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RESEARCH LETTER

ABC transporter activity is affected by the size of lipid nanodiscs

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Lipid nanodiscs have become a widely used approach for studying membrane proteins thanks to several advantages they offer. They have been especially useful for studying ABC transporters, despite the growing concern about the possible restriction of the conformational changes of the transporters due to the small size of the discs. Here, we performed a systematic study to determine the effect of the nanodisc size on the ATPase activity of model ABC transporters from human, plant, and bacteria. Our data confirm that the activity of the transporters and their response to regulatory molecules is affected by the nanodisc size. Our findings suggest the use of larger membrane scaffold proteins (MSPs), such as MSP2N2 nanodiscs, to minimize alterations caused by the commonly used small MSP1D1.

Keywords: ABC transporter; ATPase activity; liposomes; MSP1D1; MSP2E3D1; MSP2N2; nanodiscs

Lipid nanodiscs are small and soluble membrane environments that have become a widely used tool for the *in vitro* study of purified membrane proteins since this technology was introduced nearly two decades ago [1,2]. The diameter of the nanodiscs is determined by the length of the membrane scaffold protein (MSP) used during reconstitution. The most used MSPs are MSP1D1 and MSP1E3D1, which give discs of ~10 nm and 12 nm diameter, respectively. Other varieties are also available, including the MSP2N2, which produces larger nanodiscs (with a diameter of ~16 nm) although those have been rarely used. These discs have many advantages since the protein is embedded in a phospholipid lipid bilayer that can resemble their native environment without the complications offered by the traditional reconstitution in liposomes. Nowadays, it is very common to find structural models of proteins in nanodiscs determined by cryo-electron microscopy (Cryo-EM) and many biochemical and biophysical studies are also making use of the nanodiscs technology. However, over the years there have been growing concerns about the possible influence of the restricted size of the small discs on the conformational dynamics of the proteins inserted in them [3]. This concern has been becoming especially louder in the field of ATP-binding cassette (ABC) transporters, which are proteins that can have long helical domains protruding outside of the membrane, connecting to their large globular nucleotide binding domains (NBDs) that hydrolyze ATP. Substrate binding to the transmembrane domain allosterically regulates the ATPase activity on the NBDs, commonly leading to an increase in hydrolysis rate [4,5]. Numerous studies of ABC transporters have reported their large flexibility, and some models suggest that the ability of these proteins to undergo large conformational changes is essential for their appropriate transport function whereas

Abbreviations
ABC, ATP-binding cassette; BV, biliverdin; CHS, cholesteryl hemisuccinate; DDM, dodecylmaltoside; GSH, reduced glutathione; GSSG, oxidized glutathione; MSP, membrane scaffold protein; NBD, nucleotide binding domains; PL, proteoliposomes.

there is also concern about the possibility that some available structural models represent non-physiologically relevant conformations trapped by certain experimental conditions such as the use of detergent micelles, the crystal lattice or nanodiscs [6–8]. Reports of different ATPase activity for some ABC transporters in nanodiscs *versus* detergent are available and often a higher ATP hydrolysis rate in nanodiscs has been interpreted as a positive effect of having a more stable protein thanks to the presence of the lipid bilayer [9–12]. But are these effects on the ATPase activity due to their reconstitution in nanodiscs a reliable feature that will improve our understanding of the molecular mechanisms of these proteins?

ABC transporters belong to a superfamily of proteins found in all kingdoms of life and these proteins have played an important role in the evolution of bacteria, plants, and animals [13]. Therefore, we decided to do a systematic study evaluating the effect of different membrane mimetic systems on the ATPase activity of ABC transporters from human, plant, and bacteria. We have found that although each protein displays different susceptibility to different mimetic systems, they all have in common a decreased response to regulatory molecules when the transporters were reconstituted in the small MSP1D1 nanodiscs. Our data confirm an effect of the size of the nanodiscs on the activity of ABC transporters, possibly due to alterations in the conformational equilibrium of these proteins. These observations are likely to apply to related transporters.

Materials and methods

ABC transporters expression and purification

Genes for human ABCB10 and LmrA from *Lactococcus lactis* were synthesized by GenScript (NJ) and inserted in the pET19 plasmid. *Arabidopsis thaliana* Atm3 in pET21a+, was a gift from Dr. Douglas Rees [14]. ABC transporters were expressed in Rosetta2 (DE3) *E. coli* cells in autoinduction media with appropriate antibiotics [15]. Purifications were done with standard Ni-NTA affinity chromatography. Protein concentration was determined by BCA assay or estimated by gel electrophoresis of purified samples compared to known concentrations of standard albumin samples. Gels were stained with Coomassie blue and quantified using IMAGEJ [16].

Production of membrane scaffold proteins

Plasmids for membrane scaffold proteins (MSPs) of different sizes were a gift from Dr. Stephen Sligar [17–19]. MSP proteins were produced following standard protocols as described earlier for these proteins [2], with small modifications.

Reconstitution

Purified proteins were reconstituted in nanodiscs of different sizes or in liposomes, as described in detail in supporting information. Briefly, for nanodiscs, the purified proteins were reconstituted with *E. coli* polar lipids extract (Avanti Polar Lipids) as described earlier [20]. For reconstitution in liposomes, we followed the method described earlier for insertion of ABC transporters into liposomes, with minor modifications [21]. Importantly, we performed our reconstitutions in all these different systems with *E. coli* polar lipids from the same batch, in parallel with each other. Therefore, our comparisons between reconstitution systems in single experiments were done with an identical lipid composition. For control experiments with ABCB10 in liposomes, we also followed similar procedures but using soy phospholipids (Sigma-Aldrich, St. Louis, MO, USA) instead of *E. coli* polar lipids.

ATPase activity measurements

ATPase activity was determined by a colorimetric assay measuring released Pi [22]. Briefly, the protein sample was incubated in MgATP containing buffer at the desired temperature and time, and then, the reaction was stopped by addition of SDS, followed by color development. Absorbance (850 nm) was measured in a microplate reader (SpectroStar Nano, BMG). For ABCB10 and LmrA the buffer was 140 mM KCl, 20 mM Tris/HCl pH 8, with a final concentration of 2 mM ATP and 4 mM $MgCl_2$ (for ABCB10) or 5 mM ATP and 10 mM $MgCl_2$ (for LmrA) and the buffer was supplemented with 0.06% DDM and 0.006% CHS for samples in detergent. For both ABCB10 and LmrA, we incubated the reaction at 37 °C, because this is the physiological temperature for humans and the optimal temperature reported for biomass growth of *Lactococcus lactis* [23]. The substrates (Biliverdin, Daunorubicin, and Verapamil) were made as concentrated stocks in dimethyl sulfoxide (DMSO). For *At*Atm3 we used the same approach, but following the conditions previously reported for this transporter [14]. ATPase assays at 25 or 37 °C were performed in 100 mM KCl, 20 mM Tris/HCl pH 7.5, 5 mM ATP, and 10 mM $MgCl_2$. The substrates, GSSG and GSH were added to the buffer. For experiments in detergent, the buffer was supplemented with 0.02% DDM. For Michelis-Menten kinetics, and substrate binding effect, the experiments were performed with the respective buffers supplemented with the indicated ATP or substrate concentrations.

Statistical analysis and data presentation

Data points represent the average and standard deviation of experiments performed with at least two fully independent purifications for ABC transporter, as indicated in each figure. Experiments were done by duplicate or triplicate for each sample. Comparisons between conditions were made

with a paired t-test in Origin Lab and the P values <0.05 are shown in the figures. Curves to determine the effect of [ATP] were fitted by Michaelis–Menten equation, whereas a Hill equation with an offset start was used to determine the effect of different concentrations of substrate. Plots and fitting were done in Origin Lab.

Further details of all methodology can be found in the supporting information.

Results

Human inner mitochondrial membrane transporter ABCB10

We first analyzed the dependence of the ATPase activity of a purified human transporter and the activation by substrate in the traditional systems represented by detergent micelles and liposomes. ABCB10 is a transporter in the inner mitochondrial membrane, where it transports biliverdin [24]. Our data (Fig. 1A) show no significant difference in basal ATPase activity for ABCB10 in detergent and proteoliposomes, but a clear difference in how much the substrate biliverdin (BV) activates. In detergent, BV increases the ATPase activity around twice, whereas in liposomes the activity is increased by a factor of almost four. This difference could reflect a sensitivity of the transporter to the biophysical properties of detergent micelles *versus* liposomes. For the detergent micelles, we have used a combination of DDM and CHS, as previously reported [25]. These detergent-sterol micelles have a bicelle-like architecture that can help stabilize membrane proteins [26]. For reconstitution we have used lipids from *E. coli*, which contain cardiolipin, a lipid found in the inner mitochondrial membrane, and which have been suggested to regulate the activity of ABCB10 [25,27]. However, control experiments of ABCB10 in liposomes of soy lipids (which have a different composition and lack cardiolipin) show similar basal ATPase activity and activation by BV as in *E. coli* lipids (Fig. S1). Lipids tightly bound to purified ABCB10 have been detected before, which might explain the lack of regulation in our sample if our protein co-purifies with lipids essential for its function [27].

Next, we evaluated the ATP hydrolysis activity of ABCB10 reconstituted in nanodiscs of different size, using membrane scaffold proteins small (MSP1D1, ~10 nm), medium (MSP1E3D1, ~12 nm) or large (MSP2N2, ~16 nm), and the same lipid composition used for liposomes [2]. Our data (Fig. 1B) shows that the basal activity of ABCB10 changes with the size of the nanodiscs used, with the smaller nanodiscs (D1) causing an increase in basal activity to almost twice the rate observed for larger nanodiscs (N2), or the activity measured in either liposomes or detergent micelles. The basal activity in medium size nanodiscs (E3) is intermediate and significantly different than that measured on the larger nanodiscs. Interestingly, there is no statistical difference in the ATPase activity measured at 2 mM ATP in the presence of substrate between the different-sized nanodiscs or when compared to liposomes. To further evaluate the effect of different reconstitution systems, we studied the dependence of the ATPase activity over a range of [ATP]s (Fig. 1C). We found that the measured K_m (~0.3 mM) was not significantly different between nanodiscs and liposomes nor affected by the presence of substrate. The main differences occurred in V_{max}, with the small D1 nanodiscs promoting a much-elevated basal ATPase activity, that is less activated by the presence of substrate. We also evaluated possible differences in affinity for substrate (Fig. 1D) and found that ABCB10 in N2 nanodiscs and liposomes have an identical affinity for BV (K_d 1 μM), whereas that affinity is increased in the small D1 nanodiscs (K_d ~ 0.4 μM). Tables S1 and S2 show additional details for fittings presented in Fig. 1C and D. In summary, our data indicates that the ability of the transporter to respond to substrate is decreased in the D1 nanodiscs (~2-fold lower activation than for N2 nanodiscs and liposomes), likely due to the existence of a preactivated state in basal conditions. The decreased activation by BV is not caused by a decreased affinity for the substrate, since ABCB10 in D1 nanodiscs displayed an increased affinity for BV. Thus, whereas the behavior of ABCB10 in N2 nanodiscs and liposomes follows a similar trend, with low basal ATPase activity and robust activation by substrate, the transporter in D1 nanodiscs behavior deviates from that tendency. Incorporation in small discs might alter the conformational equilibrium, promoting enhancements in basal conditions (faster ATP hydrolysis and increased affinity for BV), but undermining the net stimulation by substrate.

An obvious question that arises from our observations on ABCB10 is whether the size of the nanodiscs also affects the activity of other ABC transporters and their ability to respond to substrate or other regulatory molecules. Therefore, we have made a systematic analysis of the ATPase activity of two additional ABC transporters (one from plant and one from bacteria) to obtain a side-by-side comparison of their basal activity and the response to regulatory molecules when these transporters are in detergent micelles, liposomes, and nanodiscs of different size.

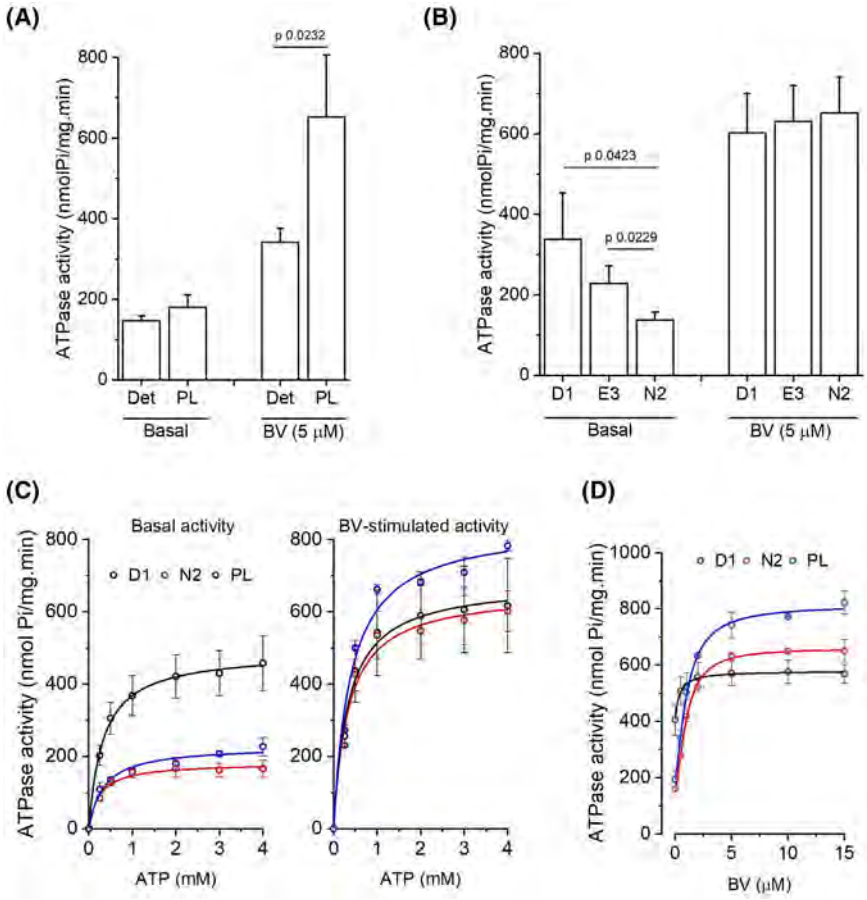

Fig. 1. ATPase measurements for ABCB10 in the absence (basal) or presence of biliverdin (BV). (A) Protein in detergent micelles (Det) or proteoliposomes (PL). (B) Protein reconstituted in nanodiscs of different size: MSP1D1 (D1), MSP1E3D1 (E3), or MSP2N2 (N2). (C) ATP concentration dependence of ABCB10 in absence (left) and presence of 5 μM BV (right). Data points were fitted to a Michelis-Menten equation (continuous lines), with K_m values of 0.33 ± 0.02, 0.24 ± 0.04, and 0.34 ± 0.07 mM for basal activity in D1 (black), N2 (red), and PL (blue), respectively. K_m values for BV-stimulated activity were 0.33 ± 0.05, 0.34 ± 0.07, and 0.39 ± 0.08 mM for D1 (black), N2 (red), and PL (blue), respectively. (D) BV concentration dependence of ABCB10 at 4 mM ATP. Data points were fitted to a Hill equation (continuous lines) with apparent K_d of 0.38 ± 0.06, 1.01 ± 0.04, and 1.06 ± 0.08 μM for D1 (black), N2 (red), and PL (blue), respectively. Additional details of fittings in parts C and D can be found in Tables S1 and S2. Data from at least 2 independent protein purifications are presented as mean and standard deviation. The figure shows *P* values between statistically different conditions.

Plant inner mitochondrial membrane transporter AtAtm3

Like ABCB10, the *Arabidopsis thaliana* Atm3 (ABCB25) is a half-transporter member of the B family also found in the inner mitochondrial membrane [28]. Although the physiological substrate for *At*Atm3 is unknown, glutathione in the reduced (GSH) or oxidized (GSSG) forms have been recently shown to increase the ATPase activity of the transporter in detergent micelles and in MSP1D1 nanodiscs at 25 °C [14]. Here, we compare the ATPase activity of *At*Atm3 in detergent micelles, proteoliposomes, and small (D1) and large (N2) nanodiscs (Fig. 2). Because the inner mitochondrial membrane of mammalian and plant cells have a similar composition [29], and to have a direct comparison with our ABCB10 results, we have used the cardiolipin-containing lipids from *E. coli* as described above. We first studied *At*Atm3 at 25 °C (Fig. 2A) and found that the basal activity of the transporter is indistinguishable for all these conditions. Interestingly, another mitochondrial ABC transporter implicated in the transport of glutathione from a thermophilic fungus (*Ct*Atm1) has very similar basal ATPase activity in detergent or in MSP1D1 nanodiscs [30]. When we analyzed the stimulation by GSSG and GSH at the optimal concentrations priorly reported for these substrates [14], we found that in the small D1 nanodiscs, there was no significant difference in

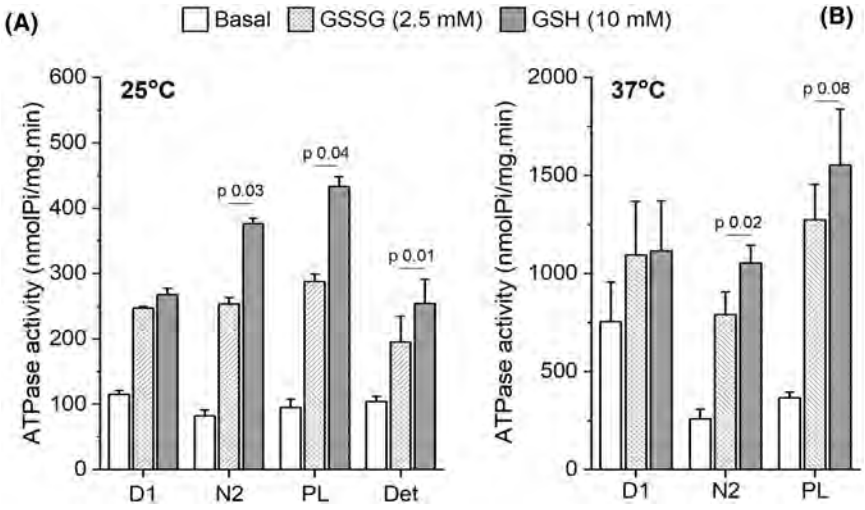

Fig. 2. ATPase activity of *At*Atm3 transporter in different membrane mimetic systems in the absence of substrate (basal) and in the presence of the indicated concentration of oxidized (GSSG) or reduced (GSH) glutathione. (A) ATPase activity measured at 25 °C in MSP1D1 (D1) or MSP2N2 (N2) nanodiscs, in proteoliposomes (PL), and in detergent micelles (Det). (B) ATPase activity measured at 37 °C in MSP1D1 (D1) or MSP2N2 (N2) nanodiscs, in proteoliposomes (PL). Data from 2 independent protein purifications are presented as mean and standard deviation. The figures show *P* values between statistically different conditions.

the activation by GSH and GSSG. However, GSH consistently had a larger stimulatory effect than GSSG for the transporter in detergent micelles, liposomes, and in the large N2 nanodiscs. The mechanistic or functional implications of this different response to reduced *versus* oxidized glutathione for this transporter are currently unclear, but our experimental data for ABCB10 and AtAtm3 show a similar tendency where the transporters in N2 nanodiscs behave similarly to those in liposomes, whereas the reconstitution in the small D1 nanodiscs causes a different trend.

The temperature affects the biophysical properties lipid bilayers of a determined lipid composition [31]. The lipids we used are extracted from *E. coli* cells grown at 37 °C (Avanti Polar Lipids), and bilayers formed with this extract are expected to have decreased fluidity at 25 °C. Therefore, we decided to also study the activity of *At*Atm3 at the same temperature we used for ABCB10 (37 °C), thus maintaining identical parameters for the membrane. *Arabidopsis thaliana* is adapted to live in a wide range of temperatures [32,33], so this more elevated temperature is still physiologically relevant for this transporter. The data (Fig. 2B) shows higher ATPase activity for all the conditions at 37 °C *versus* 20 °C, but the basal activity in D1 nanodiscs at 37 °C is ~2-fold higher than that of liposomes or N2 nanodiscs. This preactivated *At*Atm3 displays lower substrate stimulation and remains unable to distinguish between GSH and GSSG. The molecular mechanisms by which D1 nanodiscs are altering the behavior of these transporters and the specific role of the membrane's biophysical parameters remain to be elucidated. Our data suggest that, when studied under identical experimental conditions, both the human and the plant mitochondrial inner membrane transporters show elevated basal activity and diminished response to substrate in the small D1 nanodiscs.

Bacterial multidrug transporter LmrA

To determine if the nanodiscs size also affects the activity of ABC transporters in the plasma membrane, we have studied the bacterial transporter LmrA, a multidrug resistance transporter from *Lactococcus lactis*, capable of replacing the function of P-glycoprotein (ABCB1) in cultured lung fibroblasts [34]. Similarly to what has been reported before, our LmrA solubilized with DDM presents very low ATPase activity that is not stimulated by substrate, but the ATP hydrolysis rate increases significantly after reconstitution in liposomes made with *E. coli* lipids [35]. Since the ATPase activity of LmrA in detergent micelles is very low, we have focused on comparing the activity of the transporter reconstituted in liposomes and in the smallest (D1) or largest (N2) nanodiscs (Fig. 3). We evaluated the effect of various drugs and found that verapamil and daunorubicin significantly stimulated the ATPase activity of the reconstituted transporter in a concentration-dependent fashion (Fig. 3A,B,

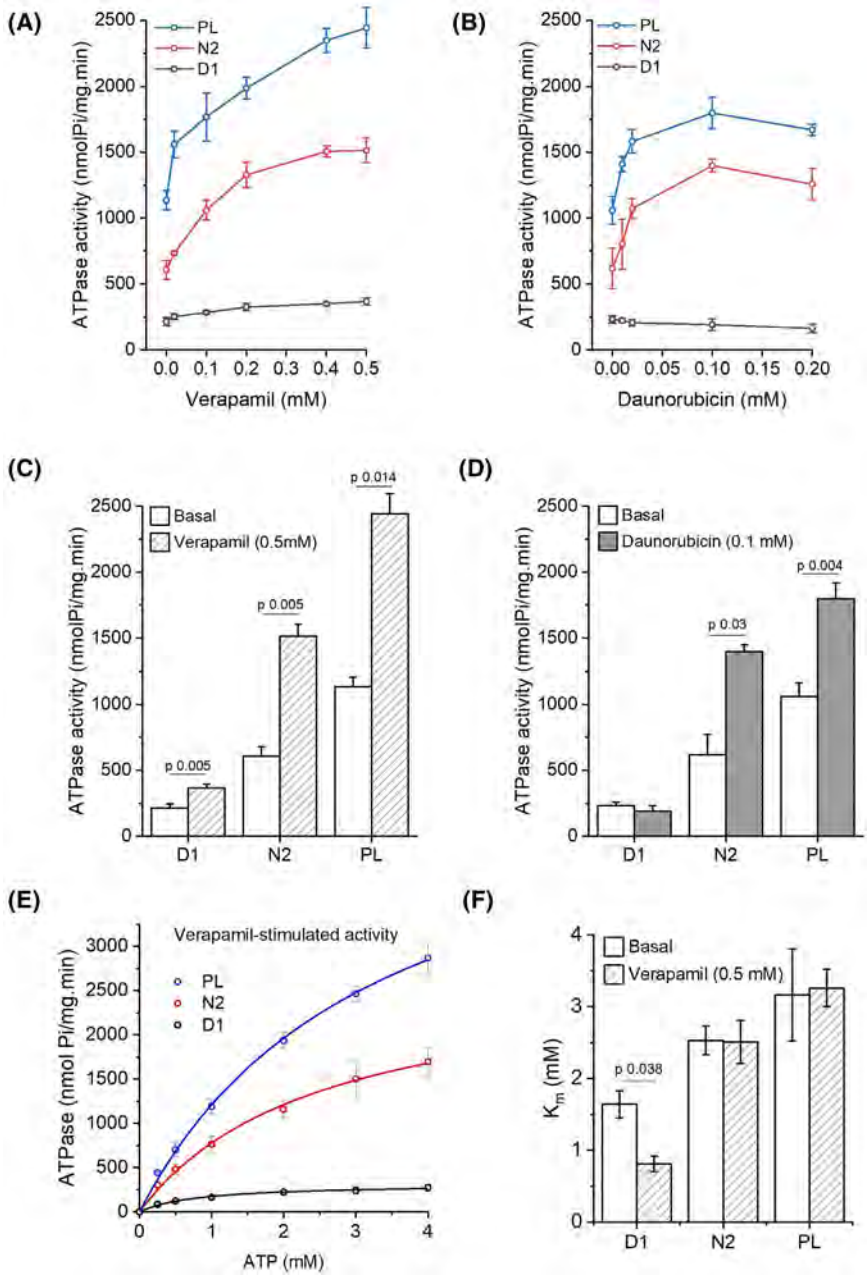

Fig. 3. ATPase activity of the bacterial transporter LmrA in MSP1D1 (D1) or MSP2N2 (N2) nanodiscs, and in proteoliposomes (PL). (A) Response to different concentrations of verapamil. (B) Response to different concentrations of daunorubicin. (C) Comparison of ATPase activity in the absence (basal) and presence of verapamil at the indicated concentration. (D) Comparison of ATPase activity in the absence (basal) and presence of daunorubicin at the indicated concentration. (E) ATP concentration dependence of LmrA's ATPase activity stimulated by 0.5 mM verapamil. Data points were fitted to a Michelis-Menten equation (continuous lines) for protein reconstituted in D1 (black) and N2 (red) nanodiscs, and PL (blue). (F) Effect of reconstitution systems on the affinity for ATP during basal and verapamil-stimulated conditions. Data from 2 independent protein purifications are presented as mean and standard deviation. The figures show *P* values between statistically different conditions.

respectively). Verapamil has been priorly shown to increase the ATPase activity of LmrA on vesicles from insect cells overexpressing the transporter and to inhibit the resistance to drugs due to overexpression of LmrA in lung fibroblasts [34]. Daunorubicin stimulation of the ATPase activity of LmrA in liposomes has

been reported before [36]. In general, our data show a dramatic effect of the reconstitution system on the specific ATPase activity of LmrA for both basal and drug-stimulated activity, with increasing values as we move from the low activity in D1 nanodiscs to the highest activity measured in liposomes. In addition, our data clearly show that the drug-activation of LmrA in the large N2 nanodiscs is closer to the effect measured in proteoliposomes, which is the traditionally used reconstitution system. For an easier comparison with our data for ABCB10 and *At*Atm3, Fig. 3C, D summarize the basal activity and the stimulated activity at a single drug concentration (0.5 mM Verapamil or 0.1 mM Daunorubicin) at 5 mM ATP and 37 °C. Verapamil increases the ATPase activity ~2-fold in liposomes and N2 nanodiscs, but only ~1.3-fold in D1 nanodiscs, whereas Daunorubicin activates LmrA in liposomes and N2 nanodiscs but fails to activate the transporters in D1 nanodiscs. Once again, our data on LmrA suggests that these ABC transporters reconstituted in either N2 nanodiscs or liposomes have a similar stimulation by regulatory molecules, whereas transporters reconstituted in the small D1 nanodiscs have a decreased response. We next studied the dependence on ATP concentrations of the basal and verapamil-stimulated ATPase activity in the different reconstitution systems (Fig. 3E) and we report the values of K_m for each condition (Fig. 3F). LmrA presents larger K_m values (above 1 mM) that tend to increase as the size of the reconstitution system increases. The K_m in N2 nanodiscs and PL were not affected by Verapamil, but Verapamil does decrease the K_m for the protein in D1 nanodiscs, suggesting that the transporter can adopt a distinct state in the small nanodiscs where verapamil leads to higher affinity for ATP. These interesting results on LmrA highlight one more time the similarity between N2 nanodiscs and PL for the study of ABC transporters and contrast them with the divergent behavior observed in D1 nanodiscs.

Discussion

It is well known that the activity of membrane proteins can be regulated by protein-lipid interactions. For example, the activity of P-type ATPases is affected by general lipid-protein interactions, where the general physicochemical properties of the lipid environment affect the conformational flexibility of the membrane protein, and by specific lipid-protein interactions, where specific molecules directly interact with specific lipid-binding sites in the protein [37]. In terms of the physicochemical properties of the lipid bilayer, membrane mimetic environments can cause perturbations in protein structure, including the effect that thickness and lateral tension of lipid bilayer mimetics can produce on the tilt and compactness of alpha-helices in the transmembrane domains [38]. Experimental and theoretical studies in nanodiscs have shown that the thickness and order parameters of the bilayer are not homogenous from the center to the edges of the discs [39] and that the nanodiscs can alter the elastic properties of the bilayer, with MSP1D1 nanodiscs showing larger stiffness in the center, whereas MSP1E3D1 and MSP2N2 nanodisc present milder spatial changes [40]. In addition, a recent Cryo-EM study has presented clear evidence of how the structure of a pentameric ligand-gated ion channel is affected by either the size of the nanodiscs or by direct interactions between the reconstituted channel and the small membrane scaffold protein that surrounds the disc [41]. The lipid composition of the nanodiscs can also induce changes in the form of the disc, with incorporation of negatively charged phosphatidylglycerol making MSP1D1 nanodiscs more elongated than discs made with only phosphatidylcholine, perhaps due to electrostatic interactions between the lipids head and the MSP [42]. Altogether, the data point to complex differences in the physicochemical properties of the phospholipid bilayer in nanodiscs of different sizes, plus the establishment of potential interactions between the phospholipids, the MSP, and our protein of interest, which can lead to additional conformational perturbations. A similar tendency has been described for the use of polymers instead of MSP-based nanodiscs. The size of the disc and/or the properties of the polymer affected the conformational changes of the G-protein-coupled receptor protein rhodopsin, with the smaller discs (SMALP and SMILP, both ~10 nm diameter) restricting the photoconversion to the fully active receptor that could be achieved with larger discs (DIBMALP, ~19 nm diameter) [43]. Our data clearly shows that ABC transporters can be very sensitive to the size of the nanodiscs. Using the activity of the transporters in liposomes as reference model, our data suggest that the smallest MSP1D1 nanodiscs do alter the basal activity of human (ABCB10), plant (*At*Atm3), and bacterial (LmrA) ABC transporters and their ability to respond to substrate, whereas the largest MSP2N2 nanodiscs seem to be a better reconstitution system, where the transporter presents good stimulation by substrate. As discussed above, the phospholipid bilayer in larger nanodiscs is expected to be more homogenous, and its properties could be more like those of the lipid bilayer in liposomes. Also, the larger diameter of the MSP2N2 nanodiscs can decrease

the chances of direct interactions between the ABC transporter and the MSP that surrounds the nanodisc.

The dependency of the ATPase activity of each transporter on the size of the nanodiscs has shown different tendencies. In ABCB10, the smallest nanodiscs increase the basal ATPase activity of the transporter, suggesting that the properties of the bilayer in this system and/or undesired interactions due to the MSP, might be affecting the dynamic equilibrium of ABCB10, favoring the transition to catalytically active conformations and promoting a distinct conformational state with higher affinity for biliverdin. In LmrA, we observed the opposite behavior, with the lowest ATPase activity in the MSP1D1 nanodiscs, whereas for *At*Atm3 the relative basal ATPase activity is elevated at 37 °C, but not at 25 °C. These observations for the basal activity of each transporter suggest that they are affected differently by the physicochemical properties of the bilayer, perhaps because each of these proteins has adapted to function under determined circumstances that might diverged between humans, plants, and bacteria. ABCB10 and *At*Atm3 are mitochondrial inner membrane transporters, while LmrA resides in the plasma membrane where it can be more exposed to environmental variations. The dramatic changes observed in LmrA could be related to ability of lactic acid bacteria to survive under a large variety of stress conditions (pH, osmolarity, temperature, etc.) [44,45] and the lower affinity of LmrA for ATP could reflect another evolutive feature, since LmrA has been shown to also function as a secondary-active multidrug transporter using energy from a sodium electrochemical gradient [46]. Our results can also imply possible differences in the individual molecular mechanism of each transporter. For example, ABCB10 transports the small and hydrophobic biliverdin and *At*Atm3 has been shown to transport the small and hydrophilic glutathione, whereas LmrA is a multidrug resistance transporter homolog to P-glycoprotein, which transports a large variety of hydrophobic molecules. In addition, any possible regulatory effect of specific lipids in each of these transporters remains to be investigated.

The nanodiscs technology represents a very useful tool for biophysical and biochemical studies of membrane proteins. Detergent micelles seem to be a good mimetic model for certain proteins, whereas other membrane proteins can be highly unstable outside of a phospholipid bilayer. In the case of ABC transporters, our data show that ABCB10 has stable ATPase activity and some activation by substrate in detergent micelles, whereas LmrA essentially lacks ATPase activity in detergent and becomes very active after reconstitution. However, our data strongly suggests that the MSP must be carefully selected for the membrane protein to be studied. Most available Cryo-EM structural models of ABC transporters have been obtained for transporters reconstituted in MSP1D1 nanodiscs and now it seems likely that this small nanodisc might have caused perturbations on the structure of those proteins. Our data highly recommends the use of larger MSPs, such as MSP2N2, which consistently supported basal and stimulated ATPase activities closer to those values observed for the ABC transporters reconstituted in liposomes. In the study of ABC transporters in lipid nanodiscs, the size does matter.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

ANB designed study, conducted experiments, analyzed data, and edited manuscript. LGC conducted experiments and edited the manuscript. MEZ designed study, conducted experiments, analyzed data, and wrote manuscript.

Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/1873-3468.15096.

Data accessibility

The data that support the findings of this study are available from the corresponding author mzoghbi@ucmerced.edu upon reasonable request.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. The ATPase activity of ABCB10 reconstituted in liposomes of different lipid composition.

Table S1. Michaelis–Menten fittings of ABCB10 in different reconstitution systems, for basal and biliverdin (5 μM) stimulated ATPase activity.

Table S2. Response of ABCB10 to biliverdin in different reconstitution systems.