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The P-glycoprotein multidrug transporter: interactions with membrane lipids, and their modulation of activity

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Introduction

Lack of response to chemotherapy is one of the major reasons for treatment failure in many human cancers, including colon, kidney and breast carcinomas, leukaemias and multiple myeloma. This lack of response is often caused by resistance to multiple chemotherapeutic agents, known as multidrug resistance (MDR). Drug resistance may be intrinsic to the tumour at the outset, or it may be acquired after one or more rounds of chemotherapy treatment. One of the leading causes of such drug resistance is the overexpression of a 170 kDa plasma-membrane glycoprotein, designated P-glycoprotein (Pgp). This protein, also known as the multidrug trans-

Abbreviations used: DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DSC, differential scanning calorimetry; MDR, multidrug-resistant; PC, phosphatidycholine; PE, phosphatidylethanolamine; Pgp, P-glycoprotein; PI, phosphatidylinositol; PMPC, 1-palmitoyl-2-myristoylphosphatidylcholine; PS, phosphatidylserine; SM, sphingomyelin.

porter, is proposed to operate as an ATP-driven efflux pump for a wide variety of hydrophobic drugs (for reviews, see [1–3]). Drugs falling within the MDR spectrum include the Vinca alkaloids (vinblastine, vincristine), colchicine, anthracyclines (doxorubicin, daunorubicin) and taxol. These compounds are structurally unrelated, and have different intracellular targets and mechanisms of action, but share the common features of being large hydrophobic natural products. Many clinical studies have linked the expression of Pgp in human cancers to low response to chemotherapy, and poor overall prognosis [4].

Pgp is also displayed in large amounts on the surface of cell lines selected for growth in vitro in high levels of cytotoxic drugs (for example, see [5]). Such MDR cell lines have proved invaluable in the study of the MDR phenomenon over the last two decades. Compared with the drug-sensitive parent cell line, MDR cells show decreased accumulation and increased efflux of drugs, which is dependent on metabolic energy. More recently, drug-selected MDR cells have provided the starting material for
puriﬁcation of functional Pgp by several research groups [6–8], and subsequent characterization of its biochemical properties.

Molecular biological studies have indicated that Pgp exists as a small multigenic family, with three isoforms in rodents (Classes I, II and III) and two isoforms in human (Classes I and III). Both Class-I and -II Pgps confer MDR, as shown by transfection experiments, whereas Class-III Pgp does not. Class-III Pgp is expressed exclusively at the apical surface of liver canaliculus cells in rodents, and a recent study using transgenic mice in which this Pgp gene was inactivated by homologous recombination (so-called Class-III Pgp knock-outs) suggested that the role of this isoform is to export phosphatidylcholine (PC) into the bile [9]. This hypothesis was corroborated by the work of Ruetz and Gros [10], who demonstrated that Class-III Pgp (but not Class-I or -II isoforms) was a PC-speciﬁc ﬂippase.

Membrane organization and function of Pgp

Determination of the cDNA sequence of the multidrug transporter proteins from human and rodents, and hydropathy analysis of the deduced amino acid sequence, led to a proposed topographical model for Pgp (Figure 1; for reviews, see [1–3]). The protein is predicted to possess two homologous halves, each with six putative hydrophobic membrane-spanning α-helices, and a cytosolic domain with the Walker A and B consensus sequences for a nucleotide-binding fold. Many of these topological features have since been conﬁrmed by biochemical mapping studies (see, for example, [11–13]). These features are not unique to Pgp; they are shared by many other proteins which make up the ABC (ATP-binding cassette [14]), or trafﬁc ATPase [15], superfamily of membrane transporters. Other members of this superfamily include components of binding-protein-dependent permeases in bacteria (e.g. HlyB, hisP, MalK), and in eukaryotes, the CFTR Cl– channel, the yeast ste-6 a-factor exporter, the Tap1/2 peptide exporters of the endoplasmic reticulum, and another protein that confers MDR, MRP. Pgp can be photoaffinity-labelled with both drug analogues and azido-ATP, and was proposed to function as an active transporter for drugs, powered by ATP hydrolysis at the two nucleotide-binding folds. Bio-

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\[ \text{Figure 1} \]

Structural and topological model of Pgp

(a) Linear sequence of the Pgp molecule: the solid boxes represent the 12 putative membrane-spanning segments of the transporter, and the Walker A and B motifs of the two cytosolic nucleotide-binding domains are indicated by hatched boxes. (b) Proposed arrangement of Pgp in the plasma membrane. The site of N-glycosylation is indicated in the first extracellular loop. This orientation is supported by both predictive hydropathy plots, and several independent studies on membrane topology of the full-length protein. Reproduced with permission from [3].

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Extracellular

\[ \text{PLASMA MEMBRANE} \]

Intracellular
chemical evidence has accumulated that this is indeed the case. Isolated Pgp has the ability to hydrolyse ATP [6–8], and when reconstituted into lipid bilayers, transports a variety of hydrophobic drugs in an active fashion [8,16].

The mechanism by which Pgp exports such a wide diversity of hydrophobic compounds and how this transport is coupled to ATP hydrolysis are currently unknown. Class-III Pgp, which is a lipid flippase, shows a high degree of sequence similarity to the two Pgp isoforms that are multidrug transporters, indicating that they may share fundamental aspects of their mechanism of action. In fact, Pgp was previously proposed to be a drug flippase that moves drugs from the inner to the outer leaflet of the plasma membrane [17]. Diffusional equilibration of the drug in each leaflet with the adjoining aqueous compartment would generate the observed transmembrane drug concentration gradient. In support of this proposal, it has been demonstrated that Pgp intercepts hydrophobic substrates before they have an opportunity to enter the cytosol [18,19], suggesting that the transporter may pump hydrophobic compounds directly out of the lipid bilayer.

Certain chemical agents have been identified that reverse MDR by inhibiting the drug-pumping action of Pgp (for reviews, see [3,20]). These compounds, termed chemosensitizers, MDR antagonists or MDR reversers, appear to compete, although in a complex and poorly understood fashion, for the drug-binding site(s) on Pgp. Some first-generation chemosensitizers that were identified include verapamil (a Ca2+-channel blocker), trifluoperazine (a calmodulin inhibitor) and cyclosporin A (an immuno-suppressant). As with Pgp substrates, chemosensitizers are also hydrophobic, and exhibit wide structural diversity. Despite a large number of studies of many different chemosensitizers, their mode of action remains poorly understood. Chemosensitizers are clearly of great clinical potential when combined with chemotherapeutic drugs for modulation of MDR, and clinical trials of several more effective second-generation chemosensitizers are currently under way.

Given the intimate relationship between Pgp, its hydrophobic substrates, and the surrounding membrane environment, it is not surprising that phospholipids appear to play an important role in modulating both the ATPase activity and transport properties of Pgp. This paper aims to present some of the more recent results obtained by our laboratory in this area, and relate them to the structure and function of the transporter.

**Model systems for the study of Pgp**

In order to examine the functions and behaviour of Pgp at the biochemical level, it was necessary to move from using intact MDR cells to much simpler model systems. We have made extensive use of plasma-membrane vesicles [21] from a series of MDR Chinese hamster ovary cells originally selected for resistance to colchicine [5,22], especially the highly drug-resistant cell lines CH'C5 and CH'B30. Plasma-membrane vesicles from these cells display high levels of Mg2+-dependent ATPase activity, 70–80% of which is attributable to Pgp [23–25]. This ATPase activity is further stimulated by certain drugs and chemosensitizers in the MDR spectrum [23,24]. In addition, on being supplied with ATP, an ATP-regenerating system and an appropriate substrate (such as radiolabelled colchicine or vinblastine), uptake of drug into the vesicle lumen can be demonstrated [26]. Drug uptake is osmotically sensitive and abolished by detergent permeabilization, confirming that it represents transfer of drug into the intravesicular space. Drug transport takes place up a concentration gradient, which indicates that Pgp is indeed an active transporter [26].

Since Pgp constitutes only approx. 10–15% of the protein in plasma-membrane vesicles from MDR cell lines, purification was necessary for further biochemical and biophysical characterization of the multidrug transporter. This was made possible by the development of a two-step protocol for differential extraction of Pgp from CH'C5 plasma-membrane vesicles using the zwitterionic detergent CHAPS [23]. The resulting detergent extract, known as the S2 fraction, is highly enriched in Pgp. Inhibitor studies indicated that all of the ATPase activity of the S2 preparation arises from Pgp. The remaining contaminants can be removed from the S2 fraction by a negative lectin-affinity selection step on lentil lectin–or concanavalin A–Sepharose to give more than 90% pure Pgp with a high level of constitutive ATPase activity [6]. This procedure has recently been improved by the use of the CH'B30 cell line, which has a very high level of both drug-resistance and Pgp expression. It is now possible to obtain 0.25 mg quantities of pure Pgp (>95%) with a very high level of ATPase activity (>2.3 μmol/min per mg) from 2 mg of CH'B30.
plasma-membrane starting material, within only a few hours [25]. This preparation is relatively concentrated, and free of ATP and added lipids, which makes it ideal for biochemical and biophysical studies of the protein.

Pgp has been reconstituted into phospholipid bilayers using detergent removal by gel-filtration chromatography. The resulting vesicles displayed high levels of ATPase activity, which could be further stimulated by drugs, and were able to carry out active ATP-dependent colchicine uptake [16]. Reconstituted Pgp also showed the expected specificity for MDR spectrum drugs. For more detailed information on the characterization and functional reconstitution of the multidrug transporter, the reader is referred to a recent review [27]. Purified ATPase-active Pgp, and functionally reconstituted Pgp in vesicles of defined phospholipids provide excellent model systems for exploration of the interactions between Pgp and membrane lipids, and their role in modulating the functional properties of the transporter.

**Endogenous membrane lipids associated with Pgp**

Many integral proteins still retain a layer of tightly bound lipids after extraction from the membrane using detergents, and subsequent purification. To investigate the size and nature of the endogenous lipids associated with purified Pgp, intact MDR CHPC5 cells were grown in the presence of [32P]Pi to metabolically label the membrane phospholipids [6]. Plasma-membrane vesicles were then isolated, and Pgp was solubilized with CHAPS and purified as outlined above. Extraction of the purified protein with hexane/propan-2-ol, followed by phospholipid quantification, showed that 53–56 phospholipids are associated with each Pgp molecule [6]. Given the size and hydrophobicity of Pgp, this quantity of lipid seems reasonable, and may be sufficient to completely surround the membrane-spanning domains of the protein, helping to preserve its native conformation and function. No attempt was made to remove endogenous bound lipids from Pgp in these experiments, in fact, delipidation was found to result in complete loss of ATPase activity (see below).

To identify the Pgp-associated phospholipids, the [32P]-labelled lipid extract was separated by two-dimensional high-performance thin-layer chromatography, visualized by both autoradiography and iodine staining, and compared with lipid standards [6]. Three major phospholipid species were associated with Pgp (Figure 2): phosphatidylethanolamine (PE) and phosphatidylserine (PS) were the most prominent. A small amount of PC was present, together with a trace of phosphatidylinositol (PI), however, no sphingomyelin (SM) was observed. In contrast, analysis of a total lipid extract of the MDR cell plasma membrane showed that PC is the most abundant phospholipid, followed by PE, with smaller amounts of SM and PS, and traces of PI. Thus the endogenous lipids associated with Pgp are not representative of the host cell membrane; in particular, the choline phospholipids PC and SM are greatly under-represented. Pgp therefore appears to associate selectively with certain phospholipids in the MDR cell plasma membrane. It is interesting that the two major phospholipids associated with the protein, PE and PS, are located preferentially in the inner leaflet in eukaryotic plasma membrane, whereas the under-represented phospholipids, PC and SM, are situated mainly in the outer leaflet.

Another unidentified phospholipid was also associated with Pgp, as shown in Figure 2 (marked X). It did not correspond to any of the phospholipid standards, and was not present in significant amounts in a lipid extract from the MDR cell membrane. This species may be a metabolic intermediate, which becomes labelled with [32P] to very high specific radioactivity because of rapid turnover.
Integral membrane proteins often require a minimum number of associated phospholipids for activity. When Pgp was treated with delipidating concentrations of either Triton X-100 or sodium deoxycholate at 4°C, ATPase activity was completely abolished. Addition of various phospholipids restored ATPase activity to differing degrees [28]. In the presence of highly fluid natural lipid mixtures containing PC, such as asolectin (soyabean phospholipids) and egg PC, high levels of Pgp ATPase activity were recovered (Table 1). Natural mixtures of PS, PE and PI, and the relatively fluid synthetic lipid dimyristoylPC (DMPC), were also able to restore substantial levels of activity. In contrast, little or no ATPase activity was recovered in the presence of lipids with high-Tc values, such as dipalmitoylPC (DPPC), dimyristoylPE (DMPE) and dipalmitylPE (DPPE). Although Pgp function was restored to higher levels after treatment with Triton X-100 compared with deoxycholate, the pattern of recovery with different lipids was very similar. These data indicate that Pgp requires fluid phospholipids in its immediate surroundings for ATPase activity. This requirement for lipids suggests that either lipid stabilization of the membrane-spanning regions of the protein is necessary for catalytic activity at the nucleotide-binding domains, or, alternatively, these domains themselves interact with the membrane bilayer, so that their integrity depends on the presence of phospholipids. Additional experiments presented below suggest that the latter hypothesis may in fact be true.

### Effects of membrane lipids on Pgp ATPase

We then investigated the effects of bulk lipids on the function of purified Pgp which retained its endogenous lipids. The result of experiments such as this provide information that is very important in the design of systems for optimal reconstitution of an integral membrane protein into lipid bilayers. Purified Pgp in CHAPS was preincubated with various phospholipids for 1 h at 4°C before measurement of ATPase activity at 37°C [6]. As shown in Figure 3, some lipids produced a large concentration-dependent activation of Pgp ATPase, whereas others caused inhibition of activity. Addition of DPPE led to the largest catalytic activation (almost 3-fold at 2 mM). Egg PC was also very effective, with 2-fold stimulation of activity seen at this concentration, whereas asolectin and DMPE gave a smaller increase. The addition of PI and PS led to substantial inhibition of Pgp ATPase. Analysis of ATP hydrolysis kinetics indicated that DPPE acted as a mixed activator of Pgp ATPase, both increasing $V_{\text{max}}$ and lowering the $K_m$ for ATP. Pgp ATPase activity can therefore be modulated to a significant extent by exogenous lipids. Pgp has been successfully reconstituted into a mixture of egg PC and DPPE, the two lipids that promote maximal activation of catalytic activity, and the resulting vesicles retain a high level of ATP-dependent drug transport and drug-stimulated ATPase activity [16].

### Differential-scanning-calorimetry (DSC) studies of reconstituted Pgp in DMPC bilayers

Purified Pgp was reconstituted into bilayers of DMPC using detergent removal by gel filtration. During these reconstitution experiments, it was noted that recovery of Pgp in the lipid bilayer vesicles was greatly improved if the reconstitution process was carried out below the $T_c$ of DMPC, perhaps suggesting that Pgp preferred to insert into gel-phase bilayers. The effects of Pgp on the phospholipid phase transition were then measured using DSC. Shown in Figure 4 are DSC scans for lipid samples containing Pgp.
reconstituted at three different DMPC/protein ratios, as well as pure DMPC vesicles, which were prepared in the same fashion as the reconstituted vesicles. As the amount of Pgp incorporated into the bilayer increased, the value of the gel-to-liquid crystalline phase-transition temperature, \( T_m \), declined, and the line-width at half-height (\( \Delta T_{1/2} \)) increased dramatically (Table 2). Pgp clearly has a large perturbing effect on the DMPC phase transition, even at relatively low lipid/protein ratios.

**Effect of lipid bilayer-phase state on Pgp ATPase**

Experiments outlined earlier suggested that the nucleotide-binding domains of Pgp were dependent on lipids for ATPase activity. Therefore we determined whether the phase state of the lipid bilayer could affect the kinetic parameters for ATP hydrolysis. Pgp was reconstituted into DMPC using detergent removal by gel filtration, to obtain bilayer vesicles with substantial

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**Figure 4**

DSC of Pgp reconstituted into DMPC bilayers

Purified Pgp [25] was reconstituted into bilayers of DMPC at 4°C using detergent removal by gel filtration [16]. DSC was carried out on 1.5 ml samples containing approx. 1 mg of phospholipid, using a Microcal MC-2 differential scanning calorimeter, with a scan rate of 1.5°C/min. Samples were prewarmed through the phase transition before analysis. Successive scans were highly reproducible.

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**Table 2**

DSC parameters for Pgp reconstituted into DMPC bilayers

The gel-to-liquid crystalline phase-transition temperature, \( T_m \), was defined as the peak temperature in the endothermic phase transition; \( \Delta T_{1/2} \) represents the width of the transition at half-height. Both parameters were determined from the DSC scans shown in Figure 4 using the Microcal Origin software programme.

<table>
<thead>
<tr>
<th>Lipid/protein mol ratio</th>
<th>( T_m ) (°C)</th>
<th>( \Delta T_{1/2} ) (°C)</th>
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<tr>
<td>DMPC alone</td>
<td>25.0</td>
<td>1.1</td>
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<tr>
<td>8777:1 (35:1, w/w)</td>
<td>24.5</td>
<td>1.4</td>
</tr>
<tr>
<td>5517:1 (22:1, w/w)</td>
<td>24.4</td>
<td>1.4</td>
</tr>
<tr>
<td>1505:1 (6:1, w/w)</td>
<td>23.0</td>
<td>3.3</td>
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ATPase activity. The rate of ATP hydrolysis by Pgp in DMPC vesicles was measured at ATP concentrations between 0 and 1.0 mM, both above and below the DMPC phase transition, at 28 and 20°C respectively. Fitting of the data to a Michaelis–Menten model for a single type of catalytic site [6], followed by extraction of kinetic parameters, showed that while the $V_{\text{max}}$ decreased by 2.6-fold at the lower temperature, the $K_m$ for ATP also decreased approx. 1.8-fold (Table 3), indicating that Pgp binds ATP with higher affinity when DMPC is in the gel phase. This effect was not observed when kinetic experiments were conducted at the two temperatures using detergent-solubilized Pgp, before reconstitution, in the absence of added lipid. In this case, the $V_{\text{max}}$ decreased 2.5-fold at the lower temperature, whereas the $K_m$ remained essentially unchanged (Table 3). Thus the lowering of $K_m$ at 20°C is observed only in the context of a gel-phase lipid bilayer. These results indicate that the catalytic activity of the ATP-binding domains of Pgp is influenced by the phase state of the bilayer, strongly suggesting that these domains may be associated in some way with the membrane surface.

The rate of ATP hydrolysis at various temperatures was measured for Pgp reconstituted into bilayers of DMPC ($T_m = 23^\circ$C) and 1-palmitoyl-2-myristoylphosphatidylcholine (PMPC; $T_m = 28^\circ$C), as well as native plasma-membrane vesicles from MDR CHB30 cells. Arrhenius plots of the data for native membrane vesicles took the form of two lines of almost identical slope, with a sharp ‘break’, or discontinuity, at approx. 23°C. The slope of the two linear regions gave an activation energy, $E_{\text{act}}$, for ATP hydrolysis in the native plasma membrane of 98 kJ/mol. Arrhenius plots of the rate data for Pgp in DMPC bilayers similarly consisted of two straight lines, with a break at 23°C. The origin of this discontinuity is currently unknown, but it is clearly an intrinsic property of the ATPase domains of the protein, since it is independent of the host membrane, occurring in both native membranes and bilayers of synthetic phospholipid. In the case of DMPC bilayers, the two linear regions of the Arrhenius plots showed a small, but reproducible, change in slope close to $T_m$ ($23^\circ$C), corresponding to a decrease in $E_{\text{act}}$ from 89 kJ/mol above $T_m$ to 71 kJ/mol below $T_m$. To avoid the problems arising from the close proximity of the inherent discontinuity to $T_m$ the same series of experiments was repeated using PMPC ($T_m = 28^\circ$C) as the host bilayer. Again, the Arrhenius plot took the form of two segments, separated by the break at 22°C. However, in this case, the high-temperature section of the plot showed a distinct change in slope at 28°C, the $T_m$ of the PMPC bilayer. Again, calculation of $E_{\text{act}}$ from the slopes indicated a substantially lower value when the bilayer was in the gel phase. This behaviour is unusual; for most membrane transporters and enzymes, the activation energy is increased in the gel phase, indicating that catalysis is less favoured in a more rigid lipid environment. The observed decrease in activation energy in this case suggests that Pgp hydrolyses ATP more efficiently when incorporated into lipid bilayers that are in the gel phase, rather than the liquid crystalline phase.

These findings confirm that the ATPase domains of Pgp are sensitive to the phase state of the bilayer into which the protein is incorporated, again suggesting that these domains may be closely associated with the bilayer. Alternatively, changes in ATPase activity may arise from a global change in the conformation of Pgp, which

<table>
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<th>Kinetic parameters for ATP hydrolysis by Pgp in gel- and liquid-crystalline-phase DMPC</th>
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<td>Pgp in DMPC vesicles</td>
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<td>Pgp in CHAPS solution</td>
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may be transmitted indirectly from the membrane-inserted regions of Pgp to the ATPase domains.

**Effect of lipid bilayer phase state on Pgp transport properties**

The ability of reconstituted Pgp to carry out drug transport was investigated in DMPC vesicles, both above and below the phase transition. Addition of [3H]colchicine and ATP resulted in rapid uptake of radiolabelled drug into the vesicle lumen, which reached an equilibrium after 2–3 min. At this point, inward pumping by Pgp up the concentration gradient is balanced by outward diffusion of the membrane-permeant drug down the concentration gradient [16,26]. Over the 15–45 s time range, the rate of drug uptake by Pgp in DMPC vesicles at 20°C was approx. 5-fold faster than drug transport measured in the same vesicles at 28°C, indicating that the transporter works more efficiently in gel-phase than liquid-crystalline-phase lipid. Similar studies conducted using native CHRB30 plasma-membrane vesicles at these two temperatures revealed a small (~12%) decrease in the rate of drug transport at the lower temperature, as expected. Pgp therefore appears to transport drugs at a substantially faster rate in gel-phase lipid. This conclusion was confirmed by carrying out analogous studies in PMPC vesicles, where the rate of drug uptake in the gel state at 24°C was again substantially faster (over 2-fold) than that observed in the liquid-crystalline state at 32°C. Our findings are supported by the data of Sinicrope et al. [29], who showed that the ability of Pgp to transport daunorubicin and vinblastine was decreased 2–4-fold by the addition of membrane fluidizers such as benzyl alcohol. The preference of Pgp for less fluid lipid is most unusual; most transport proteins prefer fluid-phase lipid, and several cease to function when the lipid matrix is below the T. The unusual preference of Pgp for a gel-phase lipid milieu may play an important, as yet unknown, role in its mechanism of action.

One important avenue that should be pursued in the future is investigation of the effects of various lipids on drug binding to Pgp. One of the immediate consequences of drug binding is the stimulation or inhibition of the ATPase activity of Pgp. The fact that drugs affect ATPase activity indicates that there is some type of communication, or coupling, between the drug-binding site, which is believed to reside within the membrane-spanning regions of the transporter, and the ATP-binding domains.

**Conclusion**

In summary, Pgp is an unusual ATP-driven transporter, the function of which appears to be modulated by lipids in a novel way. Although the two nucleotide-binding folds of Pgp are usually visualized as separately folded soluble domains on the cytosolic face of the protein, they are entirely dependent on the presence of phospholipids for catalytic activity, and their ATPase activity is modulated by the addition of exogenous phospholipids. Reconstitution of Pgp into defined lipid bilayers has shown that the protein has a very large perturbing effect on the host bilayer, even at relatively high lipid/protein ratios. Surprisingly, the transporter prefers to reconstitute into gel-phase DMPC rather than lipid in the liquid-crystalline phase. The ATPase activity of Pgp is affected by the phase state of the bilayer, and demonstrates both a higher affinity (lower $K_a$) for ATP and a lower activation energy in gel-phase lipid. These results strongly suggest that the ATP-binding folds of Pgp may, in fact, associate with the membrane in some way, perhaps by interacting with phospholipid headgroups on the surface of the inner leaflet. This proposal may also explain our finding that PE and PS, both of which are almost exclusively located on the inner leaflet of the eukaryotic plasma membrane, are the two major endogenous phospholipids remaining bound to Pgp after detergent extraction and purification. Drug transport by Pgp also appears to be favoured when the lipid matrix is below the $T_r$. The lipid matrix is below the $T_r$ The unusual preference of Pgp for a gel-phase lipid milieu may play an important, as yet unknown, role in its mechanism of action.

One important avenue that should be pursued in the future is investigation of the effects of various lipids on drug binding to Pgp. One of the immediate consequences of drug binding is the stimulation or inhibition of the ATPase activity of Pgp. The fact that drugs affect ATPase activity indicates that there is some type of communication, or coupling, between the drug-binding site, which is believed to reside within the membrane-spanning regions of the transporter, and the ATP-binding domains. Using purified Pgp specifically fluorescently labelled at a single cysteine residue within each nucleotide-binding domain, recent work in our laboratory has demonstrated the existence of the proposed conformational coupling [25]. Urbatsch and Senior [30] have shown that the pattern of stimulation/inhibition of ATPase activity by various drugs can be affected by the nature of the lipids surrounding Pgp, which strongly implies that lipids may modulate the binding of drugs to the transporter. The data presented above also suggest that membrane lipids may modulate ATP binding. We have recently developed a fluorescence method, using purified Pgp in the presence of lipids, which allows direct measurement of the $K_d$ for binding of both various drugs and chemosensitizers, and nucleotides such as ATP, to the transporter [25]. This new methodo-
logy should make it possible to address some of these issues in the near future.

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