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REVIEW ARTICLE

OSCP subunit of mitochondrial ATP synthase: role in regulation of enzyme function and of its transition to a pore

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The permeability transition pore (PTP) is a latent, high-conductance channel of the inner mitochondrial membrane. When activated, it plays a key role in cell death and therefore in several diseases. The investigation of the PTP took an unexpected turn after the discovery that cyclophilin D (the target of the PTP inhibitory effect of cyclosporin A) binds to F_OF_1 (F)-ATP synthase, thus inhibiting its catalytic activity by about 30%. This observation was followed by the demonstration that binding occurs at a particular subunit of the enzyme, the oligomycin sensitivity conferral protein (OSCP), and that F-ATP synthase can form Ca^{2+}-activated, high-conductance channels with features matching those of the PTP, suggesting that the latter originates from a conformational change in F-ATP synthase. This review is specifically focused on the OSCP subunit of F-ATP synthase, whose unique features make it a potential pharmacological target both for modulation of F-ATP synthase and its transition to a pore.

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Abbreviations

Abeta, beta amyloid protein; AD, Alzheimer disease; ANT, adenine nucleotide translocator; Bz 423, benzodiazepine 423; cryo-EM, cryo-electron microscopy; CsA, cyclosporin A; CyPD, cyclophilin D; DAPIT, diabetes-associated protein in insulin-sensitive tissue; FRET, fluorescence resonance energy transfer; IF1, inhibitor factor 1; Lrpprc, leucine-rich pentatricopeptide repeat containing protein; MnSOD, Manganese superoxide dismutase; mtDNA, mitochondrial DNA; NMR, nuclear magnetic resonance; OSCP, oligomycin sensitivity conferral protein; pdx-1, pancreatic duodenal homeobox 1; PT, permeability transition; PTP, permeability transition pore; Sirt3, Sirtuin 3; Strap, stress responsive activator p300; TSPO, translocator protein; VDAC, voltage-dependent anion channel

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The F_OF_1 ATP synthase

The **F_OF_1 (F)-ATP synthase** is a ubiquitous enzyme located in energy-converting membranes that conform to essentially the same 'building plan' across all kingdoms of life (Kühlbrandt and Davies, 2016). This enzyme comprises two distinct parts, that is, the globular, catalytic F_1 sector and the membrane-embedded F_O subcomplex, which are connected by central and peripheral stalks (Figure 1). The **oligomycin sensitivity conferral protein (OSCP**) in mitochondria (and its orthologous **δ subunit** in bacteria) is located in the upper part of the peripheral stalk which, along with the central stalk, ensures efficient energy interconversion (Devenish *et al.,* 2000; Antoniel *et al.,* 2014). Indeed, F_O and F_1 are molecular motors exerting rotational torque against one another. When the electrochemical proton gradient is sufficient to impose a large Fo torque, the enzyme catalyses the synthesis of ATP from ADP and Pi. When the phosphate potential predominates, F_1 reverses the rotation of F_O to build up a membrane potential (Noji *et al.,* 2017). The OSCP (or δ) subunit located on top of F1 ensures the structural coupling between F_O and F_1. This coupling is disrupted by the antibiotic oligomycin, which inhibits both ATP synthesis and hydrolysis.

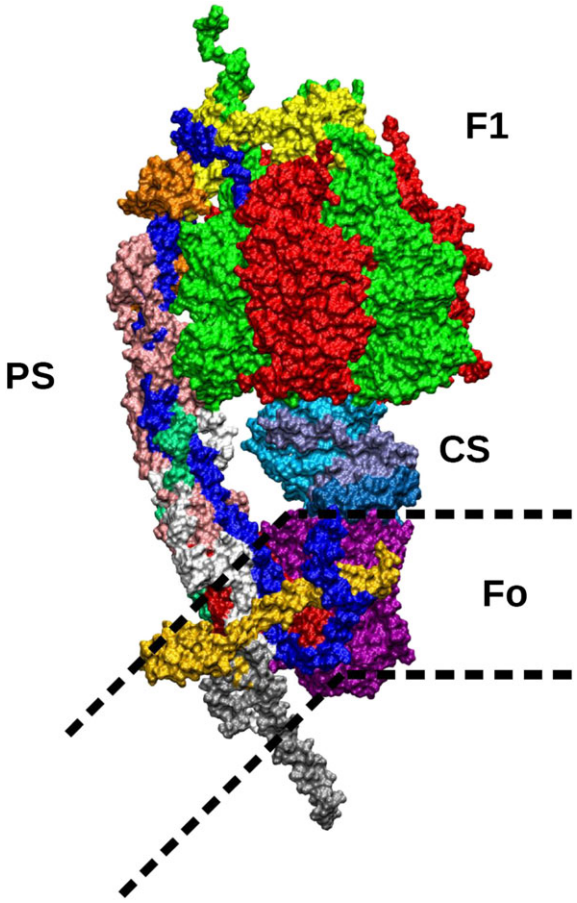

Figure 1

Molecular structure of the mitochondrial F-ATP synthase. Subunits are shown in colours as follows. In the upper part of the figure, F1 is shown with the alternating α (green) and β subunits (red). On the left, the peripheral stalk (PS), connecting the top of F1 with the membrane subunits, includes the OSCP subunit (yellow) on top of F1; the b subunit (dark blue) which can be followed from the top of F1 along the peripheral stalk down to the membrane region; the F6 subunit (orange) and the d subunit (pink). In the lower part of the PS, reaching the Fo region in the membrane, subunits f (white) and A6L (emerald, mostly covered by other subunits) can be seen. The central stalk (CS) connecting the α/β subunits to the c-ring in the membrane includes the γ (cyan), δ (blue) and ε subunits (ice blue). The membrane-embedded c-ring interacts with the CS and is composed of eight identical subunits c (purple). The Fo membrane subunits include the a subunit in contact with the c-ring (dark red, mostly covered in the picture by other subunits), the g subunit (light orange) disposed in parallel with the membrane surface and the e subunit (silver) whose helix is protruding outside the membrane on the opposite side of F1.

Bacterial F_O has the simplest subunit composition with (i) the ring of 10–15 **c subunits**, (ii) the membrane-embedded subcomplex ab2 and (iii) the peripheral stalk comprising the extrinsic part of the **b subunits** and the F_1 subunit δ (Noji *et al.,* 2017). In the mitochondrial enzyme, F_O mediates the formation of **V-shaped F-ATP synthase dimers** (Hahn *et al.,* 2016; Guo *et al.,* 2017), which are not found in bacteria, and has a far more complex structure. Besides the c-ring (formed by 8–10 subunits), the membrane sector consists of single copies of subunits **a** and **b**, which contact each other, of other four conserved proteins (**A6L**, **e**, **f**, **g**) and of two or three additional subunits, diabetes-associated protein in insulin-sensitive tissues (DAPIT) and 6.8PL in vertebrates (Lee *et al.,* 2015). Besides OSCP, the peripheral stalk was originally proposed to be formed by the membrane-distal part of subunit b and by subunits **F6** and **d** (Rees *et al.,* 2009). More recently, the C-terminal region of subunit A6L of the yeast and bovine complex (Lee *et al.,* 2015; Guo *et al.,* 2017), the N-terminal domain of subunit f (Guo *et al.,* 2017) and subunit i/j (Srivastava *et al.,* 2018) of the yeast species have been found at the base of the peripheral stalk. In all F-ATP synthases, proton translocation through the two half-channels of subunit a drives the rotation of the c-ring (Kühlbrandt and Davies, 2016).

The F_1 sector is always made up of 3**α** and 3**β** subunits that alternate around a central stalk, composed by subunits γ and ε in bacteria and by subunits **γ**, **δ** and **ε** in mitochondria. The central stalk is also bound to the c-ring, thus being able to transmit the F_O torque to the catalytic β subunits. Together, the c-ring and the F_1 central stalk constitute the rotor, while the rest of the complex acts as the stator of the molecular engine. Rotation of γ sequentially takes each of the three β subunits through the three major functional states β_{DP}, β_{TP} and β_E or *vice versa*, thereby synthesizing or hydrolysing three Mg^{2+}-ATP molecules during each 360° rotation (Walker, 2013). In bacteria, ATPase activity can be inhibited by the ε subunit (Nakanishi-Matsui *et al.,* 2016) and in mitochondria by the endogenous inhibitor IF1, which helps prevent 6dissipation of cellular ATP under conditions of respiratory impairment (Faccenda *et al.,* 2017).

As the two stepping motors F_O and F_1 differ in the number of steps during the catalytic cycle, their cooperation is smoothed by elastic power transmission rather than by fine tuning of the respective reactions, thereby increasing kinetic efficiency (Junge *et al.,* 2009). Fluctuation analysis of F_OF_1 in *Escherichia coli* established that the main flexibility is

associated with the globular portions of subunits γ and ε in contact with the c-ring (Junge *et al.,* 2009), while the peripheral stalk is rather stiff (Sielaff *et al.,* 2008). Conversely, the observation that deletion of multiple residues between bA50 and bI75 was without effect on enzyme catalysis led to the proposal that the stator in *E. coli* rather resembles a flexible rope (Dunn *et al.,* 2000).

The stiffness of the peripheral stalk is consistent with its role of helping $\alpha_3\beta_3$ resist against the mechanical torsional stress of the rotor. A major structural role is played by the high binding strength of the N-terminal domain of OSCP (δ) to the α subunits and of the C-terminal domain of OSCP (δ) to the peripheral stalk (Figure 2). The latter interaction was documented by FRET in yeast F_OF_1, showing that native OSCP conformation remains almost unaltered relative to subunit b during catalysis (Gavin *et al.,* 2003). In contrast, the enzyme complexes containing a G166 N mutation (corresponding to G161 in *Bos taurus*, a residue that is conserved in virtually all phyla) exhibited large changes in FRET, highlighting the stress that develops between F_1 and OSCP during ATP hydrolysis (Gavin *et al.,* 2003). These complexes were more susceptible to dissociation than complexes containing native OSCP and were insensitive to inhibition by oligomycin (Boyle *et al.,* 2000). As oligomycin binds to the c subunit (Symersky *et al.,* 2012), this result demonstrates that OSCP is capable of modulating the proton conductance within F_O. Interestingly, binding of **17β-oestradiol** to OSCP also promotes an uncoupled state of F-ATP synthase in rat liver mitochondria (Moreno *et al.,* 2013). These findings suggest the presence of flexible regions in OSCP that could impair the stator integrity. It is of note that electron cryo-EM did reveal combined conformational changes between the OSCP C-terminus and subunit b at its point of entrance into the membrane (Zhou *et al.,* 2015).

Structure, interactions and assembly of the OSCP subunit

Rees *et al*. (2009) were the first to define the crystal structure of the OSCP by analysing an F_1-peripheral stalk subcomplex from bovine heart. The N-terminal domain consists of six α-helices (OH1–OH6), as recently found in the crystal structure of the F-ATP synthase from *Paraccocus denitrificans* (Morales-Rios *et al.,* 2015) and in the cryo-EM structure of the F-ATP synthases from *Pichia angusta* (Vinothkumar *et al.,* 2016), *Yarrowia lipolytica* (Hahn *et al.,* 2016) and *Saccharomyces cerevisiae* (Srivastava *et al.,* 2018). Helices 1 (residues 18–26, bovine numbering) and 5 (residues 83–92) together with adjacent residues provide the binding site for residues 2–17 of subunit α_E, which occurs both through hydrophobic contacts and through electrostatic interactions between α R15/OSCP E91 and α E7/OSCP R94 (Rees *et al.,* 2009) (Figure 2A). This first interaction is present in all published structures, where the states E, TP and DP of α and β subunits may be different. For example, in the most recent published structure, this interaction involves a random coil of α_{DP} (Srivastava *et al.,* 2018). Further points of contact between the N-terminus of OSCP and F_1 have been defined. Indeed, a second interaction occurs at the N-terminus of α_{TP} around residue 50 (Rees *et al.,* 2009) (Figure 2B). Similar contacts with α_{TP} are found in other available structures, where the N-terminus of the α subunit forms a helix and interacts with the region corresponding to helices OH2, OH3 and OH4 of OSCP in the bovine complex. Based on sequence similarity, we think that this type of contact is conserved also in *B. taurus*. A third interaction occurs between the N-terminal β-barrel domain of the βDP-subunit with residues 1–14 of OSCP (Rees *et al.,* 2009) (Figure 2C). Finally, the N-terminus of α_{DP} has been seen to contact the C-terminal domain of OSCP (Figure 2D).

The structure of the C-terminal domain of bovine heart OSCP consists of a β-hairpin, which could represent a point of flexion in the peripheral stalk followed by two α-helices, OH7 and OH8 (Rees *et al.,* 2009). A similar structure is found in the *P. angusta* (Vinothkumar *et al.,* 2016) and *Y. lipolytica* enzymes (Hahn *et al.,* 2016). A flexible stretch of about 30 amino acids, not resolved in all available structures, is found between helices OH7 and OH8. In Srivastava *et al*. (2018), OH8 is mapped to a sequence region closer to OH7, thus reducing the length of the link region. In the bovine enzyme, this stretch forms a five-helix bundle with the *N*-terminal α-helix of F6 and a segment of subunit b, resulting in an extensive interface between the three subunits (Figure 2E). A similar arrangement is found in *Y. lipolytica*, *P. angusta* and *S. cerevisiae*, where the C-terminus of OSCP forms a helix bundle with subunit b, subunit h (the fungal orthologue of bovine F_6) and the N-terminus of $\alpha_{DP/TP}$ (not resolved in the bovine enzyme), which positions the F_1 sector towards the peripheral stalk. This latter interface is largely established *via* hydrophobic interactions, except for those mediated by the conserved residues αE33 and αR41 (Hahn *et al.,* 2016). It is worth mentioning that the N- and C-terminal domains of OSCP are almost independent, with only a very short region (encompassing residues V111 to V116) showing contacts with both domains. This region appears unique in its ability to control the relative position of the C- and N-terminal domains, thereby influencing the position of the peripheral stalk relative to the rest of the F-ATP synthase (the connecting region is highlighted in green in Figure 3, and detailed as sticks in Figure 3A).

OSCP can also undergo phosphorylation at the C-terminal domain (Covian and Balaban, 2012), and glycoforms of OSCP and d subunit have been detected in bovine heart (Burnham-Marusich and Berninsone, 2012), but the functional implications of these posttranslational modifications are still unknown. On the other hand, the metabolic significance of OSCP (de)acetylation has been elucidated and will be discussed further later.

OSCP is encoded by the nuclear *ATP5PO* gene and plays a key role in F-ATP synthase assembly, which occurs in a modular fashion preventing formation of intermediates that could depolarize the membrane or waste ATP (Rühle and Leister, 2015). At variance from other subunits, in rat tissues, OSCP is a low-transcript gene (Sangawa *et al.,* 1997). Pulse-labelling experiments in yeast indicate that OSCP binding is the last step in F_OF_1 assembly (Rak *et al.,* 2011). Although this pathway has been questioned, based on disruption of individual human genes for subunits e, f, g, DAPIT and 6.8PL (He *et al.,* 2018), it is consistent with the observation that a decreased OSCP level in human cells did not alter the expression of other subunits (Johnson *et al.,* 2005; Giorgio *et al.,* 2013) unlike suppression of subunit ε, which in

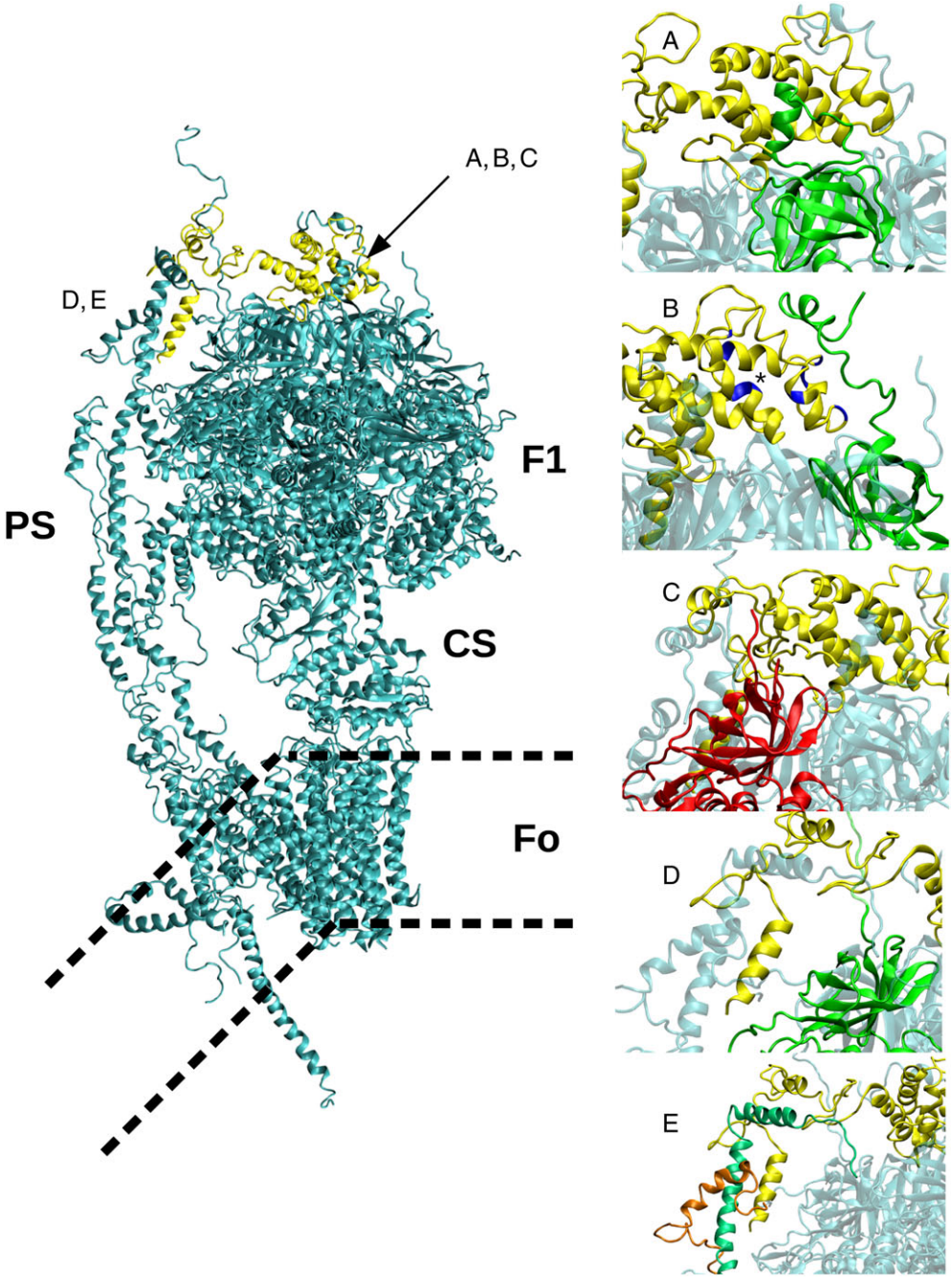

Figure 2

Structure of F-ATP synthase and its interactions with OSCP. The F_1 and F_O subunits are shown in the central panel. PS, peripheral stalk; CS, central stalk. The position of the membrane (thick dotted lines) is purely indicative because the view is not along the line of bending to better show the overall structure of the complex. Interactions between OSCP and the N-terminal region of subunits α_E (A), α_{TP} (B), β_{DP} (C), α_{DP} (D) and with subunit b and F6 (E) are reported in the right panels. In panel (B) the residues encompassing the 'shoulder' region of the Bz 423 binding site (asterisk) are in blue. Highlighted subunits are shown in colour as follows: OSCP in yellow, α subunit in green, β subunit in red, F6 in orange and b in emerald. The image has been built from available crystal structures. Missing parts that could not be modelled by homology to experimental data have been built using the software Modeller (Webb and Sali, 2016).

HEK293 cells caused a marked decrease of assembly intermediates (Havlickova *et al.,* 2010).

In summary, OSCP ensures the structural and functional coupling between F_O and F_1 (which is essential for enzyme catalysis) through its contacts with both the α3β3 hexamer and with the other subunits of the peripheral stalk. OSCP also undergoes regulatory posttranslational modifications. The possible presence of flexible regions in OSCP is intriguing, because such regions could influence the position of the peripheral stalk and thus potentially modulate the membrane-

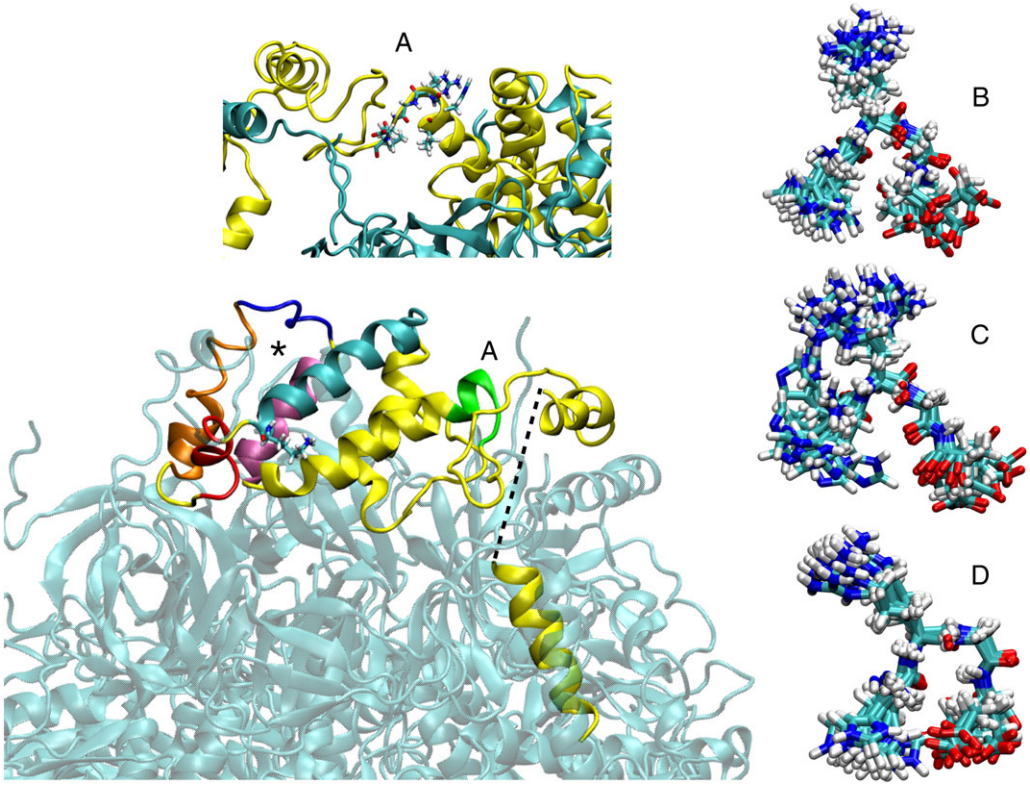

Figure 3

OSCP structure and features. OSCP is shown in the context of F-ATP synthase (transparent). The missing region joining residues 146 and 174 of OSCP and encompassing G161 is indicated by the dashed thick line. Helices OH3 (red), OH4 (orange) and OH5 (mauve) encompassing the Bz 423 binding region (asterisk) are highlighted on the structure. Helices OH2 (cyan) and OH3 (red) and the loop between OH4 and OH5 (blue) that fluctuates most in the presence of Ca^{2+} and the location of K47 (shown as sticks) possibly involved in CyPD binding are also shown. Residues V111 to V116 encompassing H112 and connecting the C-terminal to the N-terminal domains are shown in panel (A). The superposition of snapshots from molecular dynamics simulations of the OSCP subunit (residues H112–E115) is reported in the three right panels corresponding to the two neutral forms [proton on Nε (panel B) or Nδ (panel C)] and to the protonated form (panel D).

embedded Fo sector where, as we will discuss more in detail in the following paragraphs, the permeability transition pore (PTP) may form (Antoniel *et al.,* 2018; Giorgio *et al.,* 2018).

OSCP and the permeability transition pore

The PTP is a high-conductance channel located in the inner mitochondrial membrane that is modulated by a series of effectors, among which matrix Ca^{2+} is an essential permissive factor (Giorgio *et al.,* 2018). As the PTP plays a key role in cell death and in Ca^{2+} signalling, it is a viable target for therapy in a variety of diseases, most notably heart ischaemia/reperfusion injury, stroke, muscular dystrophies, neurodegenerative diseases and cancer (Bernardi *et al.,* 2015). The molecular composition of the PTP is still a matter of debate. Putative components such as the **adenine nucleotide translocator** (ANT), **VDAC** and **TSPO** have been ruled out by genetic ablation studies (Kokoszka *et al.,* 2004; Krauskopf *et al.,* 2006; Baines *et al.,* 2007; Šileikyte *et al.,* 2014). The most recent hypothesis is that the channel forms from a specific, Ca^{2+}-dependent rearrangement of F-ATP synthase, which is favoured by cyclophilin (CyP) D binding (Giorgio *et al.,* 2009, 2013). Channel formation has been observed in F-ATP synthase from beef heart (Giorgio *et al.,* 2013), human cells (Alavian *et al.,* 2014), yeast (Carraro *et al.,* 2014) and *Drosophila* (von Stockum *et al.,* 2015), but the mechanism of formation and whether this channel coincides with the PTP are still matters of debate (Bernardi *et al.,* 2015; Bernardi and Lippe, 2018).

A potential role of OSCP in modulation of the PTP is suggested by the observation that CyPD (the receptor for the PTP inhibitory effects of **cyclosporin A**) binds the peripheral stalk of F-ATP synthase at OSCP (Giorgio *et al.,* 2009, 2013; Lee *et al.,* 2016; Burstein *et al.,* 2018). CyPD is the only mitochondrial isoform of this family of peptidyl prolyl *cis-trans* isomerases and sensitizes the PTP to matrix Ca^{2+} as revealed by studies on CyPD-null mice (Baines *et al.,* 2005; Basso *et al.,* 2005; Nakagawa *et al.,* 2005; Schinzel *et al.,* 2005). The nature of the OSCP-CyPD interactions appears to be mainly electrostatic, and surface potential studies have identified two putative regions on the OSCP N-terminal domain at residues E48, D71, E76 and F78 located between helices OH3, OH4 and OH5 and at residues H112, E115, V116, E128 and E133 (Antoniel *et al.,* 2014) (Figure 3). CyPD is displaced by cyclosporin (Cs) A, a well-characterized inhibitor of the permeability transition (Giorgio *et al.,* 2010).

OSCP is also the binding site of the immunomodulatory benzodiazepine (Bz) 423 (Johnson *et al.,* 2005; Cleary *et al.,* 2007; Stelzer *et al.,* 2010), which like CyPD acts as an inducer of the PTP (Giorgio *et al.,* 2013). In striking analogy with CyPD, this molecule also mildly inhibits both ATP synthesis and hydrolysis (Johnson *et al.,* 2005) and can cause cytochrome c release and cell death (Blatt *et al.,* 2002). Bz 423 binding to OSCP displaces CyPD in a concentration-dependent manner, suggesting competition for a common binding site (Giorgio *et al.,* 2013). Consistent with a PTP modulatory role, Bz 423 promotes channel formation in electrophysiological studies performed on preparations lacking CyPD (Giorgio *et al.,* 2013). NMR studies demonstrated that Bz 423 binding to OSCP (asterisk, Figure 3) causes conformational changes that affect protein regions that are not directly involved in interactions with the drug (Stelzer *et al.,* 2010). This finding further supports the notion that OSCP is a highly flexible protein potentially prone to ligand-induced conformational changes. Indeed, the chemical shift perturbations caused by Bz 423 fall in three general regions of OSCP: (i) the binding region, which is located between helices OH3, OH4 and OH5 (the 'shoulder'), includes residues M51, L56, K65, V66, K75, K77, T82, S83 and N92 and covers the first potential CyPD binding site (Figures 2B and 3); (ii) the region comprising the C-terminal tail of helices OH1 and OH6 (the 'tail'), which includes residues V111, R113, V116, C118 and T119 and partially covers a second hypothetical binding site of CyPD (Figure 3); (iii) a third region located between the tail and the shoulder.

As already proposed (Stelzer *et al.,* 2010), the contacts of the OSCP shoulder region with the N terminus of α_{TP} (Rees *et al.,* 2009) (Figure 2B) can potentially transmit conformational changes to the F_1 part of the enzyme and thus affect catalysis. On the other hand, the tail region involving residues V111–V116 may control the position of the peripheral stalk relative to the rest of F-ATP synthase. It is tempting to speculate that the conformational changes occurring in this region, due to CyPD or Bz 423 binding to OSCP, may be transmitted through the peripheral stalk to the intramembrane portion of Fo, where the PTP should form. Molecular dynamics simulations highlighted a possible role of OSCP in this mechanical transmission. Trajectory inspection in the presence of Ca^{2+} indeed revealed that the larger van der Waals radius of this cation (compared to that of Mg^{2+}) in the catalytic sites may induce spatial rearrangements increasing overall F_1 rigidity, causing in turn fluctuations in the OSCP helices (Giorgio *et al.,* 2017). Peaks of movements included OH2 and OH3 (residues 26–55) and the loop between OH4 and OH5 (residues 74–80), that is, the same overlapping helices perturbed by Bz 423 binding (Figure 3). The mechanical energy transmitted to OSCP could then be transferred to the inner membrane through the peripheral stalk (Figure 2). Our working hypothesis is that the conformational changes of OSCP induced by CyPD or Bz 423 binding could also affect Ca^{2+} binding to the catalytic site, thus increasing the probability of PTP opening.

The relevance of the region encompassing residues V111–V116 (sticks in Figure 3A) in modulation of the PTP was confirmed by the recent finding that H112 (position in the human mature protein) is responsible for the inhibitory effect of acidic matrix pH on the pore (Antoniel *et al.,* 2018), a long-standing observation that awaited molecular clarification (Bernardi *et al.,* 1992; Nicolli *et al.,* 1993). Point mutations of H112 abolished pH-dependent inhibition of PTP-dependent swelling in permeabilized HEK293 cells and prevented H^+-induced channel block of the mega-channel studied by patch-clamp experiments (Antoniel *et al.,* 2018). Molecular dynamics simulations indicate that protonation of H112 may cause a shift in conformational preference within OSCP from more open (Figure 3B,C) to a more tight form (Figure 3D). Simulation results suggest that, upon protonation, (i) a polar interaction occurs between side chains of H112 and E115 of OSCP and (ii) the HRGE stretch 112–115 interacts with region 130–135 (as shown in the crystal structure), which in turn contacts subunit b). Both contacts are more frequent for the protonated form of H112 (Figure 3D). Interestingly, HRGE residues 112–115 in OSCP are conserved in all *Vertebrata* (from *Homo sapiens* to *Xenopus laevis*, excluding *Sauria* and most divergent sequences). The HRGE motif also shows a conformational preference for tight turn or 3–10-helix or α-helix conformations, often with a hydrogen bond or salt bridge between H and E (Wang and Dunbrack Jr., 2003). These data suggest that a direct interaction between the side chains of H112 and E115 in OSCP is likely and could establish pH-dependent interactions with the flexible C-terminal region influencing the position of the peripheral stalk relative to the rest of F-ATP synthase, as the N-terminus of OSCP is rather rigidly linked to the top of F_1 (Figure 2).

The role of OSCP in the modulation of the PTP has apparently been questioned by the results of a recent study where OSCP was genetically ablated in HAP1 haploid clones (He *et al.,* 2017). The authors found that a CsA-sensitive PT could still occur, yet PTP-dependent swelling in KCl-based media was significantly decreased, suggesting a major effect of OSCP ablation on pore size (Bernardi and Lippe, 2018). The persistence of the inhibitory effect of CsA is unexpected because several laboratories have reported the occurrence of PTP-regulatory and CsA-sensitive interactions of CyPD with OSCP (Giorgio *et al.,* 2013; Lee *et al.,* 2016; Burstein *et al.,* 2018). We suspect that inhibition by CsA in OSCP-null cells may be due to insofar undetected interactions of CyPD with additional subunits of F-ATP synthase. Subunit b appears to be a good candidate, as first suggested by Kühlbrandt and coworkers based on high-resolution structures of the *Podospora anserina* F-ATP synthase (Daum *et al.,* 2013). This putative interaction would be consistent with a faint positivity for subunit b in our immunoprecipitation experiments (Giorgio *et al.,* 2013), which we may have overlooked. Indeed, the interaction between OSCP and CyPD could have been weakened by dodecylsulfate, which was added at low concentrations to allow dissociation of the OSCP, b and d subunits (Giorgio *et al.,* 2013).

OSCP interactors

OSCP is strategically located on top of F-ATP synthase, where it is easily accessible to protein interactors (such as CyPD) and drugs. Decrease of OSCP expression with matching increase of CyPD expression and PTP activation were described in brain mitochondria from aging mice, where CyPD depletion

preserved OSCP expression and mitochondrial function (Gauba *et al.,* 2017). Intriguingly, physical interaction of OSCP with **β amyloid protein** (Aβ) and selective loss of OSCP was also observed in the brain of individuals with Alzheimer's disease (AD) and in an AD mouse model (Beck *et al.,* 2016). The predicted Aβ binding region encompasses residues 107–122 of OSCP, leading to a reduced ability to bind the F_1 sector and potentially mediating the mitochondrial impairment characteristic of AD brains. Indeed, in cultured neurons, the OSCP-Aβ interaction sensitized PTP opening and caused synaptic injury despite unaltered CyPD expression levels while OSCP overexpression in mouse and human neurons ameliorated Aβ-mediated mitochondrial and synaptic impairment (Beck *et al.,* 2016).

It is remarkable that earlier studies had already suggested an involvement of CyPD and the PTP in the pathogenesis of Aβ-dependent brain diseases. Thus, in cortical mitochondria of AD brains, as well as in the mAPP mouse model overexpressing a mutant form of human Aβ, a direct interaction of Aβ with CyPD has been described (Du *et al.,* 2008). Cortical mAPP mitochondria showed an increased ROS generation and PTP activation, which were attenuated in *Ppif*$^{-/-}$ mice (which lack CyPD). CyPD deficiency also protected neurons from synaptic impairment and improved learning and memory (Du *et al.,* 2008) also during aging (Du *et al.,* 2011). Based on the crystal structure of the CyPD-CsA complex, 45 compounds have been recently identified in the ChemDiv database, 15 of which inhibited CyPD and showed excellent protective effects against Aβ-induced mitochondrial dysfunction in neuronal cells (Park *et al.,* 2017). Although mechanistic details of how changes of expression of OSCP may affect assembly and/or stability of the F-ATP synthase complex have not been addressed in these models, these findings point to a possible PTP dysregulation mediated by OSCP and CyPD in Aβ-dependent diseases that deserves further attention.

Another condition where OSCP alterations led to F-ATP synthase dysfunction and PTP dysregulation is the French Canadian variant of Leigh Syndrome, which is characterized by low amounts of the leucine-rich pentatricopeptide repeat containing protein (Lrpprc), an RNA-binding protein involved in the stabilization of most mtDNA-encoded mRNAs. In mice with a heart conditional knockout of Lrpprc, impairment of ATP production was matched by lack of assembly of F-ATP synthase and presence of F-ATP synthase subcomplexes, which lacked subunits OSCP and A6L and catalysed oligomycin-insensitive ATP hydrolysis (Mourier *et al.,* 2014). Mice with hepatocyte-specific conditional inactivation of Lrpprc showed assembly defects of F-ATP synthase (whose activity was largely insensitive to oligomycin) with decreased formation of dimers and a remarkable inactivation of the PTP (Cuillerier *et al.,* 2017). Interestingly, in spite of increased CyPD expression, the PTP was insensitive to CsA, suggesting that alterations in the peripheral stalk could have prevented CyPD binding (Cuillerier *et al.,* 2017).

It has been reported that, unexpectedly, OSCP is a client protein of the abundant chaperone **Hsp90**. In COLO 205 colon cancer cells, Hsp90 inhibitors stabilized OSCP levels and elicited reversal of transformation and differentiation. In these cells, OSCP levels were decreased and Hsp90 inhibition increased OSCP levels more markedly than those of other F-ATP synthase subunits and promoted apoptosis *via* the mitochondrial pathway (Margineantu *et al.,* 2007), a possible PTP-dependent event that awaits to be explored and hopefully exploited therapeutically.

OSCP gene expression appears to be altered in diabetes. Up-regulation of OSCP mRNA was found in a rat beta cell line overexpressing the transcription factor pdx-1 (encoded by pancreatic duodenal homeobox gene-1) and exposed to IL-1β for 24 h (Bergholdt *et al.,* 2007) while decreased levels of OSCP-encoding *ATP5PO* transcripts were observed in skeletal muscle from type 2 diabetic patients (Rönn *et al.,* 2009). Modulation of OSCP transcription may be related to DAPIT, which was originally identified in skeletal muscle of rats made diabetic by treatment with streptozotocin (Paivarinne and Kainulainen, 2001) and later shown to be a subunit of F-ATP synthase (Chen *et al.,* 2007; Meyer *et al.,* 2007) essential for its assembly and oligomerization (Ohsakaya *et al.,* 2011; He *et al.,* 2018). The above findings suggest that F-ATP synthase is involved in diabetes, possibly through PTP formation. This hypothesis is supported by the demonstration that mutations of the *Pdx1* gene, which cause diabetes in both mice and humans, also cause increased death of beta cells through CyPD-dependent PTP opening (Fujimoto *et al.,* 2010).

p53 is an oncosuppressor regulating several target genes with diverse biological functions (Sullivan *et al.,* 2018). Although p53 is not necessary for PTP formation (Karch and Molkentin, 2012), it has been reported to trigger PTP opening by physical interaction with CyPD after import into the matrix (Vaseva *et al.,* 2012). The p53-CyPD complex appears to form during brain ischaemia/reperfusion injury, and pretreatment of mice with CsA prevented complex formation resulting in brain protection (Vaseva *et al.,* 2012). Intriguingly, a mitochondrially targeted p53 was also found to interact with OSCP and suggested to take part in the assembly or stabilization of the mature F_OF_1 complex (Bergeaud *et al.,* 2013). Mitochondrial p53 also increased oxygen consumption and decreased ROS levels, suggesting that it may play a role in mitochondrial physiology (Bergeaud *et al.,* 2013). Finally, the newly characterized protein stress responsive activator p300 (Strap), which is known to increase p53 transcripts, has been shown to interact with β subunit of F-ATP synthase decreasing ATP production (Maniam *et al.,* 2015). As cancer cells are sensitized to apoptosis by mitochondrial Strap under glucose-limiting conditions, the interaction of Strap with F-ATP synthase might contribute to the pro-apoptotic effects of p53.

OSCP interacts with the NAD^+-dependent deacetylase **Sirtuin 3** (Sirt3) (Yang *et al.,* 2016). Remarkably, decreased activity of Sirt3 in complex I deficiency favours CyPD binding through increased acetylation of OSCP K47 (Lee *et al.,* 2016) (Figure 3), providing a solid mechanistic explanation for the observation that mitochondria from hearts of complex I deficient mice are sensitized to PTP opening (Karamanlidis *et al.,* 2013). The relevance of OSCP acetylation was also studied in human cells with the 4977 bp deletion of mtDNA that causes chronic progressive external ophthalmoplegia, where ROS production induced a reduction of Sirt3 expression. Suppression of Sirt3 reduced F-ATP synthase activity, decreased ATP levels and respiratory capacity and prevented galactose utilization (Wu *et al.,* 2013). Binding of Sirt3 to F-ATP synthase

Table 1

OSCP interactors affecting F-ATP synthase function and/or PTP formation

Compound/Interactor	F-ATP synthase function	PTP modulation
17β-oestradiol	Zheng and Ramirez (1999) Moreno *et al.* (2013)	Burstein *et al.* (2018)
Bz 423	Johnson *et al.* (2005) Stelzer *et al.* (2010)	Giorgio *et al.* (2013)
Honokiol	Pan *et al.* (2014) Pillai *et al.* (2015)	Li *et al.* (2007) Tian *et al.* (2016)
Cyclophilin D	Giorgio *et al.* (2009)	Giorgio *et al.* (2013) Lee *et al.* (2016) Gauba *et al.* (2017) Burstein *et al.* (2018)
β amyloid protein	Beck *et al.* (2016)	Du *et al.* (2008) Du *et al.* (2011) Beck *et al.* (2016)
p53	Bergeaud *et al.* (2013)	Vaseva *et al.* (2012)
Sirtuin 3	Wu *et al.* (2013) Yang *et al.* (2016)	Lee *et al.* (2016)

critically involves the unique His of OSCP (Yang *et al.*, 2016), which is the same residue mediating inhibition of the PTP by acidic pH (Antoniel *et al.*, 2018). When matrix pH decreases, this pool of Sirt3 is released leading to rapid deacetylation of matrix proteins and to mitochondrial depolarization (Yang *et al.*, 2016). These results indicate that the interaction of Sirt3 with OSCP has a regulatory role in mitochondrial bioenergetics and possibly in regulation of the PTP.

Oestrogens affect both ATP synthase and PTP activity. It was shown that 17β-oestradiol binds OSCP (Zheng and Ramirez, 1999). This interaction provides a mechanistic basis for the reduced efficiency of ATP synthesis by 17β-oestradiol that could be reversed by oligomycin but not resveratrol and was attributed to increased intrinsic uncoupling of the enzyme (Moreno *et al.*, 2013). In a recent development, it has been suggested that sex differences can exist in susceptibility to PTP induction, as shown by the higher sensitivity to Ca^{2+} of mitochondria from female mouse forebrain (Burstein *et al.*, 2018). This higher sensitivity was blunted by an oestrogen receptor β antagonist, which also decreased sensitivity to inhibition by CsA. The effects appear to be mediated by the F-ATP synthase and to involve OSCP because genetic ablation of the oestrogen receptor decreased the interaction between OSCP and CyPD (Burstein *et al.*, 2018).

Honokiol, a low MW polyphenolic compound isolated from *Magnolia*, has been proposed as an anti-cancer compound. It blocks signalling in tumours with defective p53 function and promotes PTP-dependent cell death by up-regulating CyPD levels and affecting channel formation (Li *et al.*, 2007). More recently, and in line with its effect on the pore, honokiol has been found to induce apoptosis in lung cancer cells by promoting ROS production in mitochondria and by inhibiting oligomycin-sensitive respiration (Pan *et al.*, 2014) and to inhibit the mTOR signalling pathway (Tian *et al.*, 2016). Honokiol treatment also enhanced Sirt3 expression nearly twofold and further increased its deacetylase activity with a direct effect on OSCP and MnSOD (Pillai *et al.*, 2015). These effects were associated with amelioration of the Sirt3-dependent cardiac hypertrophic responses in mice, which is consistent with the reported inverse effect of Sirt3 inactivation on the CyPD-OSCP interaction (Lee *et al.*, 2016).

It has already been mentioned that OSCP is the binding site of the immunomodulatory compound Bz 423. Bz 423 was originally characterized in the search for apoptosis-inducing agents able to selectively kill autoreactive B lymphocytes (Blatt *et al.*, 2002). OSCP was identified as its target by screening a phage display library, and it was then shown that Bz 423 is an inhibitor of the F-ATP synthase (Johnson *et al.*, 2005, 2006; Stelzer *et al.*, 2010). Bz 423 competes for CyPD binding on OSCP. Like CyPD, it sensitizes the PTP to Ca^{2+} and pore opening remains sensitive to inhibition by CsA (Giorgio *et al.*, 2013). Bz 423 is thus a chemical mimic of CyPD that can be used to stimulate PTP opening in the absence of CyPD (Giorgio *et al.*, 2013).

The OSCP interactors that affect F-ATP synthase function and/or PTP formation are summarized in Table 1.

Conclusions

OSCP is a key site for the interaction of regulatory proteins and drugs with F-ATP synthase. The recent discovery that F-ATP synthase is also critically involved in the PT has placed OSCP in the spotlight as a potential site of regulation of the transition of the complex into a pore, with a postulated role in the transmission of PTP-regulatory signals to the inner membrane. Molecular definition of the interactions of OSCP with these regulators will provide novel insights into the

mechanism through which the energy-conserving enzyme is transformed into an energy-dissipating structure.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c).

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Conflict of interest

The authors declare no conflicts of interest.

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