The c ring of the F1Fo ATP synthase forms the mitochondrial permeability transition pore: a critical appraisal

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A commentary on

An uncoupling channel within the c-subunit ring of the F1Fo ATP synthase is the mitochondrial permeability transition pore

by Alavian KN, Beutner G, Lazrove E, Sacchetti S, Park HA, Licznerski P, Li H, Nabili P, Hockensmith K, Graham M, Porter GA Jr., Jonasa EA. Proc Natl Acad Sci U S A (2014) 111:10580–5. doi:10.1073/pnas.1401591111

The mitochondrial permeability transition pore (MPTP) is a non-specific pore in the inner mitochondrial membrane (IMM) whose opening is triggered by high matrix [Ca\(^{2+}\)] to which it is sensitized by [Pi] and oxidative stress. MPTP opening plays a critical role in necrotic cell death such as in cardiac ischemia/reperfusion (I/R) injury and the action of cytotoxic drugs. Indeed MPTP inhibition with cyclosporine A (CsA) protects tissues from I/R injury and the action of cytotoxic drugs. Nevertheless, CsA sensitizes the IMM to MPTP opening but that another IMM protein must also be involved. Several recent papers suggest that this may be the F1Fo ATP synthase.

CyP-D was shown to bind to the F1Fo ATP synthase and modulate its hydrolytic activity (13, 14) and in 2013, the laboratories of both Bernardi (15) and Pinton (16) presented data that implicated the ATP synthase in MPTP formation. Bernardi and colleagues (15) detected Ca\(^{2+}\)-activated channels, similar to the MPTP, in phospholipid bilayers containing reconstituted dimers of mammalian F1Fo ATP synthase. Similar channel activity was demonstrated in yeast mitochondria and this was strongly attenuated in mutants lacking the ε and γ subunits needed for ATP synthase dimer formation (17). However, high levels (0.3 mM) of Ca\(^{2+}\) were required for channel opening which, unlike MPTP opening, also required Bz-423. No data were presented on the effects of oxidative stress, CsA, or reconstituted F1Fo ATP synthase. Furthermore, Pinton and colleagues (18) pointed out that Bernardi’s laboratory had previously demonstrated MPTP opening in p\(^0\) cells, which lack the mitochondrial DNA encoding the α and A6L subunits of the ATP synthase. In addition, the ATPase inhibitor protein F1, which promotes ATP synthase dimerization, attenuates rather than promotes MPTP opening, and enhances cell survival under ischemic conditions (19). Rather, Pinton and colleagues (16) implicated the c-subunits of the Fo ATPase in MPTP formation, showing that their knockdown reduced MPTP opening in response to ionomycin or hydrogen peroxide and their over-expression enhanced opening. The c-subunits form a ring structure in the IMM, and so represent an attractive candidate for forming the MPTP, but direct evidence for this was not provided. However, the paper of Alavian et al. (20) claims to do this.

Alavian et al. (20) confirmed the observations of Bonora et al. (16), but they also reconstituted the purified c-subunit into proteoliposomes and demonstrated channel activity. Most channels conducted at ~100-pS but a few did so at 1.5–2 nS, similar to the MPTP conductance (21). However, the channels were insensitive to Ca\(^{2+}\) and CsA and were only inhibited by much higher concentrations of ATP and ADP than required to inhibit MPTP opening. The authors proposed that other F1Fo ATP synthase components are needed for MPTP regulation, which they investigated using purified monomeric F1Fo ATP synthase reconstituted into proteoliposomes. Some infrequent channel activity was observed, which was increased by addition of CyP-D and further by 100 μM Ca\(^{2+}\). These effects were prevented by 5 μM CsA, a concentration much higher than the K\(_i\) for CyP-D (2 nM). Channels sensitive to

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both Ca\(^{2+}\) and CsA could also be detected in patches from sub-mitochondrial vesicles (SMVs) enriched in F1Fo ATP synthase or IMMs and these were absent when OSCP and β subunits of the ATP synthase and bound CyP-D were removed by urea treatment, while adding back purified β subunit to reconstituted c-subunits largely abolished channel activity. However, the authors did not address whether the IMM and SMV preparations also contained ANT and PIC, which is very likely. Rather, they concluded that the sites through which Ca\(^{2+}\), ADP, and CyP-D (and thus CsA) modulate channel activity are on the Fi domain of the ATP synthase whose association with the c-subunit ring may loosen upon Ca\(^{2+}\) and CyP-D binding. This might cause expansion of the ring converting it into a high conductance channel and, using fluorescent probes, they presented evidence consistent with the c-subunits moving apart during MPTP opening. Furthermore, channel activity was greatly enhanced when glycine residues in the c-subunit transmembrane domains were replaced with valines, thus moving the packed helices further apart. However, interpretation of these data is difficult because the expressed c-subunits ran at 15 kDa on SDS-PAGE and not 7.6 kDa, the size of the mature c subunit, suggesting that the mitochondrial targeting sequence had not been removed. Indeed several studies in this paper and that of Bonora et al. (16) showed expression of 15 kDa unprocessed protein rather than the true c subunit.

In summary, when the evidence for an involvement of the F1Fo ATP synthase and more specifically its c-subunit in MPTP formation is reviewed critically, it is legitimate to conclude that it is no better than that for the involvement of the ANT and PIC. Perhaps the truth lies in a synthesis, and that an interaction between the ANT, PIC, and F1Fo ATP synthase in the ATP synthasome (22) is critical for MPTP formation, as we (2) and subsequently others (4, 18) have concluded. A scheme illustrating how the different components may interact is presented in Figure 1.

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FIGURE 1 | A hypothetical model of the MPTP that proposes an interaction between the ANT, PIC, and F1Fo ATP synthase in the ATP synthasome. It is proposed that the MPTP forms at the interface between interacting domains of the PIC, ANT, and Fo ATP synthase following calcium triggered conformational changes facilitated by the PPlase activity of CyP-D. Note that potential regulatory interactions of the MPTP with outer membrane components are not shown.
The c-ring as the MPTP

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