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Review

Mitochondrial F-ATP synthase as the permeability transition pore

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ABSTRACT

The current state of research on the mitochondrial permeability transition pore (PTP) can be described in terms of three major problems: molecular identity, atomic structure and gating mechanism. In this review these three problems are discussed in the light of recent findings with special emphasis on the discovery that the PTP is mitochondrial F-ATP synthase (mtF_oF_1). Novel features of the mitochondrial F-ATP synthase emerging from the success of single particle cryo electron microscopy (cryo-EM) to determine F-ATP synthase structures are surveyed along with their possible involvement in pore formation. Also, current findings from the gap junction field concerning the involvement of lipids in channel closure are examined. Finally, an earlier proposal denoted as the 'Death Finger' is discussed as a working model for PTP gating.

1. Introduction

The mitochondria of mammalian cells can undergo a permeability transition (PT) of the inner mitochondrial membrane (IMM) caused by the prolonged opening of the permeability transition pore (PTP) [1–3], also named after its electrophysiological equivalent, the mitochondrial megachannel (MMC) [4–9]. Opening of the PTP is elicited by matrix Ca^{2+} and stimulated by binding of cyclophilin D (CyPD), P_i, elevated concentration of reactive oxygen species (ROS), free fatty acids or a diminished transmembrane potential. Nucleotides, Mg^{2+} and the binding of CsA are known molecular inhibitors of PTP opening, whereas arginine-specific adducts of phenylglyoxal modulate PTP opening in a species and net-charge dependent manner. Short, reversible openings of the PTP are thought to be involved in Ca^{2+} homeostasis and long, irreversible openings lead to non-selective efflux of matrix solutes up to a size of 1.5 kDa and a concomitant influx of water into the matrix resulting in mitochondrial swelling, remodeling of cristae architecture and the release of pro-apoptotic factors such as cytochrome c [10].

Mitochondrial permeability transition regulated cell death plays a critical role in a whole range of pathophysiological phenomena such as cardiac ischemia and reperfusion, muscle dystrophy and neurological disorders [11]. Despite the physiological importance of the PTP and a rich body of literature on its electrophysiological and pharmacological characteristics at the mitochondrial level, the field still faces three crucial interlinked problems: What is the identity of the PTP? What is its structure? What is its gating mechanism?

In this short review I will describe the current state of these three problems and attempt to give some suggestions on how to move forward. A standard procedure to probe the electrophysiological properties of a membrane protein is to express it in Xenopus oocytes by injecting its cDNA and then performing whole cell patch-clamp recordings of the oocytes [12]. By such approaches to identify which protein underlies the well characterized currents recorded from patch-clamped mitoplasts may seem straightforward. However, oocyte expression of multisubunit membrane proteins of the inner mitochondrial membrane, like for example the F-ATP synthase, is currently not feasible. Despite these experimental problems in using standard procedures to identify multisubunit membrane proteins as the molecular origin of transmembrane currents, several studies in recent years using in situ or in vitro approaches came to the surprising conclusion that the

Abbreviations: mtF_oF_1, mitchondrial F-ATP synthase; IMM, inner mitochondrial membrane; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BN-PAGE, blue native polyacrylamide gel electrophoresis; CN-PAGE, clear native polyacrylamide gel electrophoresis; F_1, factor 1; F_o, oligomycin sensitive factor; OSCP, oligomycin sensitivity conferring protein; PT, permeability transition; PTP, permeability transition pore; PT, mitochondrial permeability transition; pmf, proton motive force; MMC, mitochondrial mega channel; kDa, 1000 Dalton; Å, 0.1 nano meter; nS, nano Siemens; pS, pico Siemens; ROS, reactive oxygen species; P_i, inorganic phosphate solubilized in water; pH, negative decadic logarithm of the proton concentration; CyPD, mitochondrial cyclophilin D; CsA, cyclosporin A; PhAsO, phenylarsine oxide; Bz-423, benzodiazepin-423; ANT, mitochondrial adenine nucleotide translocator; VDAC, voltage-dependent anion channel of the outer mitochondrial membrane; DAPIT, diabetes sensitive-protein in insulin-sensitive tissue; 6.8PL, 6.8 kDa proteolipid; PS, peripheral stalk; CS, central stalk; EM, electron microscopy; cryo-EM, cryogenic electron microscopy; cryo-ET, cryogenic electron tomography; SPA, single particle analysis; AFM, atomic force microscopy; SUV, small unilamellar vesicle; GUV, giant unilamellar vesicle; GPCRs, G protein-coupled receptors; GraDeR, gradient-based detergent removal; LMNG, lauryl-maltose-neopentyl glycol; ATP, adenosine triphosphate; ADP, adenosine diphosphate

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mitochondrial F-ATP synthase is the molecular identity of the PTP [13].

Mammalian multisubunit membrane proteins like the F-ATP synthase are often recalcitrant to 3D crystallization and as a consequence do not yield to structure determination by X-ray crystallography [14]. Although single particle analysis (SPA) cryo-EM has matured into a powerful technique in structural biology of membrane proteins [15–17], the high resolution (> 3 Å) structural analysis of a complete mammalian F-ATP synthase has yet to be achieved.

Finally, even though reliable atomic models built on the basis of high resolution density maps are an essential prerequisite for arriving at realistic models of channel gating, possible involvement of lipids or transmembrane potential (electric or chemical) imply that structural information alone is insufficient. That is, structures obtained from detergent stabilized channels, even if solved to high resolution, may not reveal the structural basis of channel gating.

2. Identity: mtF_oF_1 as PTP

F-ATP synthase is a membrane bound nano-machine that interconverts the electrochemical energy of a transmembrane proton motive force (Δpmf) and the chemical energy in the form of ATP via mechanical rotation [18]. Its division into a chemical energy driven rotary motor and an electrochemical energy driven rotary motor is reflected by its bipartite structure of the water soluble F_1 domain and the transmembrane F_o domain which are mechanically coupled via a central stalk and held together by a peripheral stalk [19]. The F_1 domain's catalytic part, either synthesizing or hydrolyzing ATP, is a hexamer of alternating α-subunits and β-subunits with the central stalk positioned in its center [20]. The F_o domain harbors the central stalk bound, transmembrane proton transporting c-ring, whose random rotary Brownian motion is biased by the Δpmf dependent protonation probability in the two half-channels of the c-ring's adjacent a-subunit [21]. In which direction energy conversion takes place is dependent on the energy balance of ATP versus Δpmf [22]. To avoid futile ATP depletion in the absence of Δpmf, in mitochondria the natural inhibitor protein IF1 senses matrix pH and inhibits ATP hydrolysis powered proton pumping across the inner mitochondrial membrane by its insertion into the matrix protruding F_1 domain [23]. In contrast to monomeric chloroplast F-ATP synthase [24], mitochondrial F-ATP synthase is dimeric and organized in oligomeric rows of dimers along regions of positive curvature of the cristae membrane [25]. The biological meaning of F-ATP synthase oligomerization is under debate with ideas ranging from space saving membrane architecture [26] over the improvement of proton availability [27,28] and the control of PTP opening probability [126].

CyPD, a peptidylprolyl *cis-trans* isomerase, is a *bona fide* binding partner of the PTP on the matrix side of the inner mitochondrial membrane [29]. Therefore, binding studies on CyPD suggesting interaction of CyPD with the OSCP subunit of the mitochondrial F-ATP synthase prompted experiments probing the electrophysiological properties of bovine F-ATP synthase [30]. Gel extractions eluted from excised band of blue native PAGE gels corresponding to ATP hydrolase activity yielded currents and pharmacological behavior equivalent to the mitochondrial megachannel (MMC) after their addition to planar lipid bilayers [31]. Since excised gel bands corresponding to a molecular weight equivalent to F-ATP synthase monomers did not yield any currents, it was also concluded that the minimal PTP forming unit is the mitochondrial F-ATP synthase dimer. These conclusions were however limited by the presence of an unknown number and amount of contaminating proteins as well as by the unknown physiological relevance and oligomeric state of the F-ATP synthase after gel elution and planar lipid bilayer reconstitution.

An effort to confirm or refute the proposal of mitochondrial F-ATP synthase (mtF_oF_1) as PTP was undertaken by employing genetic ablation of single subunits and the subsequent monitoring of permeability transition by using a mitochondrial swelling assay [32–34]. Persistent swelling of mitochondria from cells with vestigal F-ATP synthase led to the conclusion that the PTP is not formed by the mitochondrial F-ATP synthase. In the mitochondrial swelling assay mitochondria are subjected to pulses of Ca^{2+} until swelling of mitochondria is observed via a decrease in light scattering of mitochondria or of lysated cells. However, the central role of F-ATP synthase in the energetic state of mitochondria as well as the known drastic alteration of cristae architecture upon genetic ablation of F-ATP synthase subunits left the biological interpretation of these results open to debate [35,36]. Also the presence of a multitude of channels in the inner mitochondrial membrane unrelated to the PTP suggested a possible explanation of persistent mitochondrial swelling in the absence of a functional PTP [37–39]. Thus in contrast to electrophysiology, the mitochondrial swelling assay can not easily distinguish between different types of channels. Indeed, even though cells lacking a fully assembled F-ATP synthase showed persistent mitochondrial swelling, patch-clamping of their mitoplasts failed to detect typical MMC currents, indicating the observed swelling to be caused by the opening of other channel forming proteins of the inner mitochondrial membrane [40]. The use of an experimental set-up dependent on a reporter system that is sensitive to the manipulation of the subject of investigation complicates decisive interpretation.

The F-ATP synthase proton transporting c-ring is the structurally best characterized transmembrane domain of this multisubunit complex with several high resolution crystal structures described [41–48]. Its prominent hourglass shape is known to be blocked by lipids [49–51], although a recent cryo-EM study of mammalian F-ATP synthase tetramers posited protein to occupy the c_8-ring's lumen [52]. The c-ring's shape obviously lends to the idea of a pore function and despite well documented lipid occupancy of rotor rings lumena, the proposed evolutionary origin of rotor rings as DNA and peptide transporting pores supports the concept of c-rings as a conductive transmembrane protein assembly [53]. Indeed, mutations of the c-ring GxxG motifs, responsible for a tight, stabilizing packing of the hairpin c-subunits, altered PTP behavior [54,55] in HEK cells. However, given the central role of F-ATP synthase in both mitochondrial energetics and cristae architecture, the direct role of c-rings in mitochondrial permeability transition is up to debate.

Point mutations in subunits of the F-ATP synthase that strongly affect well characterized electrophysiological behavior of the MMC in patch clamped mitoplasts, while affecting neither the energetics nor the architecture of mitochondrial cristae, present a convincing tool for the identification of the protein underlying the MMC/PTP. Given the very large number of amino acids in the mammalian ATP synthase monomer (> 5000) this approach appears impractical. Notwithstanding, this strategy was pursued for the known effects of Ca^{2+} [8,9], pH [56], glyoxal adducts [57–59] on the PTP. The OSCP subunit is situated on the top of the β-barrel crown of the into the matrix protruding F_1 domain, with its hinge region connecting the peripheral stalk subunits b and F_6 with F_1 [60,61]. Mutation of a highly conserved histidine in this hinge region of OSCP in HEK cells to a glutamine completely abolished the blocking of MMC currents as recorded by patch-clamped mitoplasts at acidic pH (pH 6.5) but not at a slightly basic control pH (pH 7.3) [62]. Importantly the mutation did not affect the cells respiratory rate, indicating that side-effects not connected to the PTP were not responsible for the lack of PTP inhibition by acidic pH.

Likewise, the replacement of the highly conserved threonine 163 of the β-subunit of the catalytic site in HeLa cells engaged in either ATP synthase or ATP hydrolysis changed the Ca^{2+} concentration dependent induction of PTP opening as measured by Ca^{2+} retention assay [63], findings in line with the notion of the catalytic site of the F-ATP synthase being the Ca^{2+} sensing site of the PTP.

From studies on the influence of glyoxals on PTP behavior it was known that the PTP should posses an intercristae side reactive arginine that can form glyoxal adducts tuning the probability of PTP opening in a species dependent manner; higher for mammals, lower for yeast. By

reasoning from an observed set of conserved arginines combined with mutant screening using electrophysiology of patch clamped mitoplasts, arginine 107 of subunit g was found to be the glyoxals reactive residue [64]. Its location in the rotor ring distal, membrane bending and likely flexible domain of the b-e-g unit [65] gives a rational for how this arginine conveys sensitivity to changes in membrane potential. These observations were consistent with those of the conserved arginines in the voltage sensors of potassium channels [66–68]. Furthermore, in the same study the species-dependent influence on PTP opening probabilty could be engineered by replacing the g-subunit of yeast F-ATP synthase with its human counterpart. This feat gave strong support to the conclusion that it is indeed arginine 107 of the F-ATP synthase subunit g that conveys the sensitivity of the PTP to glyoxal adducts.

Cells and even single mitochondria are highly complex interdependent systems where the cause-effect relation of experimental input and measured output is not trivial, often leaving vast amounts of doubt to interpretational conclusions. Therefore, the swelling of mitochondria or the decrease of transmembrane potential across the inner mitochondrial membrane can easily be the consequence of PTP unrelated events. Thus, for genetic studies on the PTP, ideally by point mutations, the full preservation of house keeping functionality of mitochondria in general, and the F-ATP synthase in particular, is key. With the PTP being a non-selective membrane ion channel and with the gold standard method in ion channel research of electrophysiology being capable of very rapid quantitative measurements at the timescale of thermal motion, and a very good signal to noise ratio, it is electrophysiology that should be the main method used in the detection and molecular characterization of the PTP channel function.

Research on mammalian F-ATP synthase has a long and rich history of more than 60 years, starting with seminal papers in the 1960s [69,70]. Great advances in the understanding of F-ATP synthase at the molecular level were mainly achieved with well designed in vitro experiments on extracted and purified F-ATP synthase, yielding conclusive evidence for the proton motive force as the driving force of ATP synthesis [71] and mechanical rotation as its mode of catalysis [72]. Unfortunately, these conclusive in vitro experimental breakthroughs were mostly achieved using very stable F-ATP synthase of thermophilic origin, though note is taken on recent progress for the human F_1 subcomplex [73]. Thus, even though many pioneering studies on F-ATP synthase were conducted on mammalian F-ATP synthase, their fragility in conventional detergents and also its oligomeric assembly state prevented success in the establishment of a well working isolation procedure for intact mammalian F-ATP synthase. The field has yet to establish a protocol which allows the isolation of oligomeric pure, stable, intact and fully active mammalian F-ATP synthase at high yield and concentration for the efficient performance of in vitro experiments at ease. Such a protocol will be an important prerequisite for the advancement of the field. Novel detergent tools for solubilization and stabilization originally developed for the investigation of G protein-coupled receptors (GPCRs) give hope that the situation might improve soon [74,75].

Put in a different way, in vitro experiments on the question of mammalian F-ATP synthase being the molecular identity of the PTP suffer from three distinct problems. First and foremost, is the lack of purity of the preparation in respect to the presence of contaminating proteins or pure oligomeric state, i.e. monomer, dimer, tetramer or higher oligomer. Next is the issue of stability of this multisubunit assembly outside its physiological environment of the inner mitochondrial membrane. Finally, is the question if functionality is fully retained after isolation from mitochondria and reconstitution into an in vitro membrane system, i.e. whether rotational catalysis in the form of ATP hydrolysis, proton pumping and ATP synthesis from ADP + P_i is not impaired.

To clarify if the molecular identity of the PTP is identical with mitochondrial F-ATP synthase we undertook an in vitro study employing purified bovine F-ATP synthase and planar lipid bilayer electrophysiology [76]. In order to arrive at a conclusive answer to the question of whether mammalian F-ATP synthase alone is the molecular identity of the mitochondrial PTP/MMC, we tried to overcome the above described problems associated with in vitro experiments of mammalian F-ATP synthase. For alleviating the problem of contamination by other membrane proteins of the inner mitochondrial membrane, we employed a large scale purification scheme that uses 1.2 kg of bovine heart muscle tissue to obtain 20 mg of highly pure F-ATP synthase with only a minimal amount (< 1%) of contaminating proteins, a level of contamination that is not visible in conventional SDS-PAGE gels or in micrographs from negative stain electron microscopy [76–80]. By using the very mild, lipid like detergent LMNG [74], it was ensured that the easily dissociated subunits 6.8PL and DAPIT were retained, laying testimony to the integrity of the preparation used in these in vitro experiments.

Even though the employed large scale purification exhibited only a very low level of contamination, other integral membrane proteins with known channel activity, namely, adenine nucleotide translocase (ANT) and the voltage-dependent anion channel (VDAC), were detected by quantitative mass spectrometry [76]. Persistence of MMC like currents in the presence of the established inhibitors of ANT and VDAC channel activity (Königs Polyanion and bongkrekic acid) ruled out these two proteins as a cause of the recorded currents. However, even though the influence of ANT and VDAC could be dismissed and further confidence comes from very strong statistics of the number of conducted electrophysiological recordings in this study (n > 100), yet, the effect of an unknown trace contaminant could not be completely ruled out.

Here, a novel, GraDeR based [80] automembrane reconstitution approach provided a means for clarification. In the presence of very low amounts of lauryl maltose-neopentyl glycol (LMNG) detergent, proteoliposomes densely packed with F-ATP synthase were readily formed [76,81]. Bulk solution measurements of ATP hydrolysis mediated proton pumping into these proteoliposomes demonstrated a very high level of coupling in the liposome reconstituted F-ATP synthases and extraordinary tightness to protons allowing long duration measurements. The observed generation of proton gradients in bulk after the addition of ATP·Mg^{2+} can only be explained by the activity of bovine F-ATP synthase and not by minor contaminants. Similarly, complete breakdown of these proton gradients across the membrane of the proteoliposomes in bulk after addition of PTP inducers (Ca^{2+}, Bz-423 and PhAsO) can not be explained by minor contaminants and has to be attributed to the bovine F-ATP synthase alone. The point made here is that the ability of electrophysiology to detect single channel activity is also its Achilles' heel. Recording a minor contaminant's activity instead of that of the target protein in the preparation is possible. However, by measuring in bulk the known effectors (ATP and PTP inducers) caused changes in a transmembrane property (proton gradient) the role of contaminants as the cause behind the measured changes can be ruled out. This is a clear advantage of bulk solution measurements using well prepared, proton tight proteoliposomes. This important experiment allowed us to state with high confidence that mitochondrial F-ATP synthase is the molecular identity of the PTP/MMC [76].

The oligomeric state of channel activity exhibiting F-ATP synthase, however, was not established in these experiments and thus the question of monomer, dimer or oligomer as the active agent was not answered. Native PAGE is an excellent tool for the separation of the mitochondrial F-ATP synthase's oligomeric states [82,83] and was instrumental in the discovery and acceptance of mitochondrial F-ATP synthase being an oligomer of dimers [84,85]. Nevertheless, the same pioneers on the use of Blue Native PAGE and later Clear Native PAGE for the study of mitochondrial F-ATP synthase also showed the detrimental effects the electrophoresis itself and the relative amount and type of used detergent can have on this fragile supercomplex [127]. Therefore, while raising confidence in the absence of contaminants, the electric currents of F-ATP synthase purified by native PAGE into separate oligomeric states, gel eluted and then reconstituted into planar

lipid bilayers do not allow strong conclusions on the oligomeric, biochemical or structural state of the F-ATP synthase complexes underlying the measured currents. The absence of currents from sample eluted from monomer bands could easily be explained by the loss of contact between subunit e and the c-ring or the dissociation of the very labile subunits DAPIT and 6.8 PL. Mass spectrometry on gel eluted monomers might shed some light on this question.

A separate study employed strictly monomeric porcine F-ATP synthase that was column purified using the detergent dodecyl-maltoside (DDM) and subsequently reconstituted into small unilamellar vesicles (SUVs) for structural characterization by single particle cryo-EM [86]. The resulting density map clearly showed the reconstituted F-ATP synthase to be monomeric. Fusion of the monomeric F-ATP synthase SUVs with giant unilamellar vesicles (GUVs) and subsequent electrophysiological recordings by patch-clamp technique allowed detection of currents of a magnitude characteristic for the PTP/MMC. The use of DDM for the extraction and isolation of mammalian F-ATP synthase is known to dissociate the small transmembrane subunits DAPIT and 6.8 PL from the complex [127], so the loss of DAPIT and the somewhat weak detection of subunit e and g is not surprising. The recordings of PTP/MMC like currents in the absence of Ca^{2+} and the blocking of those currents by oligomycin, however, is surprising. For the detection of PTP/MMC currents in patch-clamped mitoplasts the presence of Ca^{2+} is obligatory [10] and the use of oligomycin in previous studies of isolated mitochondria did not impede PTP opening [87,88]. As argued in a recent review, regulated PTP opening possibly relies on the interaction of subunit e with other mitochondrial specific transmembrane subunits of neighboring complexes in the structural context of dimeric or oligomeric F-ATP synthase [13]. Here it is of interest, that in the yeast F_o dimer structure subunit k of one F_o domain is in contact with subunit e of the other F_o domain [65]. Thus the PTP/MMC currents detected by patch-clamped GUVs of monomeric F-ATP synthase might stem from an unregulated conformation of the pore. The concentration of oligomycin used in this study was five times higher than in previous studies on isolated mitochondria [87]. Taking into account that the concentration of protein in the patch-clamped GUVs is presumably significantly lower than that of patch-clamped mitoplasts, the amount of oligomycin per F-ATP synthase complex was likely significantly higher. In a crystallographic study of the yeast F-ATP synthase c-ring complexed with oligomycin it was shown that oligomycin binds tightly to the central transmembrane outer face of the c-ring and does stabilize its structure in the crystal [89]. Therefore, the effect of channel blockage by oligomycin might origin from its ability at high concentration (at least 10 times the number of c-sunits used in the experimental set-up) to stabilize the orthodox architecture of the c-ring. The greatest value of the monomer-SUV-GUV study [86] lies with the experimental strategy of combining structural characterization of SUVs reconstituted F-ATP synthase with current recordings by patch-clamp electrophysiology. If structure analysis by single particle analysis cryo-EM of SUVs can be brought to the α-helical level, doubts on subunit composition and physiological relevance of the reconstituted complex will vanish. This presents a fruitful future avenue of PTP/MMC in vitro studies with conclusive structure-function interpretation. At this stage, however, the question, if monomeric F-ATP synthase is truly sufficient to exhibit all electrophysiological characteristics of the in mitoplasts measured PTP/MMC is still unresolved and will have to await a purification method that does not harm the complex in any aspect.

3. Structure: mtF_oF_1 as PTP

It is desirable to know the atomic structures of channel forming membrane proteins, because they allow us to rationalize the properties measured for wild type and mutant channels. For instance mutants of the potassium channel selectivity filter could only be understood in the framework of its crystal structure which revealed that the backbone carbonyl oxygens and not atoms of the amino acid sidechains directly participate in the selectivity process [90,91]. Since channels reside in membranes the structural information necessary for providing a firm basis for their understanding often must include lipids or even a full membrane with transversal electric fields. This is a problem, as there are no methods for structure determination able to provide such data and thus we must rely on in silico studies [92,93]. A highly regrettable state of affairs, for all the electrophysiological measurements conducted to understand channel properties are carried out in the context of a full membrane systems under non-equilibrium conditions. This is important to bear in mind when considering the relation between channel structures *per se* and explanation of gating behavior; they are not necessarily the same.

Long term efforts to obtain high resolution structures of whole F-ATP synthase by X-ray crystallography never succeeded due to the difficulty of obtaining well diffracting 3D crystals [94]. Thanks to the 'Resolution Revolution' in cryo-EM [95] and the non-necessity of crystal contacts in single particle analysis [96], in recent years numerous cryo-EM maps of mitochondrial F-ATP synthases were published at increasing resolution, nearly allowing construction of atomic models of whole mitochondrial F-ATP synthase for the yeast and mammalian complex [52,61,65,97]. The structures of bacterial and chloroplast F-ATP synthases [98–100], reported in parallel, allow comparison to non-mitochondrial F-ATP synthases and thus the assignment of structural features specific to yeast and mammalian F-ATP synthase.

In 2012, when Paolo Bernardi presented the proposal that the mitochondrial F-ATP synthase is the molecular identity of the PTP at the EBEC in Freiburg [101] the available structural data on whole mammalian F-ATP synthase was scarce: a ~20 Å single particle cryo-EM structure visualizing a bend in the membrane part of the F_o domain [102] and a ~30 Å cryo-EM map of the V-shaped dimer including the surrounding membrane from in situ cryo-ET of the inner mitochondrial membrane of yeast mitochondria [103]. Atomic models of whole mitochondrial F-ATP synthase were not reported and the most complete atomic models of subcomplexes deposited in the PDB were those of F_1-PS_{frag} [60] and F_1-c_8 [104] and F_1-c_{10} [105,106]. In 2015 the situation improved considerably. Direct visualization of membrane bending in liposomes containing 2D crystals of monomeric bovine F-ATP synthase demonstrated the minimal membrane bending unit to lie with the monomer of mitochondrial F-ATP synthase in the absence of dimer contacts [78]. Furthermore, a ~12 Å single particle cryo-EM map of the bovine monomer indicated a novel density extending from the rotor ring distal membrane bending part of the F_o domain towards the intercristae side of the c-ring and a contact site between the peripheral stalk and the c-terminal region of the adjacent α-subunit [80]. A further single particle cryo-EM map of the bovine monomer at a resolution of ~6 Å clearly showed the novel density on the intercristae side of the complex to extend towards the protruding lipid plug and a single α-helix of subunit e was correctly assigned as its underlying protein [107]. Earlier in the same year a cryo-EM map of the *Polytomella* dimer had visualized for the first time the horizontal α-helices of the ATP synthase a-subunit wrapping around the proton transporting c-ring [108]. Strikingly, the *Polytomella* dimer did not exhibit any α-helices extending from the rotor ring distal into the intercristae space side of the complex. The year 2016 brought the first structure of a yeast dimer at α-helical resolution [97]. It was also the first dimer structure clearly visualizing e-subunits extending to the intercristae side protruding c-ring. Surprisingly, however, seemingly the c-ring contacted by subunit e is from the opposing monomer and not its own [109]. Improvement of the yeast dimer structure hopefully will clarify this interesting point in the future.

The first map of the yeast F_o domain at a resolution sufficient to build atomic models for large part of the transmembrane subunits was obtained from yeast dimers which had their F_1 domain stripped off by exposure to three molar sodium bromide prior to membrane solubilization [65]. The structure described for the first time the membrane bending b-e-g unit of F_o which strikingly appeared to have an almost

separate architecture from the proton transporting region of F_o while being firmly anchored to the peripheral stalk via the b-subunit. In this structure the e-subunit extends into the intercristae space; however, the e-subunit is not bent but straight, without suggestion of a possible contact with the c-ring. Whether this apparent detachment from the c-ring is physiological relevant or an artifact of the harsh chemical treatment used to remove F_1 remains to be seen. Interestingly, the concept of the b-e-g unit as a structurally stable unit particular to mitochondrial F-ATP synthase had already been formulated earlier in a study on the assembly of human F-ATP synthase [110].

The notion of mitochondrial F-ATP synthase as a candidate PTP started with the finding that the accepted PTP modulator CyPD can bind to the OSCP subunit of the matrix-side F_1 domain [30,31]. Therefore, the visualization of the OSCP subunit by a recent high resolution (~2.8 Å) single particle cryo-EM study of the *Polytomella* dimer is of great interest [111]. The detected flexibility among the 13 rotary substate structures determined is in support of the notion that the OSCP has the ability to serve as a binding partner capable of transmitting signals via conformational change. Together with the discovery of human OSCP His112 as a pH sensor at the hinge region and the established binding of CyPD [30,31,112], Sirt3 [113,114] and matrix p53 [115] the OSCP subunit emerges as a central signaling platform in the mitochondrial matrix for the regulation of PTP behavior.

That the natural inhibitor protein IF1 of the mitochondrial F-ATP synthase can form dimers and inhibits F_1 by insertion into the catalytic hexamer has been described in detail by X-ray crystal structures of F_1-IF1 [116] and $(IF1)_2$ [117]. Electron microscopy images also suggested that IF1 dimers are able to link two F_1 domains [118]. How this plays out under physiological conditions in the mitochondria, however, has been under debate for a considerable amount of time. By combining very mild purification conditions yielding only a crude extract (target complex < 20 %) and the collection of a record breaking large cryo-EM image data set (> 40,000 images), the first description of a mammalian IF1 stabilized tetramer of the mitochondrial F-ATP synthase at a resolution of ~6 Å [52] was achieved. The resulting structure provides an explanation for how neighboring dimers can be interlinked via the insertion of dimeric IF1 into adjacent F_1 domains effectively providing stabilization to interdimer contacts, and, as a consequence, to the oligomeric form of the mitochondrial F-ATP synthase. It is easy to picture how IF1 dimers inserted in alternating directionality along a row of F-ATP synthase dimers can provide interlinkage to a large number of dimers while preventing depletion of ATP in the absence of a proton motive force (pmf). The tetramer structure also clearly visualized the e-subunits contacting a density protruding from the c-ring lumen into the intercristae space side of the complex. Using the approach of re-extraction of monomers from tetramer coordinates and a subsequent separation into two groups of monomers (E-state and DP-state) and the separate refinement of F_1 and F_o domain, the same image data set was used to internally improve the resolution of the density map for the building of atomic models of the unassigned parts of the transmembrane F_o domain. In the resulting model c-ring lumenal density was assigned to an α-helix of the 6.8PL subunit. This contradicts the expectation that lipids fill the c-ring lumen. The absence of density for at least 18 of the 60 amino acids of 6.8PL, the novel image processing approach of 'Split-and-Recenter' and the *de novo* modeling of relatively poor density gives reason for pause. Hopefully improved maps will resolve this point in the near future.

A very interesting structure of the mitochondrial dimer from the single celled alga *Euglena gracilis* exhibited a further contact by a single α-helix from the rotor ring distal F_o domain to the intercristae space side of the c-ring [119]. Remarkably the portion of the c-ring protruding into the intercristae space that makes contact with this α-helix has a β-barrel architecture which changes into a conventional α-helical structure from the transmembrane region of the c-ring on.

Membrane protein structures are almost exclusively characterized in the absence of a closed membrane system, leaving effects of gradients across the membrane out of reach. The approach developed by Sigworth of performing single particle cryo-EM on channel proteins reconstituted into SUVs should overcome this fundamental problem in structural biology [120]. A proof that this approach can be applied to mitochondrial F-ATP synthase, is the recent low resolution reconstruction (~20 Å) of porcine F-ATP synthase monomer of SUVs employed for detection of PTP/MMC currents [86]. This gives reason for optimism that in the future higher resolution structures are possible in the context of a transmembrane potential.

The list of desirable structures for providing a framework for a better understanding of mitochondrial F-ATP synthase as PTP is naturally very long. But it might be useful to point out some priorities. Clearly the most pressing demand is after a structure that can provide a complete and reliable atomic model of the entire mitochondrial F-ATP synthase from yeast or mammalian source. This can then be used for further directed point mutations probing channel behavior by in situ and in vitro electrophysiology. If the resolution is sufficiently high (< 2 Å) the structure can also be used for the design of small molecule binding partners with improved binding properties, i.e. structure based drug design. In the last four years numerous careful studies that address the question of whether mitochondrial F-ATP synthase is the PTP have been published [40,54,55,62–64,76,86,121,122]. Nevertheless, perhaps the best experimental data to dispel remaining doubt is a high resolution structure of the PTP in an open state, preferably induced by Ca^{2+} alone. In principle single particle cryo-EM is suitable to achieve this. Cryo-ET of mitoplasts used for patch-clamp recordings may seem the most straightforward way to obtain a 3D structure of the PTP in an open state; however, the high density of the matrix in intact mitoplasts likely render this approach very challenging [123]. If an open state of the PTP demands the presence of a lipid bilayer or even a membrane with transmembrane gradients, then single particle cryo-EM of SUVs should be a promising way to visualize the PTP in an open state. Good structures of mitochondrial F-ATP synthase bound with the many known PTP binding partners (e.g. Ca^{2+}, CyPD, CsA, phenylglyoxal) would of course be very informative to explain how these achieve their known effect on PTP currents and might provide fruitful ideas for pharmacological avenues to manipulate PTP properties. Furthermore, structural description of open substates of the PTP that correspond to Ca^{2+} release rather than mitochondrial permeability transition inducing full opening might give molecular insights into the mitochondrial role of cellular Ca^{2+} homeostasis. While X-ray crystallography has failed to deliver a structure of physiologically relevant intact mitochondrial F-ATP synthase after several decades of serious efforts it is still worthwhile to try. Once the problem of how to obtain oligomeric pure mitochondrial F-ATP synthase in intact and stable form has been sorted out, the growth of well diffracting 3D crystals might be quite straightforward. The resulting structural insights would be different from that of single particle cryo-EM. After a long slump, the renewed interest in mitochondrial F-ATP synthase, preparation together with the arrival of novel detergents [74,75,124] might allow the purification problem to be solved relatively soon.

4. Gating: mtF_oF_1 as PTP

To understand the mechanism of channel gating one faces the conundrum of having to describe a dynamic process, i.e. opening and closing, at the atomic level based only on static snapshots of the underlying structure. In addition, electrophysiological recordings that define gating states are obtained from non-equilibrium membranes, while, to date, high resolution structures could only be determined under equilibrium conditions, mostly in the absence of a membrane. However, progress made in unraveling the gating mechanism of voltage gated potassium channels in the last 20 years [125] gives reason to be optimistic in regard to the possibility of understanding PTP gating. With the identity problem solved, structures of mitochondrial F-ATP synthase

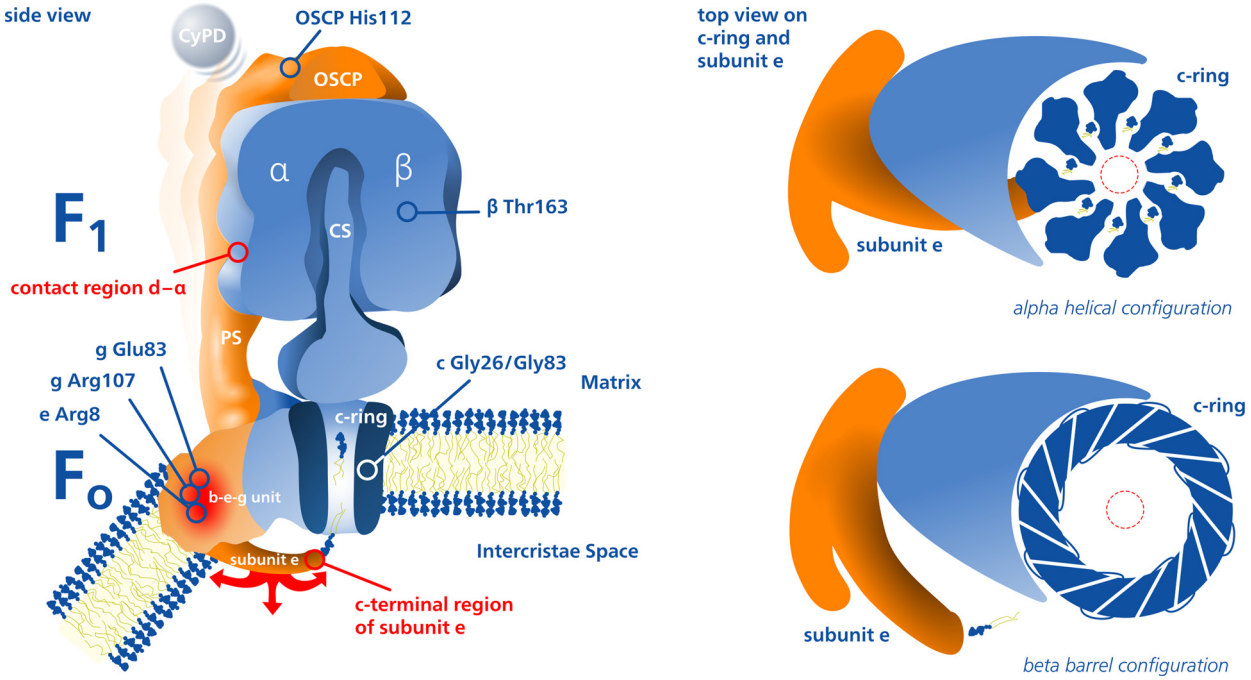

Fig. 1. Cartoon illustrating the 'Death Finger' model.
Left panel: Movement of C-terminal subunit e causes conformational changes transforming the c-ring into an ion conductive pore. Binding of subunit e at the C-terminal to lumenal lipid of the c-ring and its N-terminal anchor in the b-e-g unit of the membrane bending domain of F_o allows it to both sense changes in OSCP via subunit b of the peripheral stalk (PS) and in membrane curvature via subunit g. The structural framework transmitting PTP related changes are colored in orange with the important hinge point of the b-e-g unit marked in red. Single point mutations that have been demonstrated to have a clear effect on PTP properties are labled in blue (See section 2 for details). Regions of interest for further point mutations are labled in red.
Right side panels: Two speculative scenarios how lipid removal via movement of subunit e might be coupled to large conformational changes in the c-ring. Upper panel: In analogy to recent findings in the gap junction field lateral movement of lipids both from the c-ring lumen and embedding membrane through lateral openings of the c-ring allow the c-ring to relax into a wider conformation with low conductivity. Lower panel: Complete removal of the e-subunit bound lipid causes the c-ring to transform into a beta barrel with high conductivity.
Cartoon depiction of monomeric mitochondrial F-ATP synthase was chosen to avoid the image of certainty in the absence of complete atomic models for yeast and mammalian F-ATP synthase.

are increasingly becoming available and with structure determination in the context of membranes no longer being rare, PTP gating might be fairly well understood in less than 10 years.

4.1. 'Death Finger' model

An acceptable model for the description of PTP gating must account for typical conductance of > 1 nS, the reversibility of short openings, the presence of substates with small conductances and the molecular action of all known effectors (in particular Ca^{2+}, CypD, CsA and Bz-423). Previously, I proposed a PTP gating mechanism coined the 'Death Finger' model [126], in which an F_o subunit of unknown identity connected to the peripheral stalk reaches from the c-ring distal portion of F_o involved in membrane bending through the intercristae space to the lipid plug protruding from the c-ring (Fig. 1 left panel). The physical connection between OSCP at the top of F_1 and the intercristae side of the c-ring via the mitochondria specific membrane bending portion of F_o was envisioned to manipulate the c-ring lumenal lipid such that the complete removal of the lipid plug would cause full opening of the PTP. This model was based on several premises. First, if mitochondrial F-ATP synthase turned out to be the PTP, then the minimal structural unit should be the monomer since mammalian dimers are highly unstable [127] and would thus not function well as a stable structural basis for mitochondrial swelling during cristae remodeling. Second, the only gap junction resembling structure in the complex is the c-ring, which is assumed to be plugged by lipids. Third, the novel intercristae space side protein contacts both c-ring lipid plug and peripheral stalk for allowing communication from OSCP to the c-ring lumenal lipid plug.

When I first voiced the 'Death Finger' model at the CSH Asia Meeting on Mitochondria in Suzhou in 2015, experimental evidence on the identity of mitochondrial F-ATP synthase as PTP was still rather thin. Also structural evidence of a physical connection between rotor ring distal F_o and the c-ring lumen was limited to negative stain images of bovine F-ATP synthase dimers [128] and two medium-resolution single particle cryo-EM reconstructions of monomeric bovine F-ATP synthase [80,107]. Put simply, the 'Death Finger' model was highly speculative. However, while speculative it was not baseless, as it was able to explain functionality to membrane bending, a novel structural feature of F_o specific to mitochondrial F-ATP synthase, and also possibly how the c-ring is transformed into a conduit.

Since then, thanks to the efforts of many labs, the available data on this problem has improved dramatically. It is now clear that the c-ring contacting density is common in both mammalian and yeast F-ATP synthase and that it stems from a single α-helix of subunit e [52,65,97,107]. The structure of the yeast Fo dimer established that subunit e is part of a structural unit formed by tight interaction of subunit b, e and g; the b-e-g unit [65]. This puts subunit e in the position to sense membrane curvature through subunit g while being strongly anchored to the membrane foot of the peripheral stalk through subunit b. These unique properties allow subunit e to sense both binding of PTP effectors to OSCP as well as changes in membrane curvature and convey this directly to the c-ring lumen.

The connection of membrane bending with c-ring lumen creates a contextual link between PTP gating and the oligomeric state of mitochondrial F-ATP synthase and cristae architecture. As proposed earlier on the basis of in silico experiments the membrane tension experienced by a single dimer is higher than that of a dimer in a row of dimers [103]. This predicted self-assembly of dimers into oligomeric

rows as a consequence of the energy minimization in the oligomeric state has been verified by cryo-electron tomography of liposomes reconstituted with yeast F-ATP synthase dimers [129]. In addition, the membrane tension from membrane bending in a monomer is directed at the b-e-g unit alone, whereas in a dimer the tension is shared between the b-e-g unit and the intra-dimer contact of the monomers. Therefore, a hierarchy in levels of membrane tension experienced by the b-e-g unit might exist which rises from rows of dimers to dimers at the end points of rows over single dimers to monomers. It is tempting to speculate that the observed correlation between respiratory function and mitochondrial ultrastructure [130,131] is at least partially the result of membrane curvature sensing by the b-e-g unit and an ensuing tuning of short, reversible openings of the PTP in the form of mitoflashes [132,133]. In this scenario cristae architecture and the oligomeric state of the F-ATP synthase act as a meta-controller of PTP open probability in a synthesis of phenomena from the molecular level up to mitochondrial architecture.

The structural framework outlined here for a 'Death Finger' working model of PTP gating receives support from the effects of single point mutations on the unique histidine of the OSCP subunit [62], the phenylglyoxal sensitivity conveying arginine in subunit g [64] and the full conductance conveying conserved arginine in subunit e [122]. Also the notion of monomeric mitochondrial F-ATP synthase as the minimal structural unit capable of eliciting large currents is supported by the patch clamp recordings of GUVs fused with SUVs reconstituted with monomers only [86]. Here it is of interest to note that a recent correlative light and electron microscopy study examining the ultrastructure of mitochondria undergoing apoptotic cristae re-modelling detected only monomeric F-ATP synthase in the vicinity of the outer membrane using cryo-electron tomography [134]. Finally, remaining doubts on the molecular identity of the PTP were dispelled by combining in vitro electrophysiology and bulk solution proton pumping experiments performed on highly pure and well-characterized bovine F-ATP synthase [76]. In a very recent paper a "new bent-pull" model of PTP gating was described [135] that is basically identical to the 'Death Finger' model. New is its incorporation of the proposal that 6.8PL occupies the c-ring's lumen and is being pulled out by movement of subunit e. An already not very satisfying part of the original 'Death Finger' model is the removal of lipid from the c-ring's lumen because of the unfavorable energetics involved (see below). To place a whole hydrophobic α-helix into bulk water, however, is dissatisfying the more so.

4.2. Valid criticism

The 'Death Finger' model now appears much less speculative then when outlined four years ago and might actually point in the right direction. Nevertheless, valid criticism has been raised and must be addressed. At the EBEC in Riva del Garda in 2016 Thomas Meier rightly pointed out that the energetics of pulling out lipids into bulk water are highly unfavorable and might not even be tolerated by the strength of a possible salt bridge between subunit e and a lipid headgroup protruding from the c-ring. Indeed, the energetic penalty of pulling a single cardiolipin with four acyl chains each with 18 carbon atoms from the hydrophobic interior of the c-ring lumen into bulk water amounts to more than 30 kcal/mol [136], whereas breaking a single salt bridge amounts to less than 5 kcal/mol [137]. The energetic situation changes, however, if the c-ring lumen is less hydrophobic than expected, by for example an influx of water molecules. Or if the lumenal lipid has a small tail, which is likely the case given the c_8-ring's exceptionally small lumen. For example a lipid with only 8 carbons in its hydrophobic tail can be forced into bulk water without breaking the lipid headgroup binding salt bridge. Another way out of the energetic penalty problem is a scenario wherein the lumenal lipid is not removed by pulling it out into bulk water, but induced to move laterally into bulk lipid of the surrounding membrane through openings of the c-ring. A similar mechanism has been reported for the TRAAK channel [138,139]. In this case the energetic costs for lipid movement out of the c-ring's lumen is close to zero and only necessitates Brownian movement biased by slight movement of the bound subunit e. Note that Brownian motion of lipids is several orders of magnitude faster (10^{-7}–10^{-9} s timescale) [140,141] than that of, i.e. c-ring rotation (10^{-1}–10^{-3} s timescale), i.e. lipid movement is instant from the perspective of protein.

However, removal of lipid from the c-ring does not necessarily mean that ion conduction is possible. This point was raised in detail by an in silico study that used full atom molecular dynamic simulations to probe the question of ion conductance of c-rings in the absence of lumenal lipids [142]. In none of the simulations large ion fluxes were observed, clearly indicating that removal of lumenal lipids alone might be insufficient to cause channel activity. The simulations were performed using atomic models of the yeast c_{10}-ring and a bacterial c_{13}-ring. They did not include any conformational changes of the c-subunits themself, which certainly leaves room for further studies. It would have been interesting to see for comparison how the simulation conditions used here play out on the conductance of known channels having a hydrophobic conduit such as the actylcholine receptor or connexin gap junction channels.

4.3. Learning from gap junctions

Gap junctions are channels of large conductance formed by oligomers of connexins and innexins whose biological role is to connect neighboring cells for the fast communication of ions and small solutes [143,144]. Their hemichannels consist of six or eight subunits, each with four transmembrane helices and their conductance has been studied in depth by electrophysiology and can be very large, with e.g. ~500 pS for Connexin-50 hemichannels expressed and measured in Xenopus oocytes [145], a magnitude in electric current similar to that of the PTP/MMC. Like the PTP/MMC gap junctions exhibit a multitude of substates suggestive of a complex gating mechanism, which can be modulated by both Ca^{2+} and the transmembrane potential. Those and other shared characteristics led me to believe that the gap junction field could be very informative and inspiring for the PTP field. When it comes to the question of gating, the gap junction field faced a problem opposite to that of the PTP field. Until very recently, all reported high resolution structures determined for gap junctions by either X-ray crystallography or single particle cryo-EM were apparently in an open state [146–150]. This posed a puzzling question: how can such a large conduit be closed to ions? All open state gap junction structures were determined in detergent and in the absence of a lipid bilayer preventing visualization of a role for lipids. In a very recently published study on the problem of gap junction gating, several structures of Innexin-6 gap junction hemichannels were determined by single particle cryo-EM in the absence and presence of a lipid bilayer, i.e. in detergent or lipid nano-discs [151]. Analysis of the resulting density maps and electrophysiological recordings of wild type and N-terminal deletion mutants of Innexin-6 used in this study strongly indicate that the Innexin-6 hemi-channel is closed by lipids. In addition, it could be shown that the N-terminal domain on the lumenal side interacts with lipids and is engaged in gating the hemichannel via manipulation of lipid occupancy. The resulting model posits that the N-terminal region is up when lipids enter the hemichannel's pore and down when lipids are excluded from the channels lumen resulting in an open state. Here, it is noteworthy that the N-terminal region in connexins is thought to sense changes in the transjunctional voltage [152]. The notion that gap junctions are closed via the lateral diffusion of lipids into the channel's transmembrane lumen received support from molecular dynamic simulations of the also very recently determined structures of the related CALHM channels [153,154]. Possibly, closing via lipids rather than protein is a general motif for channels of large conductance.

In analogy to the findings on lipid mediated gating in gap junctions the PTP might be gated by lipids as well. If so, then the re-arrangement of the c-ring to an open state could involve the insertion of both

lumenal lipids and that of lipids from the embedding membrane into openings between c-subunits. As a consequence the ring's diameter would widen and possibly undergo conformational change that favors ion conductance. A complete removal of c-ring lumenal lipids might also cause more drastic conformational changes of the c-ring into an VDAC like β-barrel structure (Fig. 1 right side panels). However, the primary sequence of the c-subunit is dominated by hydrophobic residues and does not have the stretches of alternating polar and unipolar residues necessary for the formation of a classical β-barrel channel like VDAC. Hence, if this highly speculative idea is true, a non-canonical β-barrel is expected. The possibility of the α-helical c-ring subunits transforming into β-sheets has previously been suggested by in vitro experiments conducted on synthetic and extracted c-subunit polypeptides [155,156]. Coincidentally, the outer diameter of VDAC is almost identical with the outer diameter of the mammalian c_8-ring.

The microscopic world of proteins and membranes is far too different from our own macroscopic world to make surefooted guesses. Even seemingly very clear cause and effect relationships visualized by high resolution crystal structures can be misleading. Thus for example in the crystal structure of asymmetric F_1 it is seemingly obvious that the γ-subunit presses against one of the three β-subunits and thus introduces asymmetry into the catalytic $\alpha_3\beta_3$ hexamer [157]. The introduction of asymmetry could consequently be assigned to the presence of the γ-subunit in the center of the hexamer. However, later truncation studies on the role of the γ-subunit first by single molecule rotation assays on $\alpha_3\beta_3\gamma_{truncate}$ [158] and eventually high speed AFM movies of the $\alpha_3\beta_3$ hexamer alone demonstrated the γ-subunit neither to be essential for asymmetry in the $\alpha_3\beta_3$ hexamer *per se* nor for sequential, i.e. rotational, catalysis in the $\alpha_3\beta_3$ hexamer in the correct direction [159].

With this in mind, one should expect that all of the above-described gating mechanisms of the PTP are unlikely to be correct. However, they are nonetheless useful to encourage new experiments to investigate this important problem of mitochondrial biology which can then lead to better data and eventually more accurate biological interpretations.

5. Concluding remarks

In this short review I have tried to address the current state of affairs surrounding three main problems facing the PTP field: identity, structure and gating. Hopefully, it is clear that the first problem of the molecular identity of the PTP is now basically settled with very strong evidence for the mitochondrial F-ATP synthase as the sole protein complex responsible for the name giving maximum conductance of the PTP/MMC. It is also very likely that the minimal oligomeric unit of mitochondrial F-ATP synthase that can harbor a channel of large conductance is a monomer. Thanks to the maturation of cryo-EM into an extraordinary strong approach in doing structural biology on proteins which are not easily coaxed into forming 3D crystals, structures of mitochondrial F-ATP synthases are not out of reach anymore. A truly high resolution structure (< 2 Å) necessary to build a very accurate atomic model of yeast or mammalian F-ATP synthase that includes all amino acids, co-factors, lipids, waters and ions will likely remain absent for the foreseeable future, though. For deciphering the mechanism of PTP gating a combination of purification from cell cultures of wild type and mutant mitochondrial F-ATP synthase with in vitro electrophysiology and structure determination will be necessary. That is a tall order and the bottleneck here might be the establishment of a quick and reliable extraction protocol of mitochondrial F-ATP synthase from the inner mitochondrial membrane in oligomeric pure but intact form. The number of labs around the world engaging with in vitro studies on mitochondrial F-ATP synthase (i.e. using purified complexes in their experiments) had been in decline since the 1990s. In recent years this decline seems to have reversed and I hope that interest in the question of mitochondrial F-ATP synthase as PTP will help to re-activate basic research on this membrane protein complex central to eukaryotic life.

Declaration of Competing Interest

None.

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References

[1] Douglas R. Hunter, Robert A. Haworth, The Ca2+-induced membrane transition in mitochondria: I. The protective mechanisms, Arch. Biochem. Biophys. 195 (2) (1979) 453–459.
[2] Robert A. Haworth, Douglas R. Hunter, The Ca2+-induced membrane transition in mitochondria: II. Nature of the Ca2+ trigger site, Arch. Biochem. Biophys. 195 (2) (1979) 460–467.
[3] Douglas R. Hunter, Robert A. Haworth, The Ca2+-induced membrane transition in mitochondria: III. Transitional Ca2+ release, Arch. Biochem. Biophys. 195 (2) (1979) 468–477.
[4] Valeria Petronilli, Ildiko Szabò, Mario Zoratti, The inner mitochondrial membrane contains ion-conducting channels similar to those found in bacteria, FEBS Lett. 259 (1) (1989) 137–143.
[5] Ildikó Szabó, M. Zoratti, The giant channel of the inner mitochondrial membrane is inhibited by cyclosporin A, J. Biol. Chem. 266 (6) (1991) 3376–3379.
[6] Kathleen W. Kinnally, Zorov Dmitry, Yuri Antonenko, Sean Perini, Calcium modulation of mitochondrial inner membrane channel activity, Biochem. Biophys. Res. Commun. 176 (3) (1991) 1183–1188.
[7] Ildikó Szabó, Mario Zoratti, The mitochondrial megachannel is the permeability transition pore, J. Bioenerg. Biomembr. 24 (1) (1992) 111–117.
[8] Ildiko Szabo, Paolo Bernardi, M. Zoratti, Modulation of the mitochondrial megachannel by divalent cations and protons, J. Biol. Chem. 267 (5) (1992) 2940–2946.
[9] Paolo Bernardi, Stefano Vassanelli, Paolo Veronese, Raffaele Colonna, Ildiko Szabo, Mario Zoratti, Modulation of the mitochondrial permeability transition pore. Effect of protons and divalent cations, J. Biol. Chem. 267 (5) (1992) 2934–2939.
[10] Paolo Bernardi, Andrea Rasola, Michael Forte, Giovanna Lippe, The mitochondrial permeability transition pore: channel formation by F-ATP synthase, integration in signal transduction, and role in pathophysiology, Physiol. Rev. 95 (4) (2015) 1111–1155.
[11] Andrea Rasola, Paolo Bernardi, Mitochondrial permeability transition in Ca2+-dependent apoptosis and necrosis, Cell Calcium 50 (3) (2011) 222–233.
[12] Walter Stühmer, Electrophysiologic recordings from xenopus oocytes, Methods in Enzymology vol. 293, Academic Press, 1998, pp. 280–300.
[13] M. Carraro, A.A. Carrer, P. Bernardi, Molecular nature and regulation of the mitochondrial permeability transition pore (s), drug target (s) in cardioprotection, J. Mol. Cell. Cardiol. 144 (2020) 76–86.
[14] Membrane Proteins of Known 3D Structure; http://blanco.biomol.uci.edu/mpstruc/. Accessed 6 Jul 2020.
[15] Yifan Cheng, Single-particle cryo-EM at crystallographic resolution, Cell 161 (3) (2015) 450–457.
[16] Kutti R. Vinothkumar, Richard Henderson, Single particle electron cryomicroscopy: trends, issues and future perspective, Q. Rev. Biophys. 49 (2016).
[17] Robert M. Glaeser, How Good Can Single-Particle Cryo-EM Become? What Remains Before It Approaches Its Physical Limits? Annu. Rev. Biophys. 48 (2019) 45–61.
[18] Masasuke Yoshida, Eiro Muneyuki, Toru Hisabori, ATP synthase—a marvellous rotary engine of the cell, Nat. Rev. Mol. Cell Biol. 2 (2001) 669–677.
[19] Wolfgang Junge, Nathan Nelson, ATP synthase, Annu. Rev. Biochem. 84 (2015) 631–657.
[20] John E. Walker, ATP synthesis by rotary catalysis (Nobel Lecture), Angew. Chemie Int. Ed. 37 (17) (1998) 2308–2319.
[21] Wolfgang Junge, Holger Lill, Siegfried Engelbrecht, ATP synthase: an electrochemical transducer with rotatory mechanics, Trends Biochem. Sci. 22 (11 November) (1997) 420–423 1.
[22] Wolfgang Junge, Hendrik Sielaff, Siegfried Engelbrecht, Torque generation and elastic power transmission in the rotary F(O)F(1)-ATPase, Nature 459 (2009) 364–370.
[23] L.P. Fernandez-Cardenas, E. Villanueva-Chimal, L.S. Salinas, C. Jose-Nunez,

M. Tuena de Gómez Puyou, R.E. Navarro, Caenorhabditis elegans ATPase inhibitor factor 1 (IF1) MAI-2 preserves the mitochondrial membrane potential ($\Delta\psi_m$) and is important to induce germ cell apoptosis, PLoS One 12 (8) (2017) e0181984.
[24] Bertram Daum, Daniela Nicastro, Jotham Austin, J. Richard McIntosh, Werner Kühlbrandt, Arrangement of photosystem II and ATP synthase in chloroplast membranes of spinach and pea, Plant Cell 22 (4) (2010) 1299–1312.
[25] Richard D. Allen, Christopher C. Schroeder, Agnes K. Fok, An investigation of mitochondrial inner membranes by rapid-freeze deep-etch techniques, J. Cell Biol. 108 (6) (1989) 2233–2240.
[26] Richard D. Allen, Membrane tubulation and proton pumps, Protoplasma 189 (1–2) (1995) 1–8.
[27] Mike Strauss, Götz Hofhaus, Rasmus R. Schröder, Werner Kühlbrandt, Dimer ribbons of ATP synthase shape the inner mitochondrial membrane, EMBO J. 27 (7) (2008) 1154–1160.
[28] Bettina Rieger, Wolfgang Junge, Karin B. Busch, Lateral pH gradient between OXPHOS complex IV and F_oF_1 ATP-synthase in folded mitochondrial membranes, Nat. Commun. 5 (1 January) (2014) 1–7 29.
[29] Christopher P. Baines, Robert A. Kaiser, Nicole H. Purcell, N. Scott Blair, Hanna Osinska, Michael A. Hambleton, Eric W. Brunskill, et al., Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, Nature 434 (7033) (2005) 658–662.
[30] Valentina Giorgio, Elena Bisetto, Maria Eugenia Soriano, Federica Dabbeni-Sala, Emy Basso, Valeria Petronilli, Michael A. Forte, Paolo Bernardi, Giovanna Lippe, Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex, J. Biol. Chem. 284 (49) (2009) 33982–33988.
[31] Valentina Giorgio, Sophia Von Stockum, Manuela Antoniel, Astrid Fabbro, Federico Fogolari, Michael Forte, Gary D. Glick, et al., Dimers of mitochondrial ATP synthase form the permeability transition pore, Proc. Natl. Acad. Sci. 110 (15) (2013) 5887–5892.
[32] Joe Carroll, Jiuya He, Shujing Ding, Ian M. Fearnley, John E. Walker, Persistence of the permeability transition pore in human mitochondria devoid of an assembled ATP synthase, Proc. Natl. Acad. Sci. 116 (26) (2019) 12816–12821.
[33] Jiuya He, Holly C. Ford, Joe Carroll, Shujing Ding, Ian M. Fearnley, John E. Walker, Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase, Proc. Natl. Acad. Sci. 114 (13) (2017) 3409–3414.
[34] Jiuya He, Joe Carroll, Shujing Ding, Ian M. Fearnley, John E. Walker, Permeability transition in human mitochondria persists in the absence of peripheral stalk subunits of ATP synthase, Proc. Natl. Acad. Sci. 114 (34) (2017) 9086–9091.
[35] Patrick Paumard, Jacques Vaillier, B.énédicte Coulary, Jacques Schaeffer, Vincent Soubannier, David M. Mueller, Daniel Brèthes, Jean-Pauldi Rago, Jean Velours, The ATP synthase is involved in generating mitochondrial cristae morphology, EMBO J. 21 (3) (2002) 221–230.
[36] Johann Habersetzer, Isabelle Larrieu, Muriel Priault, B.énédicte Salin, Rodrigue Rossignol, Daniel Brèthes, Patrick Paumard, Human F1F0 ATP synthase, mitochondrial ultrastructure and OXPHOS impairment: a (super-) complex matter? PLoS One 8 (10) (2013).
[37] Paolo Bernardi, Mechanisms for Ca2+-dependent permeability transition in mitochondria, Proc. Natl. Acad. Sci. 117 (6) (2020) 2743–2744.
[38] John E. Walker, Joe Carroll, Jiuya He, Reply to Bernardi: the mitochondrial permeability transition pore and the ATP synthase, Proc. Natl. Acad. Sci. 117 (6) (2020) 2745–2746.
[39] Ildiko Szabo, Mario Zoratti, Mitochondrial channels: ion fluxes and more, Physiol. Rev. 94 (2) (2014) 519–608.
[40] Maria A. Neginskaya, Maria E. Solesio, Elena V. Berezhnaya, Giuseppe F. Amodeo, Nelli Mnatsakanyan, Elizabeth A. Jonas, Evgeny V. Pavlov, ATP synthase C-subunit-deficient mitochondria have a small cyclosporine A-sensitive channel, but lack the permeability transition pore, Cell Rep. 26 (1) (2019) 11–17.
[41] Thomas Meier, Patrick Polzer, Kay Diederichs, Wolfram Welte, Peter Dimroth, Structure of the rotor ring of F-Type Na+-ATPase from Ilyobacter tartaricus, Science 308 (5722) (2005) 659–662.
[42] Denys Pogoryelov, Özkan Yildiz, JoséD. Faraldo-Gómez, Thomas Meier, High-resolution structure of the rotor ring of a proton-dependent ATP synthase, Nat. Struct. Mol. Biol. 16 (10) (2009) 1068.
[43] Thomas Meier, Alexander Krah, Peter J. Bond, Denys Pogoryelov, Kay Diederichs, José D. Faraldo-Gómez, Complete ion-coordination structure in the rotor ring of Na+-dependent F-ATP synthases, J. Mol. Biol. 391 (2) (2009) 498–507.
[44] Denys Pogoryelov, Alexander Krah, Julian D. Langer, Özkan Yildiz, José D. Faraldo-Gómez, Thomas Meier, Microscopic rotary mechanism of ion translocation in the F_o complex of ATP synthases, Nat. Chem. Biol. 6 (12) (2010) 891.
[45] Laura Preiss, Özkan Yildiz, David B. Hicks, Terry A. Krulwich, Thomas Meier, A new type of proton coordination in an F1Fo-ATP synthase rotor ring, PLoS Biol. 8 (8) (2010).
[46] Jindrich Symersky, Vijayakanth Pagadala, Daniel Osowski, Alexander Krah, Thomas Meier, JoséD. Faraldo-Gómez, David M. Mueller, Structure of the c 10 ring of the yeast mitochondrial ATP synthase in the open conformation, Nat. Struct. Mol. Biol. 19 (5) (2012) 485.
[47] Laura Preiss, Julian D. Langer, David B. Hicks, Jun Liu, Özkan Yildiz, Terry A. Krulwich, Thomas Meier, The c-ring ion binding site of the ATP synthase from *Bacillus pseudofirmus* OF4 is adapted to alkaliphilic lifestyle, Mol. Microbiol. 92 (5) (2014) 973–984.
[48] Laura Preiss, Julian D. Langer, Özkan Yildiz, Luise Eckhardt-Strelau, Jérôme E.G. Guillemont, Anil Koul, Thomas Meier, Structure of the mycobacterial ATP synthase F_o rotor ring in complex with the anti-TB drug bedaquiline, Sci. Adv. 1 (4) (2015) e1500106.
[49] Thomas Meier, Ulrich Matthey, Fabienne Henzen, Peter Dimroth, Daniel J. Müller, The central plug in the reconstituted undecameric c cylinder of a bacterial ATP synthase consists of phospholipids, FEBS Lett. 505 (3) (2001) 353–356.
[50] Takeshi Murata, Ichiro Yamato, Yoshimi Kakinuma, Andrew G.W. Leslie, John E. Walker, Structure of the rotor of the V-Type Na^+-ATPase from *Enterococcus hirae*, Science 308 (5722) (2005) 654–659.
[51] Benjamin Oberfeld, Josef Brunner, Peter Dimroth, Phospholipids occupy the internal lumen of the c ring of the ATP synthase of *Escherichia coli*, Biochemistry 45 (6) (2006) 1841–1851.
[52] Jinke Gu, Laixing Zhang, Shuai Zong, Runyu Guo, Tianya Liu, Jingbo Yi, Peiyi Wang, Wei Zhuo, Maojun Yang, Cryo-EM structure of the mammalian ATP synthase tetramer bound with inhibitory protein IF1, Science 364 (6445) (2019) 1068–1075.
[53] Armen Y. Mulkidjanian, Kira S. Makarova, Michael Y. Galperin, Eugene V. Koonin, Inventing the dynamo machine: the evolution of the F-type and V-type ATPases, Nat. Rev. Microbiol. 5 (11) (2007) 892–899.
[54] Kambiz N. Alavian, Gisela Beutner, Emma Lazrove, Silvio Sacchetti, Han-A. Park, Pawel Licznerski, Hongmei Li, et al., An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore, Proc. Natl. Acad. Sci. 111 (29) (2014) 10580–10585.
[55] Massimo Bonora, Claudia Morganti, Giampaolo Morciano, Gaia Pedriali, Magdalena Lebiedzinska-Arciszewska, Giorgio Aquila, Carlotta Giorgi, et al., Mitochondrial permeability transition involves dissociation of F1FO ATP synthase dimers and C-ring conformation, EMBO Rep. 18 (7) (2017) 1077–1089.
[56] Annamaria Nicolli, Valeria Petronilli, Paolo Bernardi, Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by matrix pH. Evidence that the pore open-closed probability is regulated by reversible histidine protonation, Biochemistry 32 (16) (1993) 4461–4465.
[57] Ove Eriksson, Eric Fontaine, Paolo Bernardi, Chemical modification of arginines by 2, 3-butanedione and phenylglyoxal causes closure of the mitochondrial permeability transition pore, J. Biol. Chem. 273 (20) (1998) 12669–12674.
[58] Matts D. Linder, Sarune Morkunaite-Haimi, Paavo K.J. Kinnunen, Paolo Bernardi, Ove Eriksson, Ligand-selective modulation of the permeability transition pore by arginine modification opposing effects of p-hydroxyphenylglyoxal and phenylglyoxal, J. Biol. Chem. 277 (2) (2002) 937–942.
[59] Oliver Speer, Sarune Morkunaite-Haimi, Julius Liobikas, Marina Franck, Linn Hensbo, Matts D. Linder, Paavo K.J. Kinnunen, Theo Wallimann, Ove Eriksson, Rapid suppression of mitochondrial permeability transition by methylglyoxal role of reversible arginine modification, J. Biol. Chem. 278 (37) (2003) 34757–34763.
[60] David M. Rees, Andrew G.W. Leslie, John E. Walker, The structure of the membrane extrinsic region of bovine ATP synthase, Proc. Natl. Acad. Sci. 106 (51) (2009) 21597–21601.
[61] Anurag P. Srivastava, Min Luo, Wenchang Zhou, Jindrich Symersky, Dongyang Bai, Melissa G. Chambers, JoséD. Faraldo-Gómez, Maofu Liao, David M. Mueller, High-resolution cryo-EM analysis of the yeast ATP synthase in a lipid membrane, Science 360 (6389) (2018) eaas9699.
[62] Manuela Antoniel, Kristen Jones, Salvatore Antonucci, Barbara Spolaore, Federico Fogolari, Valeria Petronilli, Valentina Giorgio, et al., The unique histidine in OSCP subunit of F-ATP synthase mediates inhibition of the permeability transition pore by acidic pH, EMBO Rep. 19 (2) (2018) 257–268.
[63] Valentina Giorgio, Victoria Burchell, Marco Schiavone, Claudio Bassot, Giovanni Minervini, Valeria Petronilli, Francesco Argenton, et al., Ca^{2+} binding to F-ATP synthase β subunit triggers the mitochondrial permeability transition, EMBO Rep. 18 (7) (2017) 1065–1076.
[64] Lishu Guo, Michela Carraro, Geppo Sartori, Giovanni Minervini, Ove Eriksson, Valeria Petronilli, Paolo Bernardi, Arginine 107 of yeast ATP synthase subunit g mediates sensitivity of the mitochondrial permeability transition to phenylglyoxal, J. Biol. Chem. 293 (38) (2018) 14632–14645.
[65] Hui Guo, Stephanie A. Bueler, John L. Rubinstein, Atomic model for the dimeric F_o region of mitochondrial ATP synthase, Science 358 (6365) (2017) 936–940.
[66] Stephen B. Long, Ernest B. Campbell, Roderick MacKinnon, Voltage sensor of Kv1.2: structural basis of electromechanical coupling, Science 309 (5736) (2005) 903–908.
[67] Stephen B. Long, Xiao Tao, Ernest B. Campbell, Roderick MacKinnon, Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment, Nature 450 (7168) (2007) 376–382.
[68] Dmitriy Krepkiy, Mihaela Mihailescu, J. Alfredo Freites, Eric V. Schow, David L. Worcester, Klaus Gawrisch, Douglas J. Tobias, Stephen H. White, Kenton J. Swartz, Structure and hydration of membranes embedded with voltage-sensing domains, Nature 462 (7272) (2009) 473–479.
[69] Maynard E. Pullman, Harvey S. Penefsky, Anima Datta, Efraim Racker, Partial resolution of the enzymes catalyzing oxidative phosphorylation I. Purification and properties of soluble, dinitrophenol-stimulated adenosine triphosphatase, J. Biol. Chem. 235 (11) (1960) 3322–3329.
[70] Yasuo Kagawa, Efraim Racker, Partial resolution of the enzymes catalyzing oxidative phosphorylation X. Correlation of morphology and function in submitochondrial particles, J. Biol. Chem. 241 (10) (1966) 2475–2482.
[71] Nobuhito. Sone, Masasuke Yoshida, Hajime Hirata, Yasuo Kagawa, Adenosine triphosphate synthesis by electrochemical proton gradient in vesicles reconstituted from purified adenosine triphosphatase and phospholipids of thermophilic bacterium, J. Biol. Chem. 252 (9) (1977) 2956–2960.
[72] Hiroyuki Noji, Ryohei Yasuda, Masasuke Yoshida, Kazuhiko Kinosita, Direct observation of the rotation of F 1-ATPase, Nature 386 (6622) (1997) 299–302.
[73] Toshiharu Suzuki, Naoya Iida, Junko Suzuki, Yasunori Watanabe, Toshiya Endo, Toru Hisabori, Masasuke Yoshida, Expression of mammalian mitochondrial F1-ATP ase in Escherichia coli depends on two chaperone factors, AF 1 and AF 2,

FEBS Open Bio 6 (12) (2016) 1267–1272.
[74] Pil Seok Chae, S.øren G.F. Rasmussen, Rohini R. Rana, Kamil Gotfryd, Richa Chandra, Michael A. Goren, Andrew C. Kruse, et al., Maltose–neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins, Nat. Methods 7 (12) (2010) 1003.
[75] Pil Seok Chae, S.øren G.F. Rasmussen, Rohini R. Rana, Kamil Gotfryd, Andrew C. Kruse, Aashish Manglik, Kyung Ho Cho, et al., A new class of amphiphiles bearing rigid hydrophobic groups for solubilization and stabilization of membrane proteins, Chem. Eur. J. 18 (31) (2012) 9485–9490.
[76] Andrea Urbani, Valentina Giorgio, Andrea Carrer, Cinzia Franchin, Giorgio Arrigoni, Chimari Jiko, Kazuhiro Abe, et al., Purified F-ATP synthase forms a Ca^{2+}-dependent high-conductance channel matching the mitochondrial permeability transition pore, Nat. Commun. 10 (1) (2019) 1–11.
[77] Shintaro Maeda, Kyoko Shinzawa-Itoh, Kaoru Mieda, Mami Yamamoto, Yumiko Nakashima, Yumi Ogasawara, Chimari Jiko, et al., Two-dimensional crystallization of intact F-ATP synthase isolated from bovine heart mitochondria, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 69 (12) (2013) 1368–1370.
[78] Chimari Jiko, Karen M. Davies, Kyoko Shinzawa-Itoh, Kazutoshi Tani, Shintaro Maeda, Deryck J. Mills, Tomitake Tsukihara, Yoshinori Fujiyoshi, Werner Kühlbrandt, Christoph Gerle, Bovine F_1F_o ATP synthase monomers bend the lipid bilayer in 2D membrane crystals, Elife 4 (2015) e06119.
[79] Satoru Shimada, Shintaro Maeda, Masahide Hikita, Kaoru Mieda-Higa, Shigefumi Uene, Yukiko Nariai, Kyoko Shinzawa-Itoh, Solubilization conditions for bovine heart mitochondrial membranes allow selective purification of large quantities of respiratory complexes I, III, and V, Protein Expr. Purif. 150 (2018) 33–43.
[80] Florian Hauer, Christoph Gerle, Niels Fischer, Atsunori Oshima, Kyoko Shinzawa-Itoh, Satoru Shimada, Ken Yokoyama, Yoshinori Fujiyoshi, Holger Stark, GraDeR: membrane protein complex preparation for single-particle cryo-EM, Structure 23 (9) (2015) 1769–1775.
[81] Rubén Quintana-Cabrera, Charlotte Quirin, Christina Glytsou, Mauro Corrado, Andrea Urbani, Anna Pellattiero, Enrique Calvo, et al., The cristae modulator Optic atrophy 1 requires mitochondrial ATP synthase oligomers to safeguard mitochondrial function, Nat. Commun. 9 (1) (2018) 1–13.
[82] Ilka Wittig, Hans-Peter Braun, Hermann Schägger, Blue native PAGE, Nat. Protoc. 1 (1) (2006) 418.
[83] Ilka Wittig, Hermann Schägger, Advantages and limitations of clear-native PAGE, Proteomics. 5 (17 November) (2005) 4338–4346.
[84] Isabel Arnold, Kathy Pfeiffer, Walter Neupert, Rosemary A. Stuart, Hermann Schägger, Yeast mitochondrial F1F0-ATP synthase exists as a dimer: identification of three dimer-specific subunits, EMBO J. 17 (24 December) (1998) 7170–7178 15.
[85] Hermann Schägger, Kathy Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, EMBO J. 19 (8 April) (2000) 1777–1783 17.
[86] Nelli Mnatsakanyan, Marc C. Llaguno, Youshan Yang, Yangyang Yan, Joachim Weber, Fred J. Sigworth, Elizabeth A. Jonas, A mitochondrial megachannel resides in monomeric F_1F_O ATP synthase, Nat. Commun. 10 (1) (2019) 1–11.
[87] Annamaria Nicolli, Valeria Petronilli, Paolo Bernardi, Modulation of the mitochondrial cyclosporin A- sensitive permeability transition pore by matrix pH. Evidence that the pore open- closed probability is regulated by reversible histidine protonation, Biochemistry 32 (1993) 4461–4465.
[88] Valeria Petronilli, Carmen Cola, Steffan Massari, Raffaele Colonna, Paolo Bernardi, Physiological effectors modify voltage sensing by the cyclosporin A-sensitive permeability transition pore of mitochondria, J. Biol. Chem. 268 (1993) 21939–21945.
[89] Jindrich Symersky, Daniel Osowski, Eric D. Walters, David M. Mueller, Oligomycin frames a common drug-binding site in the ATP synthase, Proc. Natl. Acad. Sci. 109 (35 August) (2012) 13961–13965 28.
[90] Declan A. Doyle, Joao Morais Cabral, Richard A. Pfuetzner, Anling Kuo, Jacqueline M. Gulbis, Steven L. Cohen, Brian T. Chait, Roderick MacKinnon, "The structure of the potassium channel: molecular basis of K+ conduction and selectivity, Science 280 (5360) (1998) 69–77.
[91] Roderick MacKinnon, Potassium channels and the atomic basis of selective ion conduction (Nobel Lecture), Angew. Chemie Int. Ed. 43 (33) (2004) 4265–4277.
[92] Alfredo J. Freites, Eric V. Schow, Stephen H. White, Douglas J. Tobias, Microscopic origin of gating current fluctuations in a potassium channel voltage sensor, Biophys. J. 102 (11) (2012) L44–L46.
[93] Mona L. Wood, Alfredo J. Freites, Francesco Tombola, Douglas J. Tobias, Atomistic modeling of ion conduction through the voltage-sensing domain of the Shaker K+ ion channel, J. Phys. Chem. B 121 (15) (2017) 3804–3812.
[94] John E. Walker, The ATP synthase: the understood, the uncertain and the unknown, Biochem. Soc. Trans. 41 (1) (2013) 1–16.
[95] Werner Kühlbrandt, The resolution revolution, Science 343 (6178) (2014) 1443–1444.
[96] Fred J. Sigworth, Principles of cryo-EM single-particle image processing, Microscopy 65 (1) (2016) 57–67.
[97] Alexander Hahn, Kristian Parey, Maike Bublitz, Deryck J. Mills, Volker Zickermann, Janet Vonck, Werner Kühlbrandt, Thomas Meier, Structure of a complete ATP synthase dimer reveals the molecular basis of inner mitochondrial membrane morphology, Mol. Cell 63 (3) (2016) 445–456.
[98] Alexander Hahn, Janet Vonck, Deryck J. Mills, Thomas Meier, Werner Kühlbrandt, Structure, mechanism, and regulation of the chloroplast ATP synthase, Science 360 (6389) (2018) eaat4318.
[99] Hui Guo, Toshiharu Suzuki, John L. Rubinstein, Structure of a bacterial ATP synthase, Elife 8 (2019) e43128.
[100] Meghna Sobti, Robert Ishmukhametov, James C. Bouwer, Anita Ayer, Cacang Suarna, Nicola J. Smith, Mary Christie, Roland Stocker, Thomas M. Duncan, Alastair G. Stewart, Cryo-EM reveals distinct conformations of E. Coli ATP synthase on exposure to ATP, Elife 8 (2019) e43864.
[101] Paolo Bernardi, Valentina Giorgio, EBEC 2012—an energetic time in Freiburg, EMBO Rep. 14 (1) (2013) 7–9.
[102] Lindsay A. Baker, Ian N. Watt, Michael J. Runswick, John E. Walker, John L. Rubinstein, Arrangement of subunits in intact mammalian mitochondrial ATP synthase determined by cryo-EM, Proc. Natl. Acad. Sci. 109 (29) (2012) 11675–11680.
[103] Karen M. Davies, Claudio Anselmi, Ilka Wittig, José D. Faraldo-Gómez, Werner Kühlbrandt, Structure of the yeast F1Fo-ATP synthase dimer and its role in shaping the mitochondrial cristae, Proc. Natl. Acad. Sci. 109 (34) (2012) 13602–13607.
[104] Ian N. Watt, Martin G. Montgomery, Michael J. Runswick, Andrew G.W. Leslie, John E. Walker, Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria, Proc. Natl. Acad. Sci. 107 (39) (2010) 16823–16827.
[105] Daniela Stock, Andrew G.W. Leslie, John E. Walker, Molecular architecture of the rotary motor in ATP synthase, Science 286 (5445) (1999) 1700–1705.
[106] Alain Dautant, Jean Velours, Marie-France Giraud, Crystal structure of the Mg·ADP-inhibited state of the yeast F1c10-ATP synthase, J. Biol. Chem. 285 (38) (2010) 29502–29510.
[107] Anna Zhou, Alexis Rohou, Daniel G. Schep, John V. Bason, Martin G. Montgomery, John E. Walker, Nikolaus Grigorieff, John L. Rubinstein, Structure and conformational states of the bovine mitochondrial ATP synthase by cryo-EM, Elife 4 (2015) e10180.
[108] Matteo Allegretti, Niklas Klusch, Deryck J. Mills, Janet Vonck, Werner Kühlbrandt, Karen M. Davies, Horizontal membrane-intrinsic α-helices in the stator a-subunit of an F-type ATP synthase, Nature 521 (7551) (2015) 237–240.
[109] Niels Fischer, Victoria Beilsten-Edmands, Dror Chorev, Florian Hauer, Chimari Jiko, Satoru Shimada, et al., Structure and function of the intermembrane space domain of mammalian F_oF_1 ATP synthase, bioRxiv (2017) 1–27.
[110] Makoto Fujikawa, Kanako Sugawara, Tsutomu Tanabe, Masasuke Yoshida, Assembly of human mitochondrial ATP synthase through two separate intermediates, F_1-c-ring and b–e–g complex, FEBS Lett. 589 (19 September) (2015) 2707–2712 14.
[111] Bonnie J. Murphy, Niklas Klusch, Julian Langer, Deryck J. Mills, Özkan Yildiz, Werner Kühlbrandt, Rotary substates of mitochondrial ATP synthase reveal the basis of flexible F1-Fo coupling, Science 364 (6446) (2019) eaaw9128.
[112] Suzanne R. Burstein, Hyun Jeong Kim, Jasmine A. Fels, Liping Qian, Sheng Zhang, Ping Zhou, Anatoly A. Starkov, Costantino Iadecola, Giovanni Manfredi, Estrogen receptor beta modulates permeability transition in brain mitochondria, Biochimica et Biophysica Acta (BBA)-Bioenergetics 1859 (6) (2018) 423–433.
[113] Yu-Ting Wu, Hsin-Chen Lee, Chen-Chung Liao, Yau-Huei Wei, Regulation of mitochondrial FoF1ATPase activity by Sirt3-catalyzed deacetylation and its deficiency in human cells harboring 4977 bp deletion of mitochondrial DNA, Biochim. Biophys. Acta 1832 (2013) 216–227.
[114] Wen Yang, Koji Nagasawa, Christian Münch, Yingjie Xu, Kyle Satterstrom, Seungmin Jeong, Sebastian D. Hayes, et al., Mitochondrial sirtuin network reveals dynamic SIRT3-dependent deacetylation in response to membrane depolarization, Cell 167 (4) (2016) 985–1000.
[115] Marie Bergeaud, Lise Mathieu, Arnaud Guillaume, Ute Moll, Bernard Mignotte, Nathalie Le Floch, Jean-Luc Vayssière, Vincent Rincheval, Mitochondrial p53 mediates a transcription-independent regulation of cell respiration and interacts with the mitochondrial F_1F_0-ATP synthase, Cell Cycle 12 (17) (2013) 2781–2793.
[116] Elena Cabezón, Martin G. Montgomery, Andrew G.W. Leslie, John E. Walker, The structure of bovine F_1-ATPase in complex with its regulatory protein IF 1, Nat. Struct. Mol. Biol. 10 (9) (2003) 744–750.
[117] E. Cabezon, M.J. Runswick, A.G.W. Leslie, J.E. Walker, The structure of bovine IF1, the regulatory subunit of mitochondrial F-ATPase, EMBO J. 20 (24) (2001) 6990–6996.
[118] Elena Cabezón, Ignacio Arechaga, P. Jonathan, G. Butler, John E. Walker, Dimerization of bovine F_1-ATPase by binding the inhibitor protein, IF1, J. Biol. Chem. 275 (37) (2000) 28353–28355.
[119] Alexander Mühleip, Sarah E. McComas, Alexey Amunts, Structure of a mitochondrial ATP synthase with bound native cardiolipin, eLife 8 (2019).
[120] Liguo Wang, Fred J. Sigworth, Structure of the BK potassium channel in a lipid membrane from electron cryomicroscopy, Nature 461 (7261) (2009) 292–295.
[121] Michela Carraro, Vanessa Checchetto, Geppo Sartori, Roza Kucharczyk, Jean-Pauldi Rago, Giovanni Minervini, Cinzia Franchin, et al., High-conductance channel formation in yeast mitochondria is mediated by F-ATP synthase e and g subunits, Cell. Physiol. Biochem. 50 (5) (2018) 1840–1855.
[122] Lishu Guo, Michela Carraro, Andrea Carrer, Giovanni Minervini, Andrea Urbani, Ionica Masgras, Silvio C.E. Tosatto, Ildikò Szabò, Paolo Bernardi, Giovanna Lippe, Arg-8 of yeast subunit e contributes to the stability of F-ATP synthase dimers and to the generation of the full-conductance mitochondrial megachannel, J. Biol. Chem. 294 (28) (2019) 10987–10997.
[123] Karen M. Davies, Bertram Daum, Role of cryo-ET in Membrane Bioenergetics Research, (2013), pp. 1227–1234.
[124] Hyoung Eun Bae, Yang Du, Parameswaran Hariharan, Jonas S. Mortensen, Kaavya K. Kumar, Betty Ha, Manabendra Das, et al., Asymmetric maltose neopentyl glycol amphiphiles for a membrane protein study: effect of detergent asymmetricity on protein stability, Chem. Sci. 10 (4) (2019) 1107–1116.
[125] William A. Catterall, Goragot Wisedchaisri, Ning Zheng, The chemical basis for electrical signaling, Nat. Chem. Biol. 13 (5) (2017) 455.
[126] Christoph Gerle, On the structural possibility of pore-forming mitochondrial F_oF_1

ATP synthase, Biochimica et Biophysica Acta (BBA)-Bioenergetics 1857 (8) (2016) 1191–1196.
[127] Björn Meyer, Ilka Wittig, Elisabeth Trifilieff, Michael Karas, Hermann Schägger, Identification of two proteins associated with mammalian ATP synthase, Mol. Cell. Proteom. 6 (10) (2007) 1690–1699.
[128] Fernando Minauro-Sanmiguel, Stephan Wilkens, JoséJ. García, Structure of dimeric mitochondrial ATP synthase: novel F_0 bridging features and the structural basis of mitochondrial cristae biogenesis, Proc. Natl. Acad. Sci. 102 (35) (2005) 12356–12358.
[129] Thorsten B. Blum, Alexander Hahn, Thomas Meier, Karen M. Davies, Werner Kühlbrandt, Dimers of mitochondrial ATP synthase induce membrane curvature and self-assemble into rows, Proc. Natl. Acad. Sci. 116 (10) (2019) 4250–4255.
[130] Charles R. Hackenbrock, Ultrastructural bases for metabolically linked mechanical activity in mitochondria: I. Reversible ultrastructural changes with change in metabolic steady state in isolated liver mitochondria, J. Cell Biol. 30 (2) (1966) 269–297.
[131] Marta Giacomello, Aswin Pyakurel, Christina Glytsou, Luca Scorrano, The cell biology of mitochondrial membrane dynamics, Nat. Rev. Mol. Cell Biol. (2020) 1–21.
[132] J.örg Hüser, Lothar A. Blatter, Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore, Biochem. J. 343 (2) (1999) 311–317.
[133] Xianhua Wang, Xing Zhang, Di Wu, Zhanglong Huang, Tingting Hou, Chongshu Jian, Peng Yu, Fujian Lu, Rufeng Zhang, Tao Sun, Jinghang Li, et al., Mitochondrial flashes regulate ATP homeostasis in the heart, Elife. 10 (6) (2017 Jul) e23908.
[134] Nicholas R. Ader, Patrick C. Hoffmann, Iva Ganeva, Alicia C. Borgeaud, Chunxin Wang, Richard J. Youle, Wanda Kukulski, Molecular and topological reorganizations in mitochondrial architecture interplay during Bax-mediated steps of apoptosis, Elife 8 (2019) e40712.
[135] Nelli Mnatsakanyan, Elizabeth A. Jonas, ATP synthase c-subunit ring as the channel of mitochondrial permeability transition: regulator of metabolism in development and degeneration, J. Mol. Cell. Cardiol. (May) (2020) 24.
[136] Charles Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes 2d Ed, J. Wiley, 1980.
[137] Wayne J. Becktel, John A. Schellman, Protein stability curves, Biopolymers: Original Res. Biomol. 26 (11) (1987) 1859–1877.
[138] Stephen G. Brohawn, Ernest B. Campbell, Roderick MacKinnon, Physical mechanism for gating and mechanosensitivity of the human TRAAK K+ channel, Nature 516 (7529) (2014) 126–130.
[139] Stephen G. Brohawn, Zhenwei Su, Roderick MacKinnon, Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K+ channels, Proc. Natl. Acad. Sci. 111 (9) (2014) 3614–3619.
[140] Klaus Gawrisch, The Dynamics of Membrane Lipids. The structure of biological membranes. CRC Press, 2005, pp. 147–172.
[141] Helgi I. Ingólfsson, Manuel N. Melo, Floris J. van Eerden, Clement Arnarez, Cesar A. Lopez, Tsjerk A. Wassenaar, et al., Lipid organization of the plasma membrane, J. Am. Chem. Soc. 136 (2014) 14554–14559.
[142] Wenchang Zhou, Fabrizio Marinelli, Corrine Nief, José D. Faraldo-Gómez, Atomistic simulations indicate the c-subunit ring of the F1Fo ATP synthase is not the mitochondrial permeability transition pore, Elife 6 (2017) e23781.
[143] Atsunori Oshima, Structure and closure of connexin gap junction channels, FEBS Lett. 588 (8) (2014) 1230–1237.
[144] Atsunori Oshima, Potential of cryo-EM for high-resolution structural analysis of gap junction channels, Curr. Opin. Struct. Biol. 54 (2019) 78–85.
[145] Miduturu Srinivas, Jack Kronengold, Feliksas F. Bukauskas, Thaddeus A. Bargiello, Vytas K. Verselis, Correlative studies of gating in Cx46 and Cx50 hemichannels and gap junction channels, Biophys. J. 88 (3) (2005) 1725–1739.
[146] Shoji Maeda, So Nakagawa, Michihiro Suga, Eiki Yamashita, Atsunori Oshima, Yoshinori Fujiyoshi, Tomitake Tsukihara, Structure of the connexin 26 gap junction channel at 3.5 Å resolution, Nature 458 (7238) (2009) 597–602.
[147] Brad C. Bennett, Michael D. Purdy, Kent A. Baker, Chayan Acharya, William E. McIntire, Raymond C. Stevens, Qinghai Zhang, Andrew L. Harris, Ruben Abagyan, Mark Yeager, An electrostatic mechanism for Ca 2+-mediated regulation of gap junction channels, Nat. Commun. 7 (1) (2016) 1–12.
[148] Atsunori Oshima, Kazutoshi Tani, Yoshinori Fujiyoshi, Atomic structure of the innexin-6 gap junction channel determined by cryo-EM, Nat. Commun. 7 (1) (2016) 1–8.
[149] Janette B. Myers, Bassam G. Haddad, Susan E. O'Neill, Dror S. Chorev, Craig C. Yoshioka, Carol V. Robinson, Daniel M. Zuckerman, Steve L. Reichow, Structure of native lens connexin 46/50 intercellular channels by cryo-EM, Nature 564 (7736) (2018) 372–377.
[150] Wooyoung Choi, Nicolina Clemente, Weinan Sun, Du Juan, Lü Wei, The structures and gating mechanism of human calcium homeostasis modulator 2, Nature 576 (7785) (2019) 163–167.
[151] Batuujin Burendei, Ruriko Shinozaki, Masakatsu Watanabe, Tohru Terada, Kazutoshi Tani, Yoshinori Fujiyoshi, Atsunori Oshima, Cryo-EM structures of undocked innexin-6 hemichannels in phospholipids, Sci. Adv. 6 (7) (2020) eaax3157.
[152] Priscilla E.M. Purnick, Seunghoon Oh, Charles K. Abrams, Vytas K. Verselis, Thaddeus A. Bargiello, Reversal of the gating polarity of gap junctions by negative charge substitutions in the N-terminus of connexin 32, Biophys. J. 79 (5) (2000) 2403–2415.
[153] Johanna L. Syrjanen, Kevin Michalski, Tsung-Han Chou, Timothy Grant, Shanlin Rao, Noriko Simorowski, Stephen J. Tucker, Nikolaus Grigorieff, Hiro Furukawa, Structure and assembly of calcium homeostasis modulator proteins, Nat. Struct. Mol. Biol. 27 (2) (2020) 150–159.
[154] Drożdżyk Katarzyna, Marta Sawicka, Maria-Isabel Bahamonde-Santos, Jonas Zaugg, Dawid Deneka, Christiane Albrecht, Raimund Dutzler, Cryo-EM structures and functional properties of CALHM channels of the human placenta, eLife 9 (2020).
[155] Giuseppe Federico Amodeo, Brenda Yasie Lee, Natalya Krilyuk, Carina Teresa Filice, Denis Valyuk, Daniel Otzen, Sergei Noskov, Zoya Leonenko, Evgeny V. Pavlov, C subunit of the ATP synthase is an amyloidogenic channel-forming peptide: possible implications in mitochondrial pathogenesis, bioRxiv (2020).
[156] Julie E.M. McGeoch, Malcom W. McGeoch, Entrapment of water by subunit c of ATP synthase, J. R. Soc. Interface 5 (20 March) (2008) 311–318 6.
[157] Jan Pieter Abrahams, Andrew G.W. Leslie, René Lutter, John E. Walker, Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria, Nature 370 (6491) (1994) 621–628.
[158] Shou Furuike, Mohammad Delawar Hossain, Yasushi Maki, Kengo Adachi, Toshiharu Suzuki, Ayako Kohori, Hiroyasu Itoh, Masasuke Yoshida, Kazuhiko Kinosita, Axle-less F1-ATPase rotates in the correct direction, Science 319 (5865) (2008) 955–958.
[159] Takayuki Uchihashi, Ryota Iino, Toshio Ando, Hiroyuki Noji, High-speed atomic force microscopy reveals rotary catalysis of rotorless F1-ATPase, Science 333 (6043) (2011) 755–758.