated by high matrix space pH. Indeed, pioneering work by Velours et al. (1977) showed that low pH potently inhibited ultrastructural changes in isolated mitochondria that were associated with $_{sc}$MUC closure by Jung et al. (1997).

The $_{sc}$MUC and MPT pore are modulated through respiratory chain activity. While rotenone — an inhibitor of respiratory complex I — inhibits the MTP pore (Li et al., 2012), the ATP-driven $_{sc}$MUC can be suppressed with flavone by targeting the external NADH-dehydrogenase (Manon, 1999). In the case of the MPT pore, rotenone has been determined to be as potent as CsA to inhibit pore opening in tissues where CypD is less expressed. Conversely, the mechanism by which flavone inhibits the $_{sc}$MUC appears to be more related to the respiratory chain *per se* as titration with KCN can decrease pore activity (Manon, 1999). Thus, if the pore's core involves similar proteins in yeast and mammalian mitochondria, then respiratory Complex I could only be considered a MPT regulatory component, as this multi-subunit complex is remarkably absent in *S. cerevisiae* (Gutiérrez-Aguilar et al., 2014b). Indeed, Giorgio and colleagues have nicely demonstrated that Complex I does not form channels when reconstituted in lipid bilayers (Giorgio et al., 2013).

3. Relations between structure and function of the $_{sc}$MUC

In 1997, Jung and collaborators showed that $_{sc}$MUC and the MPT pore have comparable dimensions. Comparison between both pores relied on solute size exclusion experiments using polyethylene glycols of increasing molecular weight under isosmotic conditions. Such experiments revealed that the $_{sc}$MUC allowed trafficking of solutes with a MW lower than 1.5 kDa. Antithetically, the authors also showed that the swelling extent upon $_{sc}$MUC and MPT pore opening differed in magnitude potentially due to ultrastructural differences between yeast and mammalian mitochondria. This means that yeast mitochondria have relatively few cristae, thus limiting the $_{sc}$MUC-mediated increase in matrix volume and the resultant swelling profile measured with traditional methods.

One of the most known hallmarks of the MPT pore has been its modulation with selective ligands of the mitochondrial solute carrier family. In particular, it's modulation with bongkrekic acid, ADP and atractyloside. This has led several groups to propose that its protein target, the adenine nucleotide translocator (ANT) is the pore's core component (Halestrap et al., 1997). However, this hypothesis has been challenged through biochemical (Novgorodov et al., 1994) and genetic approaches *i.e.* in yeast and mice lacking ANT isoforms, MPT is still detected (Kokoszka et al., 2004; Roucou et al., 1997). This has led researchers to either modify their proposal, suggesting that the mitochondrial phosphate carrier is the actual pore-forming protein (Leung et al., 2008) or to propose that ANT ligands exert their inhibitory or stimulating function on MPT pore through inner-membrane surface potential modification (Di Lisa et al., 2011). The latter hypothesis seems more likely as yeast mitochondria completely lacking PiC and mammalian mitochondria where PiC levels were decreased through siRNA mediated protein knockdown, or tissue-specific PiC deletion still undergoes MPT (Gutiérrez-Aguilar et al., 2010, 2014a; Kwong et al., 2014; Varanyuwatana and Halestrap, 2011). Nonetheless, it is worth to mention that the pore detected in yeast presents differences when compared to its wild type counterpart. For instance, isolated yeast mitochondria from a PiC-deficient strain are resistant to mersalyl-induced, Pi-inhibited $_{sc}$MUC opening (Gutiérrez-Aguilar et al., 2010). Although we have proposed that PiC is a complement of the $_{sc}$MUC, Bradshaw and Pfeiffer (2013) have proposed that phosphate inhibits $_{sc}$MUC by binding a site on the matrix space side of the inner membrane in addition to its known effect on matrix pH (Bradshaw and Pfeiffer, 2013). Based on the article by Giorgio et al. (2013) entitled "Dimers of mitochondrial ATP synthase form the permeability transition pore", Bernardi and Di Lisa (2015) have proposed that such binding site could be ATP synthase.

As an epilogue for the "mitochondrial carrier hypothesis", it is now possible to conclude that mitochondrial solute carriers are dispensable for MPT, although some of these proteins do regulate pore opening (see Halestrap and Richardson, 2014). Evidence favoring interaction between these proteins has been reported for yeast, where VDAC, ANT and PiC can form a complex involved in the channeling of ADP/ATP (Clémençon, 2012), which are known to modulate $_{sc}$MUC activity (Uribe-Carvajal et al., 2011). However, although we previously favored the possibility that VDAC could at least modulate the pore under specific experimental conditions (Gutiérrez-Aguilar et al., 2007), VDAC has also been largely dismissed as part of the $_{sc}$MUC/MPT pore componentry (Baines et al., 2007; Krauskopf et al., 2006; Roucou et al., 1997). In mammalian mitochondria, PiC and ANT were proposed to interact with ATP synthase among other proteins (Ko et al., 2003). But some studies have failed to detect such structure in yeast mitochondria (Couoh-Cardel et al., 2010).

4. The Ca^{2+}-induced permeability transition in *S. cerevisiae*

The $_{sc}$MUC has been considered unrelated to the mammalian MPT (Halestrap, 2010). The reasons underlying such view are tangible: This transition is inhibited by Pi whereas Ca^{2+} only activates MPT in the presence of selective ionophores (Carraro et al., 2014). A closer look at these differences may be explained in evolutionary terms. *S. cerevisiae* mitochondria lack a mitochondrial Ca^{2+} uniporter (MCU) (Uribe et al., 1992). This characteristic helped to determine the identity of the core component of the uniporter complex by ruling out MCU protein