What makes you can also break you, part III: mitochondrial permeability transition pore formation by an uncoupling channel within the c-subunit ring of the F1FO ATP synthase?

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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, Satchetti 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(29):10580–5. doi:10.1073/pnas.1401591111

The c ring of the F1FOATP synthase forms the mitochondrial permeability transition pore: a critical appraisal

by Halestrap AP. Front Oncol (2014) 4:234. doi:10.3389/fonc.2014.00234

In the hunt for identifying the structural elements of the mitochondrial transition pore, the recent paper by Alavian et al. proposes that the pore is formed by an uncoupling channel within the c-subunit ring of the F1FO ATP-synthase complex (1), reaffirming previous results (2, 3) and providing exciting additional details about the potential molecular mechanism controlling pore formation.

The paper compellingly demonstrates that purified reconstituted c-subunit rings in liposomes exhibit channel activity with multiple subconductance states that are sensitive to inhibition by adenine nucleotides, recombinant F1 beta-subunit protein, and anti-c-subunit antibodies. Channel activity matched that of the mitochondrial megachannel, now known as being the permeability transition pore (PTP) (4). Moreover, by fluorescently labeling c-subunit rings in living cells, they were able to monitor the opening and closing of the ring in response to Ca\(^{2+}\) and the PTP inhibitor, cyclosporin A. By introducing site-specific mutagenesis of highly conserved glycines within the N-terminus of the \(\alpha\)-helical region of the c subunit, which are responsible for the tight packing of the ring, thus making it looser, they achieved an increase in channel conductance by an order of magnitude, accompanied by loss of sensitivity to ATP. Finally, they propose that the physical uncoupling of F1 from F0 triggers the increase in c-subunit pore conductance, providing a mechanism for induction of the permeability transition.

The papers by Alavian et al. (1) and Bonora et al. (2) provide strong evidence that the c-subunit current was extremely sensitive to inhibition by Ca\(^{2+}\) (Figure 1A), demonstrating the closure of subunit c pores due to the cooperative effect of at least four Ca\(^{2+}\) ions per ring (7). In contrast, Alavian et al. showed that the current mediated by reconstituted c subunits in lipid bilayers is not sensitive to Ca\(^{2+}\), and it is induced by Ca\(^{2+}\) when measured in submitochondrial vesicles (1), as expected by the PTP (9). McGeoch proposed that the Ca\(^{2+}\) ions mediate the closure of the ring by binding on sites in the lumen of the channel, which forms a pore with a hauntingly similar size to what has been proposed for the PTP (2–3 nm) (Figure 1C). Indeed, when presented with a water surface, c rings change their hydrogen bonding from an \(\alpha\)-helix to \(\beta\)-sheet-like configuration and move away from previous associations
adenine nucleotides as well as the Ca$^{2+}$ out, the high-affinity site(s) of the PTP for regulators. Indeed, as the authors pointed out, exposing diverse binding sites for these complexes using different sources and protocols, only the reconstituted c ring in proteoliposomes (1) and in mitochondria or the megachannel in mitoplasts, patch-clamping of the c ring in "symmetrical" solution (an experimental condition in which the solutions across the two sides of the patched membrane contain equal concentrations of the major permeant ions) should yield a current-voltage relationship similar to that depicted in Figure 1D. Briefly, the magnitude of the absolute current through the c ring would increase as the voltage became less negative; when the voltage would closely approach the "0" value (intersect), the current would also tend to become zero, simply due to the symmetrical solution. The magnitude of the current at positive potentials (gray circles) cannot be predicted because mitochondria cannot achieve a positive value of ΔΨm. However, the electrophysiological signatures of the reconstituted c ring shown in Ref. (1) and (7) and of the megachannel reviewed in Ref. (13) are far from the expected current-voltage plot shown in Figure 1D. Altogether, the discrepancy of findings regarding voltage dependence along with Ca$^{2+}$ and nucleotide sensitivity argue that the reconstituted c ring in proteoliposomes, devoid of all regulatory and other modules, is bound not to exhibit the same properties as the PTP. Likewise, the primary current

![Figure 1](image_url)
through the subunit c pore reported by McGeoch was cationic, with anionic current not being observed (5), while the mitochondrial megachannel exhibited low anion selectivity with rare switches to cation selectivity (13); Alavian et al. did not investigate anion selectivity. In our opinion, it is difficult to imagine cation vs. anion selectivity with rare switches to mitochondrial megachannel exhibited low selectivity for a 2–3 nm diameter channel, which is known to be non-selective for solutes with a molecular weight of up to 1,500 Da (4) and maximum open probability near 0 mV (13). To the above divergence of findings, the conflicting effect of \( N,N',N'' \)-dicyclohexylcarbodiimide (DCCD) can also be added; DCCD is known to interact with subunit c and inhibit the entire operation of the \( F_0 \) subunit c pore-synthese complex (14); accordingly, DCCD was found to completely inhibit the conductance of the c-subunit channel (7), but on the other hand, DCCD is an atypical inducer of the permeability transition (15).

In the same way, the apparent complexity of the molecular entities embedding the actual pore also forecast the difficulties to study the behavior of permeability transition in living cells and tissues. Here, again the authors excel to demonstrate pore opening using classical inducers such as \( \text{Ca}^{2+} \) ionophores and pro-oxidants, but in our experience, it is excruciatingly difficult to generate reproducible data in many cellular models using these tools. Thus, a further important advance of this study is paving the way for novel genetic approaches (e.g., expressing constitutively open mutant pores, or silencing subunits) for live cell/organism studies. In this respect, an important claim of the present model is the uncoupling of the \( F_1 \) and \( F_0 \) subunits as the main trigger of pore opening, which should be amenable to interventions increasing or decreasing the sensitivity of the pore to pathophysiological stimuli. As a last point, it is also important to realize that the fundamental regulatory modules of the PTP (such as the voltage sensor, \( \text{Ca}^{2+} \), and nucleotide-binding sites) are yet to be elucidated; they may turn out to be targets more amenable to pharmacological or genetic manipulations for combating diseases in which the PTP is known to have a role.

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