ABC transporters: from survival to resistance (or vice-versa)

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COLLOQUE **ABC** FRANCO-BELGE

FRENCH-BELGIAN **ABC** MEETING

Paris, France, 14 october 2009

When preparing this lecture … A large choice …

Why active efflux ?

Efflux that saved a city

slide stolen from one of the many F. Van Bambeke's presentations

An original observation with cancer cells…

[CANCER RESEARCH 37, 4629–4634, December 1977]

Decreased Retention of Actinomycin D as the Basis for Cross-resistance in Anthracycline-resistant Sublines of P388 Leukemia

Makoto Inaba[1] and Randall K. Johnson[2]

Laboratory of Chemical Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland 20014

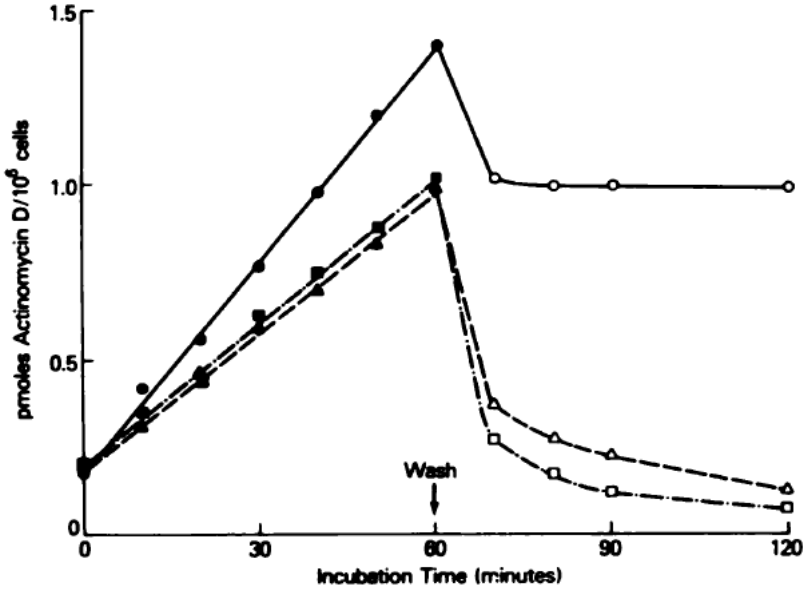
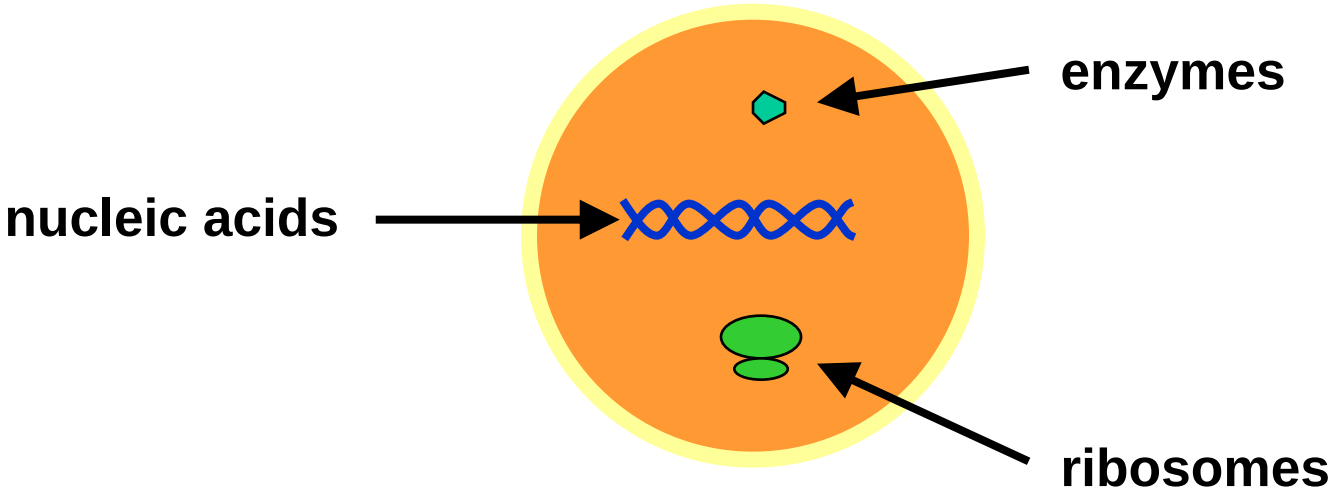


Chart 2. Time course of uptake and efflux of actinomycin D by P388/S (○, ●), P388/ADR (△, ▲) and P388/DAU (□, ■) cells. Cells were incubated in the presence of actinomycin D, 0.04 μg/ml, for 60 min, washed, and reincubated in drug-free medium for an additional 60 min. Each *point* represents the mean of 3 determinations. The coefficient of variation was less than 10%.

Most chemotherapeutic agents must reach an intracellular target…



How can these drugs reach their target inside the cells ?

Most chemotherapeutic agents must reach an intracellular target…

Table 1

Subcellular distribution of [³H]actinomycin D in P388/S and P388/ADR cells after exposure to the drug (0.1 μg/ml) for 1 hr in vitro (uptake) followed 1 h incubation in drug-free medium (retention)

	Radioactivity (dpm × 10^{-3})				
Cell line	Whole cells	Nuclear fraction	Mitochondrial fraction	Microsomal fraction	Cytoplasmic supernatant
Uptake					
P388/S	1513 ± 2[a]	1014 ± 18 (67)[b]	31 ± 1 (2)	10 ± 1 (1)	409 ± 11(27)
P388/ADR	672 ± 9	430 ± 1 (64)	41 ± 1 (6)	6 ± 0.2 (1)	198 ± 9(29)
Retention					
P388/S	1131 ± 3	766 ± 13 (68)	43 ± 1 (4)	8 ± 0.4 (1)	307 ± 8 (27)
P388/ADR	135 ± 3	88 ± 3 (65)	12 ± 3 (9)	2 ± 0.1 (1)	35 ± 1 (26)

[a] Mean ± S.D.

[b] Numbers in parentheses, percentage of total.

Conclusion #1: in order to survive to anticancer agents, cells "invented" efflux…

Inaba and Johnson, Cancer Res, 1977; 37:4629-34.

But antibiotics were first ...

1: Nature. 1963 Oct 26;200:384-5.

DISAPPEARANCE OF OXYTETRACYCLINE ACCUMULATION IN THE CELLS OF MULTIPLE DRUG-RESISTANT ESCHERICHIA COLI.

IZAKI K, ARIMA K.

PMID: 14087909 [PubMed - indexed for MEDLINE]

Who remembers that car ?

Historical observations on tetracyclines …

Biochem. J. (1965) **94**, 54

Resistance of *Escherichia coli* to Tetracyclines

BY T. J. FRANKLIN AND A. GODFREY
Imperial Chemical Industries Ltd. (Pharmaceuticals Division), Alderley Park, Macclesfield, Cheshire

(*Received* 23 *March* 1964)

1. A strain of *Escherichia coli* highly resistant to chlortetracycline and partially cross-resistant to tetracycline has been isolated. 2. The nitro-reductase system of the resistant cells was inhibited to a smaller extent by chlortetracycline than was the corresponding enzyme of sensitive cells. 3. The incorporation of leucine *in vitro* into the ribosomal protein of cell-free preparations from sensitive and resistant cells was equally inhibited by chlortetracycline. 4. Resistant cells accumulated much less chlortetracycline and tetracycline than did sensitive cells when both were cultured in the presence of these drugs. 5. The uptake of tetracycline by both sensitive and resistant *E. coli* was dependent on the presence of glucose in the medium. 6. Fractionation of cells cultured in medium containing [^{14}C]chlortetracycline indicated that the largest proportion of radioactivity in sensitive cells was in the fraction consisting mainly of cell-wall material. There was no concentration of radioactivity in any one fraction of the resistant cells. 7. No evidence could be obtained for a specific tetracycline-excretion system in the resistant cells. 8. The significance of these results in relation to current theories of the antibiotic action of and resistance to the tetracycline drugs is discussed.

However, it took 15 years to understand …

Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 3974–3977, July 1980
Biochemistry

Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*

(everted membrane vesicles/tetracycline transport/transposon Tn10/plasmids)

LAURA MCMURRY, RICHARD E. PETRUCCI, JR., AND STUART B. LEVY*

Department of Molecular Biology and Microbiology and Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

Communicated by Boris Magasanik, April 21, 1980

Conclusion #2: in order to survive to antibiotics, bacteria "invented" efflux…

Historical observations …

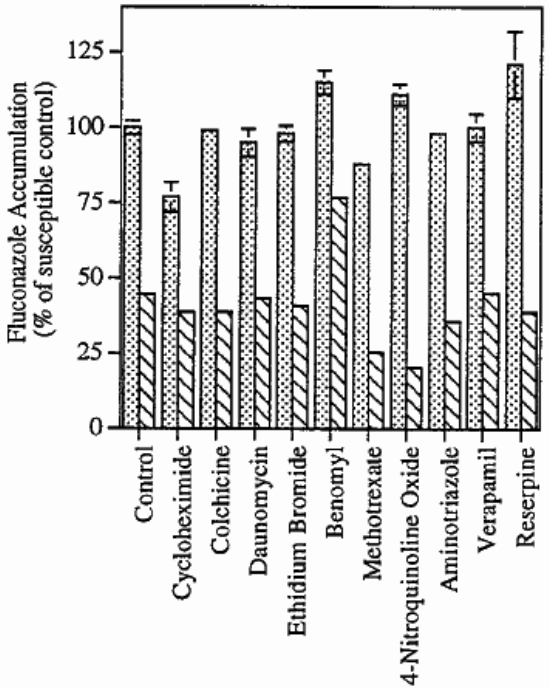


FIG. 3. Effects of MDR protein substrates or inhibitors on [^{3}H]fluconazole uptake by cells from fluconazole-susceptible (▤) and fluconazole-resistant (▨) cultures of *C. glabrata* after 80 min of incubation in the standard uptake assay; the assay was extended to 180 min for verapamil. Values are means ± standard deviations of triplicate determinations with cells from one culture.

Parkinson et al. Antimicrob Agents Chemother. 1995 Aug;39(8):1696-9

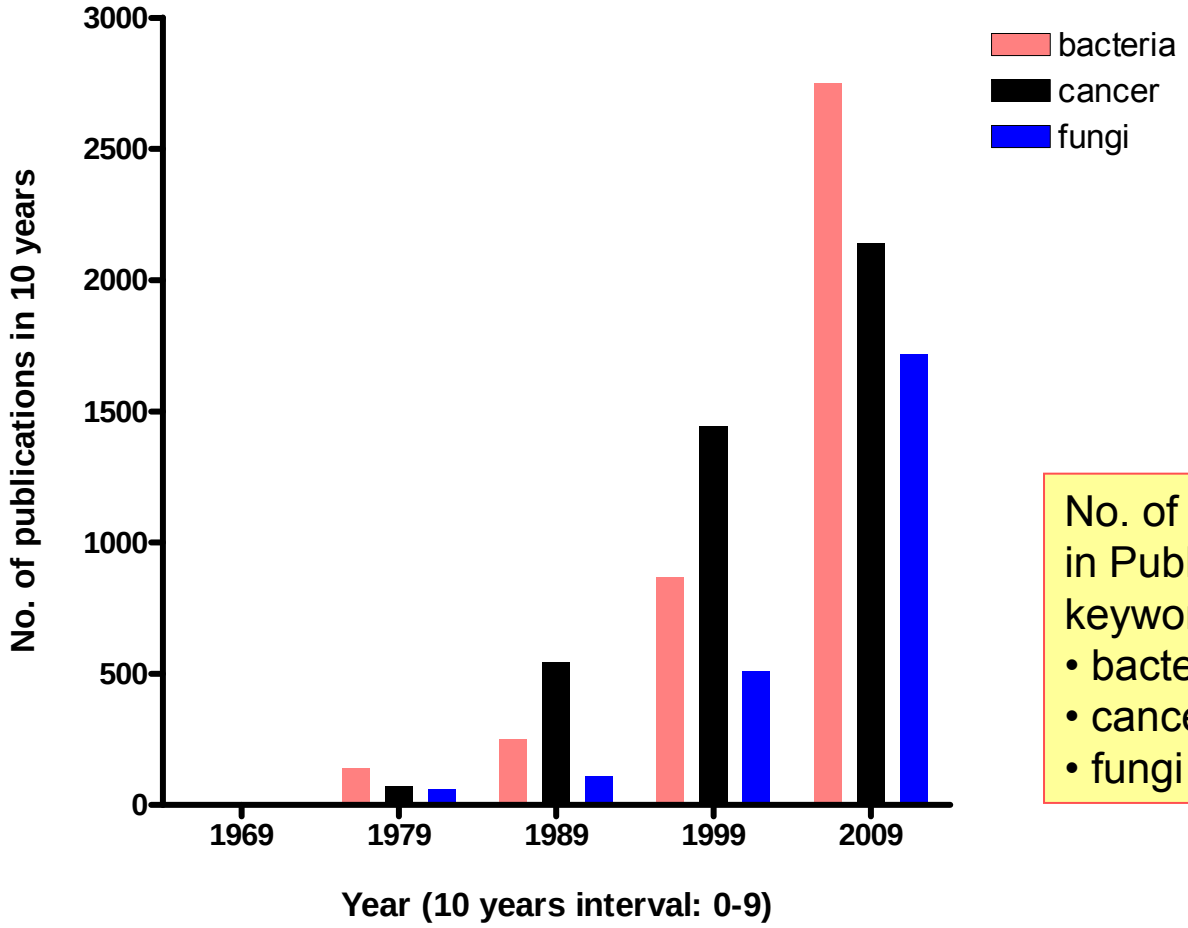
6. **De Waard, M. A., and J. G. M. Van Nistelrooy.** 1980. An energy-dependent efflux mechanism for fenarimol in a wild-type strain and fenarimol-resistant mutants of *Aspergillus nidulans*. Pestic. Biochem. Physiol. **13**:255–266.

antibiotics
1965 1980

antifungal drugs
1980 1995

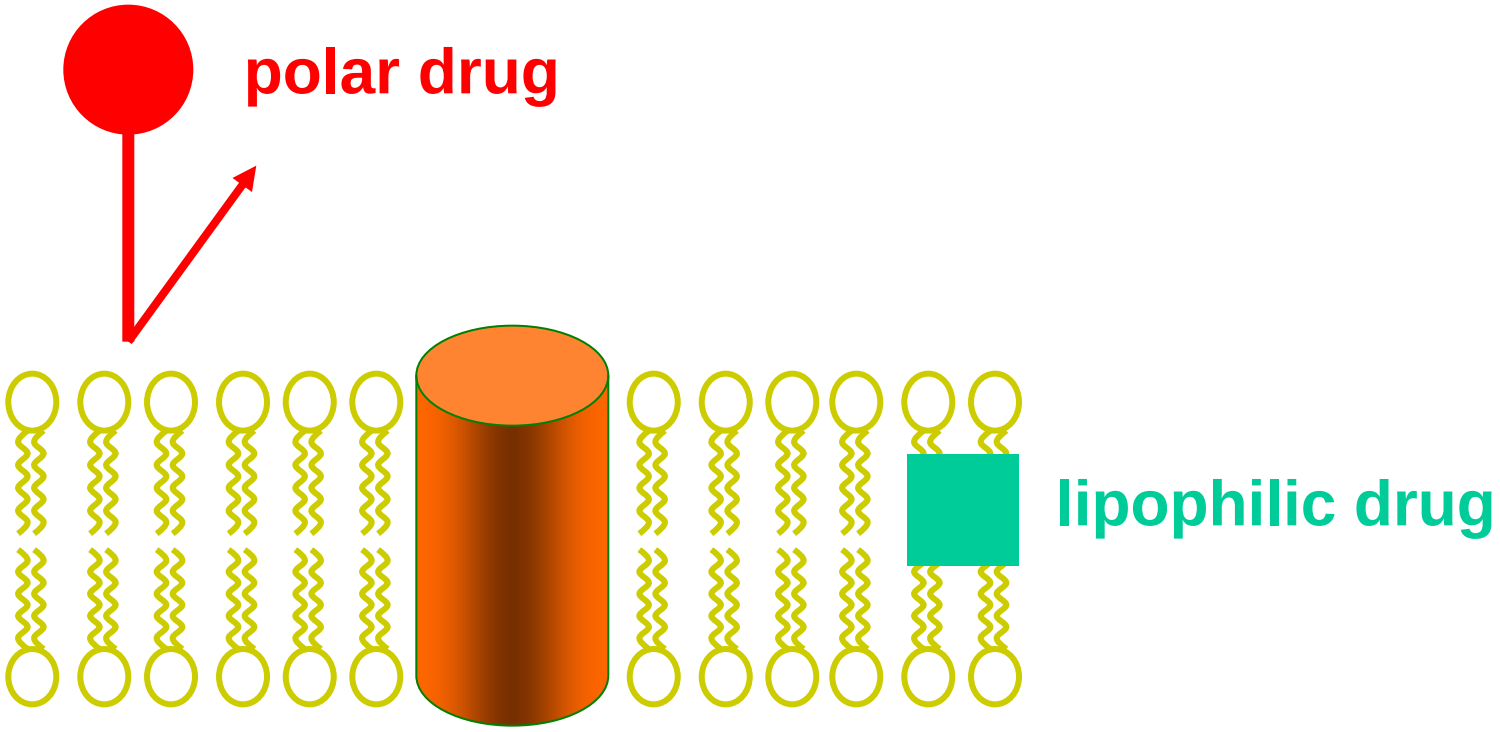
1977
anticancer drugs

Historical trends …



No. of publications in PubMed with keywords: efflux AND
- bacteria
- cancerl
- fungi

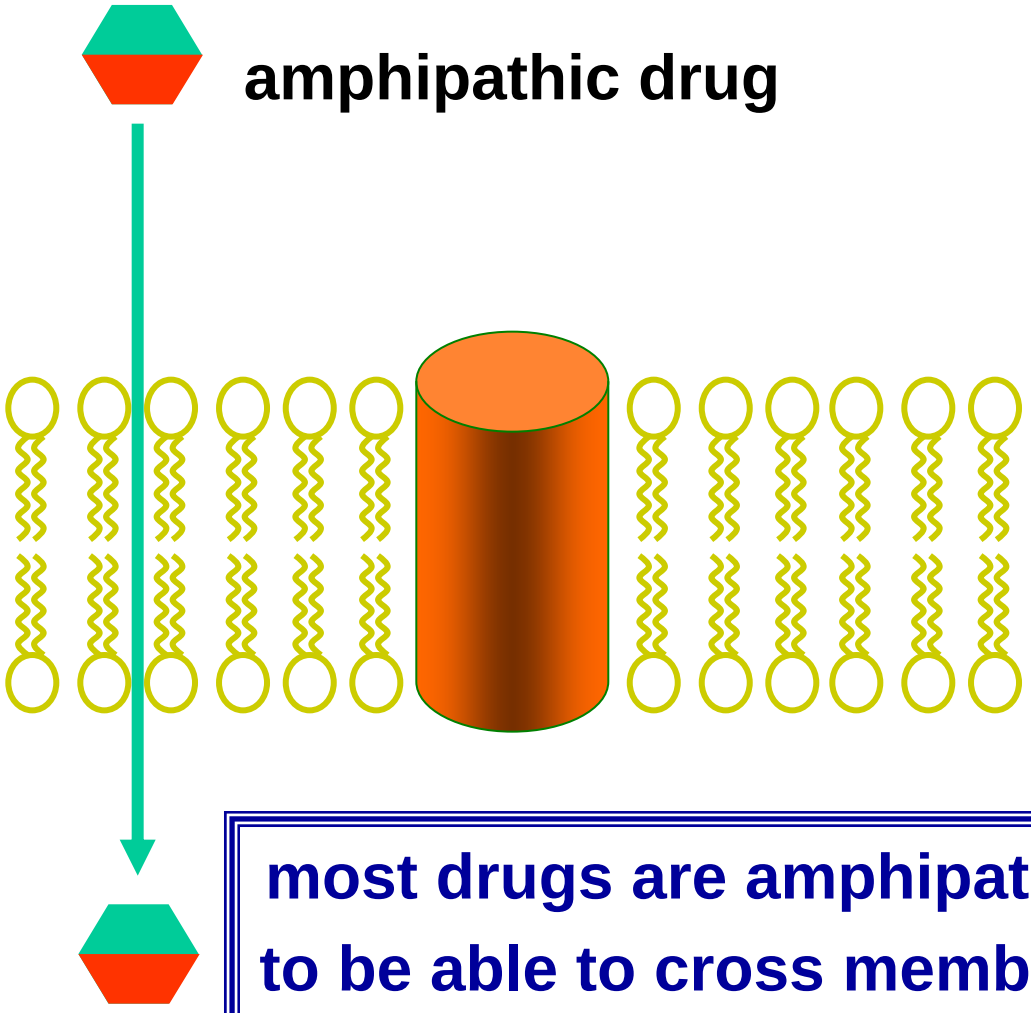
Reaching an intracellular target …



physico-chemical properties are inadequate for reaching an intracellular target !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

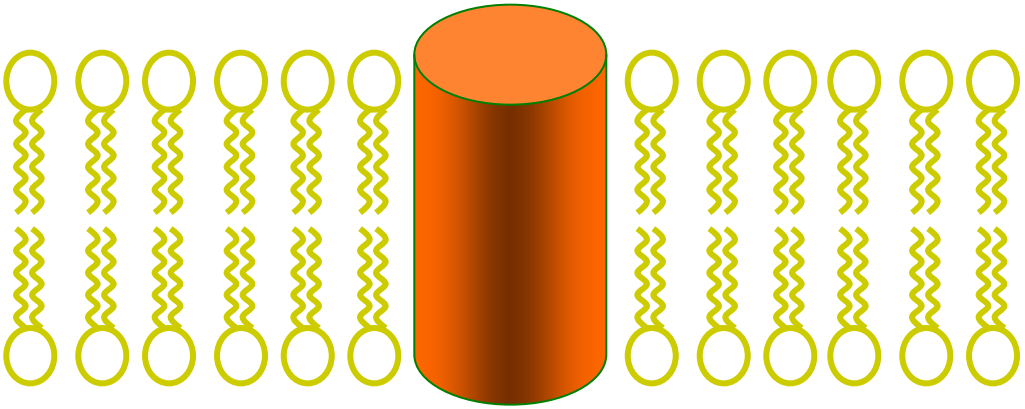
Reaching an intracellular target …

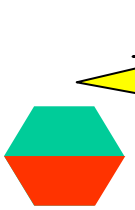


most drugs are amphipathic by design, to be able to cross membrane barriers !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Intracellular chemotherapeutic agents



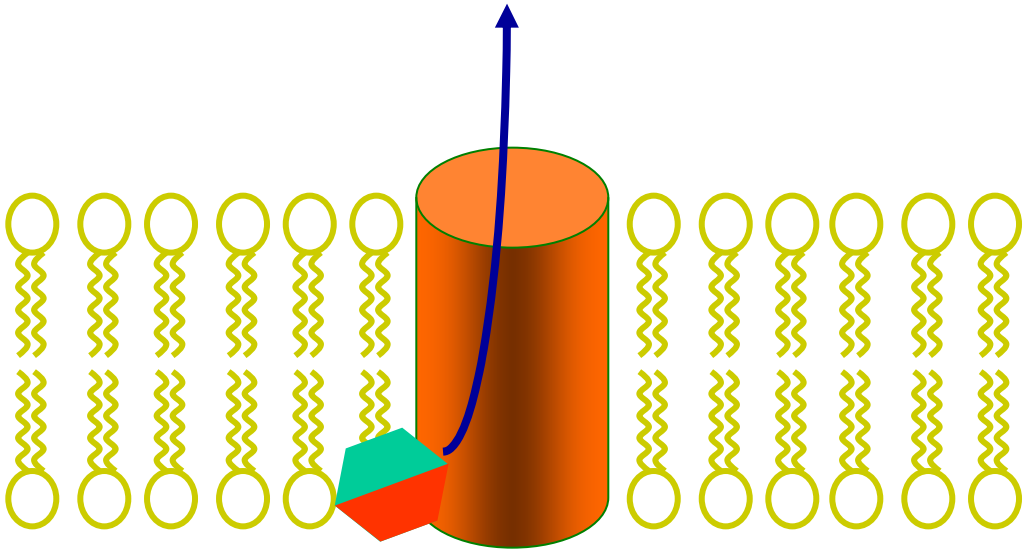


But a diffusible compound may have potentially harmful effects !

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Why efflux transporters ?

Extrusion by efflux pumps



Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Why efflux transporters ?

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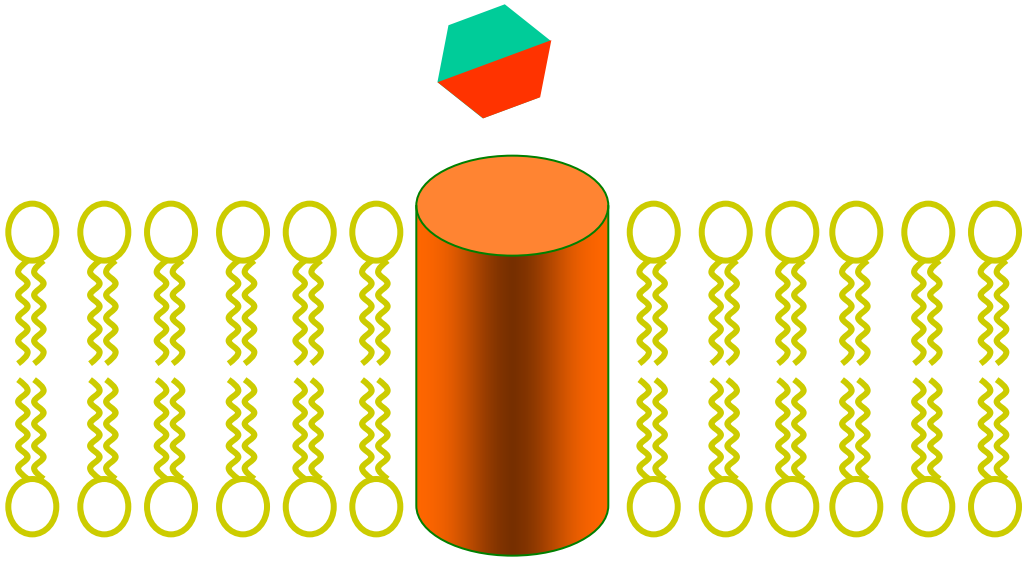
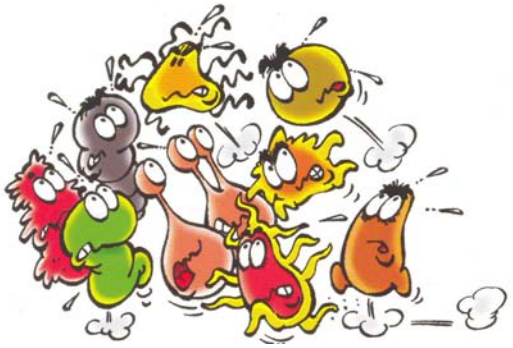


general mean of protection
against cell invasion by diffusible molecules

Van Bambeke et al., Biochem. Pharmacol (2000) 60:457-70

Typical ‘toxic’ diffusible substances as substrates for efflux pumps

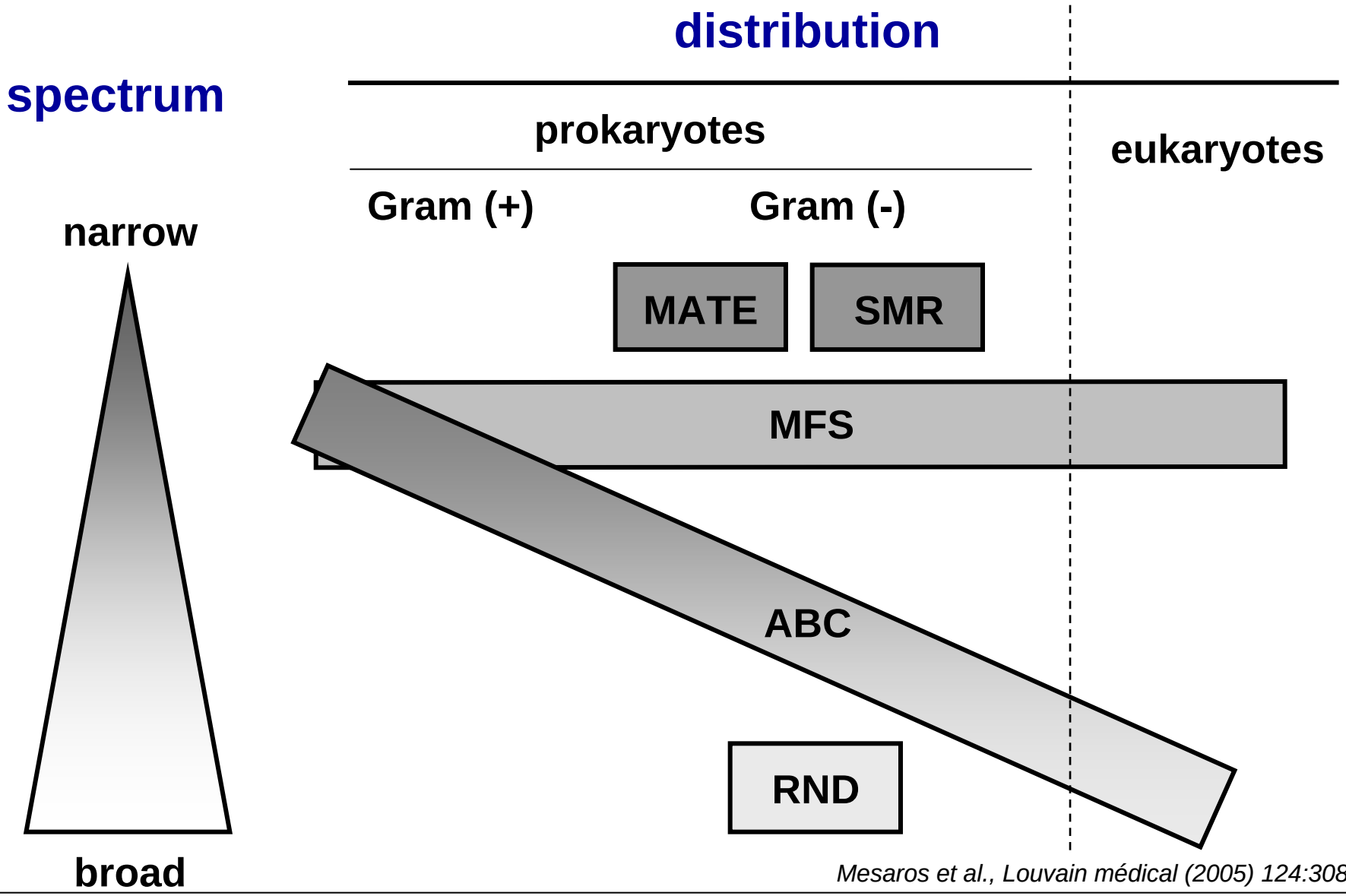
antibiotics



antifungals

anticancer agents

Antibiotic efflux transporters are ubiquitous



Mesaros et al., Louvain médical (2005) 124:308-20

All that because of the existence of a membrane…

- All cells and subcellular compartments are separated from the external milieu by lipid membranes.
- Cell survival requires the regulated and selective passage of specific molecules across these membranes, not only to acquire nutrients and excrete waste products, but also for a multitude of regulatory and other functions.
- Almost 20% of the E. coli genes so far identified are associated with transport functions (Bachmann 1990).
    - ABC transporters have received considerable attention recently because they are associated with many important biological processes in both prokaryotes and eukaryotes, as well as with clinical problems such as cystic fibrosis, antigen presentation, and multidrug resistance of cancers.
    - The designation ABC transporters recognizes a highly conserved ATP-binding cassette, which is the most characteristic feature of this superfamily
    - **Most ABC transporters have no role in multi-drug resistance and are not glycoproteins.**

Higgins CF, Annu. Rev. Cell Biol. 1992;8:67-113

ABC transporters from bacteria to man

Higgins CF, Annu. Rev. Cell Biol. 1992;8:67-113

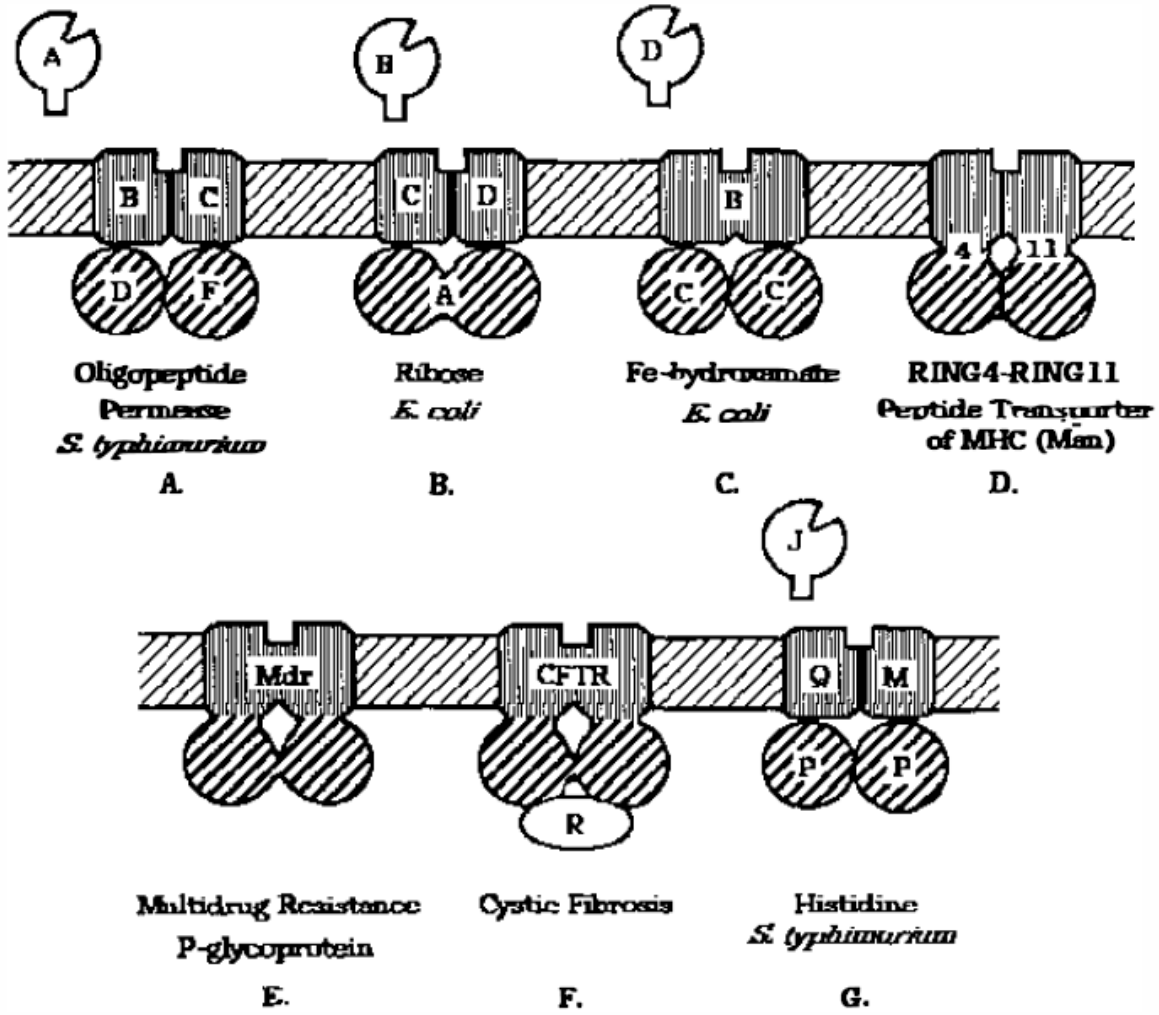


Figure 2 Domain organization of ABC transporters. A typical ABC transporter consists of four domains, two highly hydrophobic membrane-spanning domains (*shaded*), which form the translocation pathway, and two peripheral membrane domains (*shaded*), which couple ATP hydrolysis to the transport process. Certain transporters have additional domains (*unshaded*) that are not part of the core transmembrane translocation mechanism. The domains are often encoded as separate polypeptides; however, they may also be fused together in one of several alternative combinations. References to the original description of these systems are given in Table 1. See text for further details.

Bacterial ABC

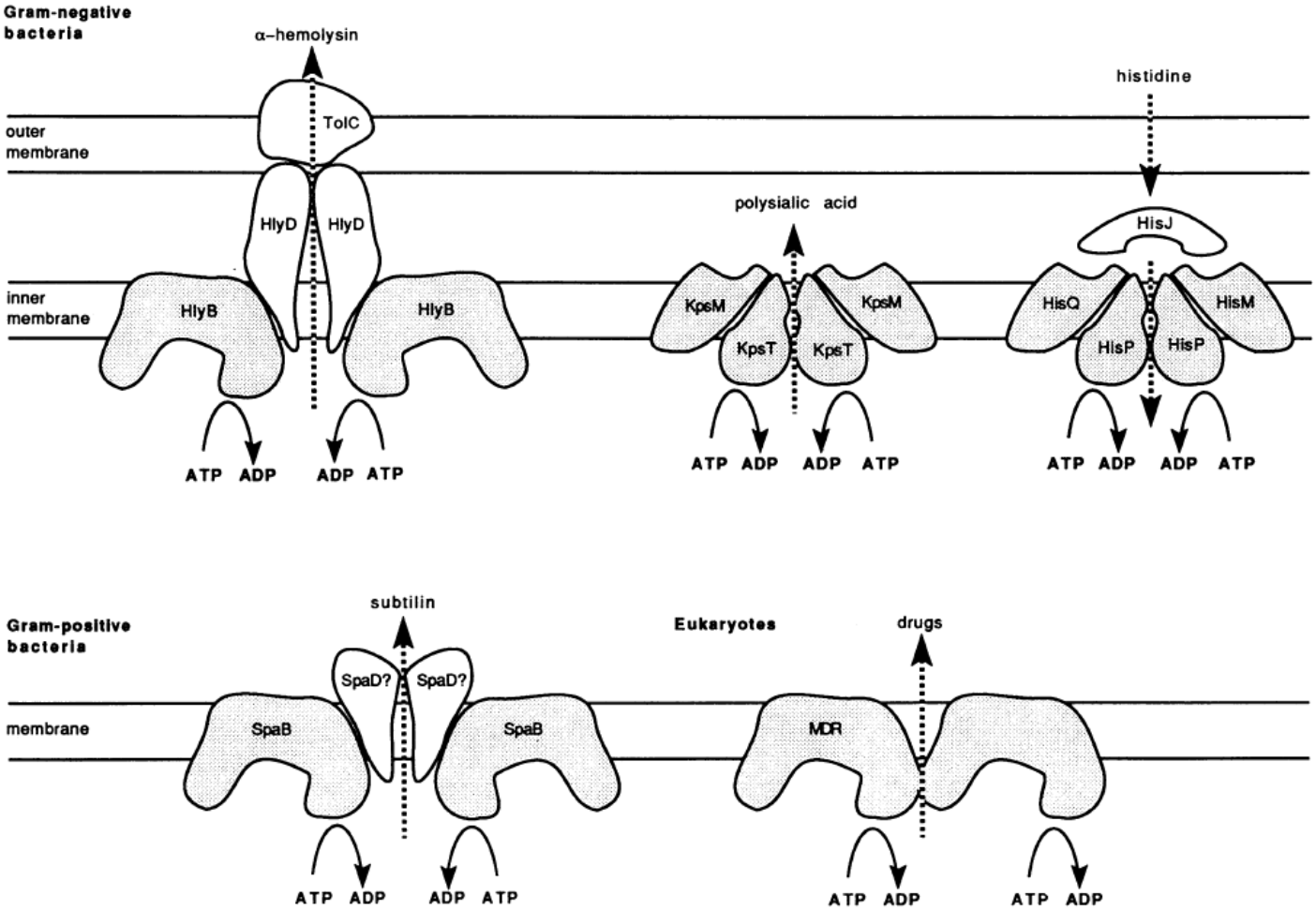


FIG. 1. Structural models of various ABC transporters. The prototype systems included are the *E. coli* alpha-hemolysin exporter, the *E. coli* polysialic acid exporter, the *S. typhimurium* histidine importer, the *B. subtilis* subtilin exporter, and the mammalian P-glycoprotein drug exporter. The bacterial exporters are drawn as dimers, consistent with the model of Higgins (80) and others, who propose a minimum of four required "core components." There is no experimental evidence that the bacterial export complexes form dimers. The core components in each complex are shaded.

Fath & Kolter, Microbiol Rev. 1993;57:995-1017

Bacterial ABC

- Proteins, peptides, polysaccharides, and many other molecules that are synthesized in the bacterial cytoplasm must often cross one or more membranes to reach their final destination.
- Many bacterial proteins are transported across the cytoplasmic membrane via the Sec pathway. But this requires an N-terminal signal sequence, which limits the type of molecule that can be transported across membranes by using this system.
- **Nonproteinaceous** secreted products as well as **extracellular proteins from Gram-negative bacteria** (which must cross both inner and outer membranes) cannot use the Sec pathway.
- In addition, there appear to be structural features of some proteins that are inherently incompatible with use of the Sec pathway.
- **Therefore many molecules must find another way to leave the cytoplasm.**
- It has become apparent from results obtained in the last few years that this problem is often solved by the e**xistence of dedicated export systems** that facilitate membrane translocation with a large degree substrate specificity

ABC are **exporters** of bacterial products …

Fath & Kolter, Microbiol Rev. 1993;57:995-1017

ABC in *E. coli*

Table 2. Inter-relationship of the ABC subfamilies with the distinct families of PBPs and TMDs.

ABC subfamily	Associated domains: TMD family	Associated domains: PBP family	Substrates
1	a	i	Sugars
2	b and c	ii	Peptides[a]
3	None	None	Unknown
4	d	iii	Amino acids
5	e and f	iv	Various
6	g	None	Unknown
7	h	v	Unknown
8	j	vi and BtuE	Iron-siderophores[b]
9	k	v	Unknown
10	l and YrbK	LivK and YhbN	Amino acids[c]
Unassigned	m	Unassigned	Various

The families of TMDs and PBPs associated with each subfamily of ABC domains is indicated. The members of each ABC, TMD and PBP family can be ascertained by cross-reference to Table 1 and Fig. 3. In many cases, specific families of TMDs and PBPs are exclusively associated with a single subfamily of ABCs (e.g. TMD family 'a' and PBP family 'i' with ABC subfamily 1). The class of substrate handled by each subfamily, when known, is indicated.

a. The nickel transporter is also a member of this family.

b. The vitamin B_{12} transporter is also a member of this family.

c. Based only on the specificity of the Liv transporter.

Linton & Higgins, Mol Microbiol. 1998; 28:5-13.

TMD: two transmembrane domains
PBP: periplasmic-binding protein

Bacterial ABC transporters

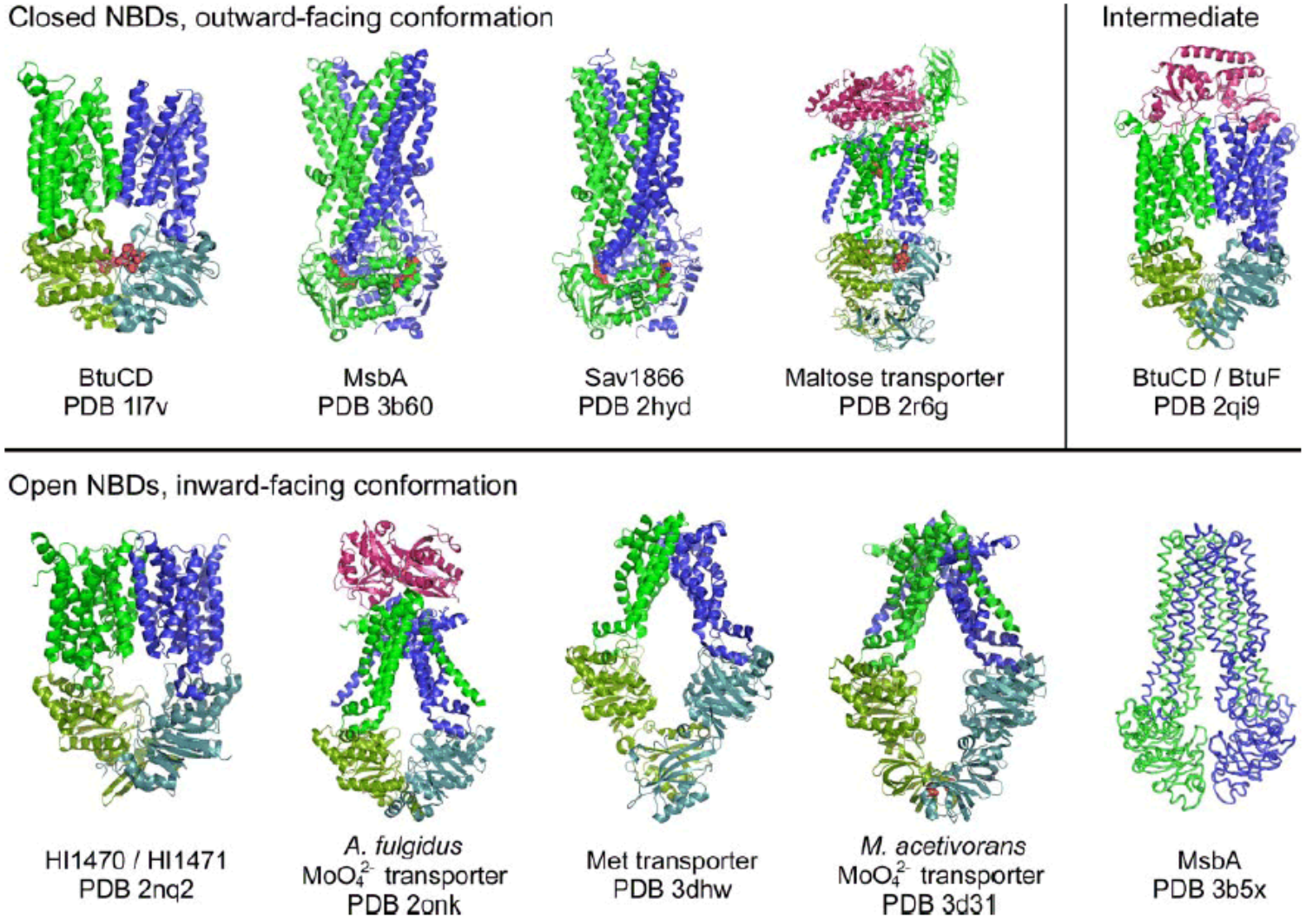


Figure 3. Crystal structures of bacterial ABC transporters. Half of each transporter is green, while the other half is blue (if NBDs and TMDs are separate polypeptides, they are different shades of green or blue); if present, periplasmic binding proteins that deliver substrate to the transporter are brick red. In each structure, TMDs are at top and NBDs are at bottom. All structures determined to date follow the same general scheme; closed NBDs are associated with an outward-facing cavity formed by the TMDs, while open NBDs are associated with an inward-facing cavity ("inward" is defined as the side of the membrane occupied by the NBDs, usually the cytosol). In the case of the maltose transporter, a maltose molecule was cocrystallized within the cavity (4), and it is likely that the cavity forms the substrate binding site in other ABC transporters. The structure of the vitamin B12 transporter BtuCD bound to its periplasmic binding protein BtuF has open NBDs and no obvious substrate-binding cavity, and is possibly an intermediate in the transport cycle (8).

How bacteria play with ABC

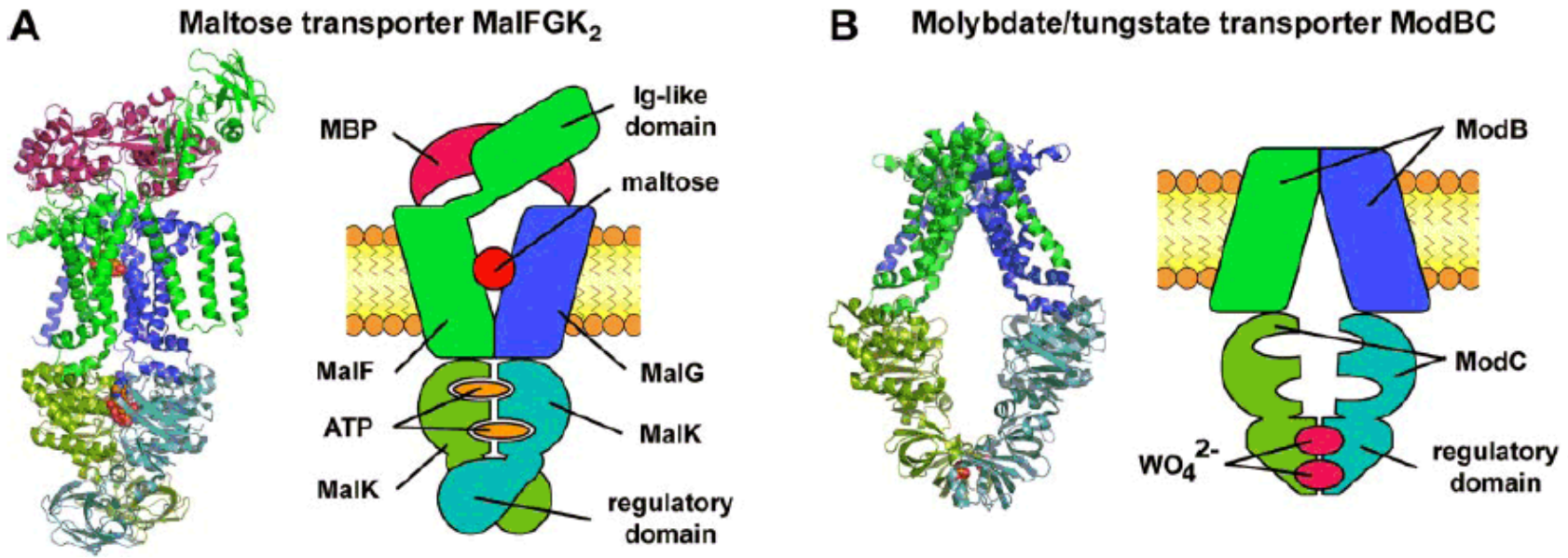


Figure 4. Accessory domains can be added to the core ABC transporter to provide additional functions. *A, B*) Architecture of 2 bacterial ABC transporters with accessory domains. In each case, the crystal structure is shown adjacent to a schematic. Colors are identical to Fig. 3. *A*) The *E. coli* maltose transporter (PDB 2r6g) in a closed, outward-facing conformation. MBP delivers maltose to the transporter and is held in place by an immunoglobulin-like domain. Cytosolic regulatory domains communicate with a transcriptional regulator. *B*) The *M. acetivorans* molybdate/tungstate transporter (PDB 3d31) locked in a trans-inhibited conformation, similar to that of the methionine transporter (PDB 3dhw) in Fig. 3. Tungstate-bound regulatory domains hold the NBDs open to prevent further import when cytoplasmic tungstate levels are high.

MBD: metabolite binding protein

At the end, what is not transported in bacteria ?

Table 1
Functional *E. coli* ABC transporters, number of components, biological function and analogous role in other bacteria

Table 1
Functional *E. coli* ABC transporters.

Transporter	Components	Substrate	Biological function	Reference
Prokaryotic-like transporters				
Als	AlsB (BP), AlsC (TM), AlsA (NB)	Allose, ribose	Monosaccharide importer	[84]
Ara	AraF (BP), AraH (TM), AraG (NB)	L-arabinose, fructose, xylose	Monosaccharide porter	[85]
Arg	ArgT (BP), HisQ (TMD), HisM (TMD), HisP (NBD)	L-lysine, L-arginine, L-ornithine	Polar amino acid transporter	[86]
ArtIMQP	ArtI (BP), ArtM (TMD), ArtQ (TMD), ArtP (NB)	L-arginine	Polar amino acid transporter	[86,87]
ArtJMQP	ArtJ (BP), ArtM (TMD), ArtQ (TMD), ArtP (NB)	L-arginine	Polar amino acid transporter	[86,87]
Btu	BtuF (BP), BtuC (TMD), BtuD (NB)	Vitamin B12	Vit B12 uptake system	[18]
Cys	CysP (BP), CysU (TMD), CysW (TMD), CysA (NBD)	Thiosulfate	Sulfate/thiosulfate importer	[88]
Ddp	DdpA (BP), DdpF (TMD) DdpD (TMD)	D,d-dipeptide	Dipeptide transporter	[89]
Dpp	DppA (BP), DppB (TMD), DppC (TMD), DppD (NB), DppF (NB)	Dipeptide, 5-aminolevulinic acid (ALA)	Dipeptide transporter	[90]
Fec	FecB (BP), FecC (TMD), FecD (TMD), FecE (NBD)	Ferric citrate	Iron porter	[91]
Fep	FepB (BP), FepD (TMD), FepG (TMD), FepC (NBD)	Ferrientero-bactin	Iron porter	[92]
Fhu	FhuD (BP), FhuB (TMD), FhuC (NBD)	Ferric hydroxamate/ferrichrome	Hydroxamate-dependent iron transport	[93]
Hi/Yec	HiY (BP), YecS (TMD), YecC (NBD)	Unknown	Putative cysteine/diaminopimelic acid transporter	[94]
Gln	GlnH (BP), GlnP (TMD), GlnQ (NBD)	Glutamine	Polar amino acid porter	[95]
Glt	GltI (BP), GltK (TMD), GltJ (TMD), GltL (NBD)	Glutamate/aspartate	Polar amino acid importer	[96]
Gsi {Yli}	GsiB {yliB} (BP), GsiC {yliC} (TMD), GsiD {yliD} (TMD), GsiA {yliA} (NBD)	Glutathione	Oligopeptide transporter	[97]
His	HisJ (BP), HisM (TMD), HisQ (TMD), HisP (NBD)	L-histidine, also arginine, lysine, ornithine	Polar amino acid transporter	[98]
LivFGHJM	LivJ (BP), LivH (TMD), LivM (TMD), LivG (NBD), LivF (NBD)	L-leucine (LivJ), L-isoleucine (LivJ), L-valine (LivJ)	Hydrophobic amino acids and amide importer	[99]
LivFGHKM	LivK (BP), LivH (TMD), LivM (TMD), LivG (NBD), LivF (NBD)	L-leucine (LivK)	Hydrophobic amino acids and amide importer	[99]
Lpt	LptA {yhbN} (BP), ?YrbK (TMD), LptB {yhbG} (NBD)	Lipo-polysaccharide	lipopolysaccharide (LPS) porter	[100]
Lsr	LsrB (BP), LsrC (TMD), LsrD (TMD), Ego (NBD)	AI-2 quorum-sensing signaling molecule	Monosaccharide porter	[101]
Mal	MalE (BP), MalF (TMD), MalG (TMD), MalK (NBD)	Maltose (malto-oligosaccharides prediced)	Disaccharide importer	[102]
Met	MetQ {MetD/YaeC} (BP), MetI {MetD/YaeE} (TMD), MetN {abc} (NBD)	D-methionine	Methionine transporter	[103]
Mgl	MglB (BP), MglC (TMD), MglA (NBD)	β-D-galactose	Monosaccharide porter	[104]
Mod	ModA (BP), ModB {ChlJ} (TMD), ModC {ChlD} (NBD), ModF {ChlD} (NBD)	Molybdate, tungsten	Molybdate transporter	[105,106]
Mpp {Opp}	MppA (BP), OppB (TMD), OppC (TMD), OppD (NBD), OppF (NBD)	Murein tripeptide	Oligopeptide transporter	[107]
Nik {hydC}	NikA (BP), NikB (TMD), NikC (TMD), NikD (NBD), NikE (NBD)	Nickel	Nickel porter	[108]
Opp	OppA (BP), OppB (TMD), OppC (TMD), OppD (NBD), OppF (NBD)	Oligopeptides	Oligopeptide porter	[109]
Phn	PhnD (BP), PhnE (TMD), PhnC (NBD)	Phosphonate, phosphites	Phosphonate importer	[110]
PotABCD	PotD (BP), PotC (TMD), PotB (TMD), PotA (NBD)	Spermidine	Spermidine importer	[111]
PotFGHI	PotF (BP), PotH (TMD), PotI (TMD), PotG (NBD)	Putrescine	Putrescine importer	[112]
Pro	ProX (BP), ProW (TMD), ProV (NBD)	Glycine, betaine, L-proline	Glycine/betaine/proline importer	[113]
Pst	PstS {nmpA} (BP), PstC {phoW} (TMD), PstB {phoT} (NBD)	Phosphate	High-affinity phosphate transport	[114]
Rbs	RbsB (BP), RbsC (TMD), RbsA (NBD)	D-ribose	Monosaccharide importer	[115]
Sap	SapA (BP), SapB (TMD), SapC (TMD), SapD (NBD), SapF (NBD)	Cationic peptide	Probable oligopeptide transporter	[116]
Spb	Sbp (BP), CysU (TMD), CysW (TMD), CysA (NBD)	Sulfate, thiosulfate	Sulfate/thiosulfate porter. TMD/NBD components from the Cys system.	[88]
Ssu	SsuA (BP), SsuC (TMD), SsuB (NBD)	Sulfonate	Aliphatic sulfonate transporter	[117]
Tau	TauA {ssiA} (BP), tauC {tssiC} (TMD), tauB {ssiB} (NBD)	Taurine	Taurine porter	[119]
Tbp Thi	TbpA {thiB} (BP), ThiP {sfuB} (TMD), ThiQ {sfuC} (NBD)	Thiamine thiamin pyrophosphate	Thiamine importer	[120]
Ugp	UgpB (BP), UgpA (TMD), UgpE (TMD), UgpC (NBD)	*sn*-glycerol 3-phosphate	Glycerol-phosphate transport protein	[121]
Xyl	XylF (BP), XylH (TMD), XylG (NBD)	D-xylose	Monosaccharide transporter	[122]
Ycj	YcjN (BP), YcjO (TMD), YcjP (TMD), YcjU (NBD)	Unknown	Putative sugar transporter	[94]
Ydc	YdcS (BP), YdcV (TMD), YdcU (TMD) YdcT (NBD)	Unknown	Putative spermidine/putrescine transporter	[94]
Yeh	YehZ (BP), YehW (YMD), YehY (TMD), YehX (NBD)	Unknown	Putative glycine/betaine/choline transporter	[123]
Ytf/Yjf	YftQ (BP), YtfT (TMD), YjfF (IMD), YtfR (NBD)	Unknown	Putative sugar transporter	[94]
Yhd	YhdW (BP), YhdX (TMD), YhdY (TMD), YhdZ (NBD)	Unknown	Putative polar amino acid transporter	[94]
Ynj	YnjB (BP), YnjC (TMD), YnjD (NBD)	Unknown	Putative thiamine transporter	[7]
Yph	YphF (BP), YphD (TMD), YphE (NBD)	Unknown	Putative sugar transporter	[94]
Yrb	YrbD (BP), YrbE (TMD), YrbF (NBD)	Unknown	Putative transporter	[7]
Znu	ZnuA (BP), ZnuC (TMD), ZnuB (NBD)	Zn^{2+}	High-affinity zinc uptake	[124]
Eukaryotic-type transporters				
Ccm {Yej}	CcmC {YejT} (TMD), CcmB {YejW} (TMD), CcmA {YejV} (NBD)	Heme	Putative Heme exporter. ccmC may act separately to ccmAB	[125]
CydBD	CydBC homodimer	Unknown	Periplasmic c-type cytochrome exporter	[126]
Fts	FtsX (TMD), FtsE (NBD)	Unknown	Putative ABC transporter involved in cell division	[127]
Lol	LolC (TMD), LolE (TMD), lolD (NBD)	Lipoproteins	Lipoprotein translocator	[128]
MacAB	MacAB {ybjYZ} homodimer	14- and 15-membered lactones	Macrolide exporter	[129]
MdlAB (ABCC family)	mldAB homodimer	Peptides of 6–21 amino acyl residues	Mitochondrial peptide exporter	[129]
MsbA (ABCB family)	msbA homodimer	Phospholipid, LPS, lipid A, vinblastine, Hoechst 33342	Lipid flippase	[130,131]
Yad	YadH (TMD), YadG (NBD)	Predicted: polyketide drugs, teichoic acid	Putative antibiotic exporter	[94]
Ybb	YbbP (TMD), YbbA (NBD)	Unknown	Putative metal exporter	[94]
Ybh	YbhR (TMD), YbhS (TMD), YbhF (NBD)	Unknown	Putative ABC transporter, unknown function	[94]
YddA	YddA homodimer	Unknown	Putative fatty acid exporter	[129]
YhhJ	YhhJ homodimer	Unknown	Putative drug exporter	[94]
YojI	YojI homodimer	Microcin J25	Drug exporter	[132]

Collated from: http://www.genome.ad.jp/kegg/pathway/eco/eco02010.html; http://www.tcdb.org/tcdb/index.php?tc=3.A.1; http://www.york.ac.uk/res/thomas/searchABC.cfm; http://ecogene.org/index.php.
Non-functional ABC transporters in *E. coli* are not listed.
Putative transporters are components listed in order from substrate BP to NBD's.
Alternative gene name is listed in brackets.

Moussatova et al. Biochim Biophys Acta. 2008; 1778:1757-71

ABC in antibiotic producing organisms…

1: FEMS Microbiol Lett. 1998 Jan 1;158(1):1-8.

ABC transporters in antibiotic-producing actinomycetes.

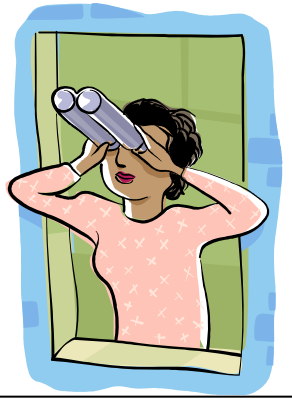
Méndez C, Salas JA.

Departamento de Biología Funcional e Instituto Universitario de Biotecnología de Asturias (I.U.B.A-C.S.I.C), Universidad de Oviedo, Spain.

Many antibiotic-producing actinomycetes possess at least one ABC (ATP-binding cassette) transporter which forms part of the antibiotic biosynthetic pathway and in most cases confers resistance to the drug in an heterologous host. Three types of antibiotic ABC transporters have been so far described in producer organisms. In Type I two genes are involved, one encoding a hydrophilic ATP-binding protein with one nucleotide-binding domain and the other encoding a hydrophobic membrane protein. In Type II transporters only a gene encoding the hydrophilic ATP-binding protein with two nucleotide-binding domains is present and no gene encoding a hydrophobic membrane protein has been found. In Type III only one gene is involved which encodes both the hydrophilic and hydrophobic components. Possibly these ABC transporters are responsible for secretion of the antibiotics outside the cells. A comparative analysis of the ATP-binding components of the different antibiotic ABC transporters and analysis of the amino acid distances between the so-called Walker motifs suggests that the three types of transporters have probably evolved from a common ancestor containing a single nucleotide-binding domain.

PMID: 9453150 [PubMed - indexed for MEDLINE]

Conclusion #4: transport is essential for survival …
and can be intrinsically linked to resistance
(it all depends how you look at it !)



ABC in yeast…

1: Acta Physiol Scand Suppl. 1998 Aug;643:297-300.

The inventory of all ion and drug ATPases encoded by the yeast genome.

Goffeau A.

Unité de Biochimie Physiologique, Université Catholique de Louvain, Louvain-la-Neuve, Belgium. Goffeau@fysa.ucl.ac.be

The 5,885 members of the yeast proteome have been screened for amino acid sequence signatures of either P-type ATPases or ABC transporters. A total of 16 P-type ATPases have been classified into six phylogenetic families which each seem to transport a specific class of substrates. In addition, a total of 16 ABC transporters comprising two nucleotide binding folds and two membrane domains were classified in two distinct phylogenetic families. Two ABC transporters of Family I (Pdr5p and Snq2p) share overlapping promiscuity for numerous hydrophobic drugs with a member of Family II (Yor1p). In this case, substrate specificity seems to have differentiated more slowly during evolution than typical phylogenetic traits reflected by amino acid sequence similarity or predicted membrane topography.

PMID: 9789573 [PubMed - indexed for MEDLINE]

ABC and transport of bile salts (with phospholipids) in hepatocytes

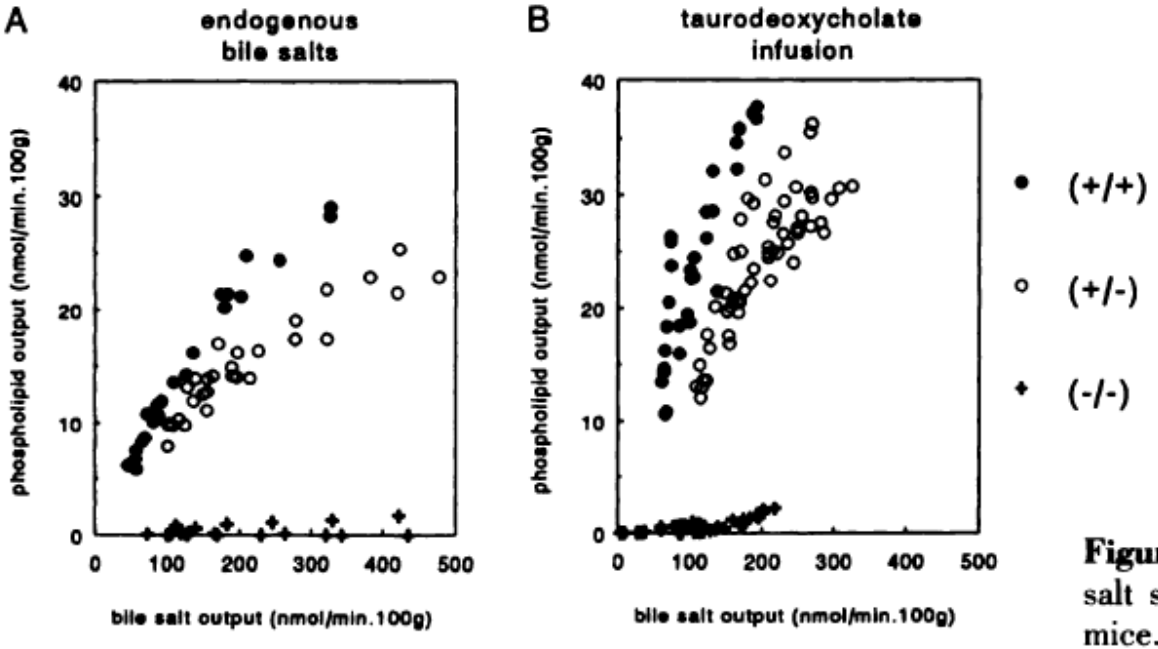
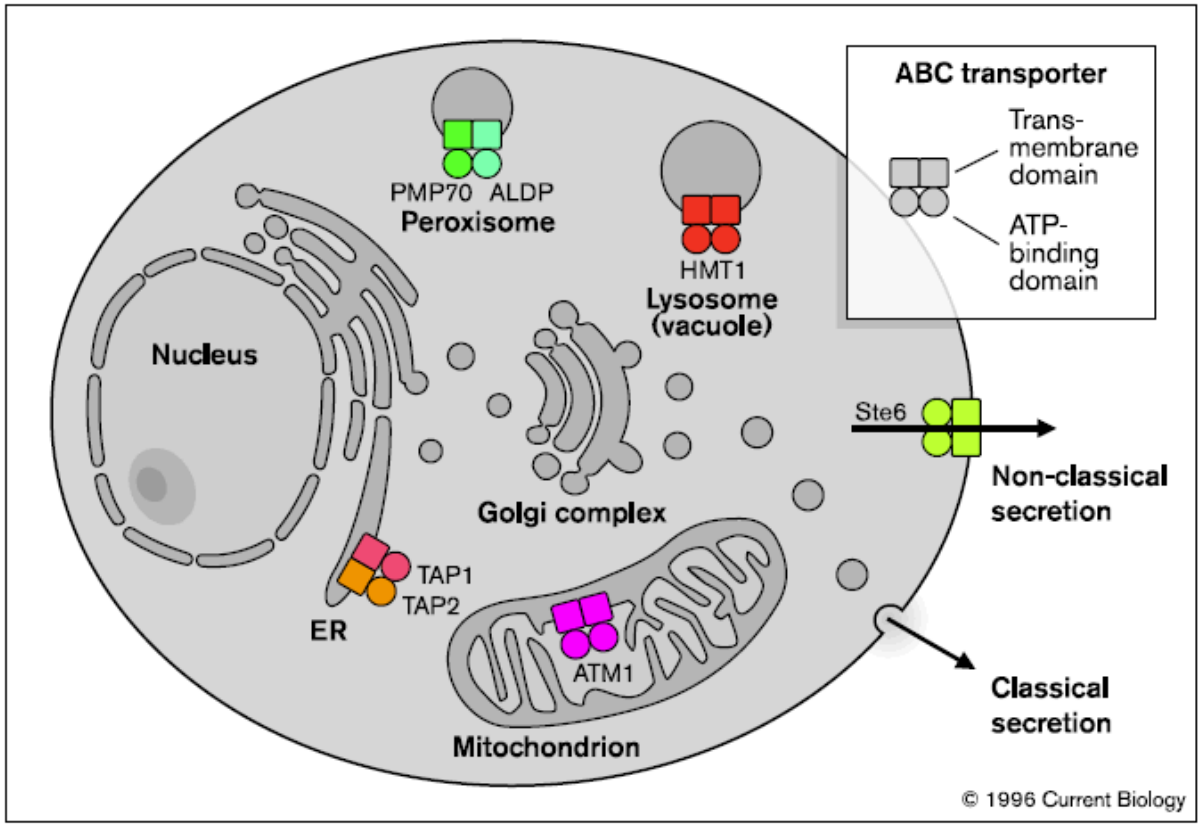


Figure 2. Relation between phospholipid and bile salt secretion in *mdr2* (+/+), (+/−), and (−/−) mice. *A)* This relation during secretion of endogenous bile salts (70% tauromuricholate and 30% taurocholate); there is a hyperbolic relation between phosphlipid and bile salt excretion. *B)* Biliary phospholipid secretion during infusion of taurodeoxycholate, which is a more hydrophobic bile salt. It is clear that the amount of phospholipid per bile salt molecule is increased during secretion of the more hydrophobic taurodeoxycholate compared to the situation with endogenous bile salts. Under both conditions phospholipid excretion is virtually absent in *mdr2* (−/−) mice and reduced in *mdr2* (+/−) mice.

Elferibck et al. FASEB J. 1997;1:19-28

ABC and intracellular transport



Examples of ABC transporters located in the organelle membranes of eukaryotic cells. A typical ABC transporter consists of two transmembrane domains that each span the bilayer six times and two ATP-binding domains. The transporters can be assembled from two half-transporter polypeptides or may be synthesized as a single polypeptide chain. See text for further details.

Cleves & Kelly, Curr Biol. 1996; 6:276-8.

ABC in mitocondria

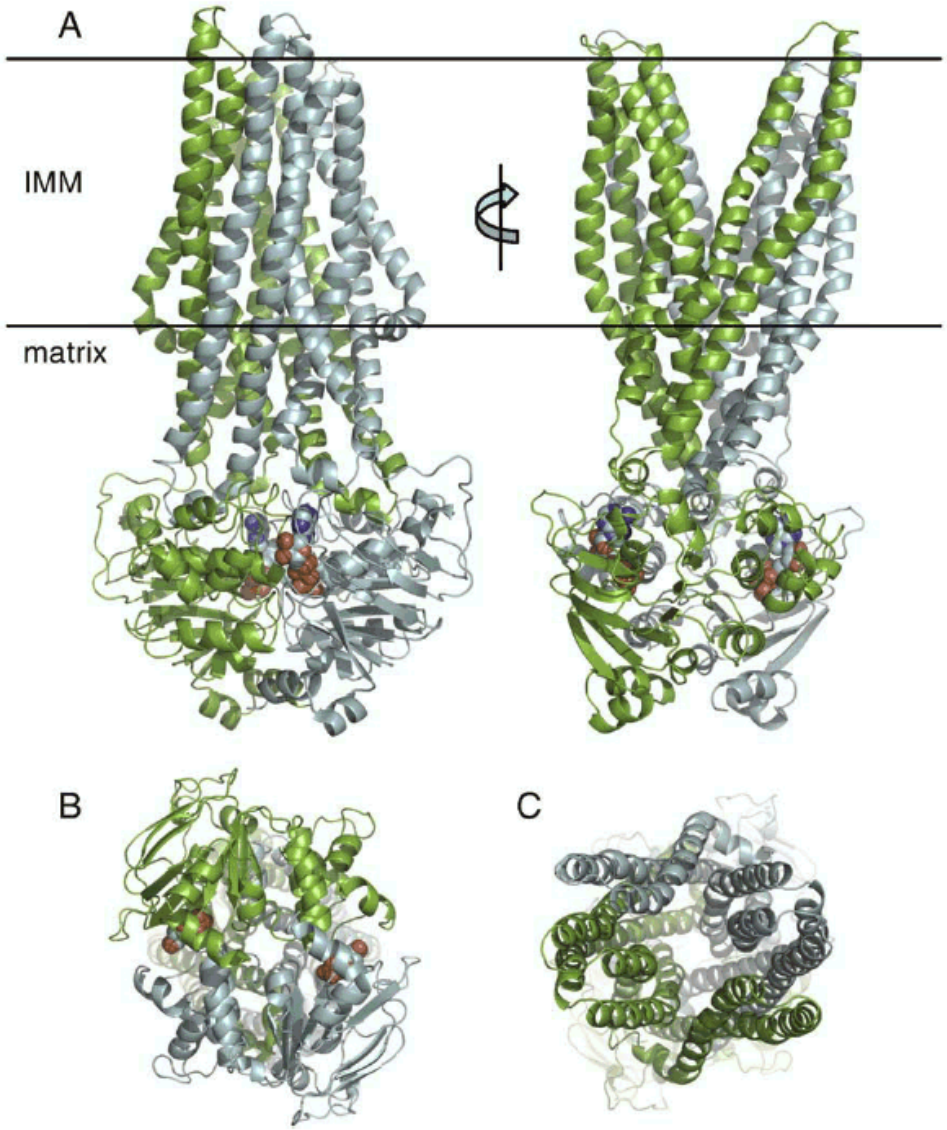


Fig. 3. Structural organization of mitochondrial ABC transporter. A 3D homology model of the homodimeric MDL1 complex was constructed based on the X-ray structure of Sav1866. Each half-transporter (cyan and green) was modeled on the corresponding subunit of the ADP-bound *S. aureus* Sav1866 homodimer (2HYD.pdb) [25]. The amino acid sequence of MDL1 and other homologous was aligned with that of Sav1866 using ClustalW2 (see Fig. 2). The alignment for mature (leader sequence-less) MDL1 [55] against Sav1866 revealed 31% sequence identity. Each MDL1 subunit was modeled separately by means of MODELLER v9.3 [88]. The half-transporters were dimerized to reproduce the Sav1866 subunit interface and refined to remove steric clashes [89]. In the 3D model (side view **A**), the NBDs form a sandwiched dimer by bound ATP (space filling model, view **B**) and the TMDs opened to the ER-luminal site, reflecting an outward-facing conformation (view **C**). The models are created by PyMOL.

ABC and early "immune" defense…

ELSEVIER

Immunology Letters 54 (1996) 215–219

immunology letters

Mini-review

The multidrug transporters—proteins of an ancient immune system

Balázs Sarkadi*, Marianna Müller, Zsolt Holló

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- With regards to general immunology, an interesting suggestion is the possible involvement of the multidrug transporters in the **cellular secretion of cytokines and/or chemokines**.
- Some of these physiologically important peptides are produced without a signal sequence and recent data indicate that the expression of MDR1 is involved in the transport of these peptides through the secreting cell membranes.
- Also, unpublished observations link the expression of multidrug transporters to the cell-mediated killing of various target cells.

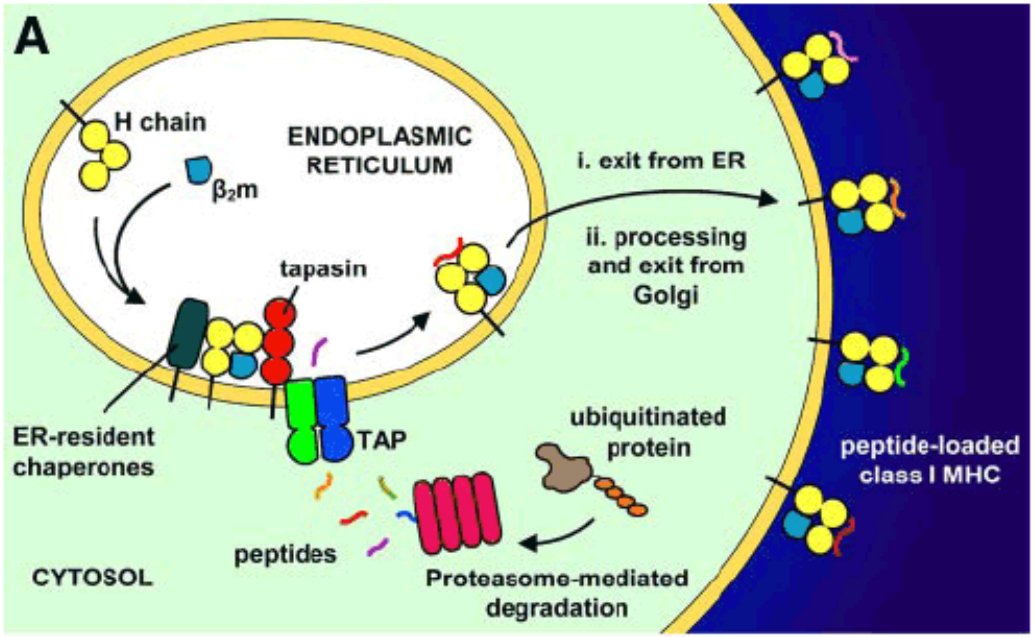
A multiplicity of exports … for protection

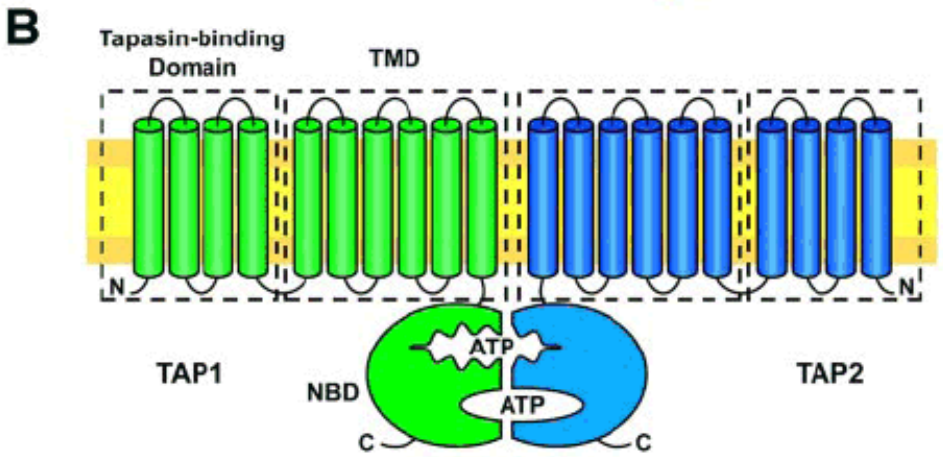
TABLE 1. Similarity Analyses and Functions of Human MRP and its Most Closely Related ABC Proteins

Protein/gene (species)	Function	% Identity	% Similarity
MRP (human)	GS-X pump, anionic conjugate transporter, multidrug resistance	100	100
mrp (mouse)	GS-X pump, anionic conjugate transporter, multidrug resistance	89.9	96.1
MOAT (human)/MRP2	GS-X pump, anionic conjugate transporter (hepatocanaliculi)	48.7	66.9
EBCR (rabbit)	Probable MOAT ortholog	48.7	66.6
C. elegans mrpl (nematode)	Heavy metal resistance	46.3	64.2
C. elegans mrp2 (nematode)	Unknown	46.6	63.6
MRP6 (human)	Unknown	42.1	60.6
YCF1 (yeast)	Cadmium resistance, vacuolar GS-X pump	40.2	59.9
AtMRP1 (*Arabidopsis*)	GS-X conjugate pump	36.0	55.0
SUR1 (human)	Sulfonylurea receptor, K^+ channel regulator (pancreas)	33.1	53.2
sur2 (rat, mouse)	Sulfonylurea receptor, K^+ channel regulator (brain, heart)	32.5	53.1
YOR1/YRS1 (yeast)	Oligomycin resistance	30.3	50.0
LtpgpA (leishmania)	Resistance to antimonial and arsenical oxyanions	30.0	47.9

Sequences were aligned along their entire length with MRP using CLUSTAL W(1.6) multiple sequence alignment. Sequence data were obtained using the following accession numbers: MRP, L05628/P33527; mrp, AF022908/1488428; MOAT, U49248/U63970; EBCR, 1430907/Z49144; *C. elegans* mrp1, U66260; *C. elegans* mrp2, U66261; MRP6, U91318; YCF1, L35327/Z48179; AtMRP1, AF008124; SUR1, L78207/U63421; sur2, D83598/D86037; YOR1/YRS1, Z73066; LtpgpA, X17154. Several additional MRP-related proteins were not included because their complete cDNA sequences have not yet been published.

Antigen processing…



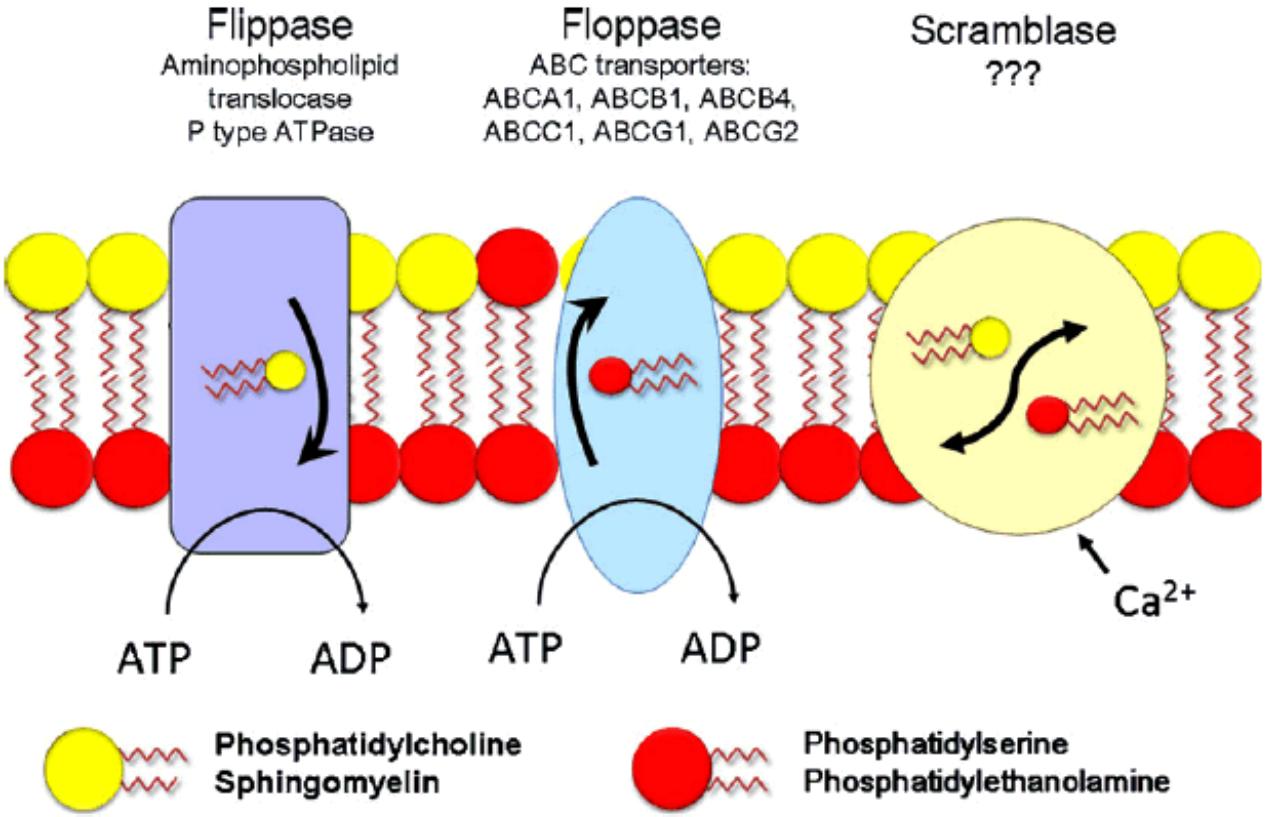


TAP: transporter associated with antigen processing

Figure 2. TAP and antigen processing. *A*) Intracellular proteins are turned over by the ubiquitin-proteasome pathway. Some of the peptides produced during protein degradation are transported by TAP into the ER, where the peptides associate with class I MHC molecules. Peptide-bound class I MHC is released by ER-resident chaperones, including the chaperone tapasin. The heterotrimeric class I complex exits the ER and Golgi toward the cell surface. *B*) TAP is a heterodimer of homologous TAP1 (green) and TAP2 (blue) proteins, which each contain an N-terminal tapasin-binding accessory domain of likely 4 TM helices (41, 102), a 6-helix TMD, and a C-terminal NBD.

Procko et al. FASEB J. 2009 May;23(5):1287-302

Lipid transmembrane transport



Regulators of plasma membrane lipid asymmetry. Translocation of lipids is carried out by the flippases (active translocation to the cytoplasmic leaflet), floppases (active translocation to the exoplasmic leaflet) and scramblases (promote equilibrium through a Ca^{2+}-dependent mechanism).

Aye et al. Chem Biol Interact. 2009; 180:327-39.

Lipid transmembrane transport: where do ABC not play a role ?

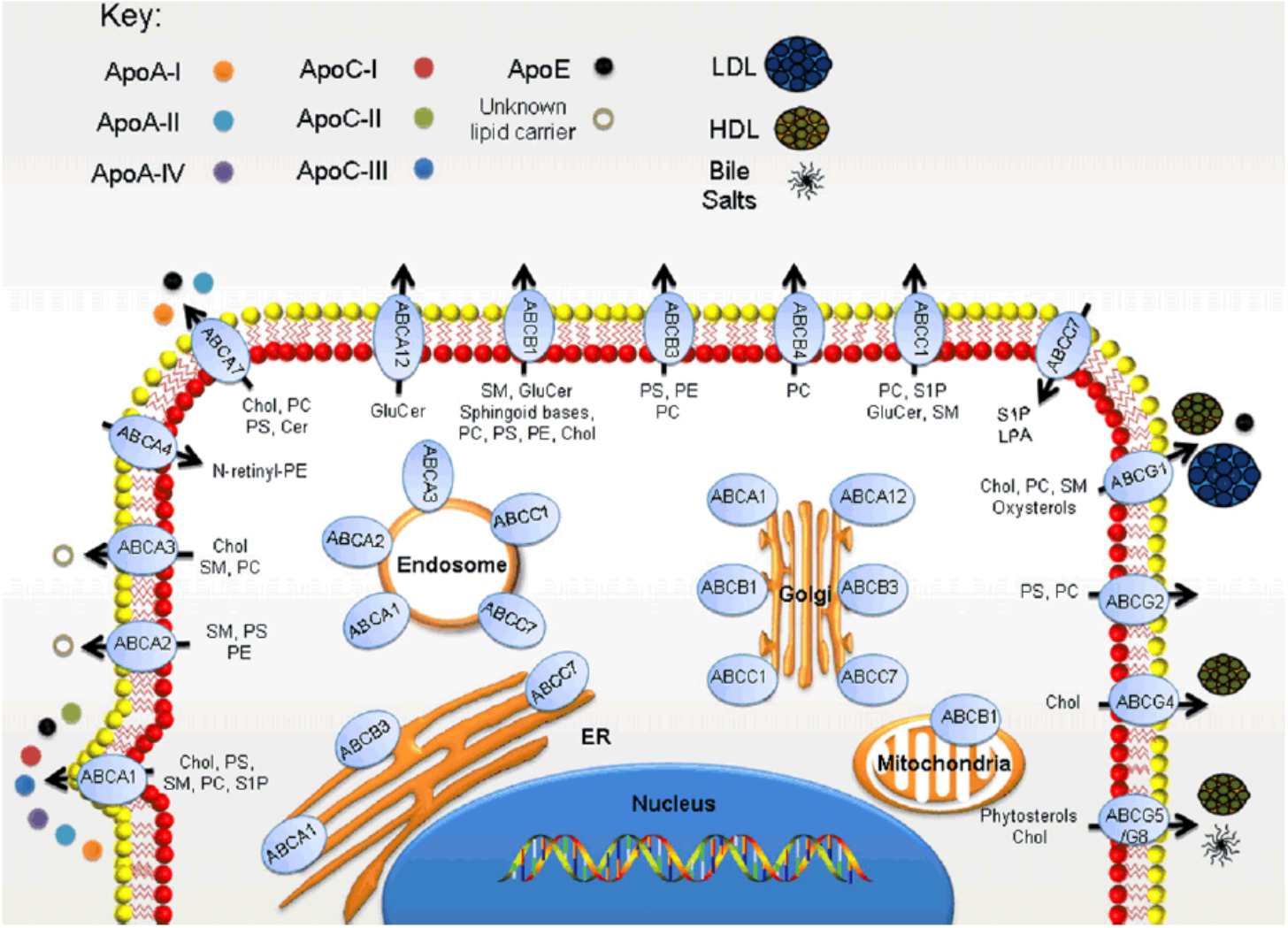


Fig. 2. Overview of ABC transporters involved in lipid efflux. Schematic representation of subcellular ABC transporter localization, known acceptors and direction of transport. Black arrows represent transport direction at the plasma membrane. Vectorial transport by intracellular transporters has not been firmly established. Apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; Chol, cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Cer, ceramide; *N*-retinyl-PE, *N*-rentinyl phosphatidylethanolamine; GluCer, glucosylceramide; S1P, sphingosine-1-phosphate; SM, sphingomyelin; LPA, lysophosphatidic acid.

Aye et al. Chem Biol Interact. 2009; 180:327-39.

Controlling cholestrol uptake

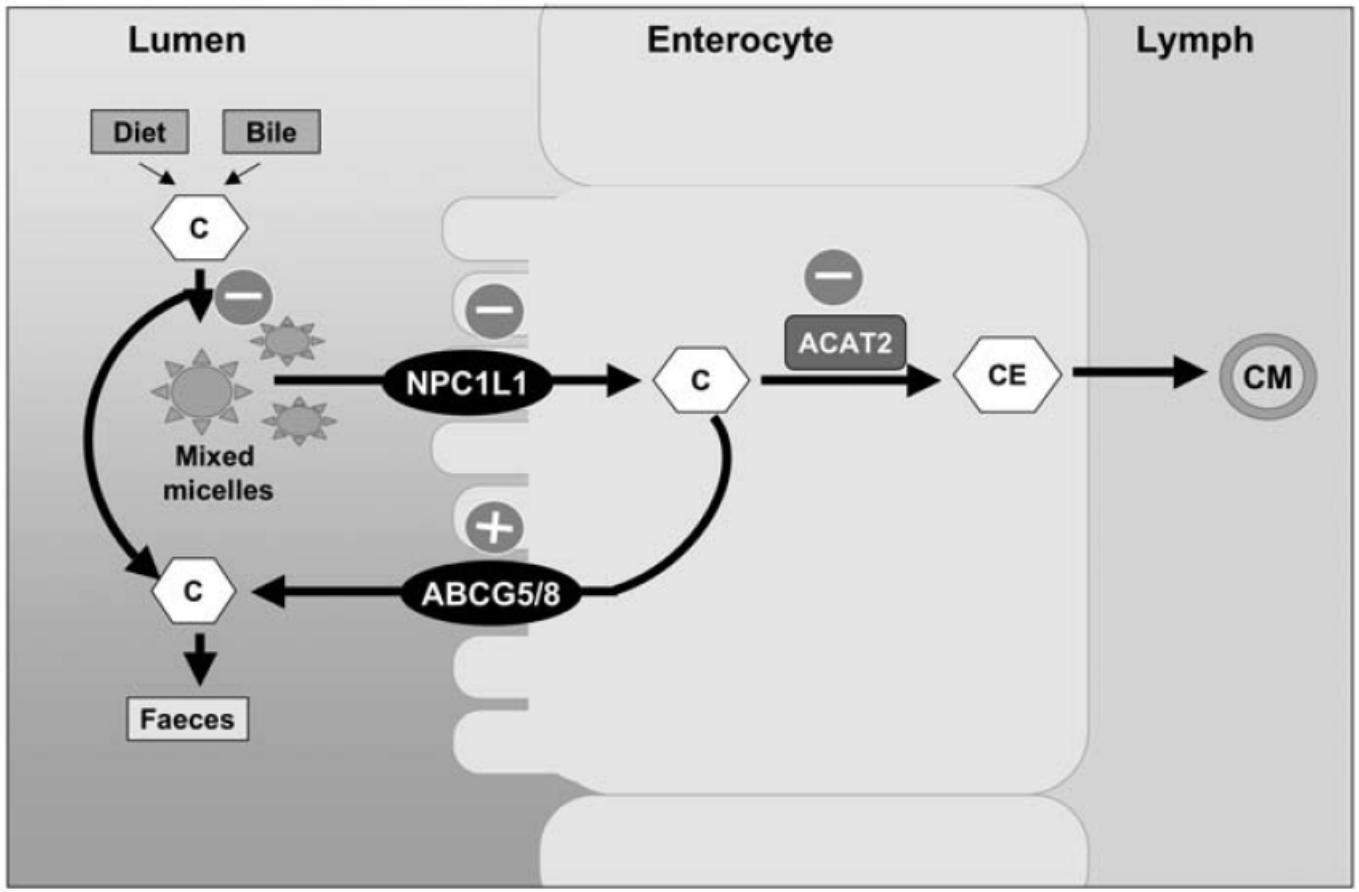


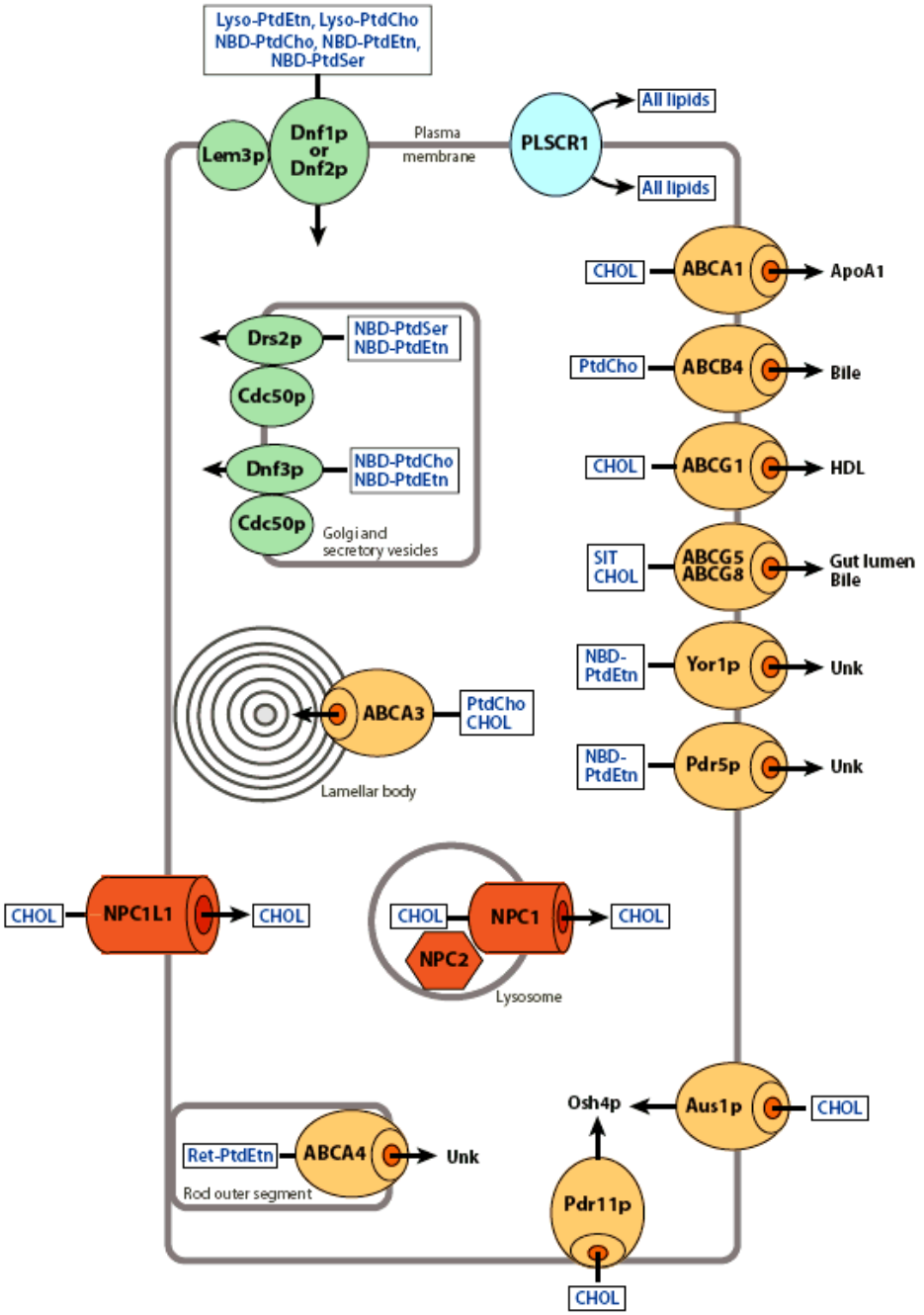
Fig. 1. *Multistep process of intestinal cholesterol absorption and potential cholesterol-lowering mechanisms of action of phytosterols.*
C, free cholesterol; CE, cholesteryl esters; CM, chylomicrons; NPC1L1, Niemann-Pick C1-like 1; ACAT2, Acyl-coenzyme A cholesterol acyltransferasa isorform2; ABCG5/8, ATP-binding cassette transporters G5/G8.

Sanclemente et al. J Physiol Biochem, 2009; 65:87-98

Transmembrane cholesterol movements

Summary of intramembrane transporters.

- A schematic summary of the locations and substrates for P-type ATPases, ATP-Binding Cassette (ABC) transporters, scramblases (PLSCR1), and Niemann-Pick (NPC) family proteins.
- The transport effected by the “Defective in ribosome synthesis 2 and Neomycin resistance 1 family” (Dnf) of proteins is from the exoplasmic to the cytoplasmic leaflet of the bilayers.
- The lipid transport by ABC family proteins results in export of the lipid from the membrane harboring the transporter to apolipoprotein A1 (ApoA1), or high-density lipoprotein (HDL) particles, or hydrophobic environments such as bile, or the interior of the lipid storage organelle known as the lamellar body.
- Lipid transport to the intracellular oxysterol-binding protein homolog 4 (Osh4p) also occurs.



Voelker D, Annu. Rev. Biochem. 2009; 78:827-856

Lipid ABC transporters

Human ABC lipid transporters, their tissue expression, lipid substrates, modulators, acceptors and associated genetic diseases.

Gene Name	Major sites of expression	Lipid Substrates	Lipid Modulators	Acceptors	Associated genetic disorders	References
ABCA1 (ABC1)	Ubiquitous	Chol, PS SM, PC, S1P, 25-OH-chol	Cer (stimulates) LacCer (inhibits)	Apo-AI, AII, E, CI, CII, CIII, AIV	Tangier disease, familial hypoalphalipoproteine-mia (ischemic heart disease)	[52,189,200,236,237]
ABCA2	Brain	Chol, SM, PS, PE			(Alzheimer's disease)	[63,66,133,238]
ABCA3	Lung, brain, heart, pancreas	Chol, SM, PC			Fetal/neonatal lung deficiency	[67,69,134,135,239]
ABCA4 (ABCR)	Retinal photoreceptors	*N*-retinyl-PE		(Cytosolic)	Fundus flavimaculatis; retinitis pigmentosum 19; cone-rod dystrophy (age-related macular degeneration)	[136]
ABCA7	Myelolymphatic system, brain, skin	Chol, PS, PC Cer		ApoA-I (ApoA-II?)		[156,159,240,241]
ABCA12	Skin keratinocytes	GluCer			Harlequin ichthyosis	[133,158]
ABCB1 (MDR1)	Brain, liver, kidneys, GI, placenta	SM, GluCer, sphingoid bases, PC PS, PE, Chol, PAF, corticosteroids, androgens, estrogens, progestins	Sph, Cer, S1P (stimulates) SM GluCer, GalCer (inhibits)			[34,37,104,120,148,170,197,242–244]
ABCB3 (TAP-2)	Ubiquitous	PS, PE, PC			(Immune deficiency)	[37,116,121,122]
ABCB4 (MDR3)	Liver, bile canilicular membrane, placenta	PC			Progressive familial intrahepatic cholestasis	[245–248]
ABCC1 (MRP1)	Brain, liver, kidneys, GI, placenta	LTC_4, GluCer, SM, PC, S1P, GSH, UGT, steroid conjugates				[105,109,127,150,193,249,250]
ABCG1	Ubiquitous	Chol, PC, SM, 7β-OH-chol, 7-keto-chol	SM (stimulates)	HDL (LDL?)		[83,94,225,251,252]
ABCG2 (BCRP)	Placenta, breast, liver, GI	PC, PS Sulphated steroids	Chol (stimulates)			[39,190,192,253,254]
ABCG4	Macrophage, brain, eye, spleen, liver	Chol				[93,95,97]
ABCG5 & ABCG8	Liver, GI	Plant sterols, Chol		HDL Bile salts	Sitosterolemia	[99,101–103,255]

Abbreviations: Apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; Chol, cholesterol; 7β-OH-chol, 7β-hydroxycholesterol; 25-OH-chol, 25-hydroxycholesterol; 7-keto-chol, 7-ketocholesterol; PC, phosphatidylcholine; PE, phosphatidyl-ethanolamine; PS, phosphatidylserine; *N*-retinyl-PE, *N*-rentinyl phosphatidylethanolamine; Cer, ceramide; GalCer, galactosylceramide; GluCer, glucosylceramide; LacCer, lactosylceramide; Sph, sphingosine; S1P, sphingosine-1-phosphate; SPK1, sphingosine kinase-1; LPA, lysophosphatidic acid; LTC_4, leukotriene C_4, PAF, platelet activating factor; GSH, glutathione; UGT, glucuronide; GI, gastrointestinal.? indicates uncertainty.

I.L.M.H. Aye et al. / Chemico-Biological Interactions 180 (2009) 327–339

A variety of substrates

Table 2. Examples of range of allocrites transported by ABC-systems

Export		Import		Export			Import	
ABC	Allocrite	ABC	Allocrite	ABC	Organism	Allocrite	ABC	Allocrite
HlyB-	HlyA toxin	HisP	Histidine	Mdr1 (Pgp)	Man	Antitumour drugs hydrophobic	ALDP	Fatty acids? (peroxysomes)
Prt	Protease	MalK	Maltose			Analgesics		
LipB	S-capsule	RsbA	Ribose			β-amyloid peptide		
PilH	Pilin?	Aap	L-amino acids			Lipids		
LcnC	Peptide/ antibiotics	OppF	Oligopeptides			Detergents		
LmrA	Multidrugs	PstB	Phosphate			Hormones		
DrrAB	Antibiotics	CysA	Sulphate			Cholesterol		
NodJ	Lipo-oligo saccharide	PhnC	Phosphonate			Antihistamine		
KpsT	Polysialic acid	BtuB	B12	Mrp (1-7)	Man	Organic ion conjugated and		
CcmA	Haem	SfuC	B1			non-conjugated drugs		
TagH	Teichoic acids	PotA	Putrescine	Mdr3	Man	Long chain PC		
		NikD,E	Nickle			Distinct drugs		
		ModC	Molybdenum	MXR	Man	Bile Salts		
		FepC	Enterochelin	Spgp	Man	PS?		
		FhuC	Fe-ferrichrome	ABC-1	Man	Retinol?		
		SapDF	(K^+) oligopeptides	ABC-r	Man	Cl^-		
				CFTR	Man	Glucouronate (Na^+)		
				SUR	Man	(K^+)		
				TAP1,2	Man	MHC peptides		
				ABCX	Plants (MT)	Haem		
				MRP-Like	Plants	Herbicides Pigments Flavenoids		
				PgpA	*Leishmania*	GST-arsenic		
				Pdr12	Yeast	Weak organic acids		
				Ste6	Yeast	Pheromone-peptide		

Holland & Blight, J. Mol. Biol. 1999; 293:381-399

The word allocrite, loosely adapted from the Greek, means heterogeneous (compounds) transported or excreted. This is specifically applying to ABC-transporters

ABC and pharmacokinetics…

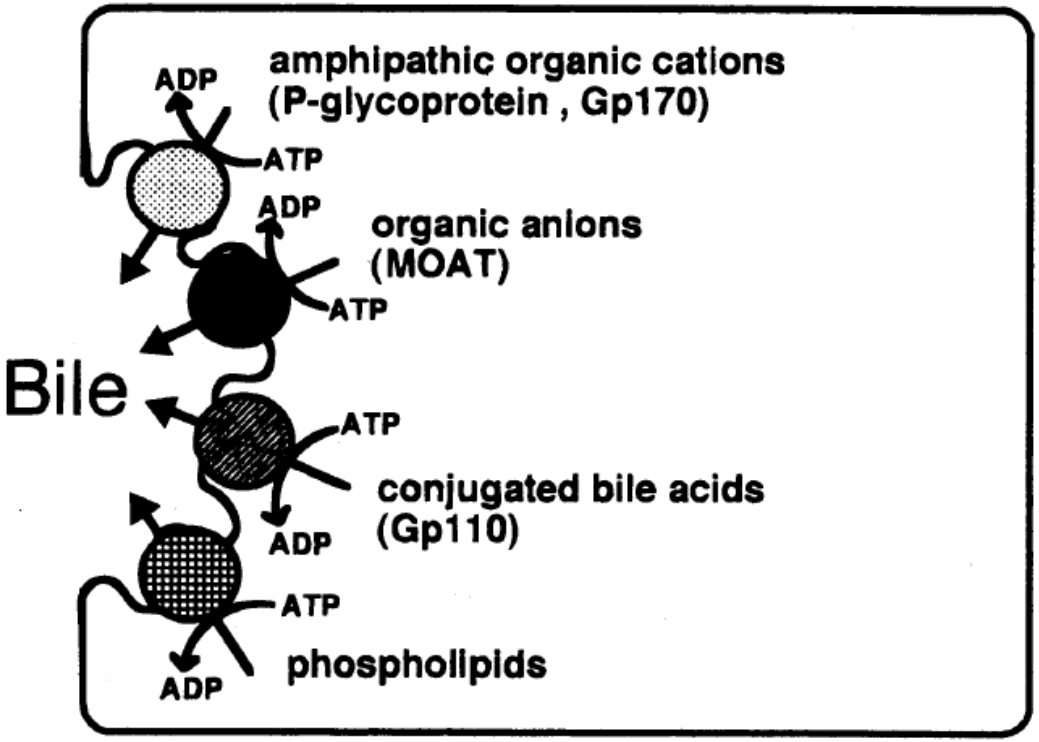


Fig. 3. ATP-dependent primary active transport carriers in the canalicular membrane involved in the biliary excretion of amphipathic organic cations, organic anions, conjugated bile acids and phospholipids.

Yamazaki et al. Pharm Res. 1996; 13:497-513

Regarding biliary excretion, we illustrate

- the possible contribution of the ABC transporters to the biliary excretion of xenobiotics.
- the multiplicities in both hepatic uptake and biliary excretion mechanisms.

This helps in our understanding of the physiological adaptability of the living body in terms of the removal (detoxification) of xenobiotics

Pharmacokinetics and HIV

Table 2. Variability in Antiretroviral Drug Disposition[89–97]

Gene (Protein)	Single Nucleotide Polymorphism (SNP) Studied	Drug	Frequency of Genotype	Effect Observed	Experimental Model Used
ABCB1/MDR1 (P-gp)	C3435T	Lopinavir, ritonavir, nelfinavir, indinavir, saquinavir, amprenavir, nevirapine, efavirenz	Significant differences in P-gp induction between genotypes not observed (CC, CT, and TT)[91]	Increased P-gp expression was observed with all drugs except incase of Amprenavir with which increased P-gp expression was not observed even at a higher concentration of 100 μM[91]	Humans, peripheral blood mononuclear cells
		Nelfinavir, efavirenz	25% TT, 50% CT, 25% CC in Caucasians.[89] 67–83% CC, 2–5% TT in African-Americans[90]	MDR1 3435 TT genotype associated with low P-gp expression and low plasma drug concentrations[89]	Humans, peripheral blood mononuclear cells
		Indinavir		The genotype affected the absorption constant of indinavir[94]	Humans (HIV-infected patients)
	C3435T, G2677T	Lopinavir, atazanavir	C/T, G/T alleles at the MDR1 C3435T and G2677T loci—equally frequent in Caucasians; wild-type alleles—more prevalent in African-Americans[92]	Trough drug plasma concentrations did not correlate with the variant T allele[92]	Humans
	G1199A	Lopinavir, ritonavir, indinavir, saquinavir, amprenavir		Significantly lower trans-epithelial permeability ratio in cells expressing wild-type MDR1 cells compared to G1199A variant[93]	Recombinant epithelial cells expressing wild-type MDR1 or G1199A variant

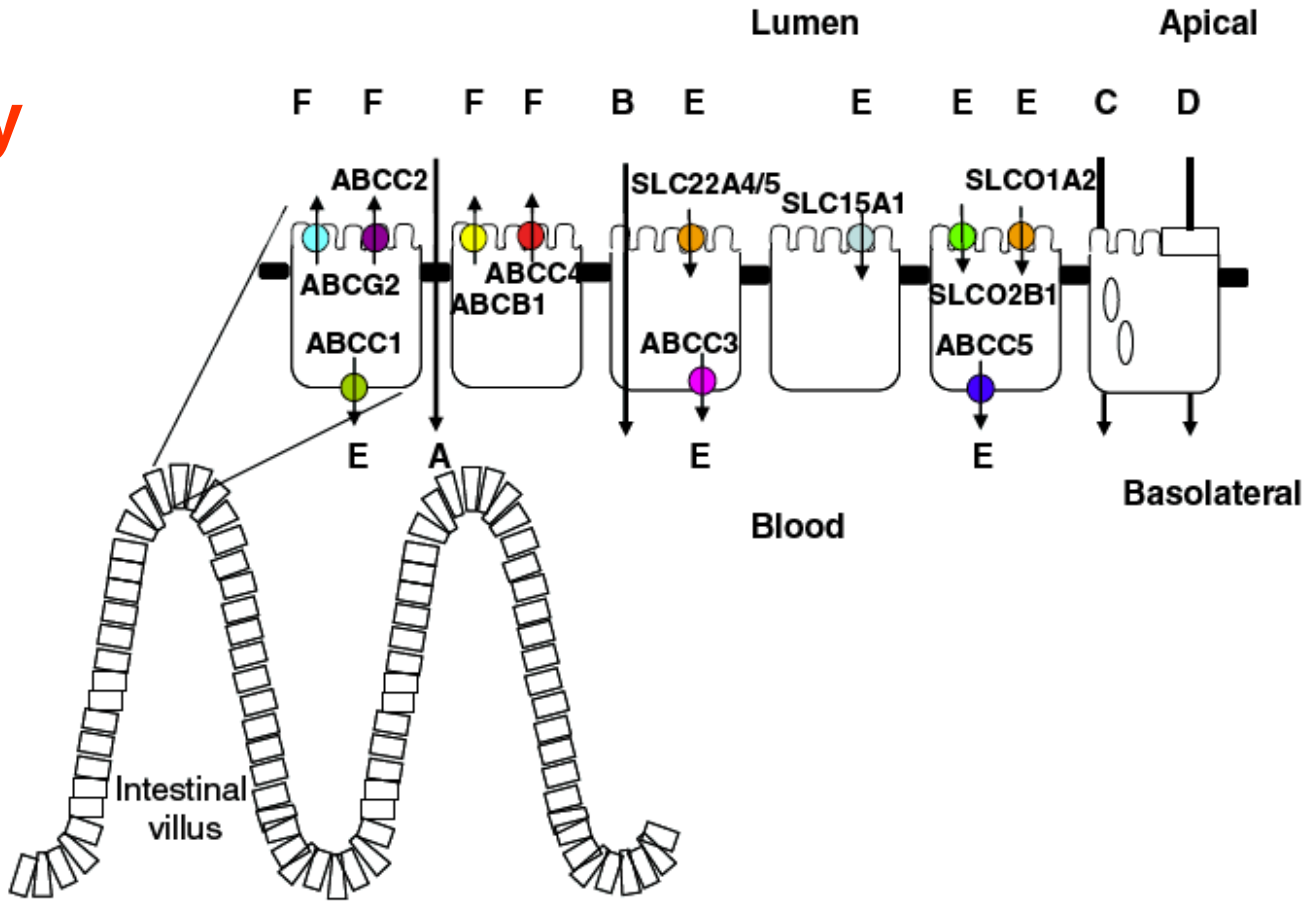
Gulati and Gerk, J Pharm Sci. 2009; 98:2317-35

Pharmacokinetics and HIV

ABCC2 (MRP2)	−24C/T	Indinavir	MRP2-24C/T variant carriers had 24% faster indinavir oral clearance[96]	Humans
	G1249A	Indinavir	No correlation observed between G1249A variant carrier status and pharmacokinetics or pharmacodynamics of indinavir[96]	Humans
		Saquinavir	Threefold higher drug concentrations in patients with MRP2 G1249A GG genotype compared to variant carriers[97]	Humans (HIV-infected patients)
ABCC4 (MRP4)	T4131G	Lamivudine	Drug concentrations—20% elevated in MRP4 T4131G variant carriers[96]	Humans
	G3724A	Zidovudine	Trend for elevated zidovudine concentrations in MRP4 G3724A variant carriers; relationship not statistically significant[96]	Humans
ABCG2 (BCRP)	C421A, G34A	Lamivudine, zidovudine	None of the BCRP variants associated with drug concentrations[96]	Humans
ABCB1/MDR1 (P-gp), ABCC1 (MRP1), ABCC2 (MRP2), ABCG2 (BCRP)	A comprehensive evaluation of 39 SNPs in MDR1, 7 in ABCC1, 27 in ABCC2, and 16 in ABCG2	Nelfinavir	No significant association between cellular nelfinavir AUC and SNPs or haplotypes at ABCC1, ABCC2, ABCG2. Association with cellular exposure for two loci in strong linkage disequilibrium: MDR1 3435C > T; $AUC_{TT} > AUC_{CT} > AUC_{CC}$[95]	Peripheral blood mononuclear cells from individuals receiving nelfinavir

Gulati and Gerk, J Pharm Sci. 2009; 98:2317-35

Bioavailability



Intestinal epithelia

Mechanisms of transport through the intestinal epithelium and localization of ABC and SLC drug transporters; ABCB1, ABCG2, ABCC1-5, SLCO1A2/2B1, SLC22A4/5 and SLC15A1
(A) passive diffusion via tight junctions; (B) passive diffusion; (C) endocytosis; (D) carrier-mediated transport; (E) carrier-mediated uptake; (F) carrier-mediated efflux.

Oostendorp et al. Cancer Treat Rev. 2009; 35:137-47

Bioavailability

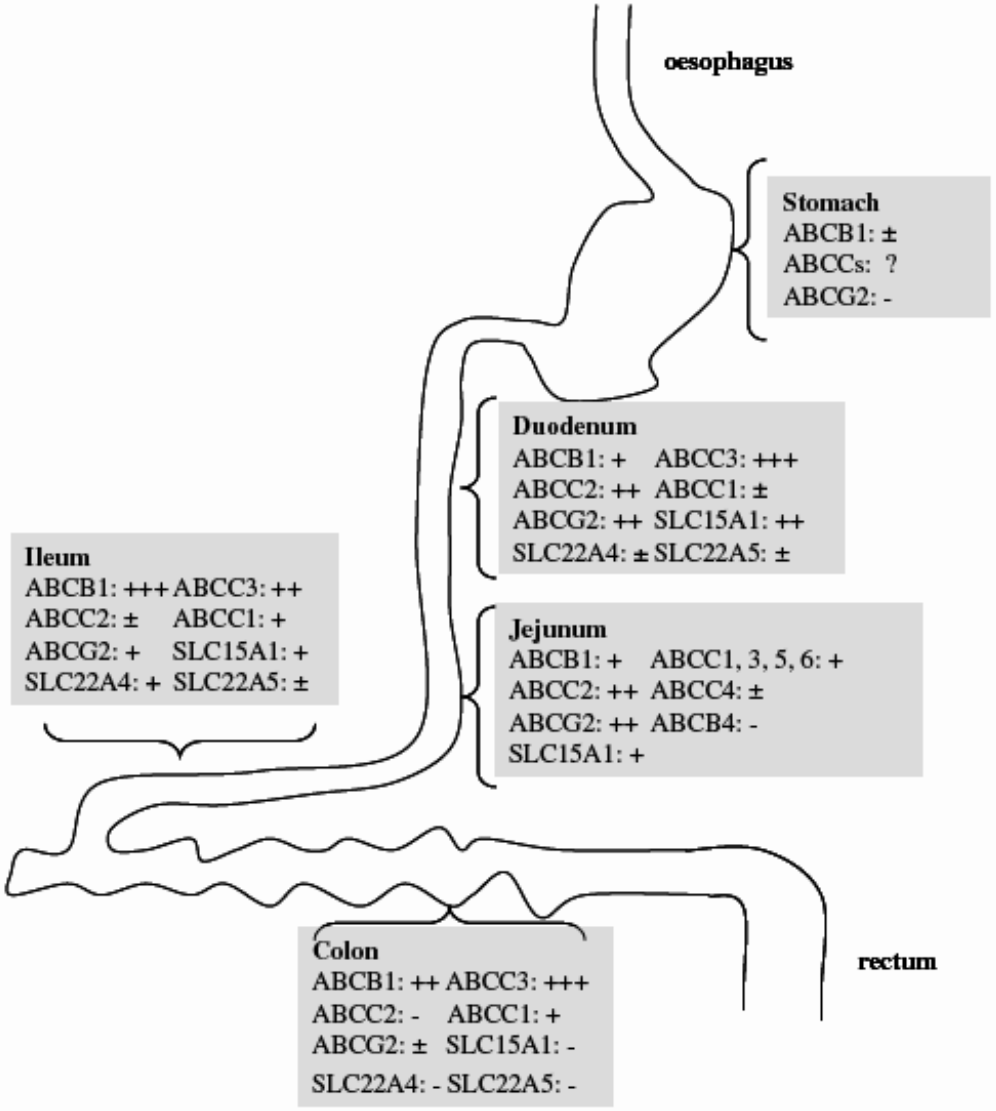


Figure 2. Expression levels of ABC and SLC transporters in different parts of the human intestine.

Oostendorp et al. Cancer Treat Rev. 2009; 35:137-47

Drugs with intestinal resorbtion influenced by ABC transporters inhibitors

Table 1
The effect of absence or inhibition of ABCB1, ABCG2 and ABCC2 on the preclinical and/or clinical pharmacology of different drugs with high affinity for ABCB1, ABCG2 and ABCC2

Oral drugs	Inhibitor	Effect measured	References
Preclinical studies:			
In vitro			
Vinblastine	–	10-Fold ↑ transport in ABCB1 over-expressing cells	[35]
Vinblastine	Verapamil	Reduced transport of vinblastine in ABCB1 over-expressing cells	[35]
Ranitidine	–	Three-fold ↑ transport in ABCB1 over-expressing cells	[37]
Ranitidine	Cyclosporin A or verapamil	Reduced transport of ranitidine in ABCB1 over-expressing cells	[37]
Indinavir	–	↑ Transport in ABCB1 over-expressing cells	[38]
Nelfinafir	–	↑ Transport in ABCB1 over-expressing cells	[38]
Saquinavir	–	↑ Transport in ABCB1 over-expressing cells	[38]
Indinavir	PSC833	Reduced transport of indinavir in ABCB1 over-expressing cells	[38]
Nelfinafir	PSC833	Reduced transport of nelfinafir in ABCB1 over-expressing cells	[38]
Saquinavir	PSC833	Reduced transport of saquinavir in ABCB1 over-expressing cells	[38]
Topotecan	–	↑ Transport in ABCG2 over-expressing cells	[68]
Topotecan	GF120918	Reduced transport of topotecan in ABCG2 over-expressing cells	[68]
Topotecan	Pantoprazole	Reduced transport of topotecan in ABCG2 over-expressing cells	[71]
SN-38	–	↑ Resistance in ABCG2 over-expressing cells	[58]
SN-38	GF120918	Reversed resistance to SN-38 in ABCG2 over-expressing cells	[58]
SN-38	Gefitinib	Reversed resistance to SN-38 in ABCG2 transduced cells	[75]
PhIP	–	↑ Transport in ABCG2 over-expressing cells	[87]
In Vivo			
Paclitaxel	–	↑ Oral bioavailability in ABCB1 knockout mice	[6]
Paclitaxel	PSC 833	↑ Oral bioavailability in wild-type mice by ABCB1 inhibition	[54]
Paclitaxel	Cyclosporin A	↑ Oral bioavailability in wild-type mice by ABCB1 inhibition	[55]
Paclitaxel	GF120918	↑ Oral bioavailability in wild-type mice by ABCB1 inhibition	[57]
Docetaxel	–	↑ Oral bioavailability in ABCB1 knockout mice	[52]
Docetaxel	Cyclosporin A	↑ Oral bioavailability in wild-type mice by ABCB1 inhibition	[52]
Docetaxel	Ritonavir	↑ Oral bioavailability in wild-type mice by ABCB1/CYP3A4 inhibition	[52]
Etoposide	–	↑ Oral bioavailability in ABCB1 knockout mice	[51]
Etoposide	GF120918	↑ Plasma levels by inhibition of ABCB1	[51]
Indinavir	–	↑ Oral bioavailability in ABCB1 knockout mice	[38]
Nelfinafir	–	↑ Oral bioavailability in ABCB1 knockout mice	[38]
Saquinavir	–	↑ Oral bioavailability in ABCB1 knockout mice	[38]
Digoxin	–	ABCB1 contributed to direct elimination of digoxin	[50]
Talinolol	Verapamil	↑ Oral bioavailability in wild-type rats by ABCB1 inhibition	[139]
Topotecan	–	↑ Oral bioavailability in ABCG2 knockout mice	[70]
Topotecan	GF120918	↑ Oral bioavailability in ABCG2 knockout mice	[68]
Irinotecan	Gefitinib	↑ Oral bioavailability in wild-type mice by ABCG2 inhibition	[73]
PhIP	–	↑ Oral bioavailability in ABCG2 knockout mice	[77]
PhIP	–	↑ Oral bioavailability in ABCC2 deficient rats	[88]
Methotrexate	Pantoprazole	↓ Clearance in wild-type mice by ABCG2 inhibition	[71]
Clinical studies:			
Digoxin	Quinidine	↑ Oral bioavailability in humans by ABCB1 inhibition	[125]
Digoxin	Talinolol	↑ Oral bioavailability in humans by ABCB1 inhibition	[140]
Paclitaxel	Cyclosporin A	↑ Oral bioavailability in humans by ABCB1 inhibition	[127]
Paclitaxel	GF120918	↑ Oral bioavailability in humans by ABCB1 inhibition	[135]
Docetaxel	Cyclosporin A	↑ Oral bioavailability in humans by ABCB1 inhibition	[134]
Topotecan	GF120918	↑ Oral bioavailability in humans by ABCB1 inhibition	[136,137]
Methotrexate	Omeprazole/lansoprazole	↓ Clearance in humans by ABCG2 and/or other transporter inhibition	[72]
Cyclosporin A	–	Correlation between oral exposure and ABCB1 expression/inter-individual variation	[28,29]
Talinolol	–	Correlation between oral exposure and ABCB1 expression	[141,142]
Tacrolimus	–	↓ Absorption by intestinal ABCB1	[53]
Digoxin	–	↓ Absorption by intestinal ABCB1	[143]
Fexofenadine		↓ Absorption by intestinal ABCB1	[144]

Oral bioavailability indicates apparent oral bioavailability under all circumstances.

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Phase III of xenobiotic metabolism

- The human ABC transporters (ABCG2, e.g.) are regarded as a member of the **phase III system** for xenobiotic metabolism, and it has been suggested that this efflux pump is responsible for protecting the body from toxic xenobiotics and for removing metabolites.

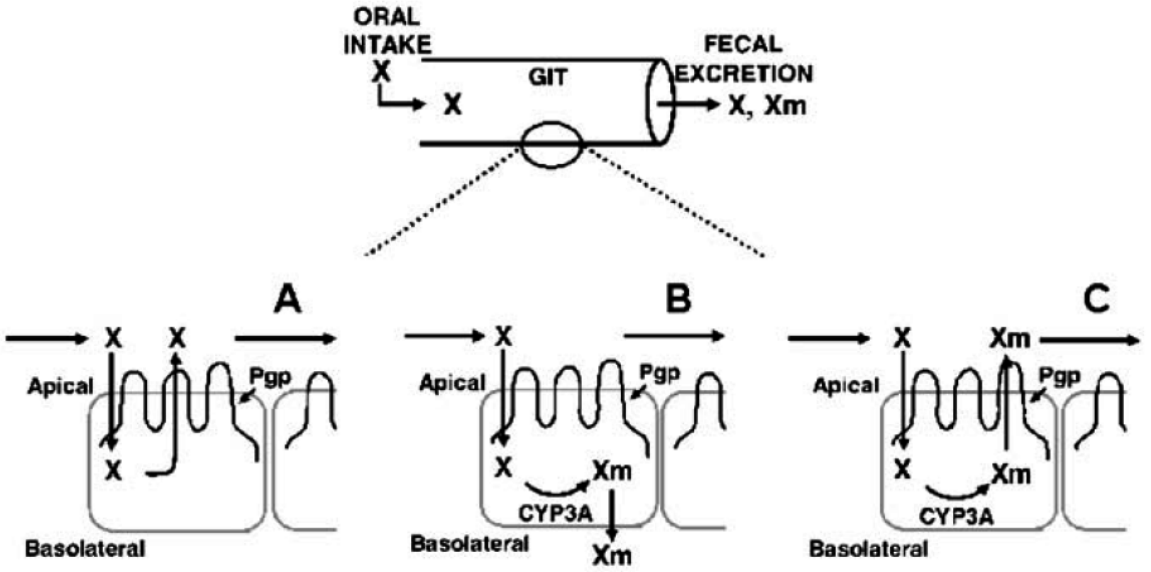
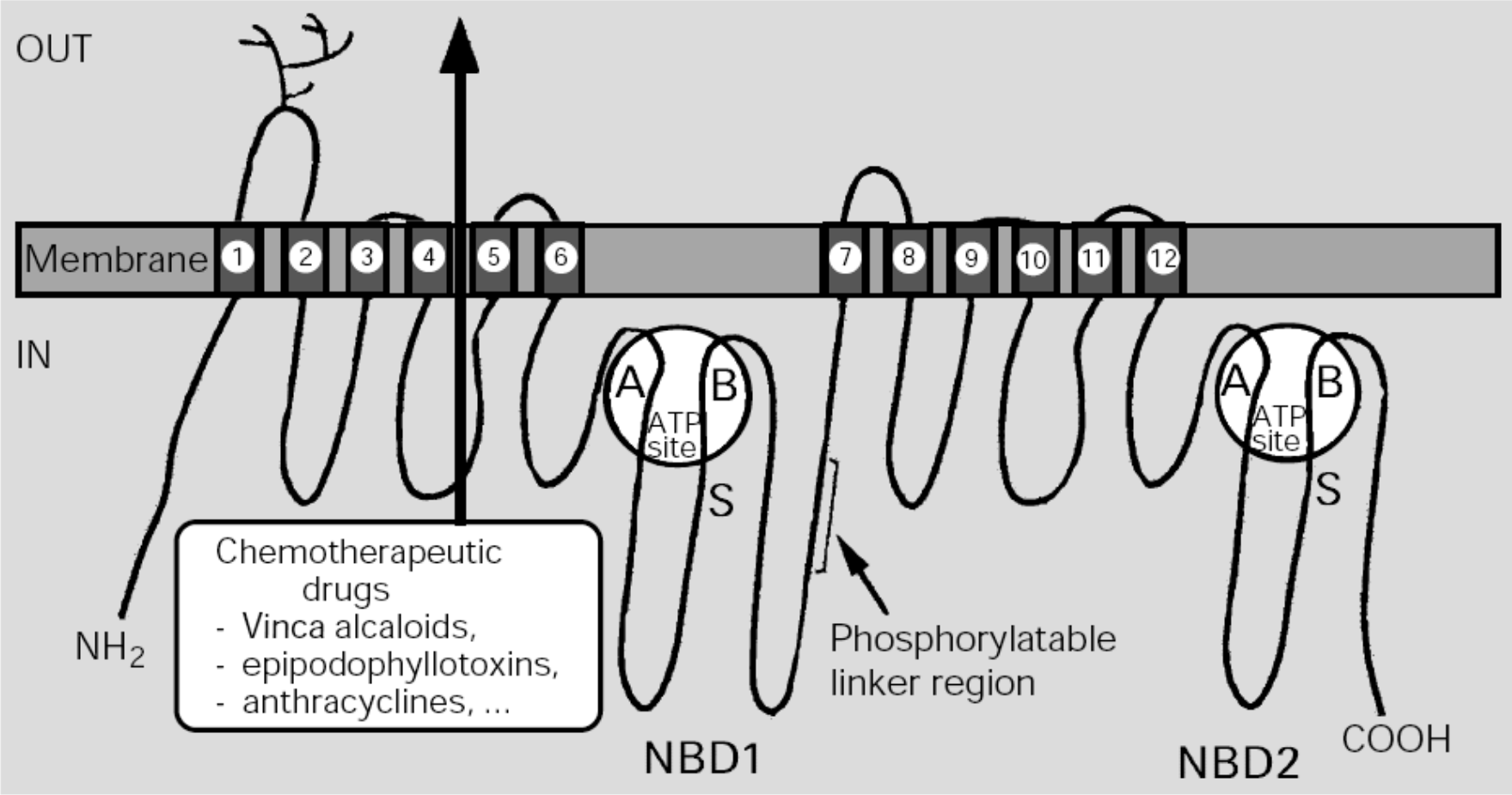


Fig. 1 Schematic representation of the functional role of P-glycoprotein (P-gp) and cytochrome P-450 3A (CYP3A) in regulating the absorption of ingested xenobiotics (X, i.e., drugs or plant secondary metabolites) in the gastrointestinal tract (GIT). P-gp and CYP3A can regulate the absorption of ingested xenobiotics (X) and detoxification metabolites (Xm) resulting in fecal excretion of these molecules via three scenarios. Ingested xenobiotics that are substrates of P-gp can be directly effluxed out of cells by P-gp (A). CYP3A metabolizes xenobiotics, which may result in absorption of the xenobiotic metabolite across the basolateral membrane (B). Finally, if the xenobiotic metabolite is a P-gp substrate, P-gp can efflux the metabolite out of cells (C)

- The diets of herbivores are laden with toxic plant secondary metabolites (PSMs)…
- We describe how efflux transporters in the gut may play a critical role in regulating the absorption of PSMs in herbivores and dictating diet selection.
- Efflux transporters may be as critical as detoxification enzymes to help herbivores to survive...

J Chem Ecol. 2006 Jun;32(6):1181-96

Structure: an overall view…



Schematic structural organization of P-glycoprotein.

Braz J Med Biol Res 32(8) 1999

Towards a tri-dimensional model…

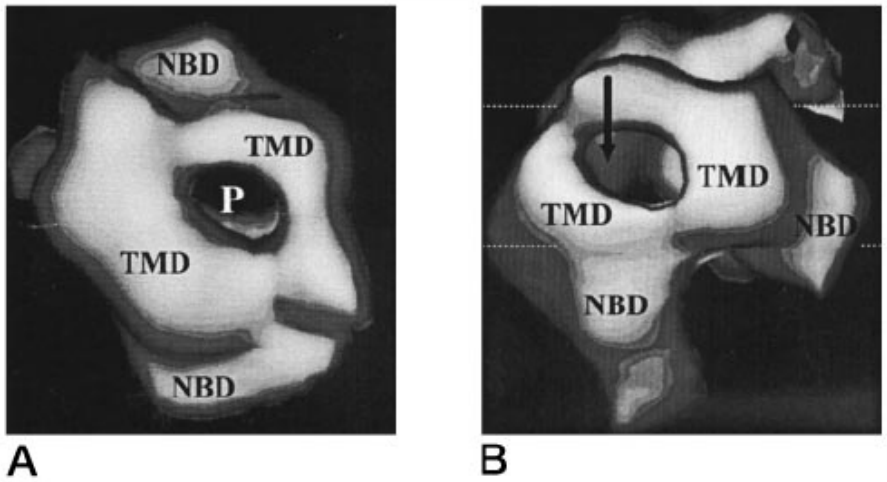


FIG. 6. **Structure of P-gp.** Projections of the three-dimensional reconstruction of P-gp from Fig. 5 are shown enlarged and annotated. *P*, aqueous pore open at the extracellular face of the membrane. *TMD*, two thumbs, each of which probably corresponds to one of the two transmembrane domains. *NBD*, 3-nm lobes projecting from the structure at the cytoplasmic face of the membrane, probably corresponding to the two nucleotide binding domains. *A*, view perpendicular to the extracellular surface of the lipid bilayer, corresponding to projection of Fig. 5*A*. The NBDs seen in this reconstruction are below the plane of the membrane and so were not visualized in two-dimensional projections of solubilized P-gp (*e.g.* Fig. 3*C*). *B*, side view of P-gp, corresponding to projection of Fig. 5*B*. The approximate position of the lipid bilayer is indicated by the two *horizontal dashed lines*. *Arrow*, asymmetric opening providing access from the lipid phase to the aqueous core of the protein. *Bar*, 1.7 nm.

Rosenberg et al. J. Biol. Chem. 1997; 272: 10685–10694

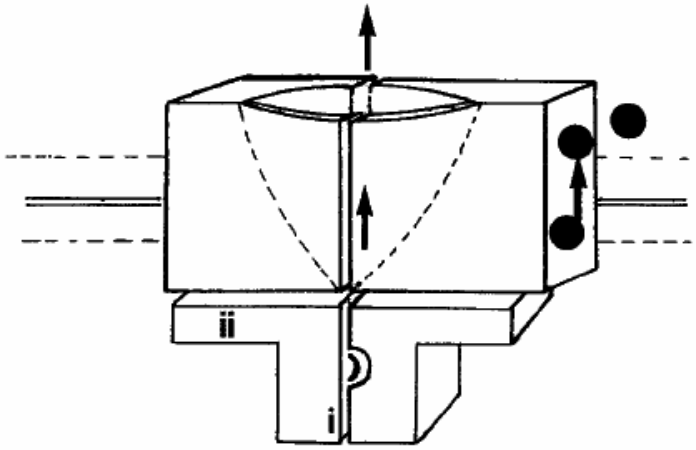


Figure 3. Representative cartoon of a ubiquitous ABC-transporter (exporter). The model is loosely based on the EM pictures by Rosenberg *et al.* (1997), FRET measurements by Sharom *et al.* (1998), and evidence discussed in the text for close interaction on the one hand between the ABC domains (presented as a dimer) and between the ABC and MD domains in the bilayer. The model emphasises at the top a water-filled chamber with walls formed by the transmembrane domains of an MD dimer. The cartoon also indicates two possible transport pathways depending upon the particular system and the type of allocrite; an aqueous pathway with access from the cytosol and a hydrophobic pathway, with lateral access to the translocator, in this case drug molecules, picked up from the inner leaflet are visualised being expelled to the exterior.

Holland & Blight, J. Mol. Biol. 1999; 293:381-399

From importers to exporters

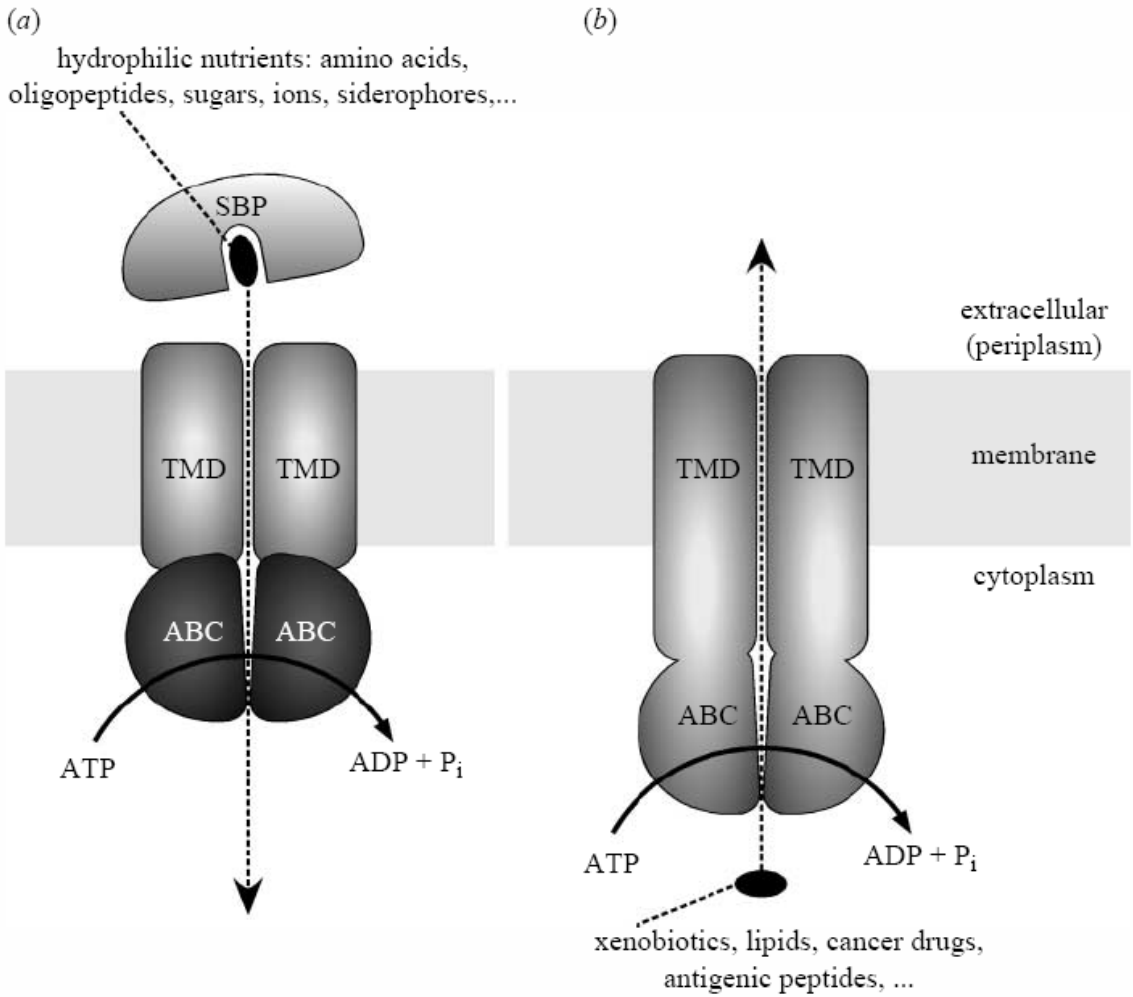


Figure 1. Schematic of ABC transporter function. (*a*) ABC importers, which require a substrate binding protein (SBP) that feeds the hydrophilic substrates into the translocation pathway formed by the TMDs. The ABCs (or NBDs) are separate subunits. (*b*) ABC exporters, which typically have their TMDs fused to the ABCs.

Locher P, Philos Trans R Soc Lond B Biol Sci. 2009; 364:239-45

Can we really prevent efflux-mediated resistance ?

Drug resistance is killing patients …

Table 1 ABC transporters involved in drug resistance.

Gene	Protein/alias	Chemotherapeutic drugs effluxed by transporter	Other drugs and substrates
ABCA2	ABCA2	Estramustine	
ABCB1	PGP/MDR1	Colchicine, doxorubicin, etoposide, vinblastine, paclitaxel	Digoxin, saquinivir,
ABCC1	MRP1	Doxorubicin, daunorubicin, vincristine, etoposide, colchicine, camptothecins, methotrexate	Rhodamine
ABCC2	MRP2	Vinblastine, cisplatin, doxorubicin, methotrexate	Sulfinpyrazone
ABCC3	MRP3	Methotrexate, etoposide	
ABCC4	MRP4	6-MP and 6-TG and metabolites, methotrexate	PMEA, cAMP, cGMP
ABCC5	MRP5	6-MP and 6-TG and metabolites	PMEA, cAMP, cGMP
ABCC6	MRP6	Etoposide	
ABCC11	MRP8	5-fluorouracil	PMEA, cAMP, cGMP
ABCG2	MXR/BCRP	Mitoxantrone, topotecan, doxorubicin, daunorubicin, CPT-11, imatinib, methotrexate	Pheophorbide A, Hoechst 33342, rhodamine

6-MP 6-mercaptopurine, *6-TG* 6-thioguanine, *PMEA* 9-[2-(phosphonomethoxy)ethyl]adenine, *cAMP* cyclic adenosine monophosphate, *cGMP* cyclic guanine monophosphate, *CPT-11* irinotecan

Dean M, J Mammary Gland Biol Neoplasia. 2009; 14:3-9

Characteristics of the ideal EPI

« to do » list for a winning molecule :

- ✓ Enhance activity of the drug under study in cells overproducing the transporter by inhibiting efflux
- ✓ Not affecting the drug activity in cells lacking efflux pumps
- ✓ Not potentiating activity or toxicity of drugs that are not effluxed
- ✓ Not affecting the physiological functions of the target transporter if looking for an host transporter

Where are we ?

- Over the past 20 years of research on ABC exporters many mechanistic and structural features of the transport cycle of ABC exporters have been discovered.
- As always, new findings raise many more new and unanswered questions.
- Snapshots of novel conformational states of ABC exporters by X-ray crystallography or other methods will be indispensible to further elucidate the molecular and mechanistic details of the transport cycle.
- One long-term goal of the research on ABCB1 and related transport systems in pathogenic microorganisms is the rational and selective inhibition of this clinically important class of proteins.
- Tremendous efforts have been made to find highly specific modulators of ABCB1 over the past 30 years, but effective drugs have proven to be too toxic for human use so far.

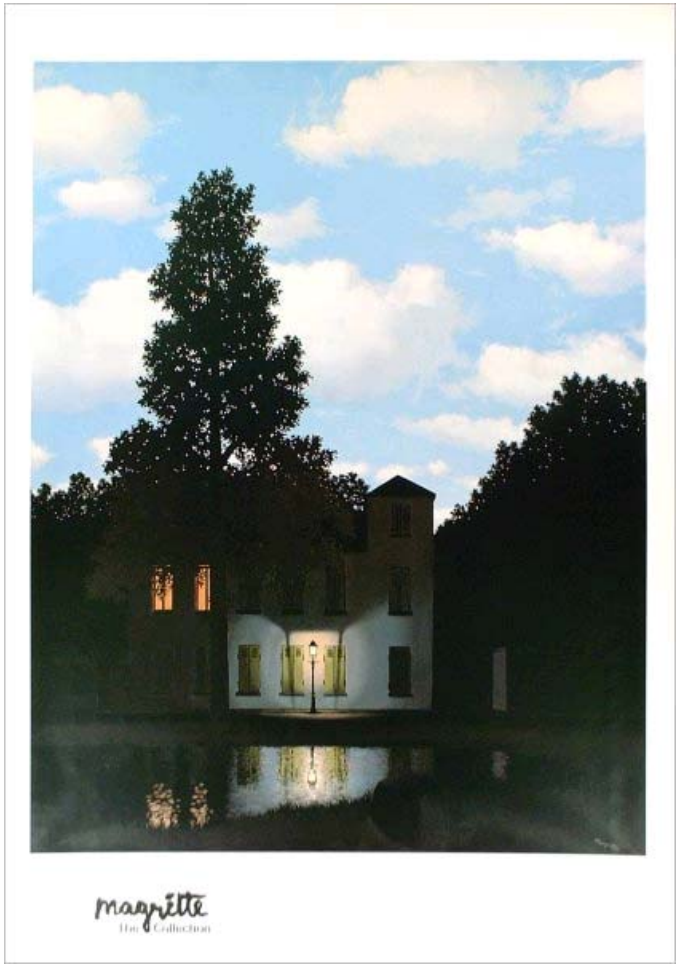
Seger & Van Veen, Biochim Biophys Acta. 2009; 1794:725-37

Potential interests of efflux pumps inhibitors for therapeutic developments ?

This ?

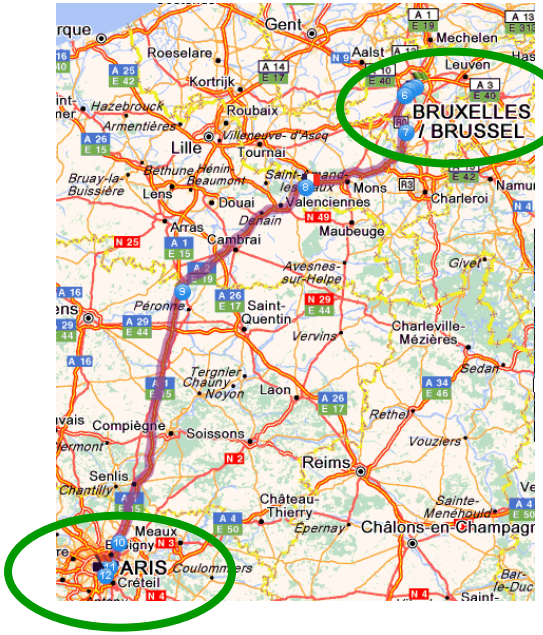
Potential interests of efflux pumps inhibitors for therapeutic developments ?

A still uncertain future for EPI ….



Still a lot of work ahead ….

And it may be tougher than we thought …



Unless we have some good ideas…