Pumps (almost) everywhere:

Impact on resistance and pharmacokinetics

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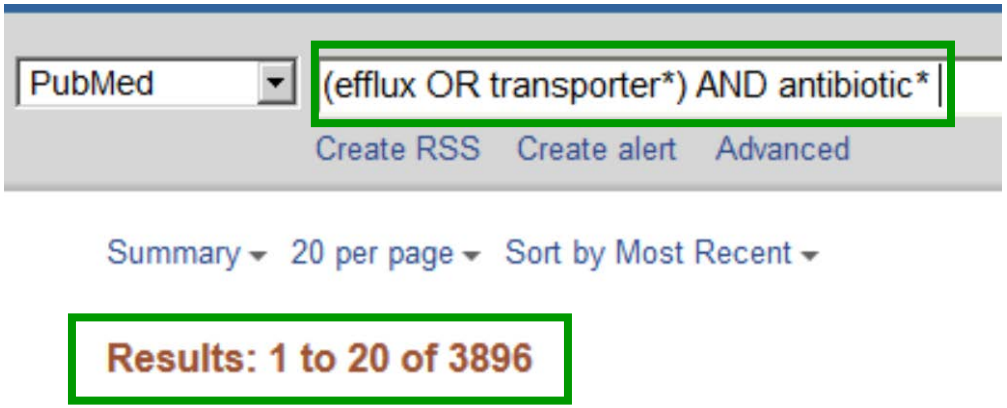
"Médecine de Soins Intensifs"

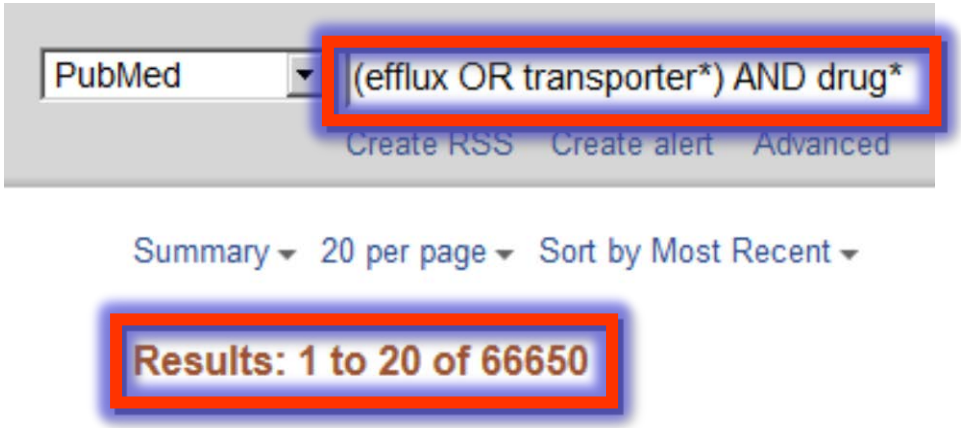
Coordonné par les Cliniques universitaires St Luc

Bruxelles

www.facm.ucl.ac.be

When preparing this lecture … A large choice …





The Menu …

- Why active efflux … or the origins of the discovery
- A survey of transporters in prokaryotic cells
  - *RND: the nightmare for the microbiologist…*
- From bacteria to eukaryotic cells
  - *ABC (and some of the others)*
    - bioavailability, intestine, blood-brain-barrier, kidney
    - cancer …
- Why we still fail …

Why 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

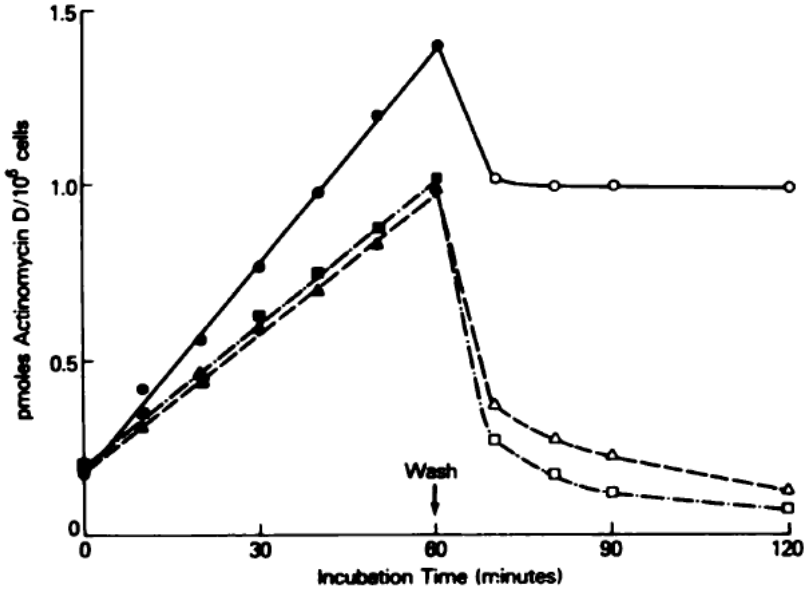
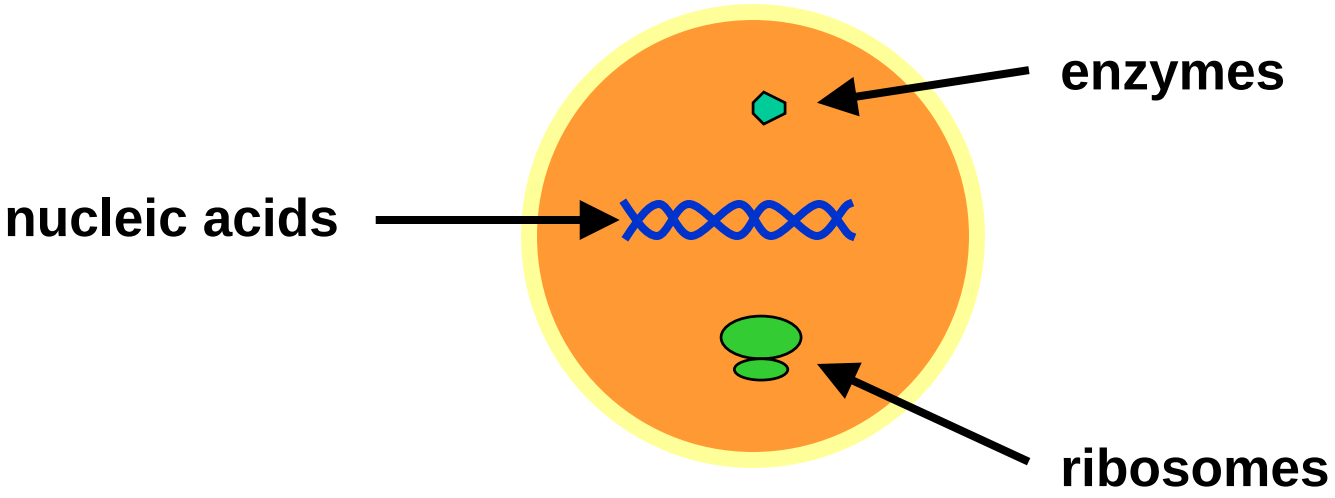


Chart 2. Time course of uptake and efflux of actinomycin D by P388/S (○, ●), P388/ADR (△, ▲) and P388/DAU (□, ■) cells. Cells were incubated in the presence of actinomycin D, 0.04 μg/ml, for 60 min, washed, and reincubated in drug-free medium for an additional 60 min. Each *point* represents the mean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

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

In order to survive to anticancer agents, eukaryotic cells "invented" efflux…

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

In order to survive to antibiotics, bacteria "invented" efflux…

Historical observations …

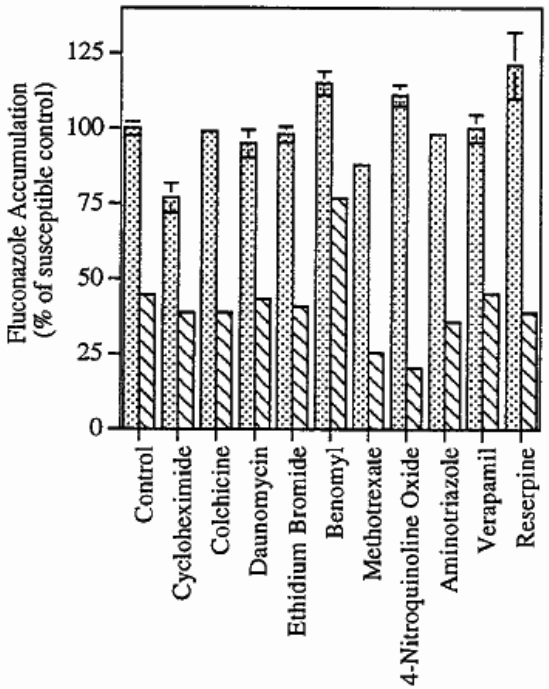


FIG. 3. Effects of MDR protein substrates or inhibitors on [^{3}H]fluconazole uptake by cells from fluconazole-susceptible (▨) and fluconazole-resistant (▧) cultures of *C. glabrata* after 80 min of incubation in the standard uptake assay; the assay was extended to 180 min for verapamil. Values are means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

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

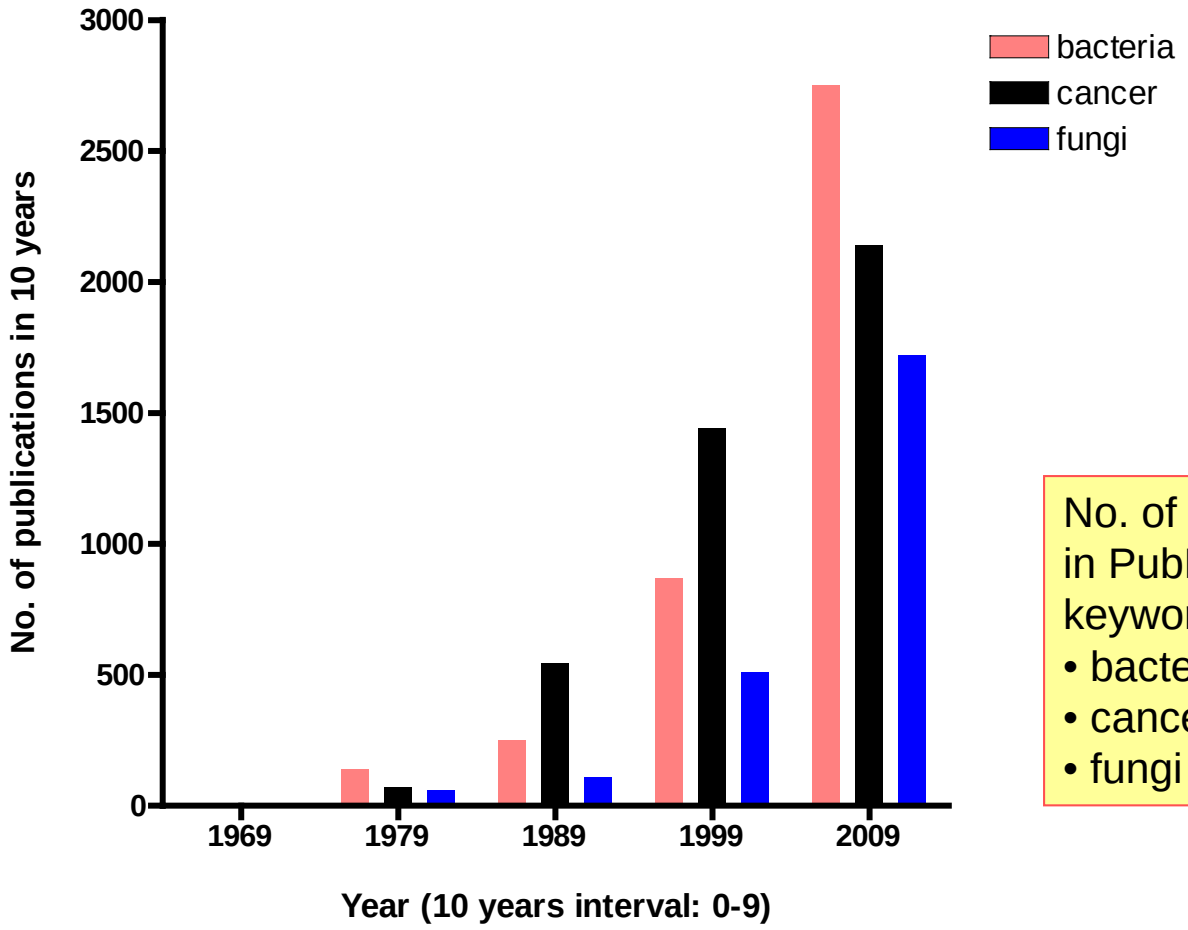
6. **De Waard, M. A., and J. G. M. Van Nistelrooy.** 1980. An energy-dependent efflux mechanism for fenarimol in a wild-type strain and fenarimol-resistant mutants of *Aspergillus nidulans*. Pestic. Biochem. Physiol. **13**:255–266.

antibiotics
1965 1980

antifungal drugs
1980 1995

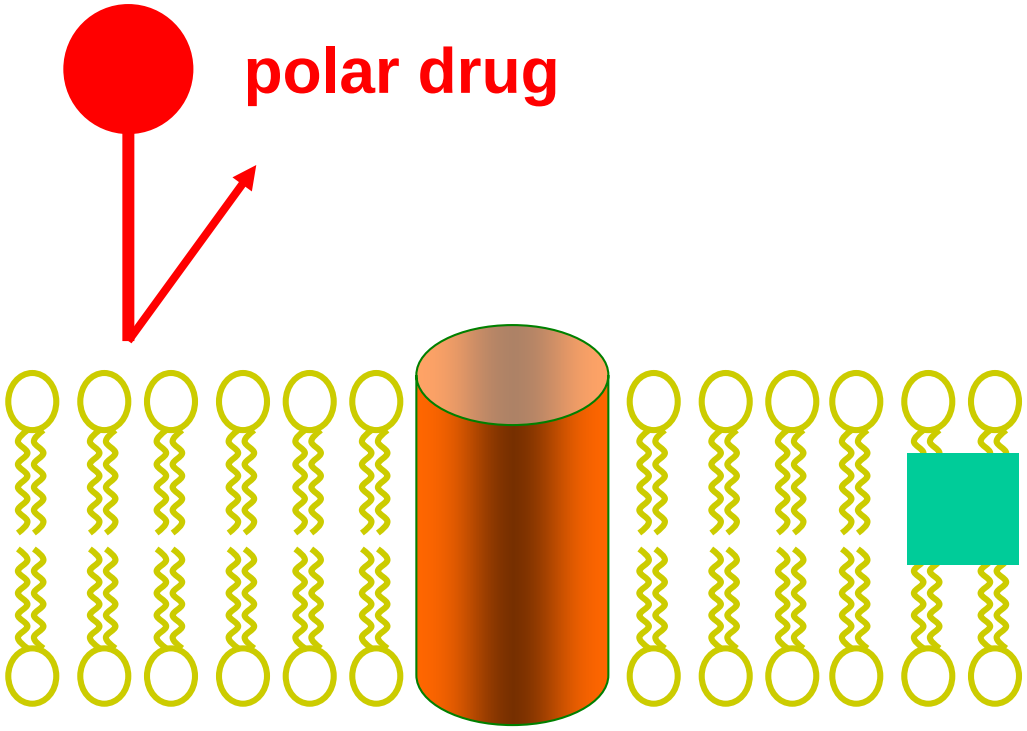
1977
anticancer drugs

Historical trends …



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

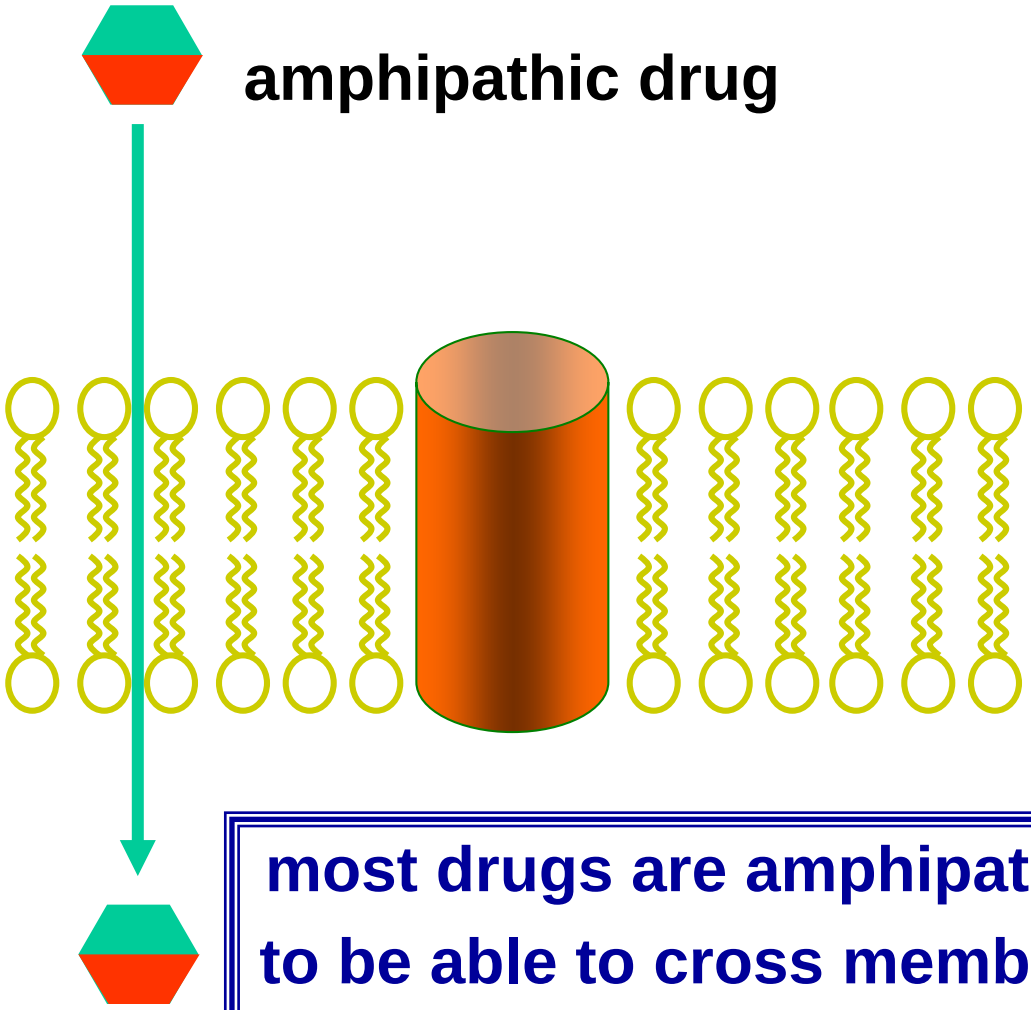
Reaching an intracellular target …



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

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

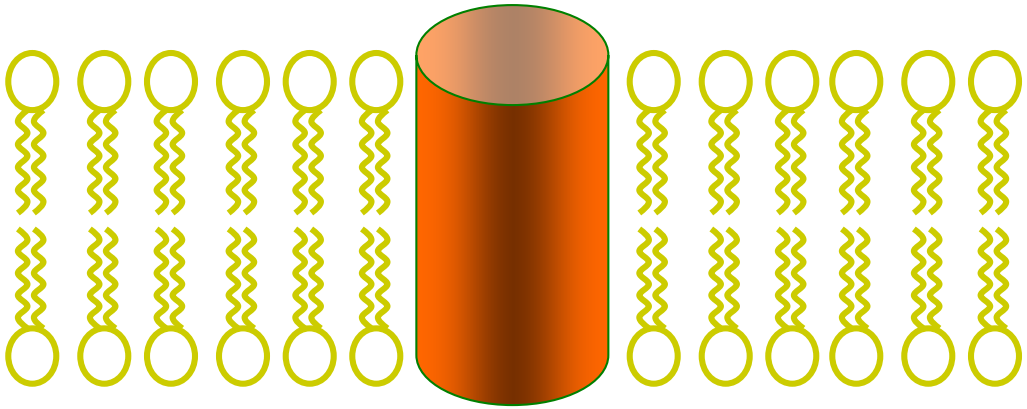
Reaching an intracellular target …



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

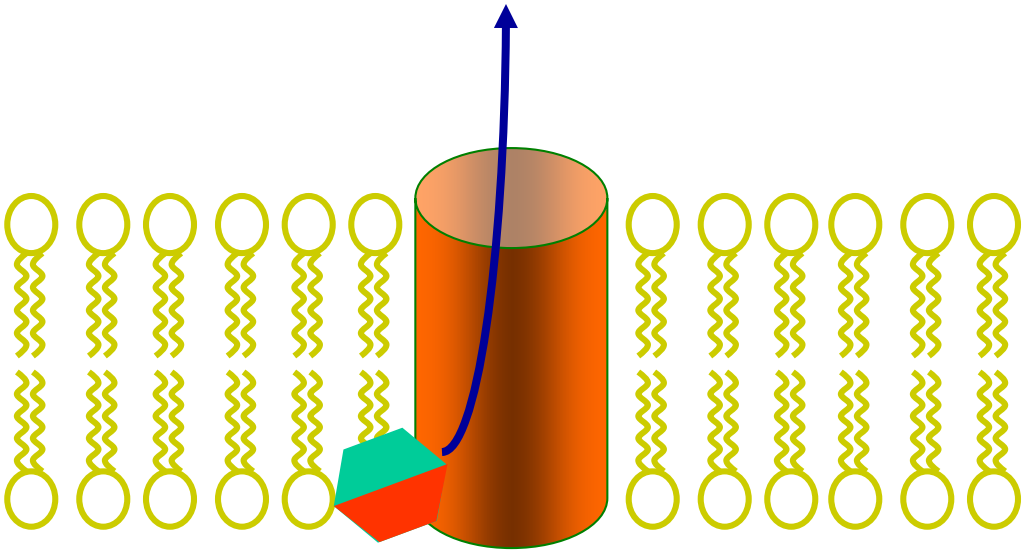
may have

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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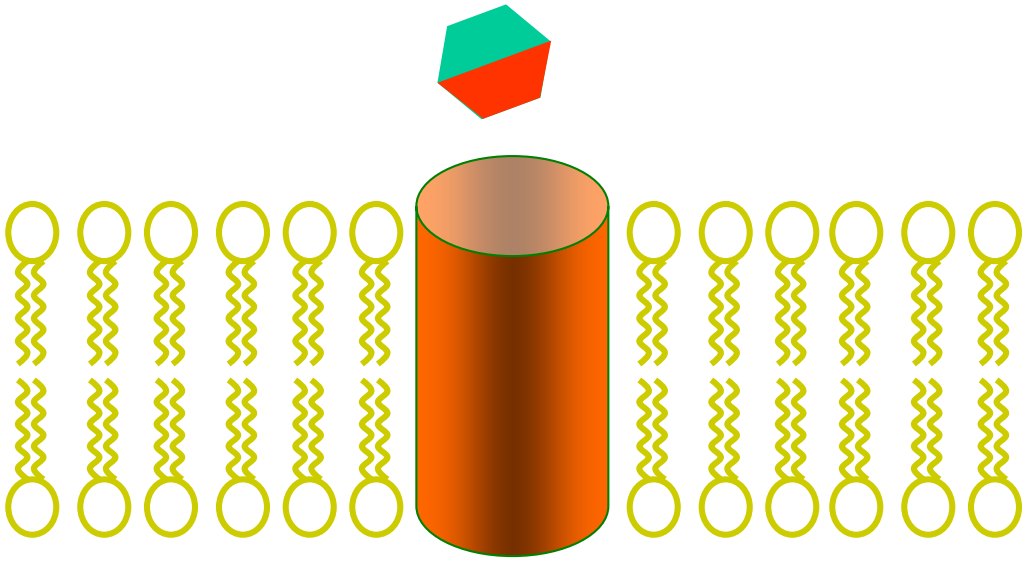
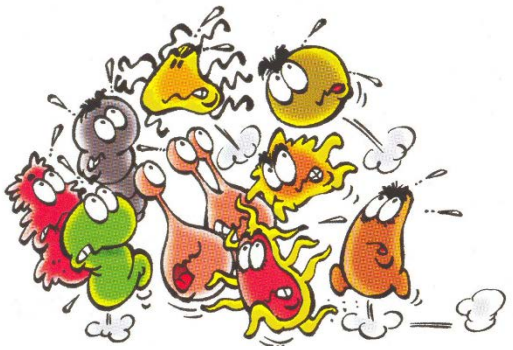


general mean of protection
against cell invasion by diffusible molecules

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

Typical ‘toxic’ diffusible substances as substrates for efflux pumps

antibiotics



antifungals

anticancer agents

Today, there are even people believing that ALL drugs get in and out of cells by transporters…

frontiers in
PHARMACOLOGY

REVIEW ARTICLE
published: 31 October 2014
doi: 10.3389/fphar.2014.00231

How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion

***Douglas B. Kell*[1,2]* *and Stephen G. Oliver*[3,4]**

[1] *School of Chemistry, The University of Manchester, Manchester, UK*
[2] *Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK*
[3] *Department of Biochemistry, University of Cambridge, Cambridge, UK*
[4] *Cambridge Systems Biology Centre, University of Cambridge, Cambridge, UK*

Today, there are even pec
drugs get in and out of c

frontiers in
PHARMACOLOGY

How drugs get into cells: tested and testable p
help discriminate between transporter-mediat
and lipoidal bilayer diffusion

Douglas B. Kell[1,2]* and Stephen G. Oliver[3,4]

[1] *School of Chemistry, The University of Manchester, Manchester, UK*
[2] *Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK*
[3] *Department of Biochemistry, University of Cambridge, Cambridge, UK*
[4] *Cambridge Systems Biology Centre, University of Cambridge, Cambridge, UK*

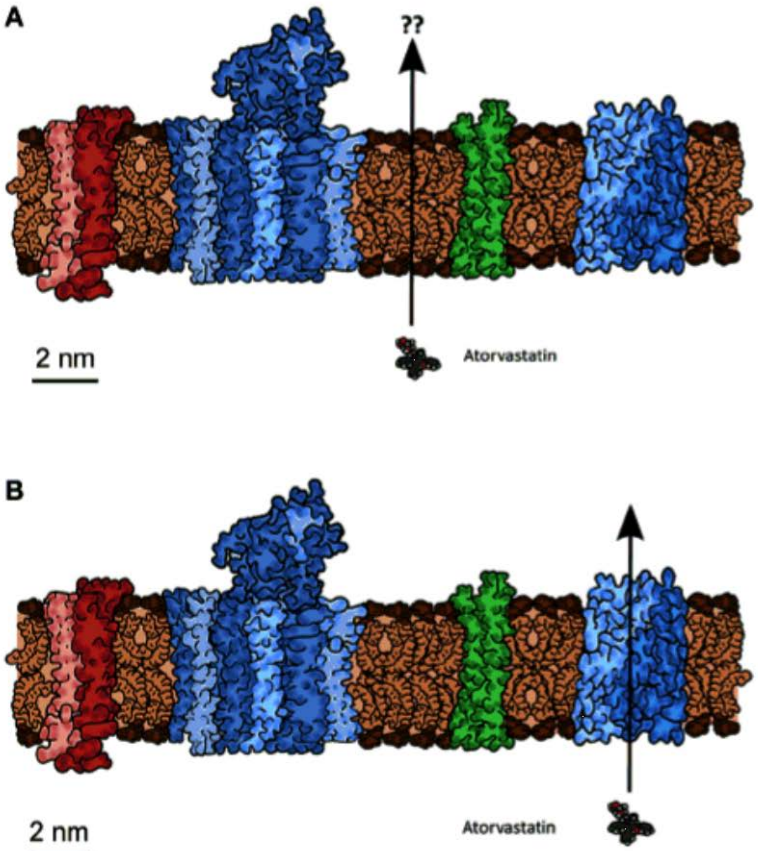


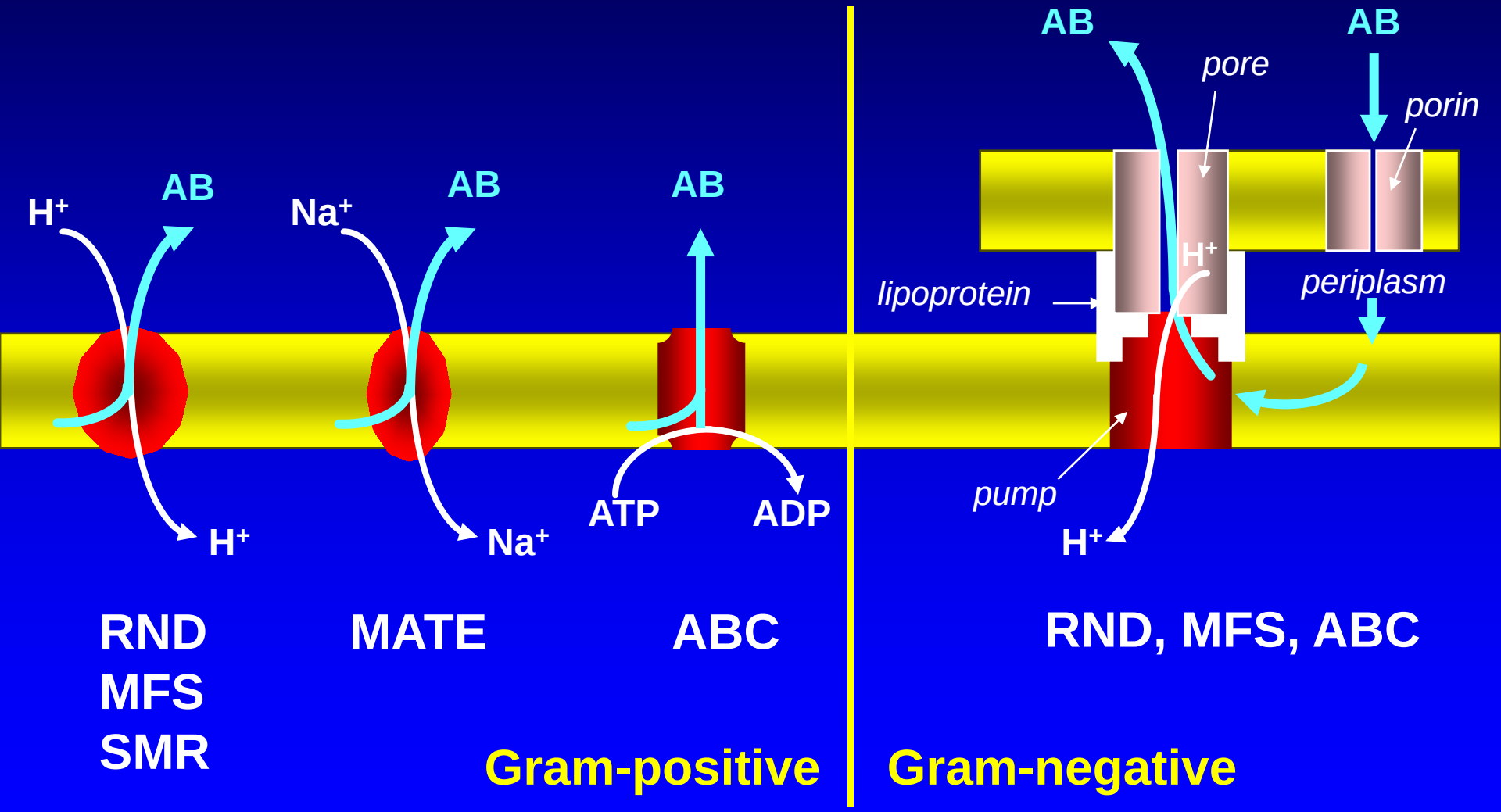
FIGURE 5 | A typical biomembrane drawn roughly to scale, indicating the typical protein:lipid mass ratio and the possible means by which a small molecule drug (atorvastatin, also drawn to scale) might cross, including (A) bilayer lipoidal diffusion through bilayer areas nominally unaffected by the presence of proteins, or (B) by hitchhiking a lift on transporters (Schlessinger et al., 2013b) normally present for the purposes of intermediary metabolism. At issue is the question of whether there is any untrammeled bilayer that might let the atorvastatin leak across, and whether biophysical properties such as log D or log P can account for this. Typically atorvastatin is in fact transported by a bile acid transporter known as OATP1B1 (SLCO or SLC21 family) (e.g., Hagenbuch and Stieger, 2013; Higgins et al., 2014).

The Menu …

- Why active efflux … or the origins of the discovery
- **A survey of transporters in prokaryotic cells**
  - *RND: the nightmare for the microbiologist…*
- From bacteria to eukaryotic cells
  - ABC (and some of the others)
    - bioavailability, intestine, blood-brain-barrier, kidney
    - cancer …
- Why we still fail …

Structure of efflux pumps in procaryotic cells

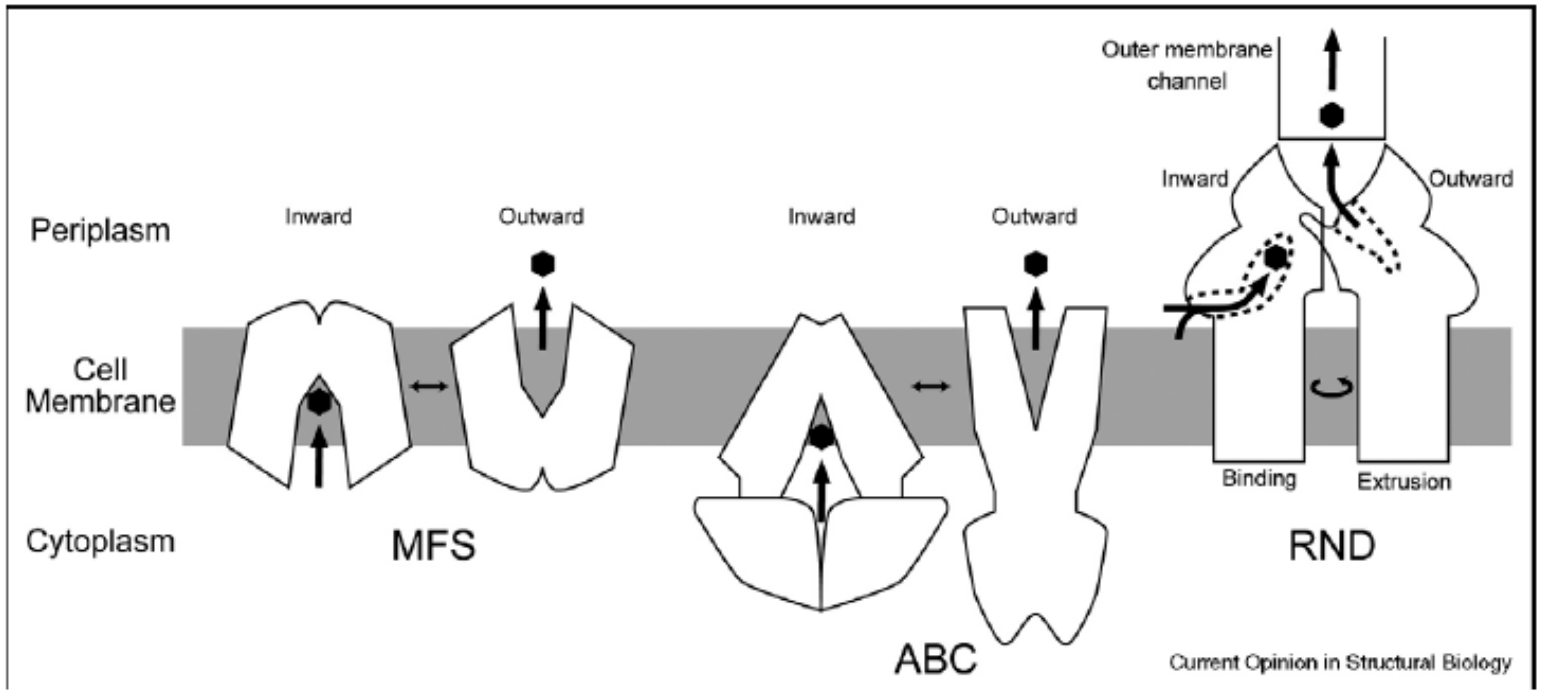
Van Bambeke et *al*. JAC (2003) 51: 1055-1065



Some abbreviations

- **ABC**: **A**TP **B**inding **C**assette
- **MATE**: **M**ulti **A**n**T**imicrobial **E**xtrusion
- **MFS**: **M**ajor **F**acilitator **S**uperfamily
- **RND**: **R**esistance **N**odulation **D**ivision
- **SMR**: **S**mall **M**ultidrug **R**esistance

Mechanisms of transport in bacteria



Alternating access mechanism of transporter families. (*from left to right*) Schematic illustrations of MFS, ABC and RND transporter families. In the case of the RND, only two monomers ('Binding' and 'Extrusion') in the trimer are depicted. In each transporter, the inward-facing and outward-facing conformations are illustrated left and right, respectively.

Murakami S., Current Opinion in Structural Biology 2008, 18:459–465

A brief survey of the many (bacterial) transporters (2003)

Journal of Antimicrobial Chemotherapy (2003) **51**, 1055–1065
DOI: 10.1093/jac/dkg224
Advance Access publication 14 April 2003

JAC

Leading articles

Antibiotic efflux pumps in prokaryotic cells: occurrence, impact on resistance and strategies for the future of antimicrobial therapy

F. Van Bambeke[1]*, Y. Glupczynski[2], P. Plésiat[3], J. C. Pechère[4] and P. M. Tulkens[1]

[1]Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Brussels; [2]Laboratoire de Microbiologie, Cliniques Universitaires de Mont-Godinne, Université Catholique de Louvain, Yvoir, Belgium; [3]Laboratoire de Bactériologie, Centre Hospitalier Universitaire Jean Minjoz, Besançon, France; [4]Département de Microbiologie, Université de Genève, Geneva, Switzerland

Keywords: antibiotic, efflux, transporters, prokaryotes, resistance

A brief survey of the many transporters (2003)

1. Gram +

Table 1. Main efflux transporters as observed in clinically important human pathogens with their corresponding antibiotic substrates[a]

				Antibiotics																	
				β-lactams														Q			
Pathogen	Transporter	Super-family	TC number[b]	peni	ceph	carb	m-bac	inhib β-ase	FA	AG	Tet	OX	ML	SG	LM	CHL	RIF	NAL	FQ	SM	TMP
S. aureus	NorA[7]	MFS	2.A.1.2.10													+[58]			+[58]		
	TetK-L[59]	MFS	2.A.1.3.6								+[31]										
	MdeA[60]	MFS								+[60]											
	MsrA[6]	ABC	3.A.1.121.1										+[6]								
S. pneumoniae	MefE[61]	MFS											+[61]								
	PmrA[62]	MFS																	+[62]		
	TetK-L	MFS									+[31]										
Streptococcus pyogenes	MefA[63]	MFS	2.A.1.21.2										+[63]		+[63]						
L. monocytogenes	MdrL[23]	MFS		–[23]	+[23]					–[23]	–[23]		+[23]		+[23]						
	Lde[64]	MFS																	+[64]		
	TetK-L	MFS									+[31]										
Mycobacterium tuberculosis	Mmr[65]	SMR	2.A.7.1.2.										+[65]								
	TetK-L	MFS									+[31]										
	DrrB[66]	ABC	3.A.1.105.1																+[66]		
Enterococcus spp.	Mef?[67]	MFS											+[67]								
	TetK-L	MFS									+[31]										
	EmeA[68]	MFS											+[68]		+[68]				+[68]		
	Lsa[69]	ABC												+[69]	+[69]						

A brief survey of the many transporters (2003)

2. Gram - (part #1)

Table 1. Main efflux transporters as observed in clinically important human pathogens with their corresponding antibiotic substrates[a]

				Antibiotics																		
				β-lactams															Q			
Pathogen	Transporter	Super-family	TC number[b]	peni	ceph	carb	m-bac	inhib β-ase	FA	AG	Tet	OX	ML	SG	LM	CHL	RIF	NAL	FQ	SM	TMP
H. influenzae	TetB, K	MFS									+[31]										
	AcrB-like	RND											+[70]				+[70]				
Neisseria gonorrhoeae	MtrD[71]	RND	2.A.6.2.5	+[72]					+[72]		+[72]		+[72]			+[72]	+[72]				
Salmonella spp.	AcrB[73]	RND		+[74]	+[74]				+[74]		+[74]		+[74]			+[74]	+[74]	+[74]	+[74]		
	TetA-D	MFS									+[31]										
	FloR[75]	MFS														+[75]					
Shigella dysenteriae	TetA-D	MFS									+[31]										
E. coli	EmrE[76]	SMR	2.A.7.1.3								+[77]		+[77]							+[77]	
	YdhE[78]	MATE	2.A.66.1.3													+[79]			+[79]		+[79]
	TetA-E[80]	MFS	2.A.1.2.4								+[31]										
	Bcr[81]	MFS	2.A.1.2.7								+[79]									+[82]	
	MdfA[83]	MFS	2.A.1.2.19							+[79,83]	+[83]		+[83]			+[79,83]	+[83]		+[79,83]		+[79]
	YceL[84]	MFS	2.A.1.2.21																+[79]		
	YidY[84]	MFS	2.A.1.2.22													+[79]					
	EmrB[85]	MFS	2.A.1.3.2								–[85]					–[85]		+[85]	–[85]		
	YebQ[84]	MFS	2.A.1.3.17																		+[79]
	SetA[86]	MFS	2.A.1.20.1							+[87]											
	Fsr[88]	MFS	2.A.1.35.1																		+[79]
	AcrB[89]	RND	2.A.6.2.2	+[20,90]					+[72]		+[72,90]	+[91]	+[72]			+[72,90]	+[72,90]	+[90]	+[72]		+[79]
	AcrD[84]	RND	2.A.6.2.7							+[79,92]											
	AcrF[89]	RND		+[90]					+[90]		+[90]		+[90]				+[90]				
	YegN	RND	2.A.6.2.12															+[79]	+[79]		
	YhiV	RND	2.A.6.2.13										+[79]								
	MacB[93]	ABC	3.A.1.122.1										+[93]								

A brief survey of the many transporters (2003)

2. Gram - (part #2)

Table 1. Main efflux transporters as observed in clinically important human pathogens with their corresponding antibiotic substrates[a]

				Antibiotics																	
				β-lactams													Q				
Pathogen	Transporter	Super-family	TC number[b]	peni	ceph	carb	m-bac	inhib β-ase	FA	AG	Tet	OX	ML	SG	LM	CHL	RIF	NAL	FQ	SM	TMP
Stenotrophomonas maltophilia	SmeE[94]	RND								+[95]	+[95]		+[95]			+[95]			+[95]		
P. aeruginosa	CmlA[96]	MFS	2.A.1.2.3													+[96]					
	TetA,C,E	MFS									+[31]										
	MexB[97]	RND	2.A.6.2.6	+[98,99]	+[99]	+[98]	+[99]	+[100]	+[72]		+[72]		+[72]		+[101]	+[72]	+[72]		+[72]	+[72]	+[72]
	MexD[102]	RND		+[101]	+[72]	+[101]					+[72]		+[101]		+[101]	+[72]			+[72]		+[72]
	MexF[103]	RND				–[104]	–[104]	+[100]								+[72,104]			+[72,104]		+[72,104]
	MexK[105]	RND									+[105]		+[105]								
	MexY[106]	RND		+[101]	+[101]	+[101]				+[101]	+[101]		+[101]		+[101]	+[101]			+[101]		

ABC, ATP binding cassette superfamily; MATE, multi-antimicrobial extrusion; MFS, major facilitator superfamily; RND, resistance nodulation division; SMR, small multidrug resistance; peni, penicillins; ceph, cephalosporins; carb, carbapenems; m-bac, monobactams, inhib β-ase, inhibitors of β-lactamases; FA, fusidic acid; AG, aminoglycosides; Tet, tetracyclines; OX, oxazolidinones; ML, macrolides; SG, synergistins, LM, lincosamides; CHL, chloramphenicol; RIF, rifampicin; Q, quinolones; NAL, nalidixic acid; FQ, fluoroquinolones; SM, sulfamides; TMP, trimethoprim.

[a] +, occurrence; –, absence (in both cases, through functional studies).

[b] According to the classification of Saier.[2]

A brief survey of the many transporters (2009)

Published in final edited form as:
Drugs. 2009 August 20; 69(12): 1555–1623. doi:10.2165/11317030-000000000-00000.

Efflux-Mediated Drug Resistance in Bacteria: an Update

Xian-Zhi Li[1] and **Hiroshi Nikaido**[2]

[1] Human Safety Division, Veterinary Drugs Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario K1A OK9, Canada

[2] Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3202, USA

809 references

A brief survey of the many transporters: *S. aureus*

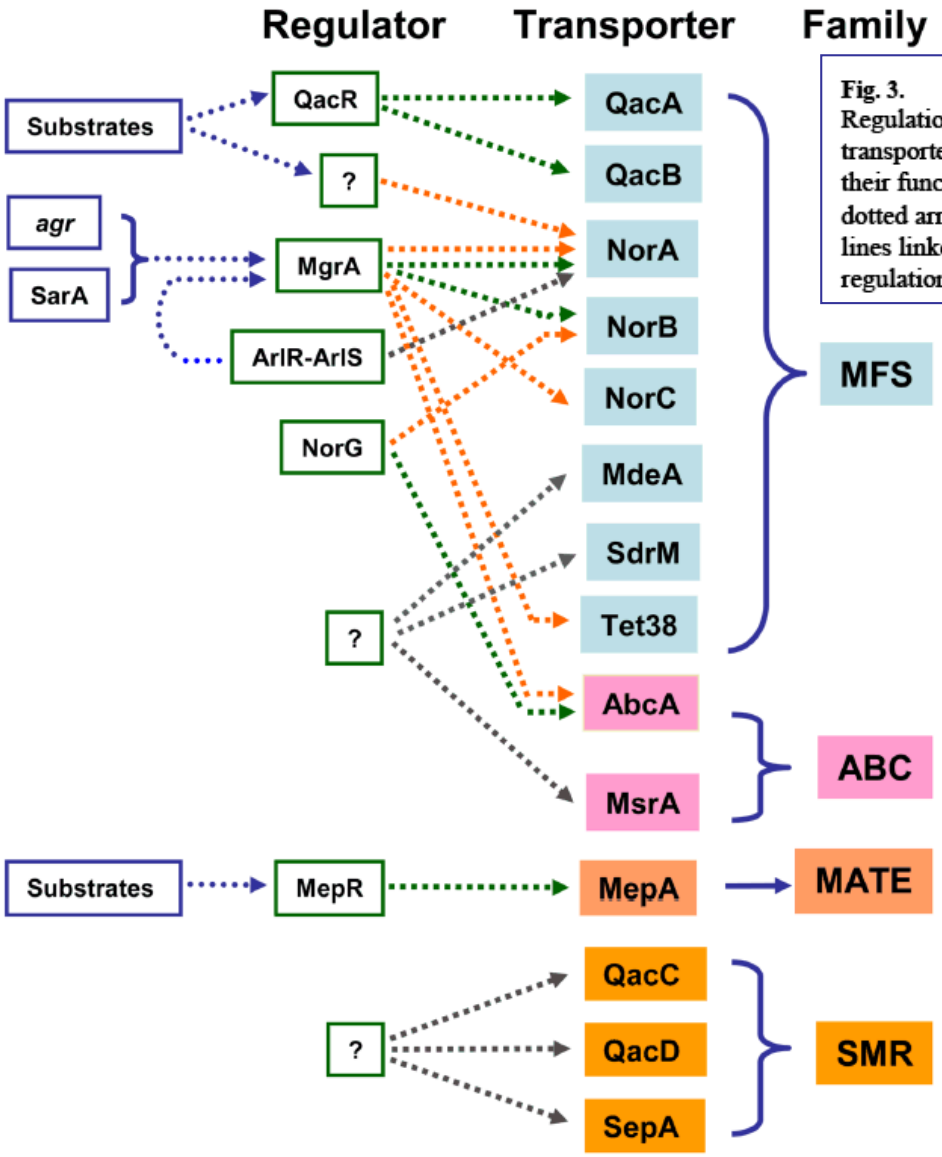


Fig. 3.
Regulation of multidrug or drug-specific efflux transporters of *S. aureus*. The efflux transporters are shown in colour blocks. All regulators are presented in the green boxes, and their functions as repressor or activator are indicated, respectively, by the green or orange dotted arrows. Unknown regulators are marked with a question mark (?) with the dotted grey lines linked to the relevant transporters. See text and relevant references for details of the regulation.

14 distinct transporters for S. aureus (only) in 2009 *vs.* 4 in 2003

Efflux and resistance in pathogenic bacteria

1 bacteria → several pumps → multiresistance

1 pump → several classes of antibiotics → crossresistance

1 class of antibiotics → several pumps → efficacy of inhibitors ?

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- From bacteria to eukaryotic cells
  - ABC (and some of the others)
    - bioavaibility, intestine, blood-brain-barrier
    - cancer …
- Why we still fail …

General structure of an RND (AcrAB-TolC in *Enterobacteriacae*)

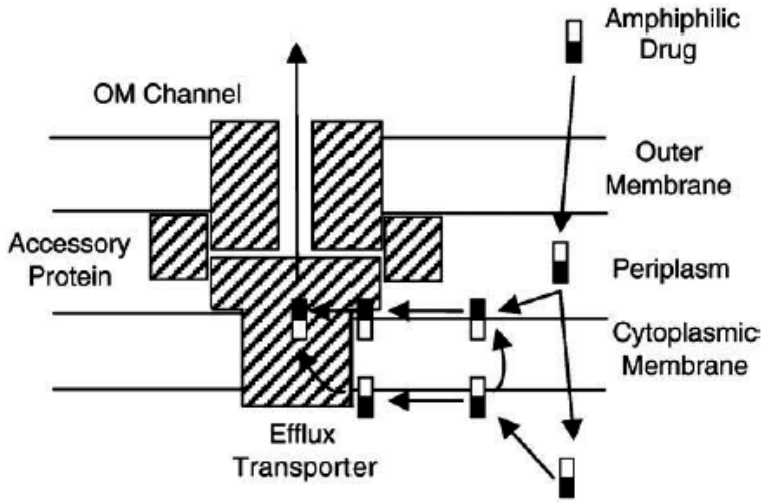
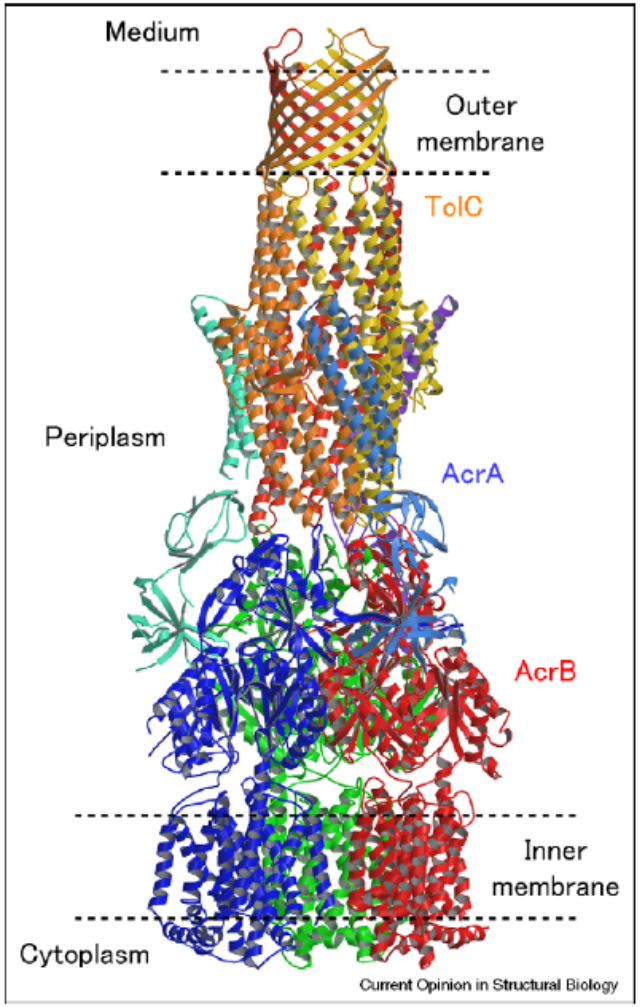


Fig. 1. An early schematic view of the tripartite pump complex. Note that amphiphilic substrates (empty and filled-in rectangles represent hydrophobic and hydrophilic parts of the molecule) are hypothesized to be captured either from the periplasm (or the periplasm–plasma membrane interface) or from the cytosol (or the cytosol–membrane interface). For the latter process, two possible pathways are envisaged: either the substrate is flipped over to the outer surface of the membrane first and then follows the regular periplasmic capture pathway, or it follows a different capture pathway from the cytosol. From [5].

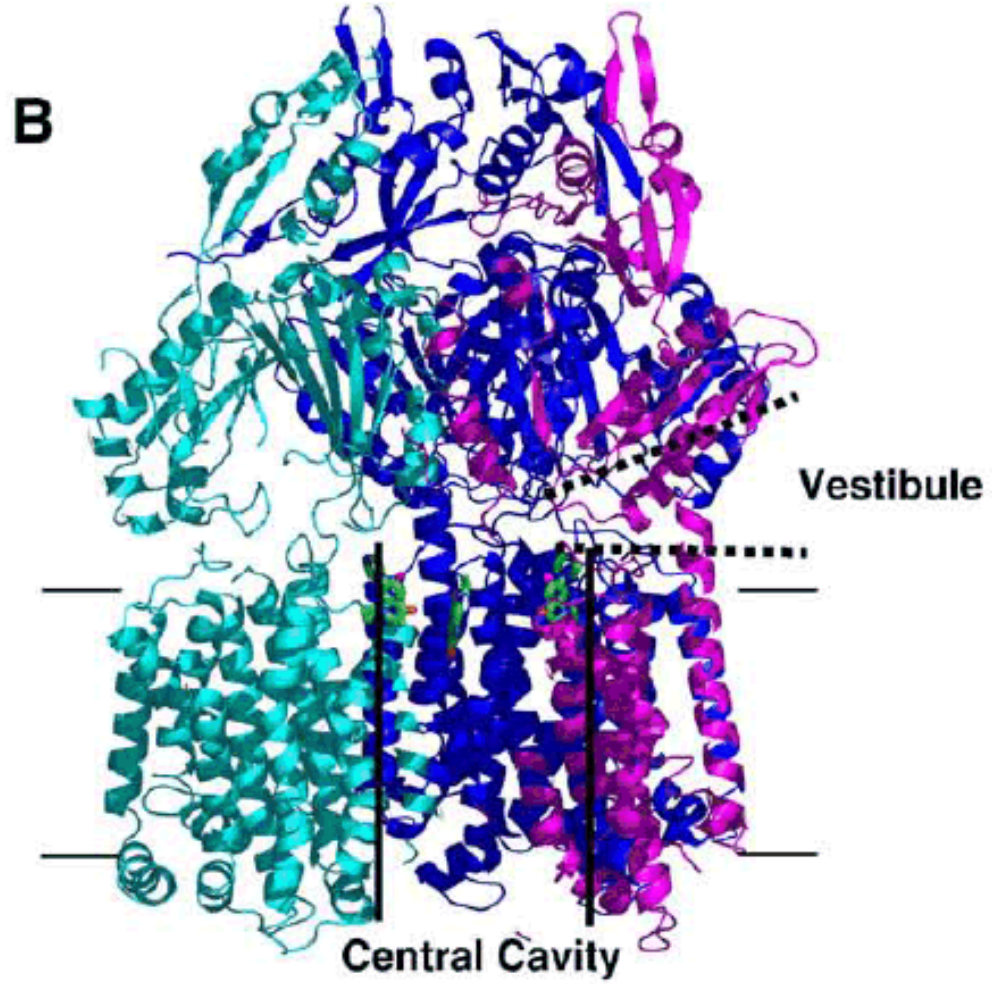
Nikaido & Takatsuka, Biochimica et Biophysica Acta 1794 (2009) 769–781



Proposed model of the AcrA–AcrB–TolC complex. Structures of AcrA [14] and TolC [12] are manually docked to AcrB with inspection according to engineered cysteine cross-linking study between AcrB–TolC [9] and AcrA–TolC [50].

Murakami S., Current Opinion in Structural Biology 2008, 18:459–465

AcrB in more details



(B) AcrB trimer. Each protomer is shown in cyan, mauve, and blue. The large central cavity (thick black lines) is connected to the periplasm through vestibules (thick dotted lines) between protomers. Substrate molecules (ciprofloxacin) bound to the ceiling of the central cavity are shown in green stick models. Proximal portion of the structure was cut away to reveal the presence of vestibule. Drawn by using PyMol with Protein Data Bank coordinate 1OYE.

Nikaido & Takatsuka, Biochimica et Biophysica Acta 1794 (2009) 769–781

Proposed AcrB drug / H^+ exchange

Pos K. Biochimica et Biophysica Acta 1794 (2009) 782–793
Seeger et al. Curr. Drug Targets 9 (2008) 729–749.

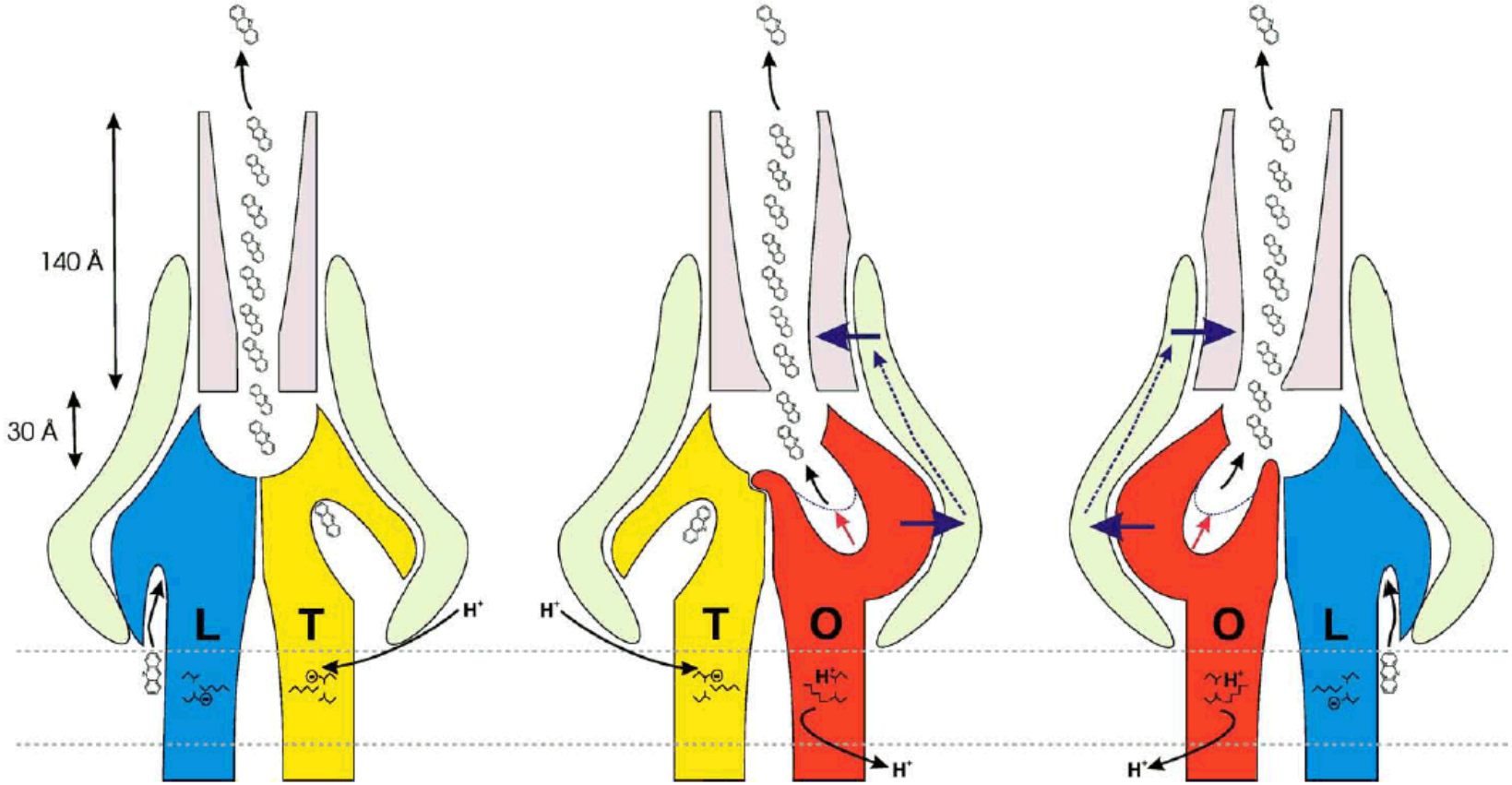


Fig. 10. Schematic representation of the AcrB alternating site functional rotation transport mechanism. The conformational states loose (L), tight (T), and open (O) are colored blue, yellow and red, respectively. Only two of the three monomers of the AcrB trimer are shown in side-view. AcrA and TolC are indicated in light green and grey, respectively. The proposed proton translocation site (D407, D408, and K940) is indicated in the membrane part of each monomer. In the first state of the cycle (from left to right), a monomer binds a substrate (acridine) in its transmembrane domain (L conformation), subsequently transports the substrate from the transmembrane domain to the hydrophobic binding pocket (conversion to T conformation) and finally releases the substrate in the funnel toward TolC (O conformation). Peristaltic transport of drugs through the AcrB tunnels (indicated by the red arrow) and through TolC in combination to the line up of drug molecules inside the AcrB funnel and the TolC channel would account for a strict unidirectional movement towards the outside of the cell. The conversion from the T monomer to the O monomer conformation is suggested to be the major energy-requiring (proton motive force-dependent) step in this functional rotation cycle and requires the binding of a proton to the proton translocation site (D407, D408, and K940) from the periplasm. The release of a proton from the proton translocation site to the cytoplasm might occur during conversion from the O monomer to the L monomer (as depicted) or from the latter to the T monomer. AcrA is expected to participate in the transduction of the conformational changes from AcrB to TolC (indicated by black arrows), which results in the movement of the proximal part of TolC and the facilitation of drug extrusion to the outside of the cell. From Seeger et al. [11] with permission.

AcrAB-TolC is a multidrug transporter

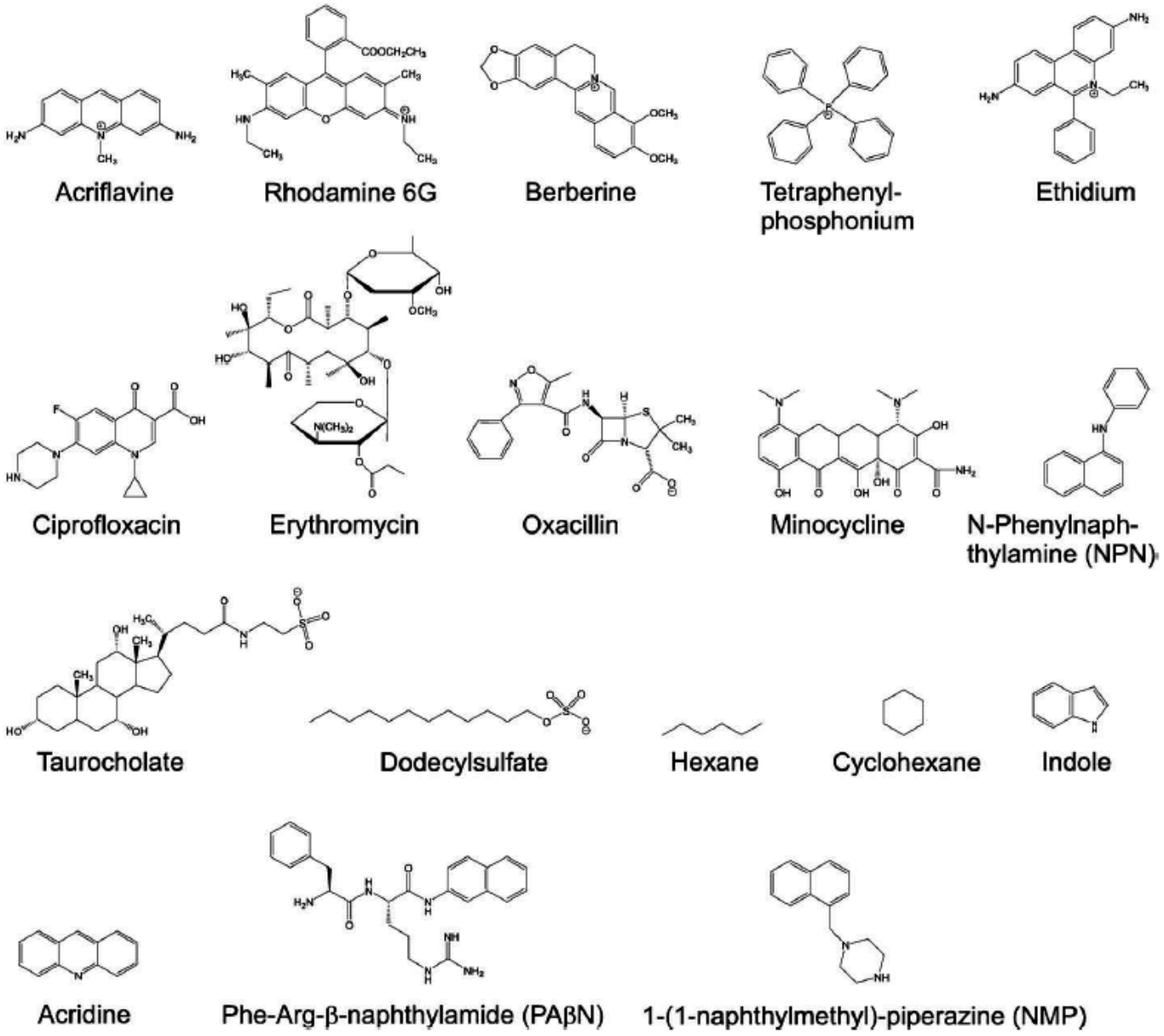


Fig. 1. Substrates and inhibitors of the AcrAB–TolC efflux system. The system confers resistance to a wide variety of noxious substances like dyes, different classes of antibiotics, detergents, bile salts and small organic molecules. Phe-Arg--naphthylamide and 1-(1-naphthylmethyl)-piperazine (NMP) inhibit RND/MFP/OMF efflux systems. From Seeger et al. [11] with permission.

Pos K. Biochimica et Biophysica Acta 1794 (2009) 782–793 / Seeger et al. Curr. Drug Targets 9 (2008) 729–749.

AcrB has multiple entry ports…

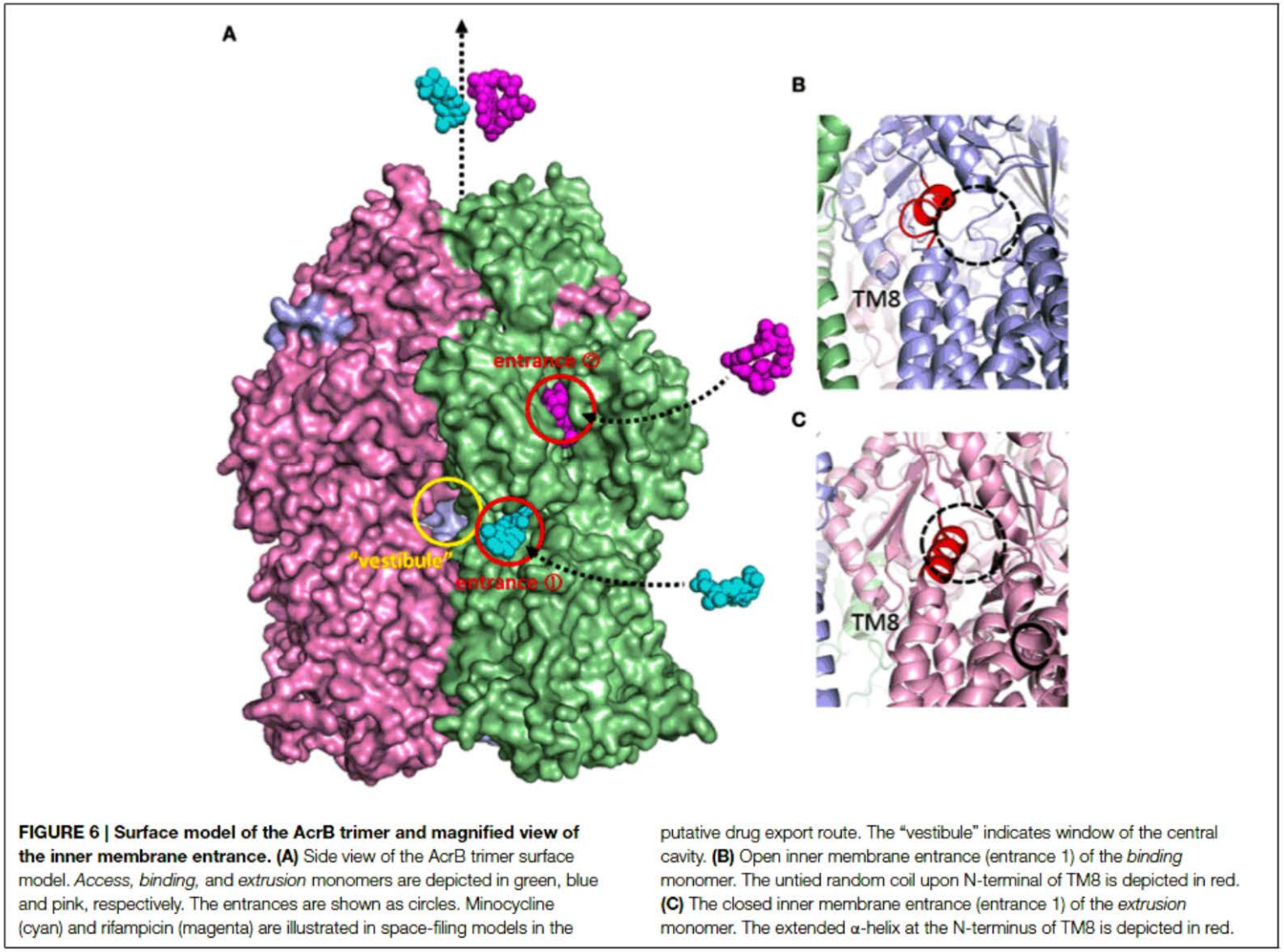


FIGURE 6 | Surface model of the AcrB trimer and magnified view of the inner membrane entrance. (A) Side view of the AcrB trimer surface model. *Access*, *binding*, and *extrusion* monomers are depicted in green, blue and pink, respectively. The entrances are shown as circles. Minocycline (cyan) and rifampicin (magenta) are illustrated in space-filing models in the putative drug export route. The "vestibule" indicates window of the central cavity. **(B)** Open inner membrane entrance (entrance 1) of the *binding* monomer. The untied random coil upon N-terminal of TM8 is depicted in red. **(C)** The closed inner membrane entrance (entrance 1) of the *extrusion* monomer. The extended α-helix at the N-terminus of TM8 is depicted in red.

Yamaguchi et al.(2015) Frontiers in Microbiology - 20 April 2015
http://dx.doi.org/10.3389/fmicb.2015.00327

Interplay of RND and porins

Structure, Function and Regulation of Outer Membrane Proteins
The Open Microbiology Journal, 2013, Volume 7 23

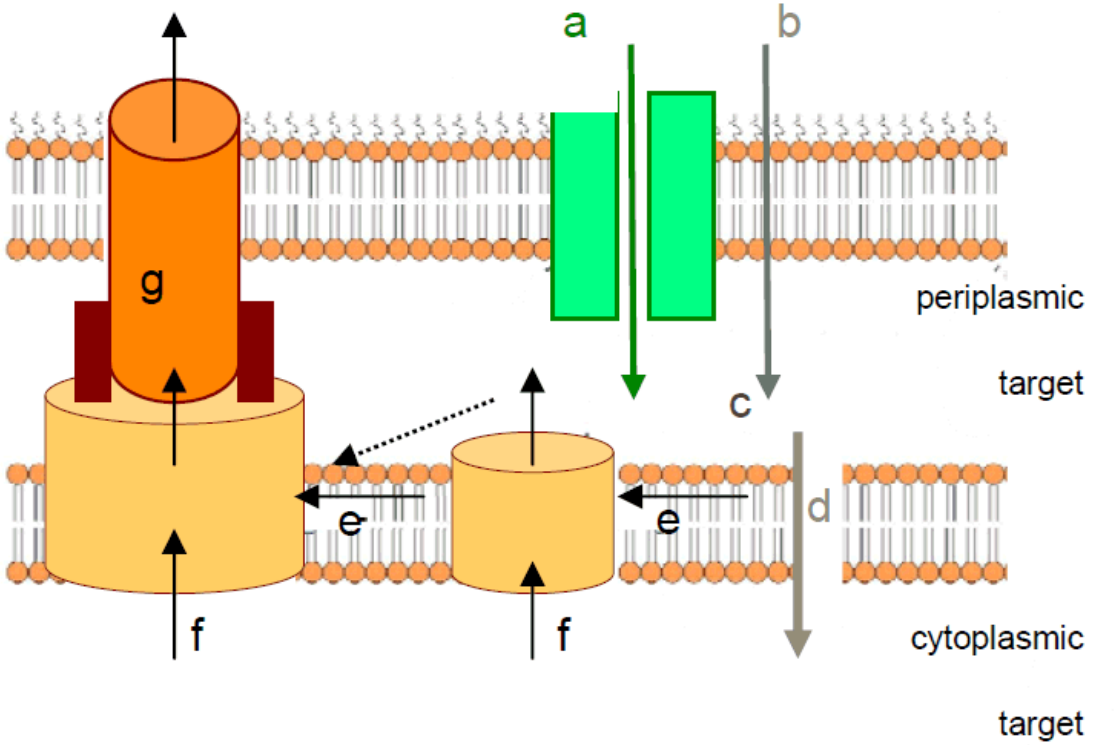
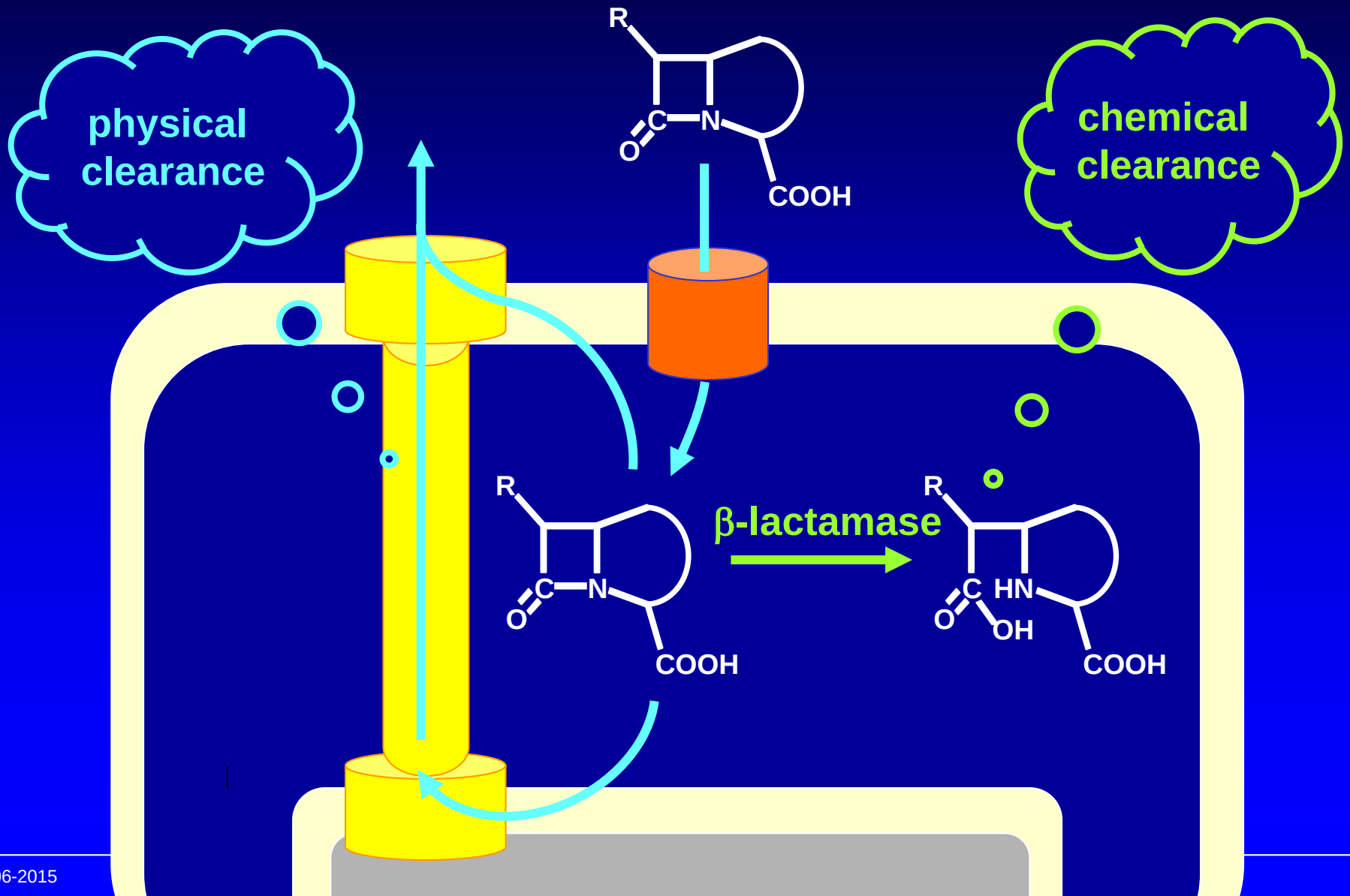


Fig. (1). Antibiotic transport through the membranes of Gram-negative bacteria (reproduced from [168]).

Rosner JL, Martin RG.
J Bacteriol 2009; 19: 5283-92

Efflux cooperates with other mechanisms of bacterial resistance



Efflux cooperates with other mechanisms of resistance

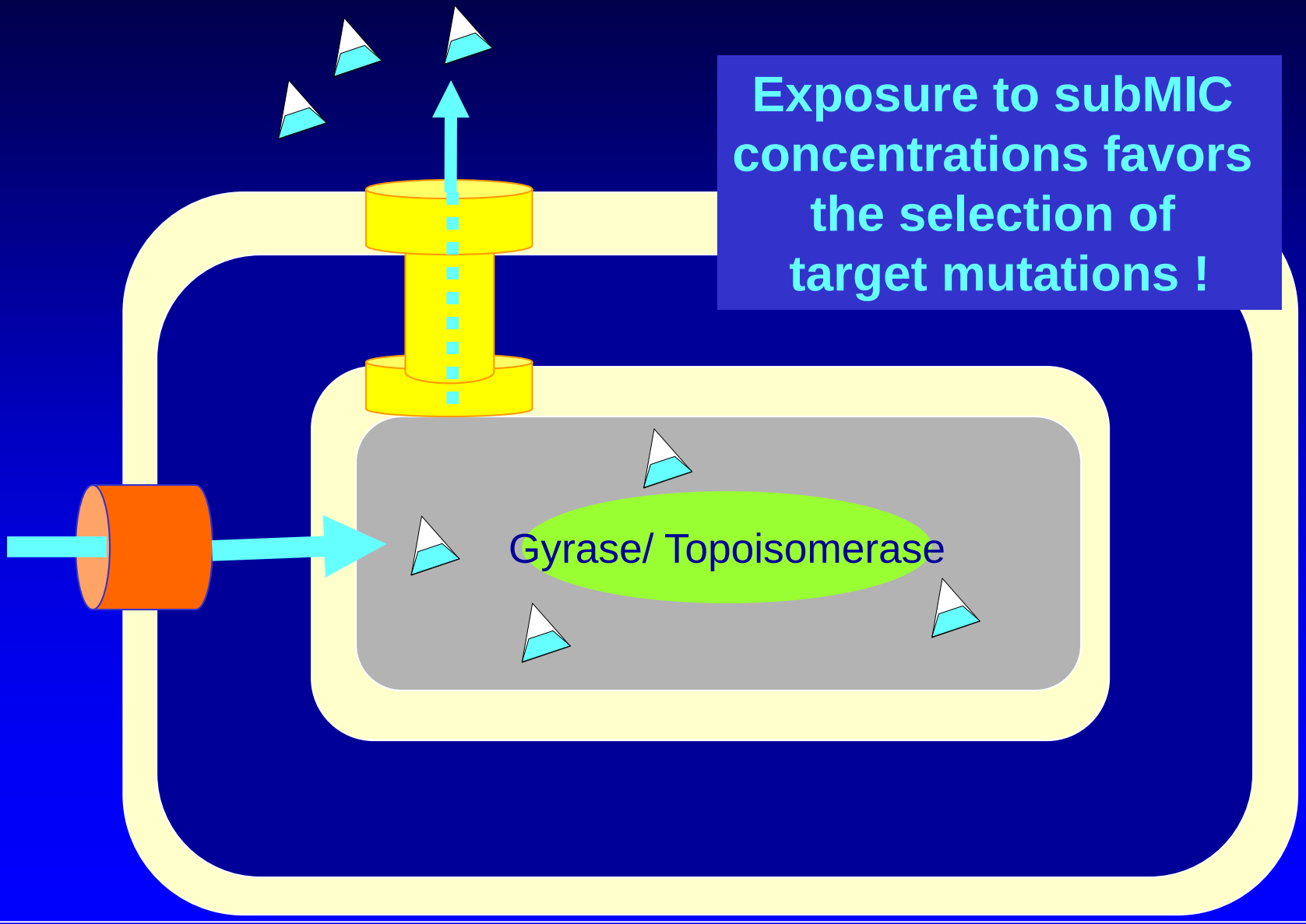
Contributions of the AmpC β-lactamase and the AcrAB Multidrug Efflux System in Intrinsic Resistance of *E. coli* to β -lactams

Efflux	β-lactamase	CMI carbenicillin	CMI ofloxacin
-	-	0.2	0.05
+	**-**	**12.5**	**0.2**
+++	-	**50**	1.56
-	+	100	0.05
+	+	200	0.39
+++	**+**	**400**	1.56

WT: intrinsic resistance !

Mazzariol *et al*, AAC (2000) 44:1387-1390

Efflux and selection of resistance to FQ



Efflux and selection of resistance

Frequency of Levofloxacin-resistant mutants in *Pseudomonas aeruginosa* with deletions of the efflux pump operons

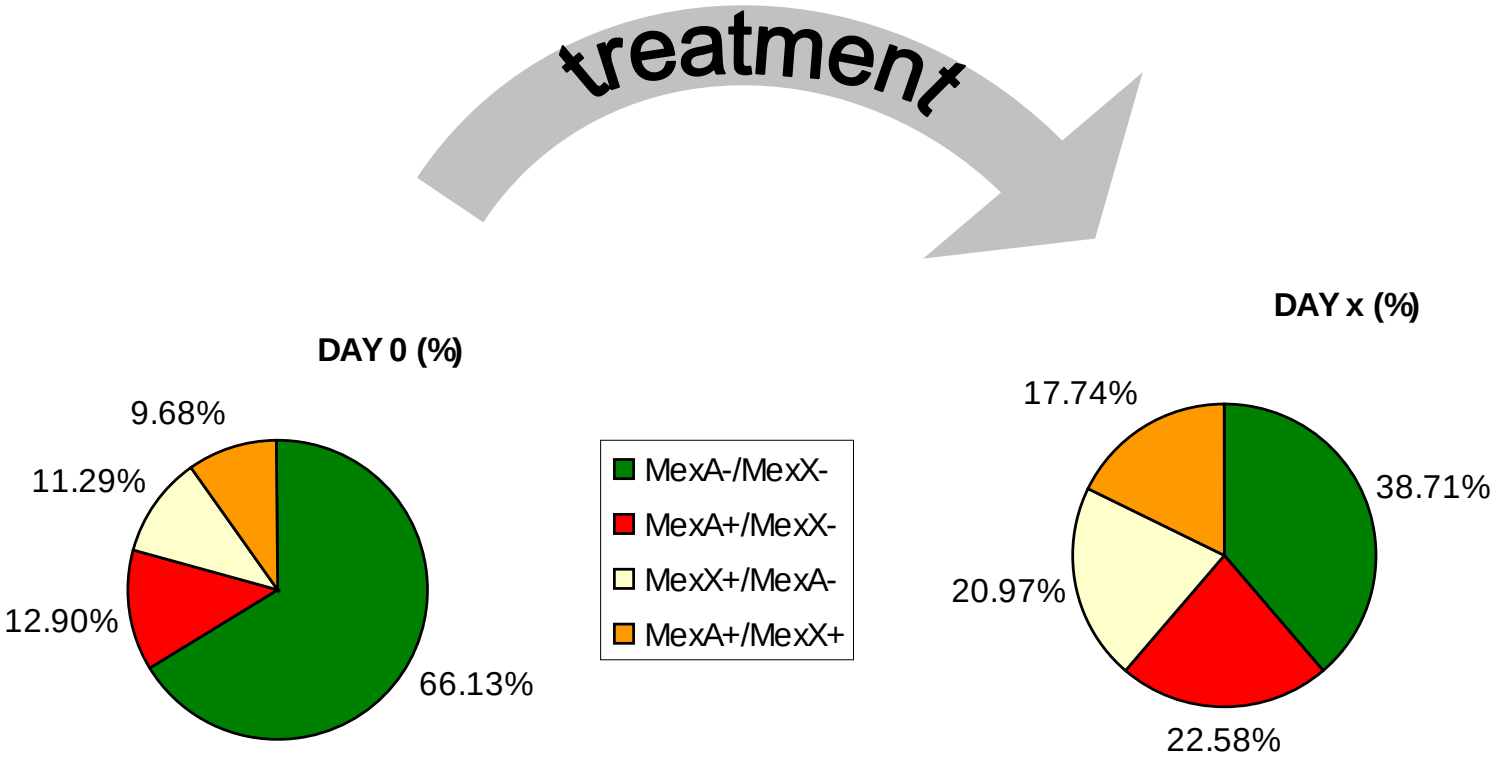
Pump status	LVX MIC	Frequency of LVX-resistant mutants
WT	0.25	2×10^7 - 4×10^7
Δ mexAB-oprM	0.015	2×10^7 - 4×10^7
Δ mexCD-oprJ	0.25	2×10^7 - 4×10^7
Δ mexEF-oprN	0.25	2×10^7 - 4×10^7
Δ mexAB-oprM;Δ mexEF-oprN	0.015	2×10^7 - 10^7
Δ mexCD-oprJ; Δ mexEF-oprN	0.25	2×10^6
Δ mexAB-oprM; Δ mexCD-oprJ	0.015	1×10^9
Δ mexAB-oprM; Δ mexCD-oprJ; Δ mexEF-oprN	0.015	**$<1 \times 10^{11}$**

Lomovskaya *et al,*
AAC (1999) 43:1340-1346

Selection of mutants in FQ target undetectable if ALL pumps are disrupted

Increase of *P.* aeruginosa MICs during treatment: involvement of efflux …

Prevalence of MexA* and MexX* overexpressers in 62 phylogentically-related pairs of *P. aeruginosa* isolated from ICU patients (VAP)



* Parts of the MexAB-OprM and MexXY-Oprm (RND pumps in P. aeruginosa)

Riou et al, ECCMID 2010
Riou et al. submitted for publication

Early diagnosis could be implemented in the clinics

RND efflux pumps in *P. aeruginosa*: an underestimated resistance mechanism

An adequate initial antibiotic therapy is a key determinant of therapeutic success in *Pseudomonas aeruginosa*-infected patients. Antibiotic efflux is an underestimated resistance mechanism because it may occur in strains categorized as susceptible. It is rarely or not at all diagnosed in routine laboratories and often masked by high-level resistance mechanisms.

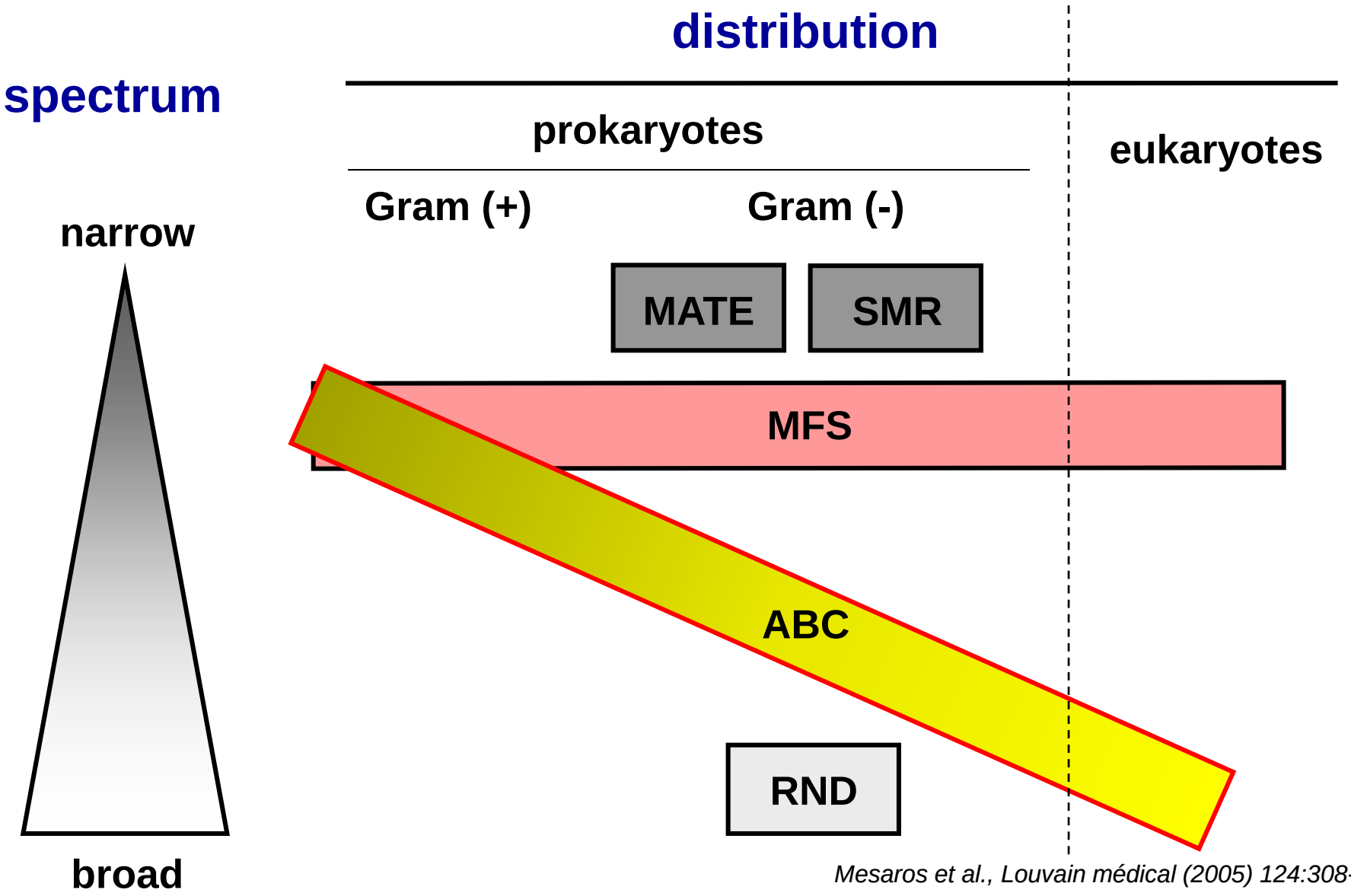
by Dr Laetitia Avrain, Dr Pascal Mertens and Professor Françoise Van Bambeke

Early diagnosis could be implemented in the clinics

The Menu …

- Why active efflux … or the origins of the discovery
- A survey of transporters in prokaryotic cells
  - *RND: the nightmare for the microbiologist…*
- **From bacteria to eukaryotic cells**
  - *ABC (and some of the others)*
    - bioavailability, intestine, blood-brain-barrier, kidney
    - cancer …
- Why we still fail …

Antibiotic efflux transporters are ubiquitous



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

ABC in bacteria and eucaryotic cells

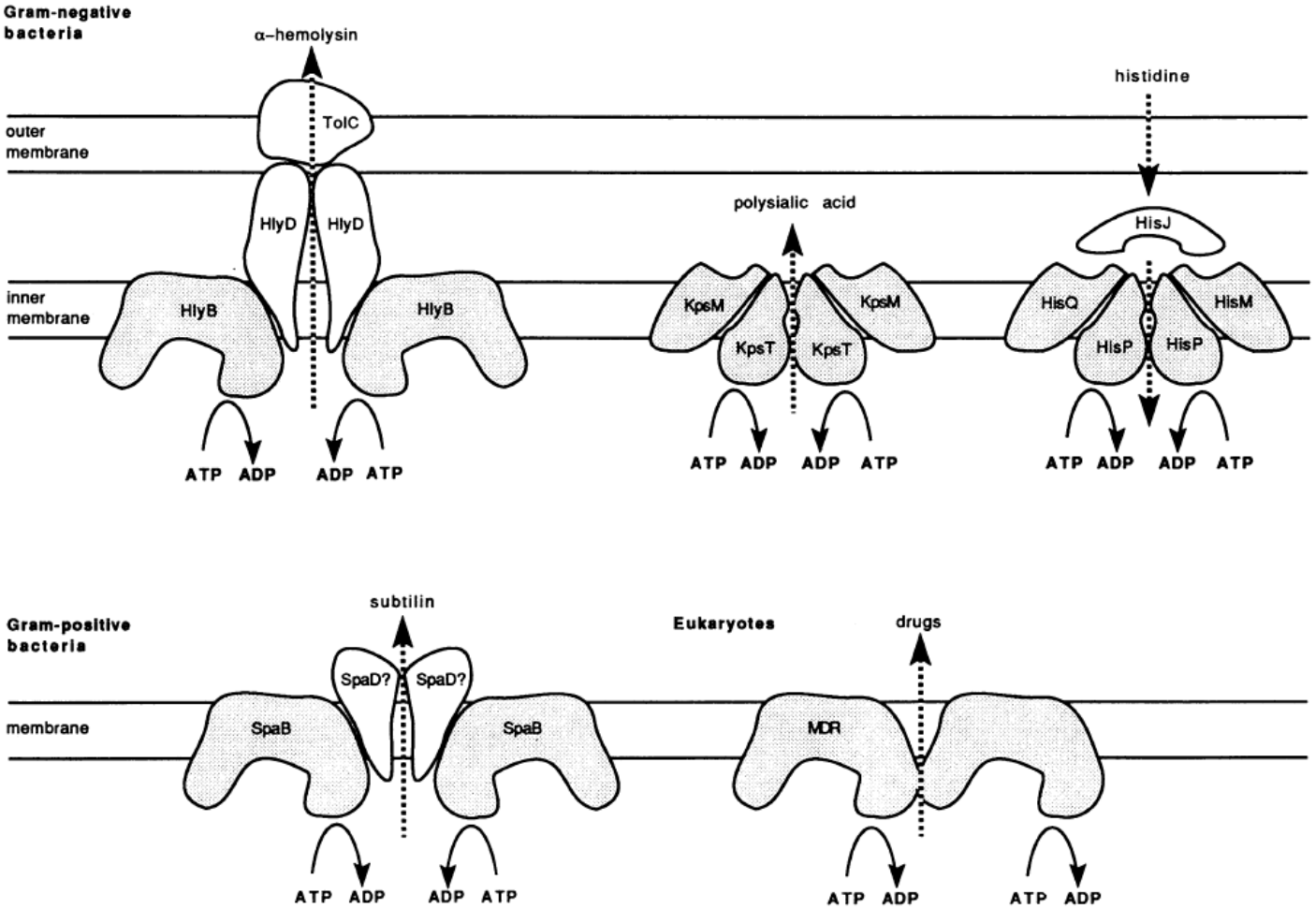


FIG. 1. Structural models of various ABC transporters. The prototype systems included are the *E. coli* alpha-hemolysin exporter, the *E. coli* polysialic acid exporter, the *S. typhimurium* histidine importer, the *B. subtilis* subtilin exporter, and the mammalian P-glycoprotein drug exporter. The bacterial 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

ABC in antibiotic producing organisms…

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

ABC transporters in antibiotic-producing actinomycetes.

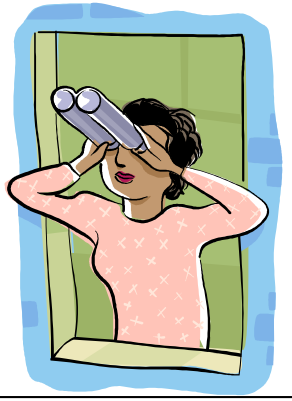
Méndez C, Salas JA.

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]

Transport is essential for survival of these organisms …
and can be intrinsically linked to resistance
(it all depends how you look at it !)



ABC transporters in bacteria could also be involved in pathogenicity

Protoplasma (2012) 249:919–942
DOI 10.1007/s00709-011-0360-8

REVIEW ARTICLE

The role of ATP-binding cassette transporters in bacterial pathogenicity

Victoria G. Lewis • Miranda P. Ween •
Christopher A. McDevitt

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DOI 10.1007/s00709-011-0360-8

REVIEW ARTICLE

The role of ATP-binding
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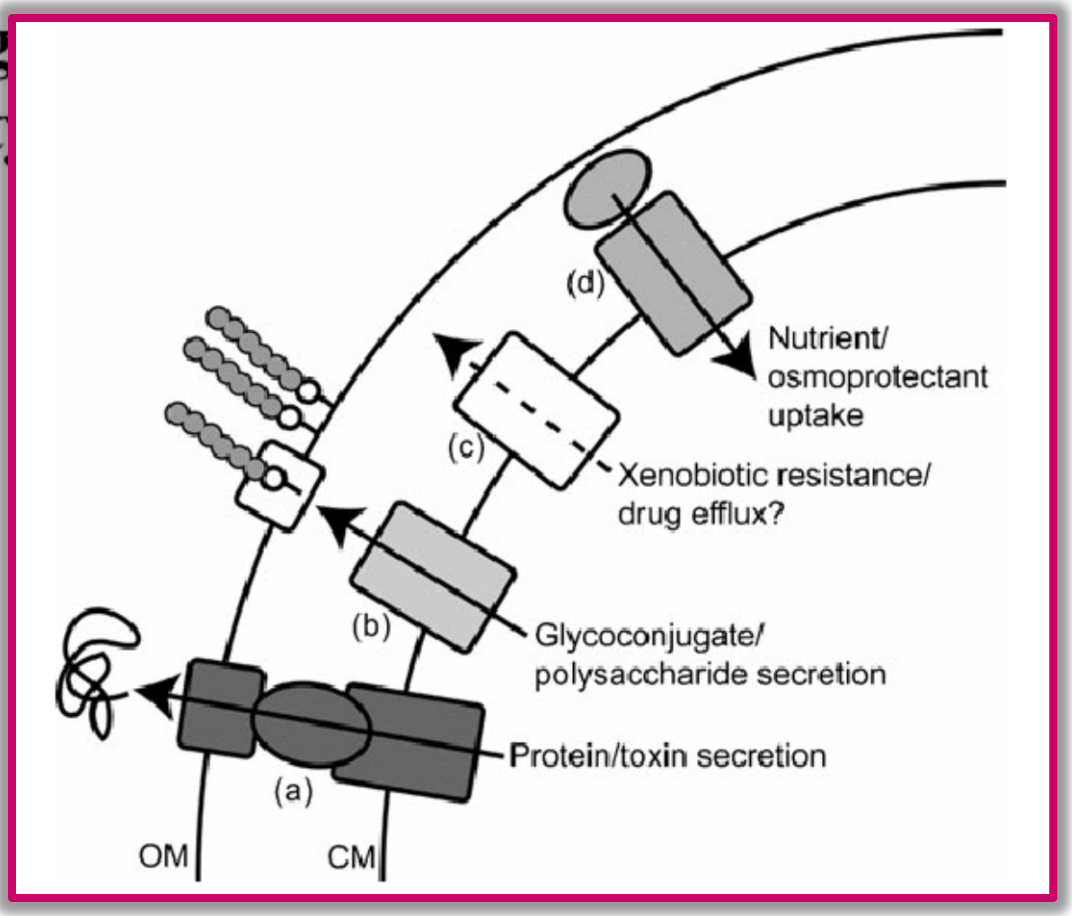


Fig. 2 Roles associated with ABC transporters involved in bacterial pathogenicity in a model Gram-negative cell. ABC exporters include (*a*) Type I secretions systems associated with toxin, S-layer protein, siderophore, hydrolytic enzyme or antimicrobial peptide secretion, which have roles in adhesin and colonization of the host; (*b*) glyco-conjugate and polysaccharide biogenesis pathways, which are involved in membrane biogenesis and immune evasion; and (*c*) xenobiotic efflux pathways involved in host environment tolerance and with postulated roles in antibiotic efflux. ABC importers (*d*) are associated with processes such as nutrient acquisition (e.g. metal ions, amino acids, vitamins and oligopeptides) and osmoprotection, which have crucial roles for successful colonization and promulgation in the host environment

ABC in Fungi (with others…)

frontiers in
PHARMACOLOGY

REVIEW ARTICLE
published: 29 August 2014
doi: 10.3389/fphar.2014.00202

Efflux pump proteins in antifungal resistance

Rajendra Prasad* and Manpreet K. Rawal

Membrane Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

a typical ABC in Fungi
(coworking with an MFS)

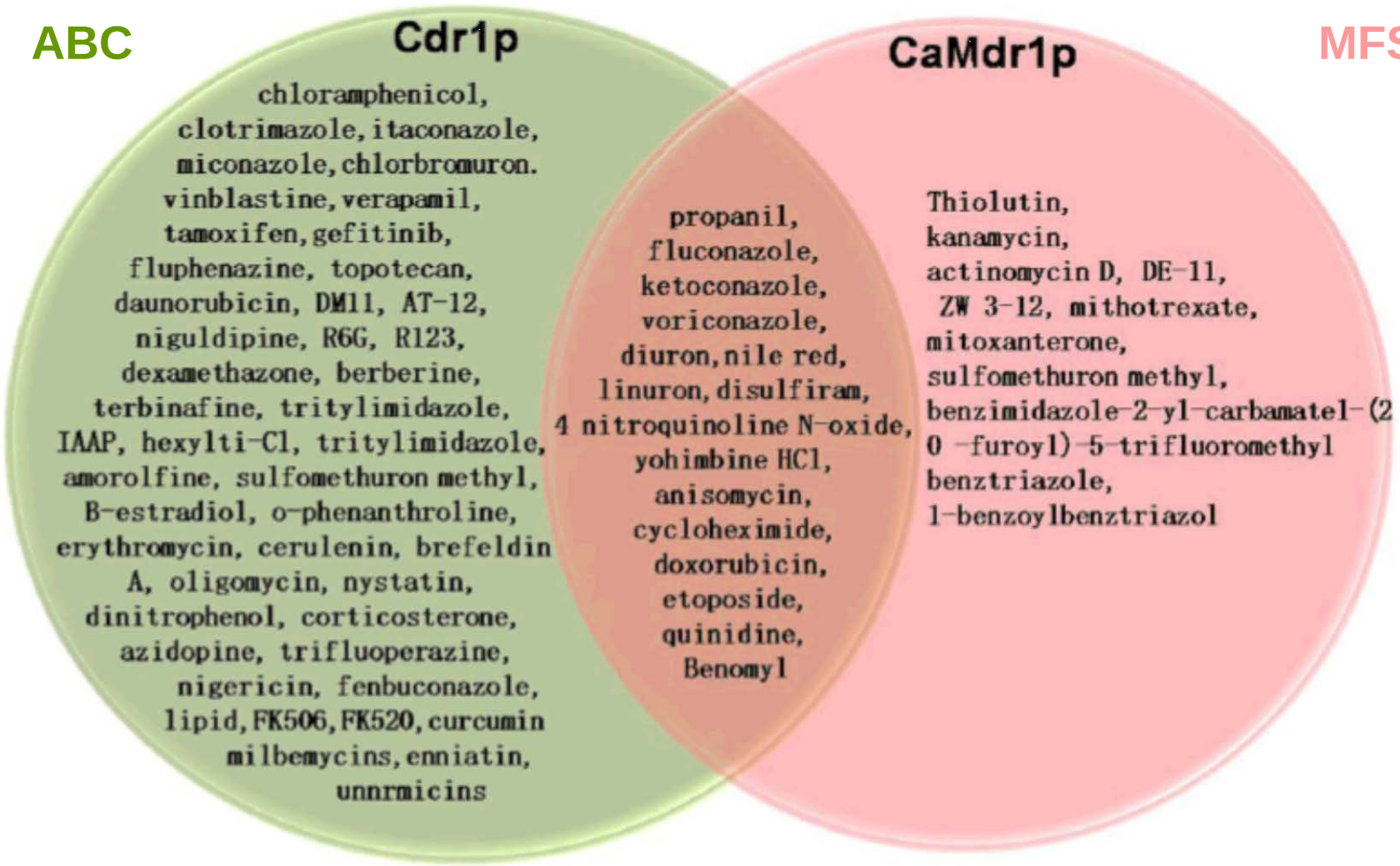


FIGURE 1 | Venn diagram showing substrates which are common and distinct for Cdr1p and CaMdr1p.

ABC transporters from bacteria to man

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

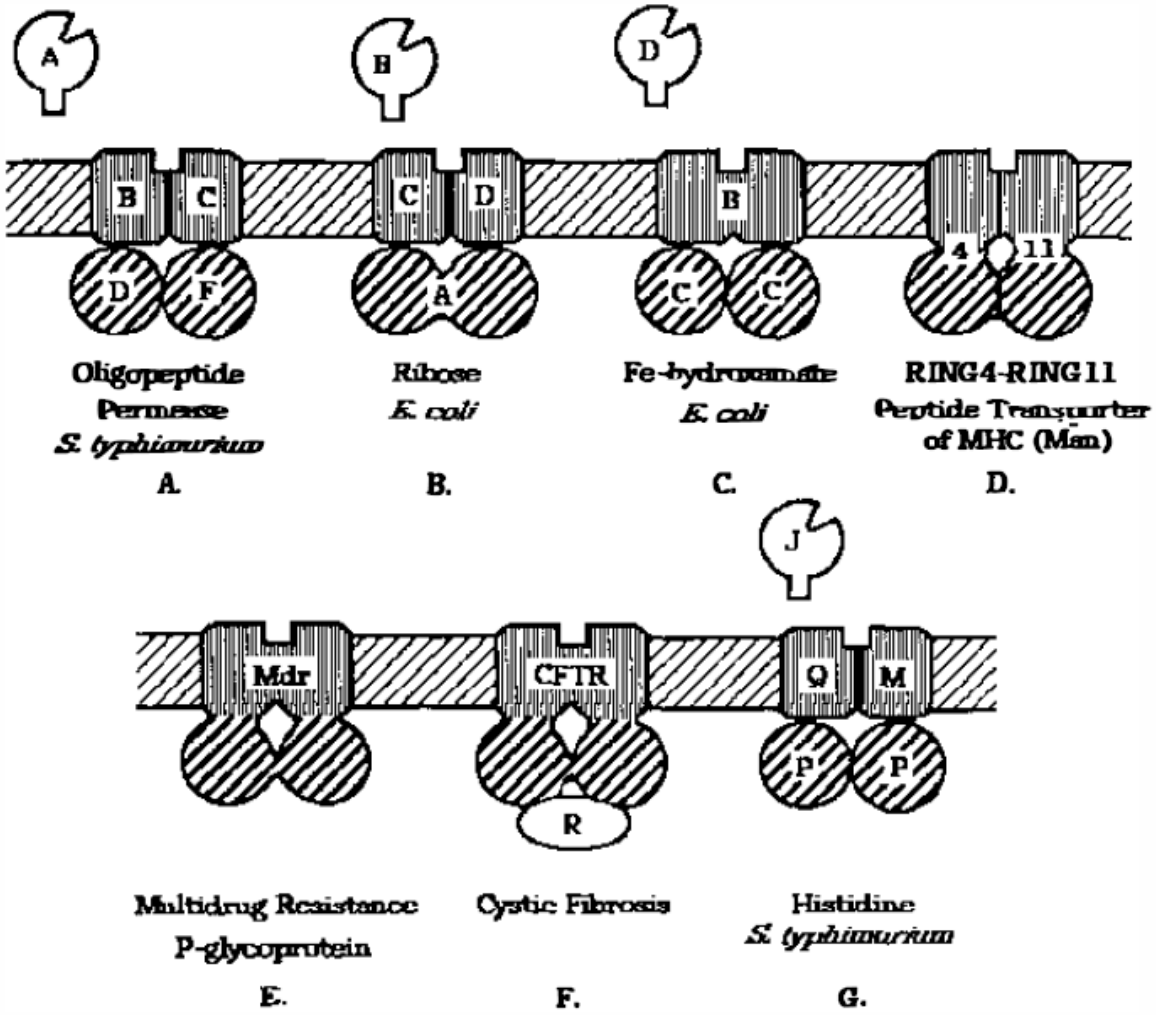
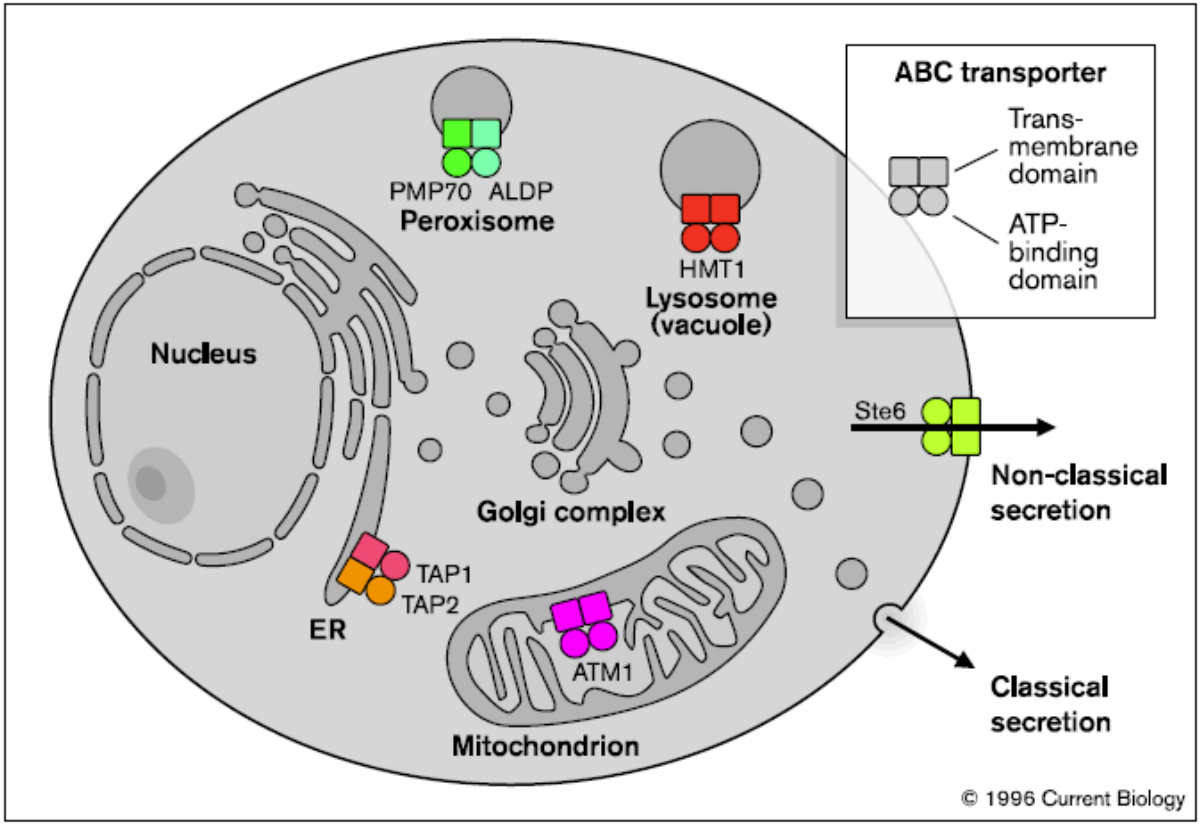


Figure 2 Domain organization of ABC transporters. A typical ABC transporter consists of four domains, two highly hydrophobic membrane-spanning domains (*shaded*), which form the translocation pathway, and two peripheral membrane domains (*shaded*), which couple ATP hydrolysis to the transport process. 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.

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.

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 mrpI (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.

ABC and biliary excretion

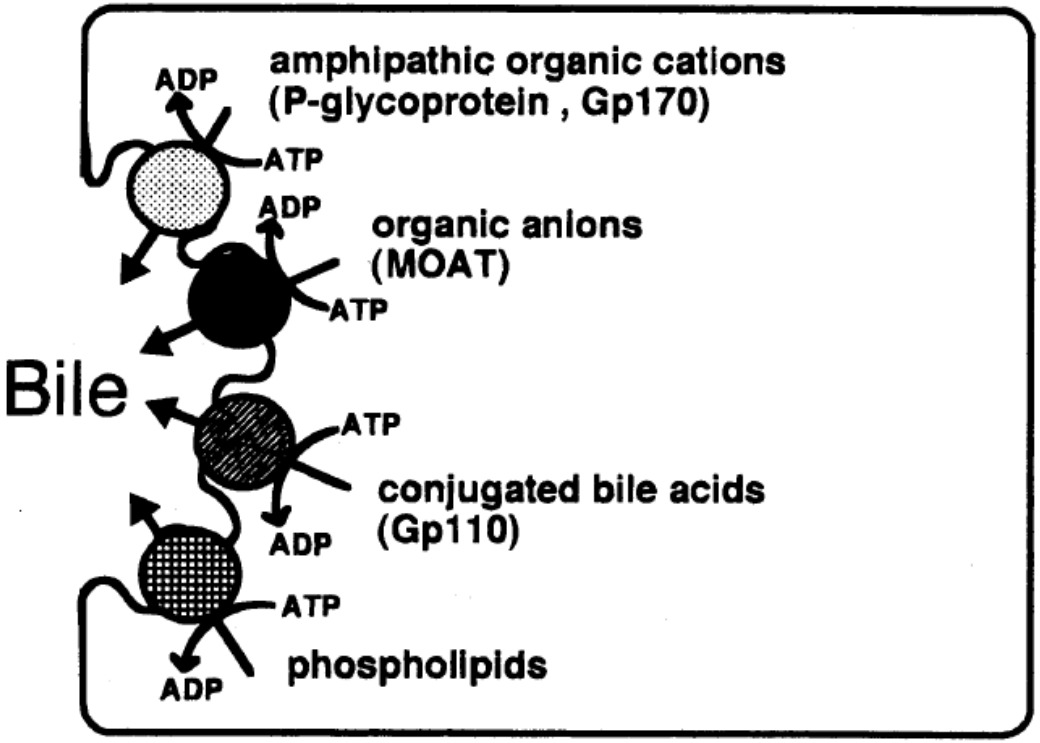


Fig. 3. ATP-dependent primary active transport carriers in the canalicular membrane involved in the biliary excretion of amphipathic organic cations, organic anions, conjugated bile acids and phospholipids.

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

Cooperation between pumps for effective extrusion of amphipathic substances from the hepatocyte

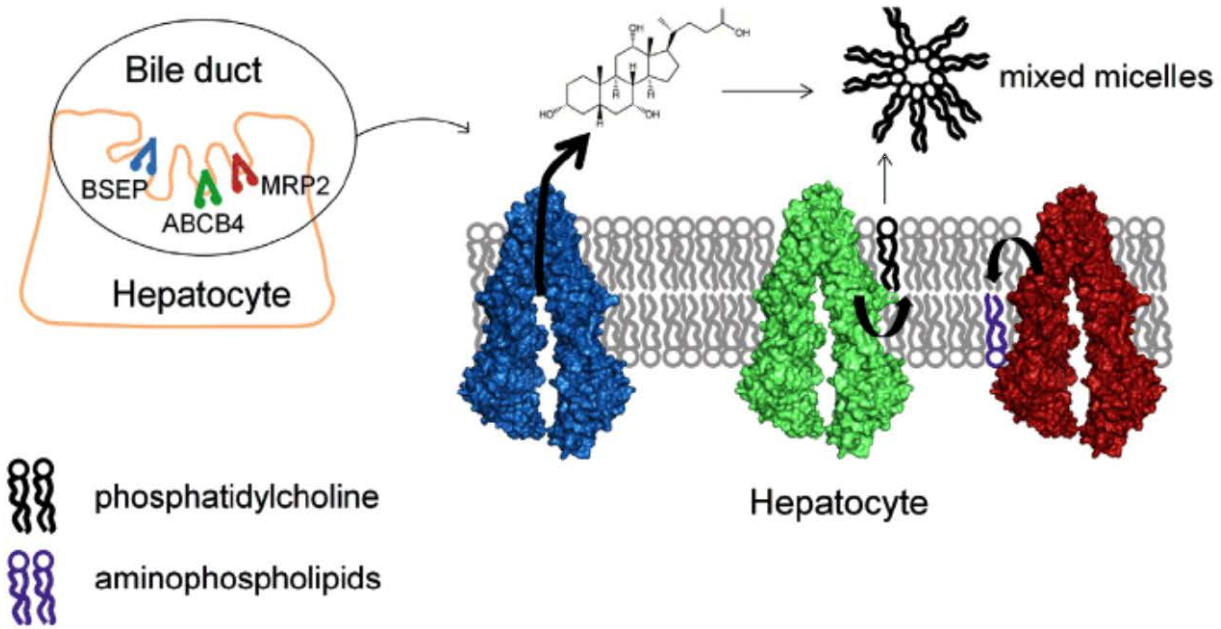


Fig. 1. Cooperation of BSEP, ABCB4 and MRP2 in the canalicular membrane of hepatocytes. BSEP (blue) exports the bile salts, ABCB4 (green) flips phosphatidylcholine to the outer leaflet of the membrane, where it is recruited by bile salts to form mixed micelles. MRP2 (red) maintains the asymmetry in lipid composition by flipping aminophospholipids to the inner leaflet of the membrane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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

But beware ! These pumps existed before the arrival of human-made drugs

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

National Institute of Haematology and Immunology, Membrane Research and Immunopathology Group of the Hungarian Academy of Sciences, Daróczi u. 24, 1113 Budapest, Hungary

- 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.

In eukaryotes, ABC transporters have multiple physiological functions

Table 1
Examples of potential signaling molecules that are substrates of ABC transporters.

Compound	Functions	Transporter(s)
Platelet activating factor (PAF)	Mediator of inflammation	ABCB1 (Pgp)
Phospholipids [e.g., phosphatidylcholine, phosphatidylserine (PS) phosphatidylethanolamine]	Membrane integrity, cell cycle regulation; cell signaling; schistosome PS and lyso-PS polarize DCs	ABCB1, ABCB3, ABCA1, ABCA2, ABCG2 (BCRP)
Cyclic nucleotides	Regulate inflammatory responses, monocyte polarization, maturation	ABCC4, ABCC5, ABCC11
Sphyngosine-1-phosphate (S1P)	T-cell homing; immunosuppression	ABCB1
Leukotriene LTC_4	Mediator of inflammation; DC migration	ABCC1, other ABCCs (MRPs)
LTB_4, LTD_4, LTE_4	Mediators of inflammation; DC migration	ABCC1, ABCC4
Prostaglandins PGE_1, PGE_2, $PGE_{2\alpha}$	DC migration/maturation; immune suppression	ABCC4
Sphingomyelin, glycolipids, cholesterol	Multiple	ABCB1, ABCAs
Peptides	Antigen presentation	ABCB2/3 (TAP1/2)
dsRNA	TLR3 activation in DCs by schistosome eggs	*C. elegans* ABCA, ABCBs, ABCD, ABCGs

Examples of some of the signaling molecules shown to be substrates of ABC transporters, and their possible functions, with an emphasis on those with relevance to immunomodulation.

Greenberg R International Journal for Parasitology: Drugs and Drug Resistance 4 (2014) 301–309

And they control physiological functions …

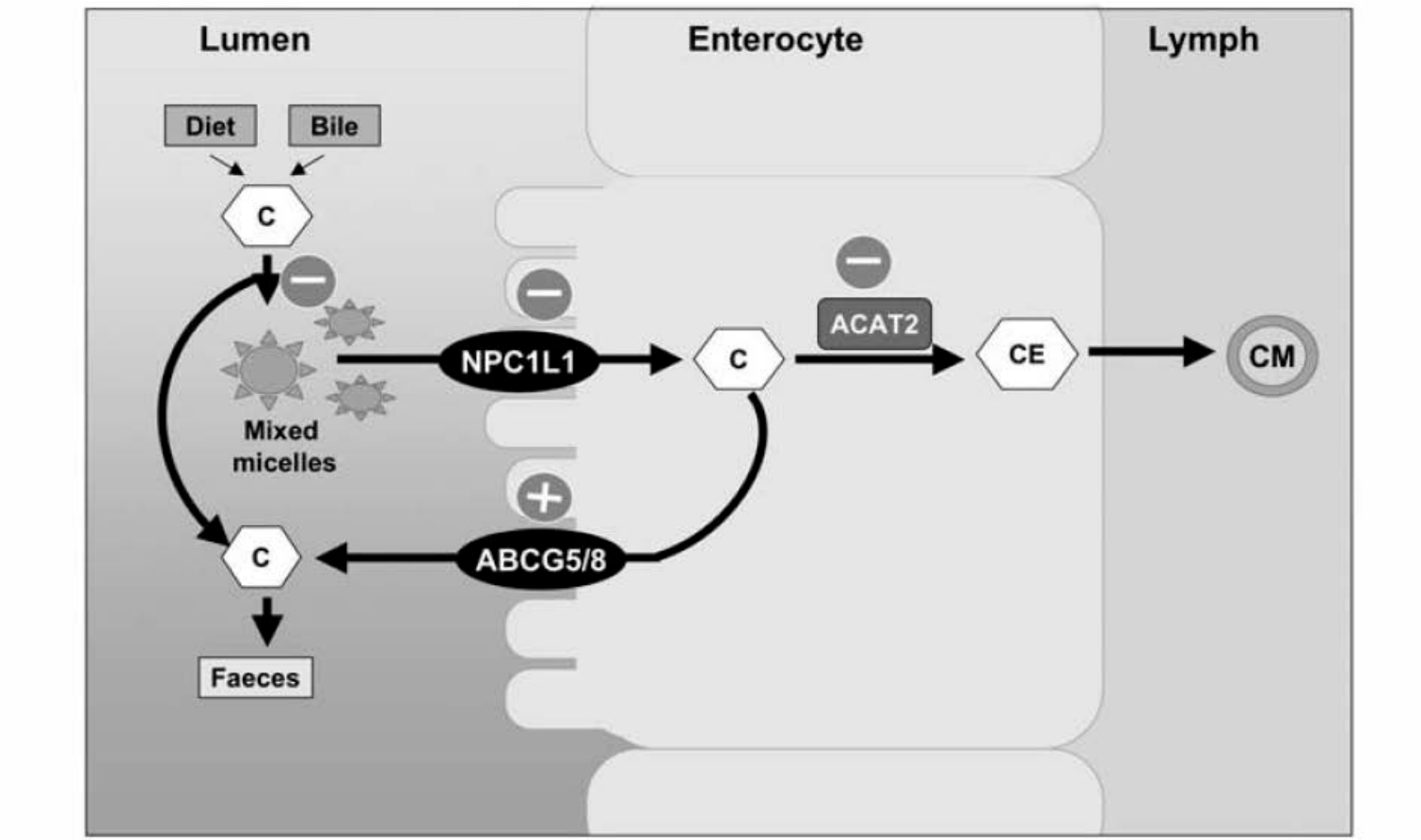


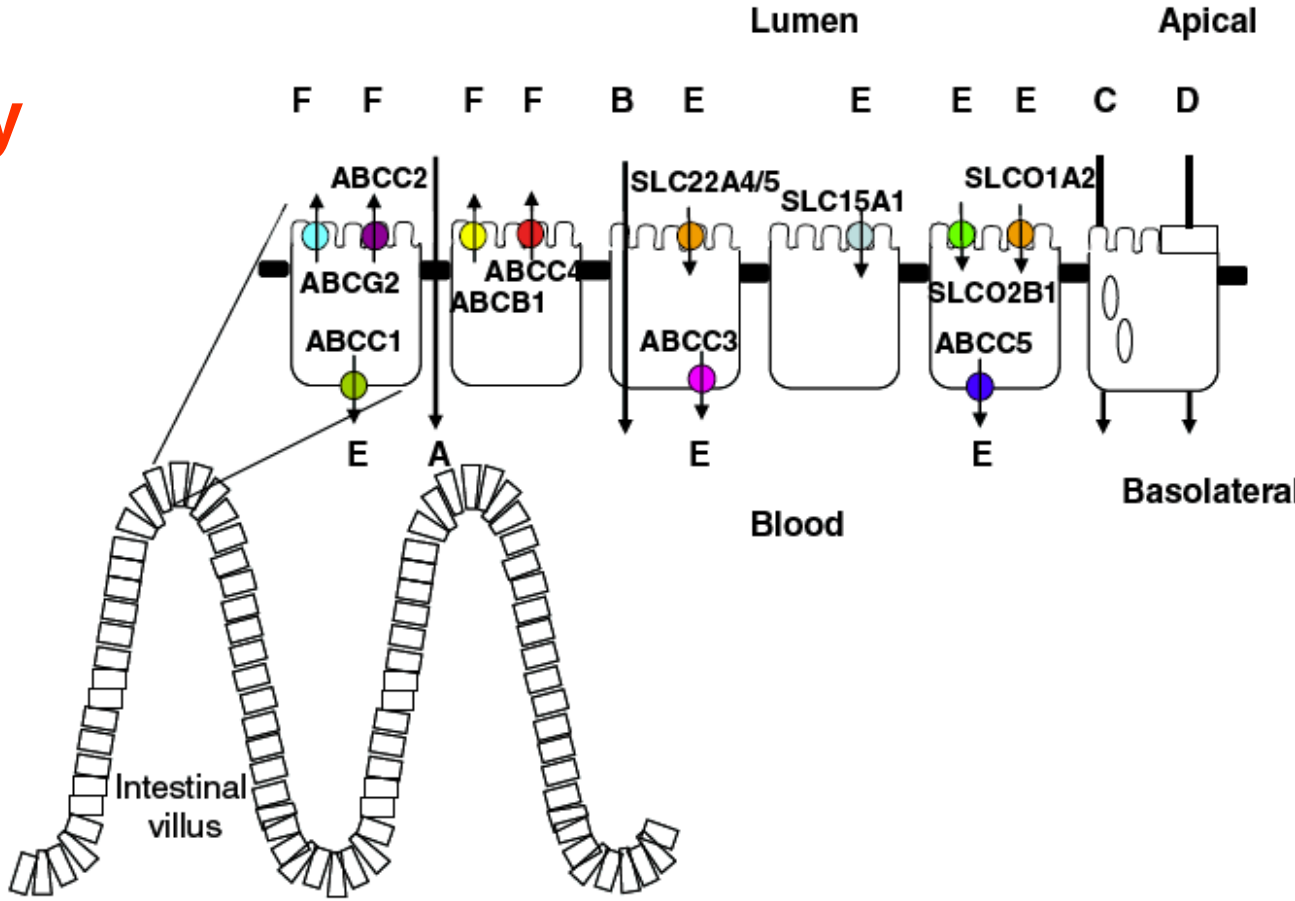
Fig. 1. *Multistep process of intestinal cholesterol absorption and potential cholesterol-lowering mechanisms of action of phytosterols.*
C, free cholesterol; CE, cholesteryl esters; CM, chylomicrons; NPC1L1, Niemann-Pick C1-like 1; ACAT2, Acyl-coenzyme A cholesterol acyltransferasa isorform2; ABCG5/8,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

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

The Menu …

- Why active efflux … or the origins of the discovery
- A survey of transporters in prokaryotic cells
  - *RND: the nightmare for the microbiologist…*
- From bacteria to eukaryotic cells
  - *ABC (and some of the others)*
    - **bioavailability, intestine, blood-brain-barrier, kidney**
    - **cancer …**
- Why we still fail …

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

The family of intestinal transporters

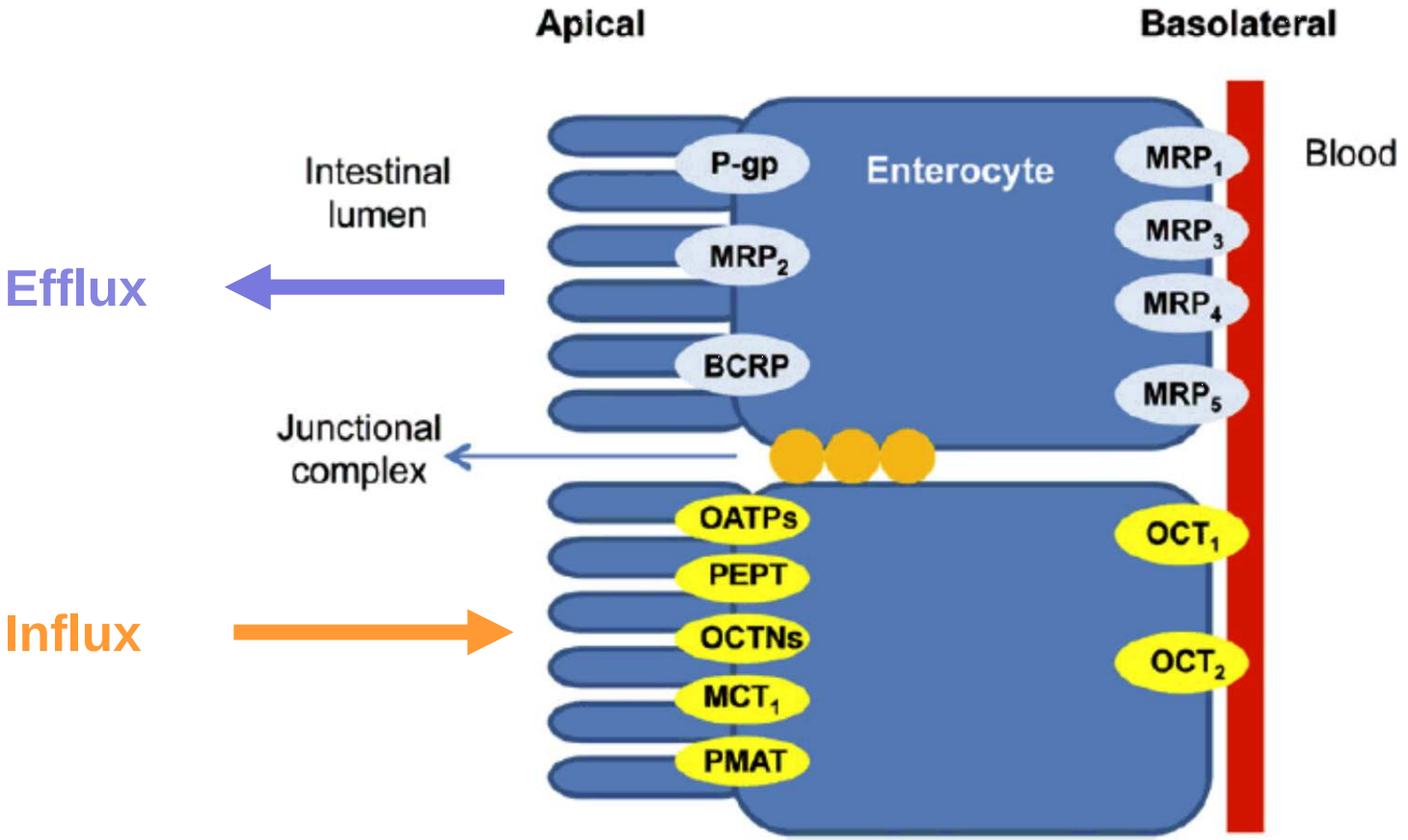


Fig. 1. Diagram of major drug transporters proteins expressed at the intestinal epithelia including intestinal uptake (yellow) and efflux (light blue) transporters. Multidrug resistance protein (MDR1, P-glycoprotein), multidrug resistance associated protein (MRP), breast cancer resistance protein (BCRP), monocarboxylate transporter protein (MCT), peptide transporter protein (PEPT), organic anion transporting polypeptide (OATP), organic cation transporter (OCT), carnitine/organic cation transporter (OCTN), and plasma membrane monoamine transporter (PMAT).

Estudante *et al.* Advanced Drug Delivery Reviews 65 (2013) 1340–1356
http://dx.doi.org/10.1016/j.addr.2012.09.042

Intestinal efflux transporters: substrates and inhibitors

Table 1
Drug efflux transporters identified in the intestine.

Drug transporter	Gene	Intestinal localization	Substrate specificity	Substrates	Inhibitors	Inducers	Polymorphisms
MDR1/ P-gp	*ABCB1*	Apical	Broad subtrate specificity; preference for hydrophobic, amphipathic or cationic molecules	[a]	Verapamil, immunosuppressive agents, SDZ PSC 833, LY335979, GF120918 (GG918), grapefruit juice, cyclodextrin, PEG 400, Tween 80 and Cremophor EL	St. John's wort, rifampicin	Yes
BCRP/MXR	*ABCG2*	Apical	Broad substrate specificity Acids and drug conjugates	[b]	Estrone, 17-β-estradiol, GG918, flavonoids, herb extracts, gefitinib, imatinib, tamoxifen, novobiocin, nelfinavir, ritonavir, dipyridamole, fumitremorgin C (FTC), Ko143, cyclosporine	Efavirenz	Yes
MRP1	*ABCC1*	Basal	Hydrophobic drugs, conjugates to glutathione, glucuronic acid or sulfate	Vinca alkaloids, anthracyclines, etoposide, teniposide, mitoxantrone, methotrexate	MK571, LTC_4, sulfinpyrazone, benzbromarone, probenecid		
MRP2	*ABCC1*	Apical	Glutathione, glucuronide, sulfate and heavy metals conjugates Unconjugated organic anions	[c]	LTC_4, MK571, phenolphthalein glucuronide, fluorescein methotrexate, probenecid, furosemide, indomethacin, grapefruit juice	Rifampin, 1,25(OH)2D3, spironalactone	Yes

[a] Steroid hormones, bile salts, glycocholate, tauroursodeoxycholate, doxorubicin, daunorubicin, reserpine, vincristine, vinblastine, valinomycin, cyclosporine, tacrolimus, tandutinib, aldosterone, hydrocortisone, dibucaine, talinolol, digoxin, TPP, ivermectin, paclitaxel, grepafloxacin, indinavir, nelfinavir, saquinavir, grepafloxacin, colchicine, darunavir, Rhei Rhizoma extract, flavonoids, glyburide, imatinib, methotrexate, mitoxantrone, prazosin, temocapril and SN-38.

[b] Topotecan, irinotecan and its active analog SN-38, mitoxantrone, doxorubicin, daunorubicin, imatinib, gefitinib, tandutinib, statins, prazosin, glyburide, dipyridamole, quercetin, temocapril, sulfate conjugates, porphyrins, nitrofurantoin, fluoroquinolones, zidovudine, lamivudine, efavirenz, ciprofloxacin, rifampicin, sulfasalazine, quercetin, flavonoids, phytoestrogens, porphyrins, estrone 3-sulfate, PhIP, resveratrol conjugates, naringenin glucuronides, methotrexate, 7-hydroxymethotrexate, ezetimibe, gefitinib, 9-aminocamptothecin, diflomotecan, rosuvastatin, atorvastatin, fluvastatine, simvastatin lactone.

[c] Leukotrienes glutathione, 2,4-dinitrophenyl-S-glutathione, bromosulfophthalein, conjugates of bile salts and heavy metals, resveratrol conjugates, naringenin glucuronides, vinblastine, reduced folates, irinotecan and its metabolite SN-38, pravastatin, ceftriaxone, ampicillin, grepafloxacin, sulfasalazine, fexofenadine, lopinavir, fosinopril, ochratoxin A, epicatechin, PhIP, phenols, colchicine, darunavir, Rhei Rhizoma extract, flavonoids, methotrexate, 7-hydroxymethotrexate, ezetimibe.

Estudante *et al.* Advanced Drug Delivery Reviews 65 (2013) 1340–1356
http://dx.doi.org/10.1016/j.addr.2012.09.042

The interplay between efflux and metabolism

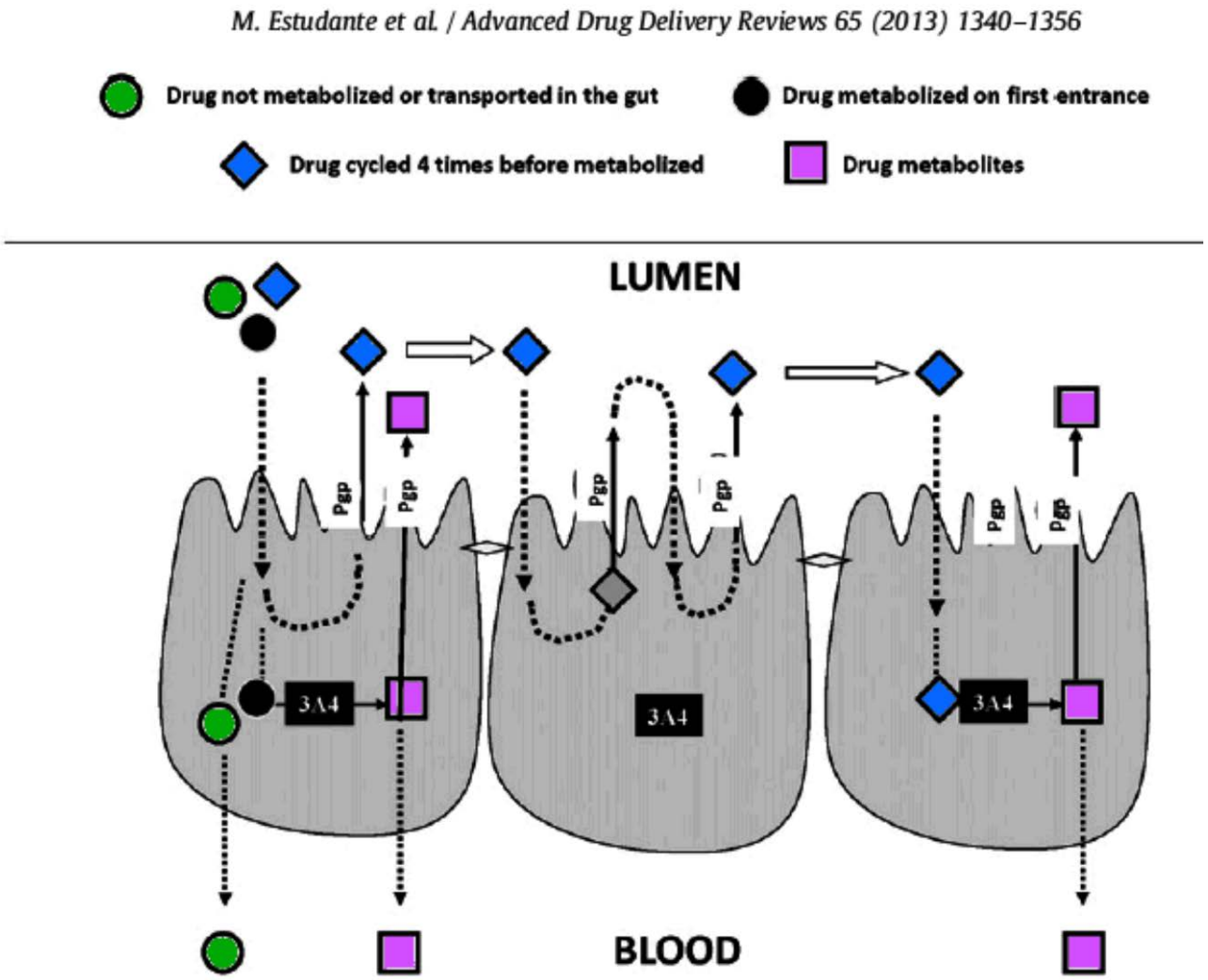


Fig. 2. Cartoon depicting CYP3A4 and P-glycoprotein interplay in the enterocytes. In Mol Pharm. 2009 [34].

Estudante *et al.* Advanced Drug Delivery Reviews 65 (2013) 1340–1356
http://dx.doi.org/10.1016/j.addr.2012.09.042

Intestinal efflux and drug interactions…

Table 4
Other drug P-glycoprotein (P-gp) interactions described in the intestine.

Affected drug	Inhibitor	Impact on affected drug
Ivermectin	Verapamil	Reduced intestinal luminal accumulation
Paclitaxel	GG918	Increase in AUC and C_{max}
Cyclosporine	Grapefruit juice	55% increase in AUC and 35% in C_{max}
Talinolol	Verapamil	Reduced intestinal luminal accumulation
	Grapefruit juice	Increase in AUC and C_{max}
Docetaxel	R101933	8.47% decrease in dose excreted in the feces
Paclitaxel	Cyclosporine	8-Fold increase in AUC
Affected drug	**Inducer**	**Impact on affected drug**
Talinolol	Rifampicin	35% decrease in AUC
Cyclosporine	St. John's Wort	1.9-Fold decrease in AUC
Fexofenadine	St. John's Wort	1.6-Fold decrease in AUC

Estudante *et al.* Advanced Drug Delivery Reviews 65 (2013) 1340–1356
http://dx.doi.org/10.1016/j.addr.2012.09.042

Bioavailability is largely related to local transport

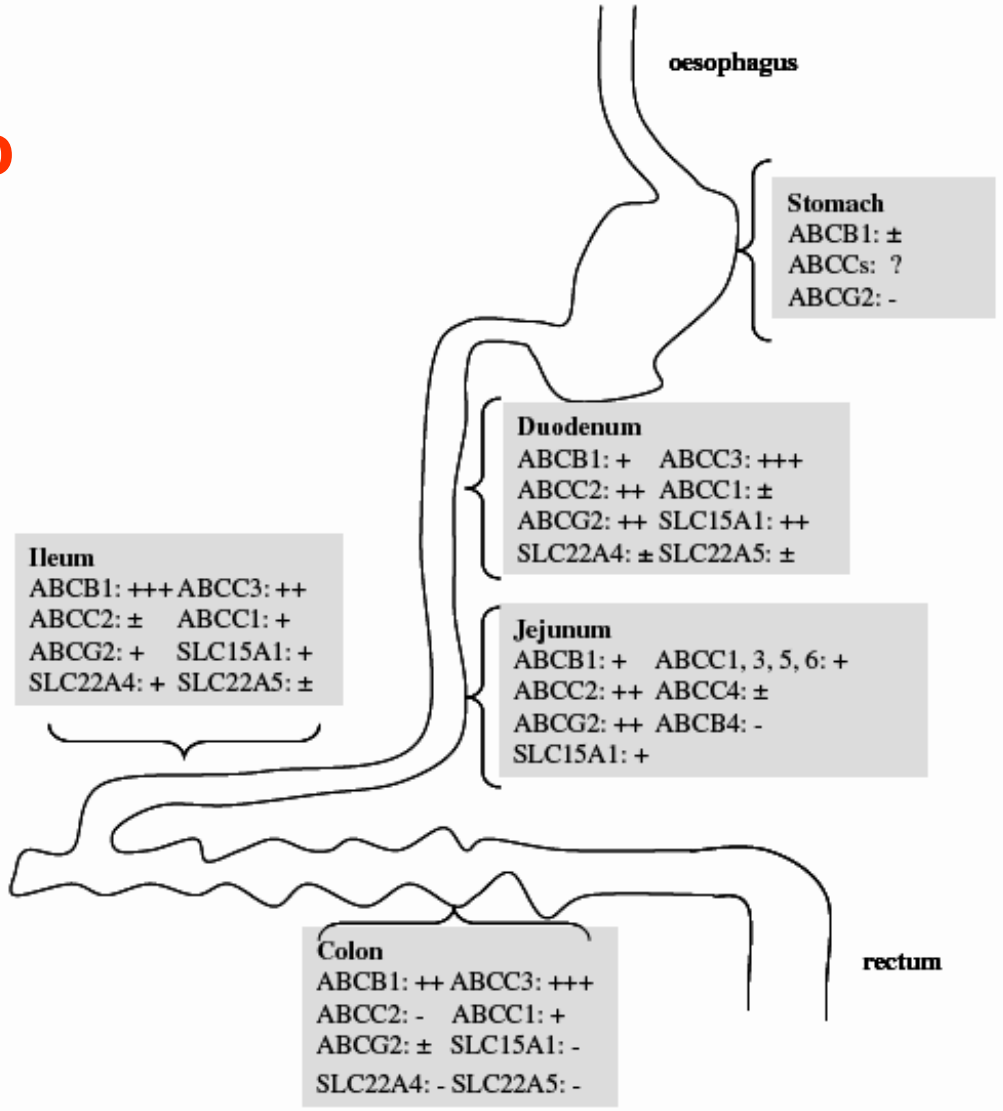
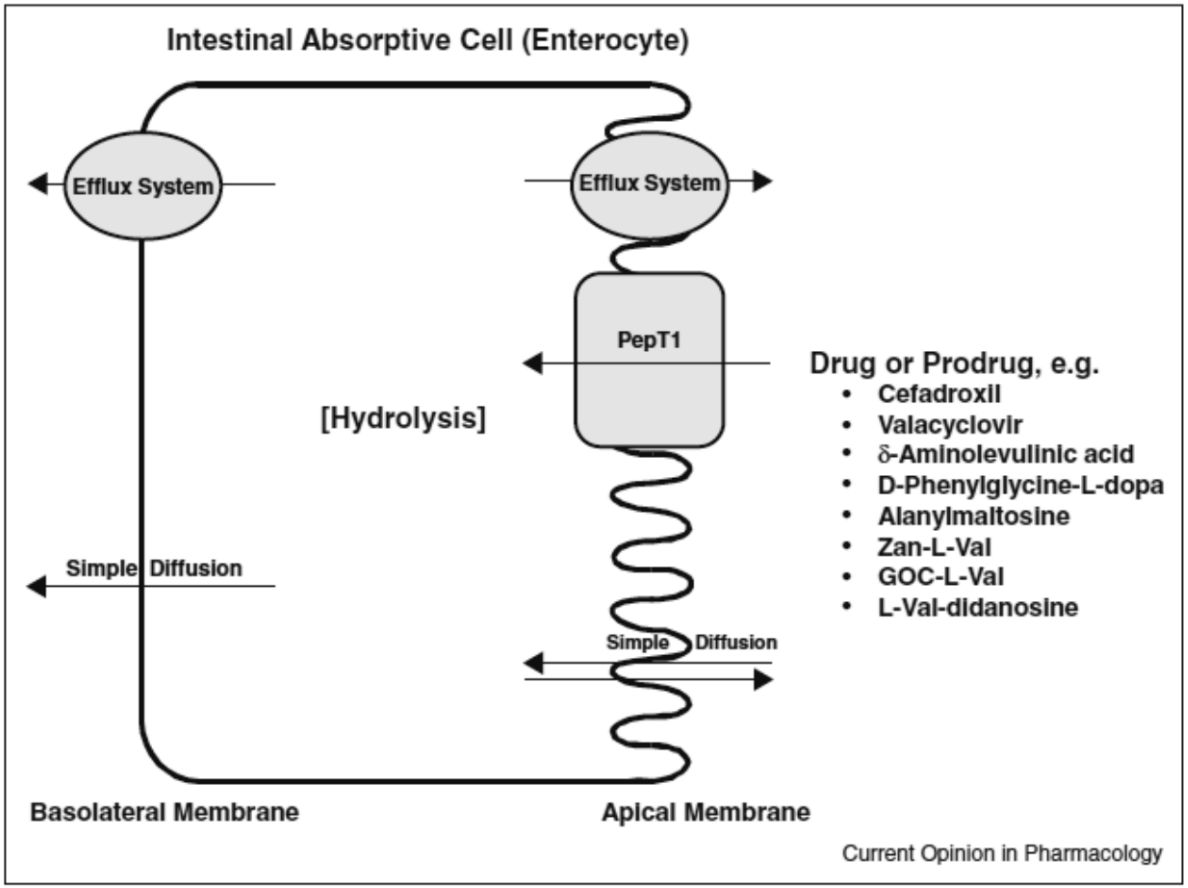


Figure 2. Expression levels of ABC and SLC transporters in different parts of the human intestine.

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

Examples of drugs transported by one intestinal inward peptide transporter



Recent examples of drug transport by the intestinal proton-coupled peptide transporter PepT1. Following uptake via PepT1, plus simple diffusion, drugs may then be transported out of the cell by basolateral solute carriers, channels or pumps that function as efflux transporters. In addition, the compounds can leave the cells also by simple diffusion along their concentration gradient. Backflux can occur across the apical membrane to the lumen. Zan-L-Val, L-valyl prodrug of zanamivir, GOC-L-Val, L-valyl prodrug of guanidine oseltamivir carboxylate.

Brandsch M. Current Opinion in Pharmacology 2013, 13:881–887
http://dx.doi.org/10.1016/j.coph.2013.08.004

More about inward intestinal transporters

Table 2
Drug uptake transporters identified in the intestine.

Drug transporter	*Gene*	Intestinal localization	Substrate specificity	Substrates	Inhibitors	Inducers	Polymorphisms
PEPT1	*SLC15A*	Apical	Di–tri-peptides	Cephalosporins, penicillins, enalapril, alacepril, oseltamivir, renin inhibitors, thrombin inhibitors, betastin, L-alpha-methyldopa-phenylalanine, D-phenylglycine-L-alpha-methyldopa, L-val-acyclovir, 5′-O-L-valyl didanosine	Gly-Sar, zinc ions, lisinopril, JBP485		
OCTN1	*SLC22A*	Apical	Carnitine and organic cations	TEA, quinidine, verapamil, ergothioneine, pyrilamine	Levofloxacin, grepafloxacin	Oxidized fats	
OCTN2	*SLC22A*	Apical	Carnitine and organic cations	TEA, quinidine, verapamil, ergothioneine, pyrilamine, cephaloridine, imatinib, ipatropium, valproic acid, spironolactone.	Levofloxacin, grepafloxacin	Oxidized fats, clofibrate	
OCT1/ OCT2	*SLC22A*	Basal	Low molecular weight organic cations	TEA, metformin, acyclovir, zalcitabine, memantine, ranitidine			
PMAT	*SLC29*	Apical	Organic cations	1-Methyl-4-phenylpyridinium (MPP+), tetraethylammonium, serotonin, dopamine, epinephrine, norepinephrine, guanidine, histamine, metformin	Decynium-22, GBR12935, fluoxetine, desipramine, cimetidine, quinidine, quinine, verapamil, rhodamine123		
OATP2B1	*SLCO*	Apical	Organic anions	Estrone-3-sulfate, BSP, pravastatin, DHEAS, rosuvastatin, atorvastatin, pitavastatin, fexofenadine, mesalazine, glyburide, taurocholate, aliskiren	Grapefruit juice, green tea, sirolimus, everolimus, budesonide, cyclosporine, rifampin.		Yes
OATP1A2	*SLCO*	Apical	Organic anions	Bile salts, BSP, steroid sulfates, thyroid hormones, prostaglandin E2, fexofenadine, opioid peptides, N-methylquinine, N-methylquinidine, ouabain, BQ-123, talinolol, CRC-220, celiprolol, atenolol, ciprofloxacin	Grapefruit, orange and apple juices, green tea, sirolimus, everolimus, budesonide, cyclosporine, rifampin.		Yes
MCT1	*SLC16A*	Apical	Unbranched aliphatic and substituted monocarboxylates	Acetate, pyruvate, lactate, acetoacetate, β-hydroxybutyrate, p-aminohippuric acid, benzoic acid, foscarnet, mevolonic acid, salicylic acid, carbenicillin indanyl sodium, phenethicillin, propicillin.			

Modulation of bioavailability by excipients

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

International Journal of Pharmaceutics 393 (2010) 17–31

journal homepage: www.elsevier.com/locate/ijpharm

Review

The effects of excipients on transporter mediated absorption

Jonathan Goole[a,b,*], David J. Lindley[a], Wyatt Roth[a], Stephen M. Carl[a], Karim Amighi[b], Jean-Michel Kauffmann[b], Gregory T. Knipp[a]

[a] Department of Industrial and Physical Pharmacy, College of Pharmacy, Nursing and Health Sciences, Purdue University, West Lafayette, IN, USA
[b] Department of Pharmaceutical Sciences, Université Libre de Bruxelles, Brussels, Belgium

Modulation of bioavailability by excipients

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The effects of excipients on transporter me

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[a] Department of Industrial and Physical Pharmacy, College of Pharmacy, Nursing and Health Sci
[b] Department of Pharmaceutical Sciences, Université Libre de Bruxelles, Brussels, Belgium

Cremophor™ RH40, a surfactant widely used as a solubilizer in pharmaceutical preparations, inhibits both P-gp and the subfamily CYP3A in a concentration-dependent manner, which explains the increase in bioavailability of P-gp substrates *in vivo*…

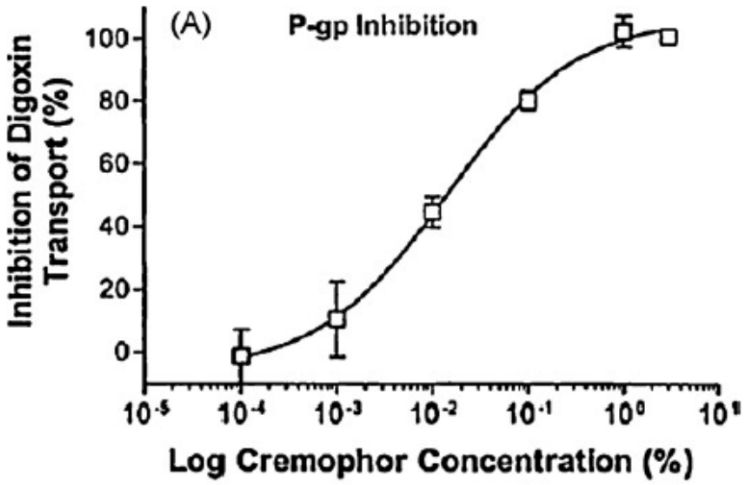

Fig. 3. Inhibition of P-gp mediated, tritium-labeled digoxin transport in Caco-2 cells (A) and CYP3A-mediated nifedipine oxidation in human liver microsomes (B) in the presence of increasing concentrations of Cremophor™ RH40 ($n = 3$) (from Wandel et al., 2003).

Blood-Brain Barrier … multiple drug transporters

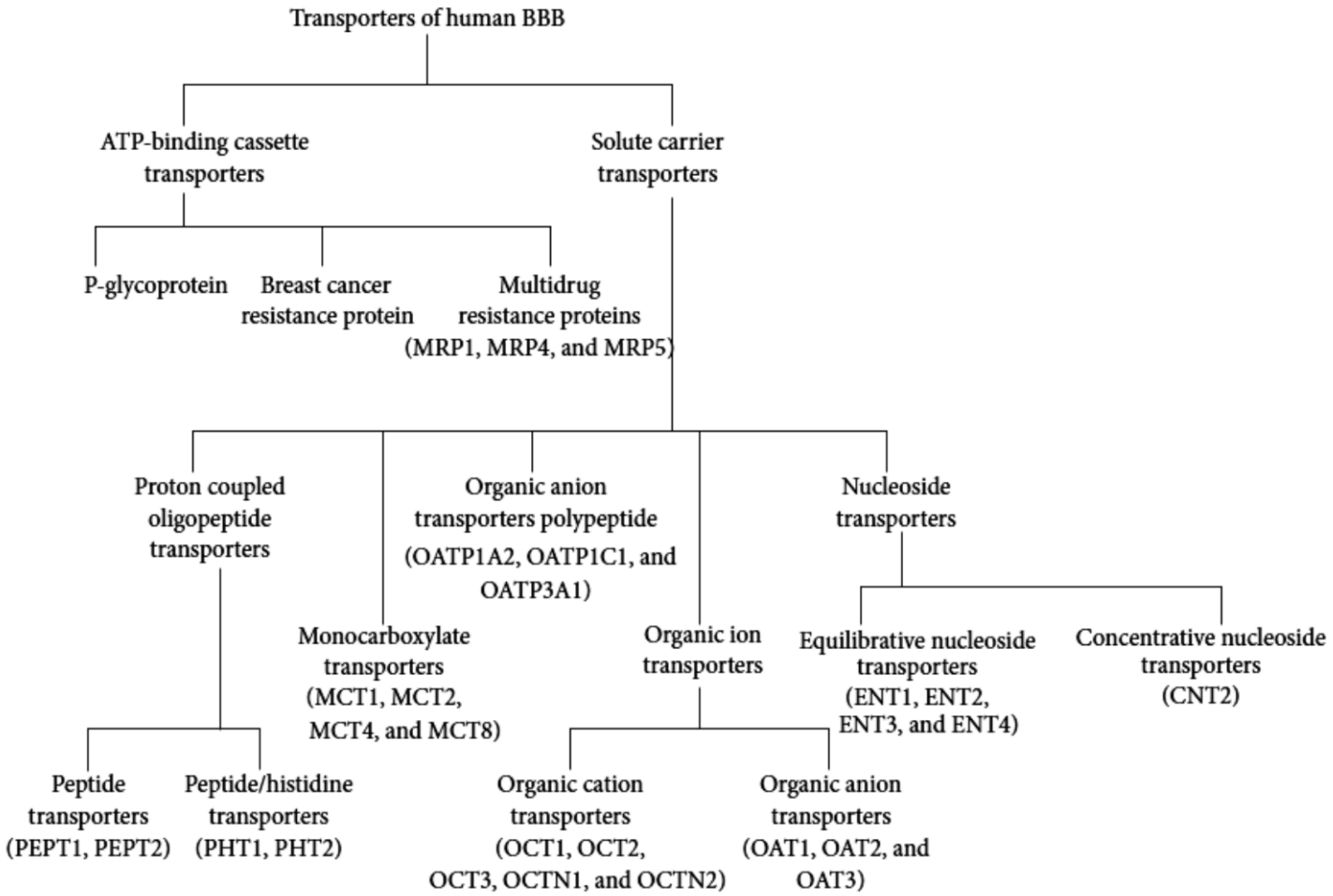

FIGURE 2: Schematic classification of transporters of human BBB. Two main classes of drug transporters are ATP-binding cassette (ABC) transporters and solute carrier transporters. Each of them is further classified into several other transporters mentioned in the flowchart. More information about each of the transporters is mentioned in the text.

BioMed Research International (2015) 2015, Article ID 320941
http://dx.doi.org/10.1155/2015/320941

Blood-Brain Barrier … transporters are protective

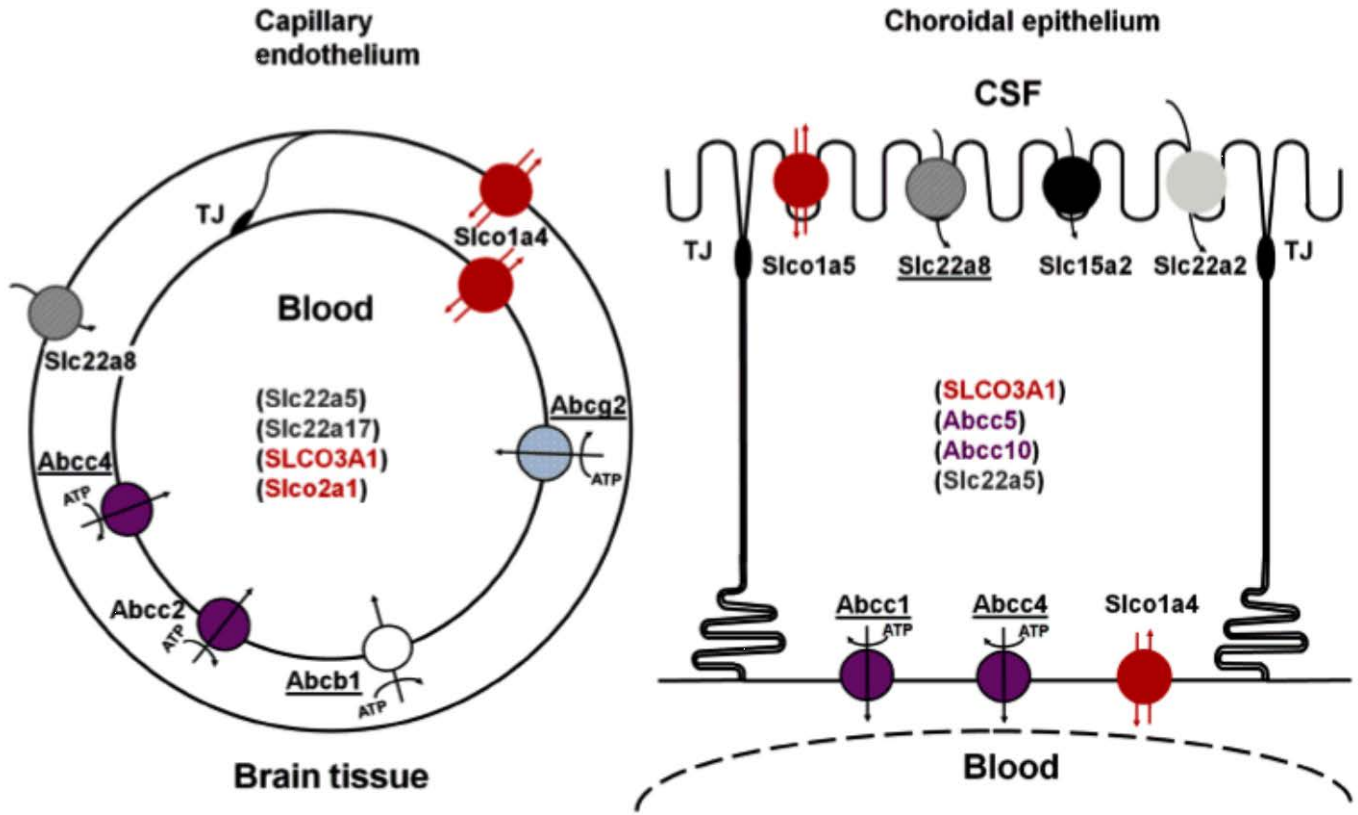

FIGURE 1 | Schematic representation of the main multispecific transporters involved in neuroprotective efflux at the blood-brain (left) and blood-CSF (right) barriers. Underlined names represent transporters for which evidence has also been reported in human. The ATP-dependency is shown only for primary ATP-dependent transporters. Transporters in parenthesis are carriers for which evidence of their involvement in CNS-blood efflux processes is still limited. See text for references. TJ, tight junction.

Strazielle & Ghersi-Egea (2015) Frontiers in Neuroscience 9: article 21
http://dx.doi.org/10.3389/fnins.2015.00021

One slide on kidney … or the story of tenofovir

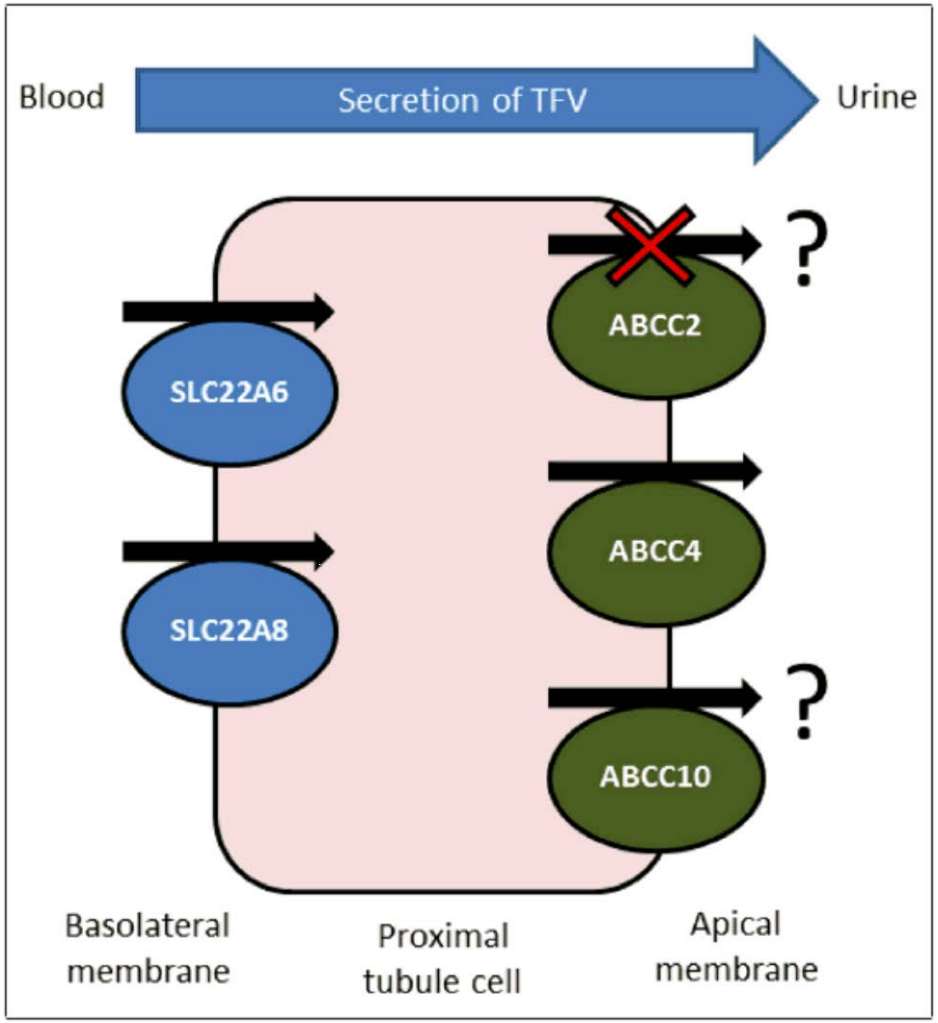

FIGURE 1 | Confirmed and potential transporters involved in active tubular secretion of tenofovir into urine. Tenofovir is removed from the circulating blood and enters the proximal tubule cells by the actions of basolaterally expressed SLC22A6 and, to a lesser extent, SLC22A8. Tenofovir is then removed into the tubular lumen by apically expressed ABCC4. ABCC2 does not transport tenofovir *in vitro* but pharacogenetics suggests ABCC2 has a role in tenofovir-induced renal toxicity. The orientation of ABCC10 in proximal tubule cells is unknown, but *in vitro* and pharmacogenetic data suggest that expression may be localized to the apical membrane, facilitating tenofovir secretion.

ABCC2 polymorphism has been associated with kidney damage phenotypes in patients taking tenofovir

Warning:
the kidney has > 26
drug transporters

Moss et al. Frontiers in Pharmacology - 11 November 2014
http://dx.doi.org/10.3389/fphar.2014.00248

A global overview of transporters in eukaryotic cells

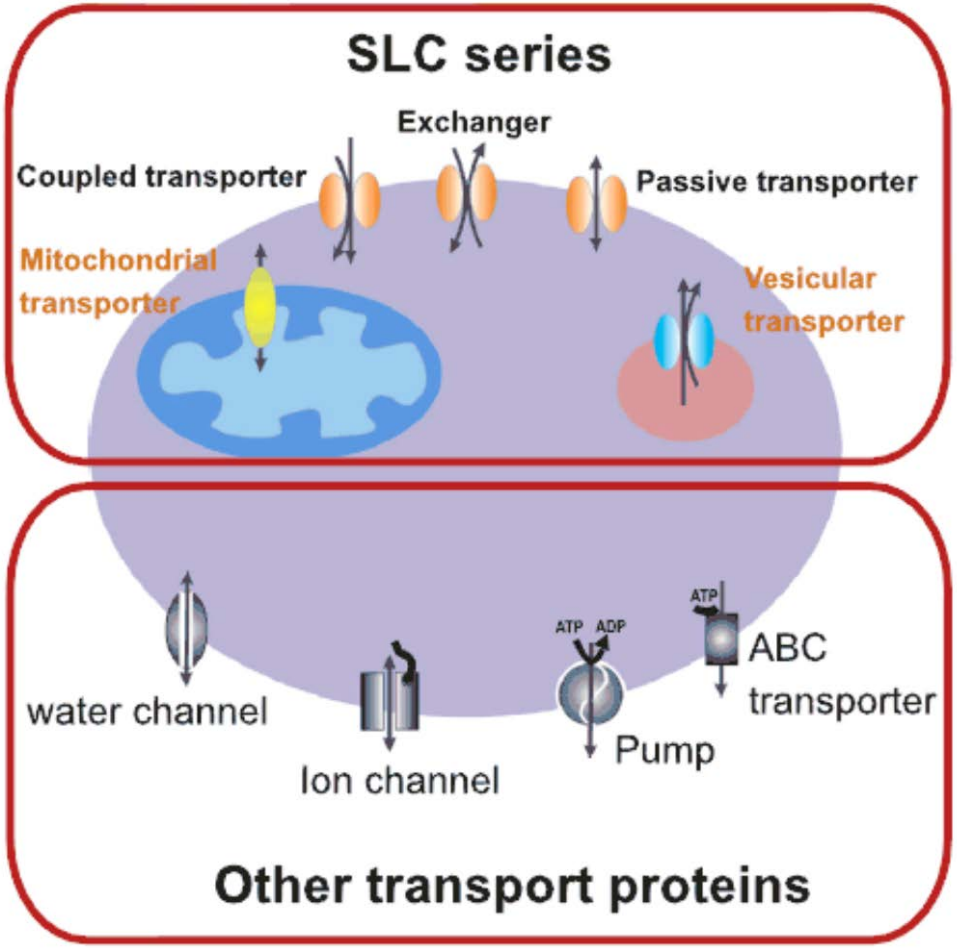

Fig. 1. Cartoon showing a cell with SLC and non-SLC transporters expressed in the plasma membrane or in intracellular compartments. Note that the non-SLC transporters are also expressed in intracellular compartments.

Hediger et al. Molecular Aspects of Medicine 34 (2013) 95–107

The Menu …

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- From bacteria to eukaryotic cells
  - *ABC (and some of the others)*
    - bioavailability, intestine, blood-brain-barrier
    - **cancer … and a surprise…**
- Why we still fail …

Efflux-related 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

An (unanticipated ?) type of resistance in plants…

Review

SCI

Received: 5 November 2013 Revised: 17 January 2014 Accepted article published: 25 January 2014 Published online in Wiley Online Library: 12 March 2014

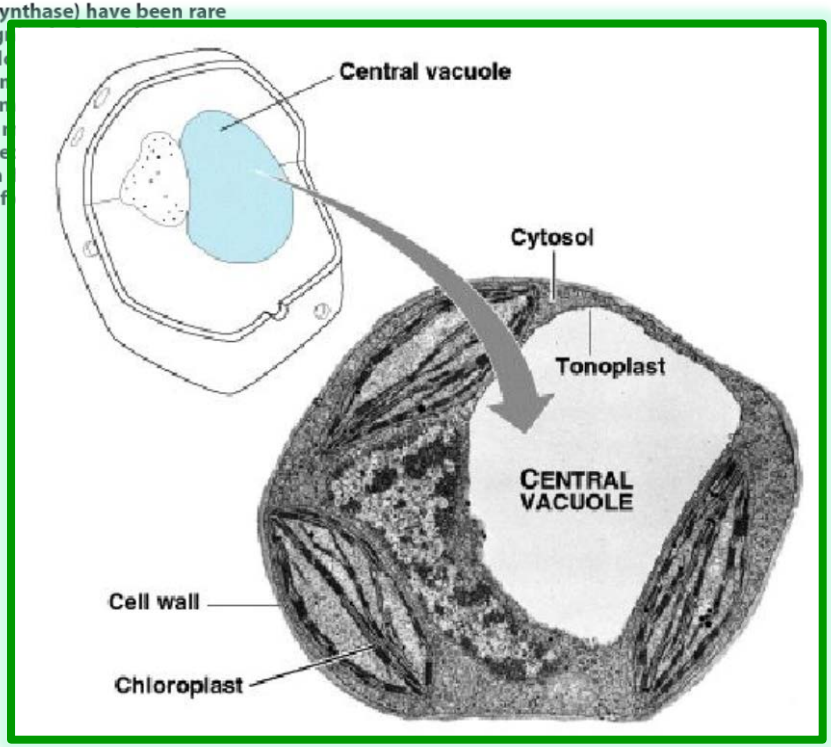
(wileyonlinelibrary.com) DOI 10.1002/ps.3743

Glyphosate resistance: state of knowledge

Robert Douglas Sammons[a*] and Todd A Gaines[b]

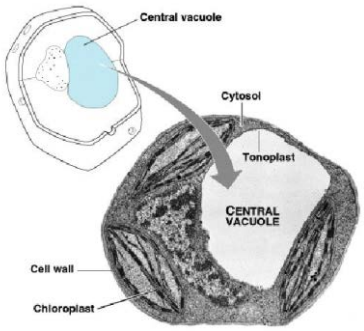
Abstract

Studies of mechanisms of resistance to glyphosate have increased current understanding of herbicide resistance mechanisms. Thus far, single-codon non-synonymous mutations of *EPSPS* (5-enolypyruvylshikimate-3-phosphate synthase) have been rare and, relative to other herbicide mode of action target-site mutations, unconventionally weak in mag glyphosate. However, it is possible that weeds will emerge with non-synonymous mutations of two cod an enzyme endowing greater resistance to glyphosate. Today, target-gene duplication is a commo mechanism and could become a fundamental process for developing any resistance trait. Based on co selectivity studies in several species, rapid vacuole sequestration of glyphosate occurs via a transporter as the chloroplast requires transporters for uptake of important metabolites, transporters associate membranes may separately, or together, successfully block glyphosate delivery. A model based on and limiting time required for chloroplast loading sets the stage for understanding how uniquely dif contribute to overall glyphosate resistance.



From Dave Krupp's lectures on plants
http://krupp.wcc.hawaii.edu/BIOL101/present/celllect/sld019.htm

An (unanticipated ?) type of resistance in plants…



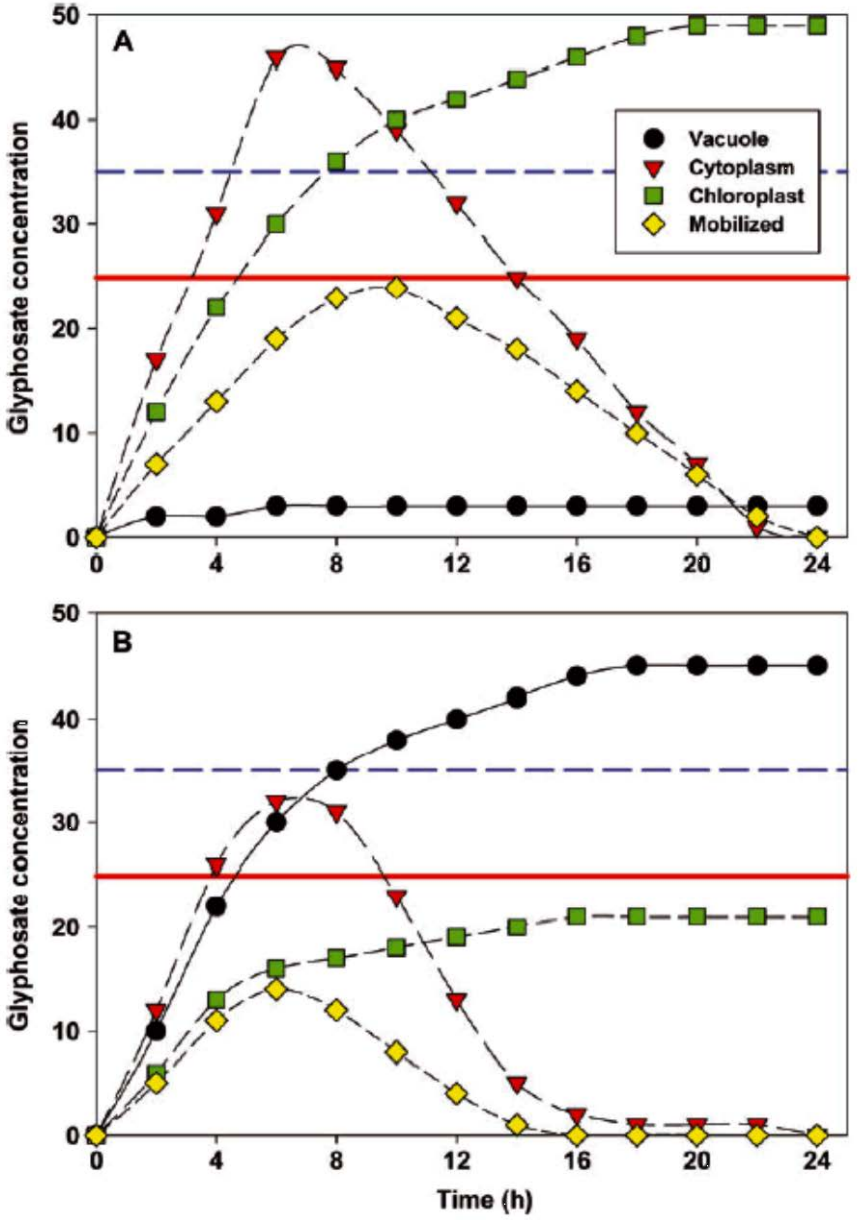


Figure 1. A theoretical cellular-level model of glyphosate uptake and distribution for (A) a normal, glyphosate-susceptible source cell and (B) a glyphosate-resistant source cell using the vacuole to sequester glyphosate. The units for glyphosate are relative concentration, with a theoretical chloroplast minimum inhibitory concentration (25) indicated by a red line, and a chloroplast glyphosate concentration (35) consistent with saturated inhibition indicated by a dashed blue line.

ATP-binding cassette (ABC) transporters and a tonoplast intrinsicprotein (TIP) may be responsible for diversion of glyphosate into the vacuole and decreasing its concentration in the chloroplasts.

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    - bioavaibility, intestine, blood-brain-barrier
    - cancer … and not a surprise…
- **Why we still fail …**

Can we really prevent efflux-mediated resistance ?

Characteristics of the ideal EPI

http://www.louisethompson.com/to-do-list/

« 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

Examples of active … but toxic molecules

Against bacterial RND…

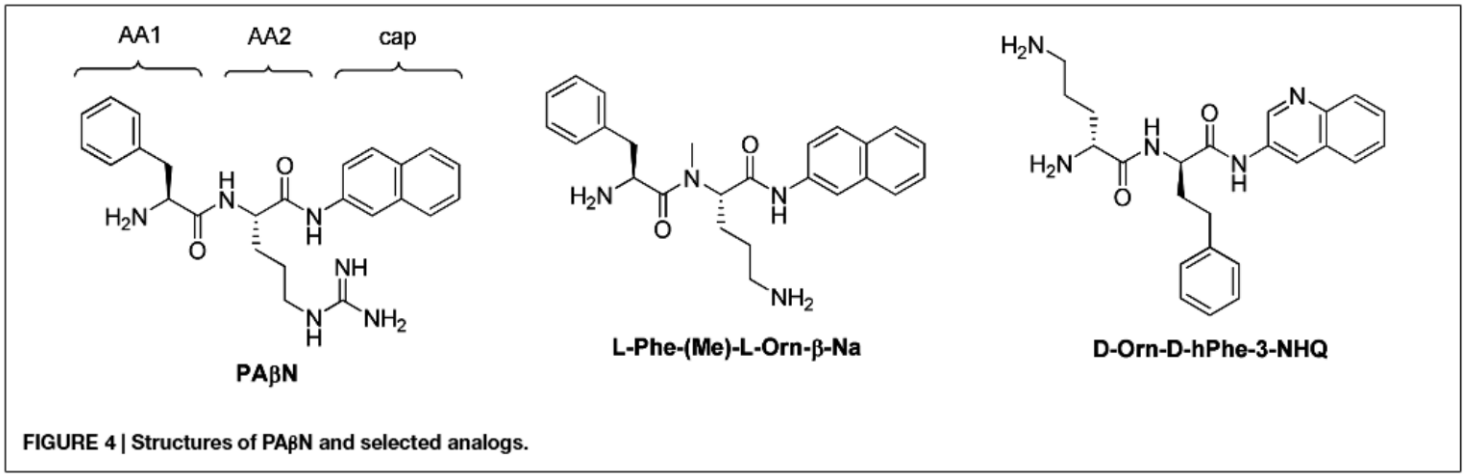
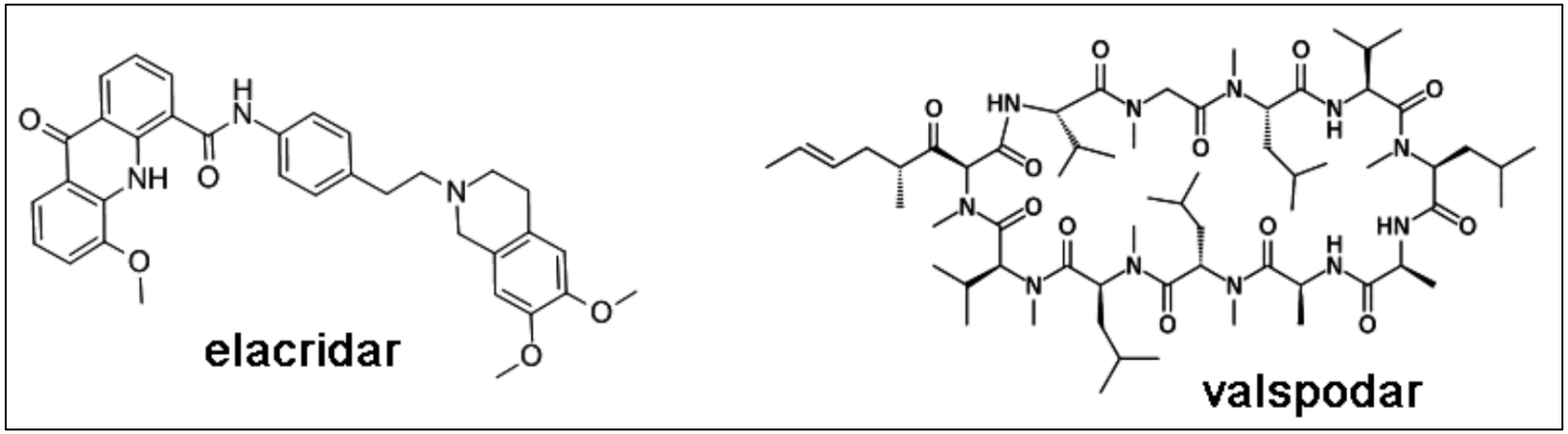


FIGURE 4 | Structures of PAβN and selected analogs.

Against human PgP (ABC)…



An example of failure

CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B Study 9720

Maria R. Baer, Stephen L. George, Richard K. Dodge, Kieran L. O'Loughlin, Hans Minderman, Michael A. Caligiuri, John Anastasi, Bayard L. Powell, Jonathan E. Kolitz, Charles A. Schiffer, Clara D. Bloomfield, and Richard A. Larson

Blood. 2002;100:1224-1232

An example of failure …

CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leuken

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Bayard L. Powell, Jonathan E. Kolitz, Charles A. Sc

Blood. 2002;100:1224-1232

ADE: etoposide alone
ADEP: etoposide + valspodar

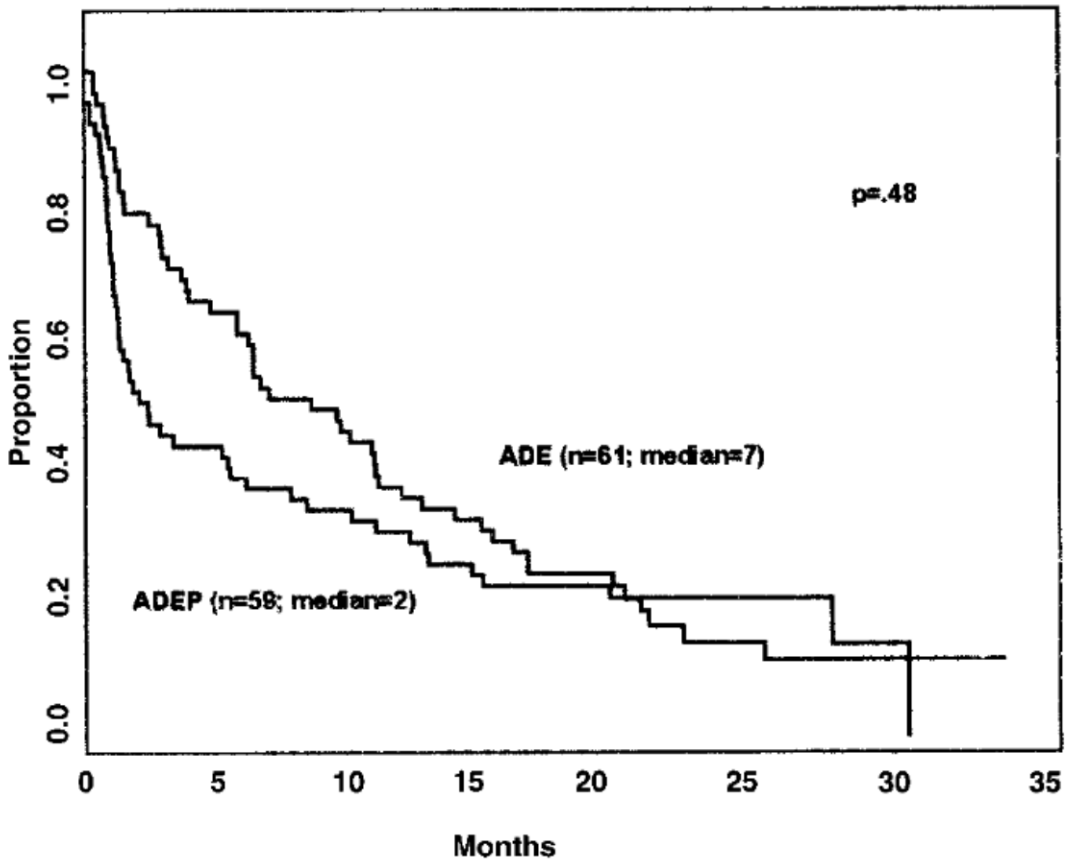

Figure 1. Survival by arm. Survival of patients treated with ADE and with ADEP.

Potential interests of efflux pumps inhibitors for therapeutic developments ?

This ?

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But then, wat to do ? … Checking, please !

Table 1 | Recommendations for drug transporter testing as outlined in the EMA Guideline on Investigation of Drug Interactions, July 2012, and the FDA Draft Guidance on Drug Interaction Studies, February 2012.

			Inhibition studies		Substrate studies	
	Transporter	Other name	EMA	FDA	EMA	FDA
Efflux	**ABCB1**	**P-gp**	Yes	Yes	Consider	Yes
	ABCG2	**BCRP**	Yes	Yes	Consider	Yes
	ABCB11	**BSEP**	Preferred	Consider	Consider	Consider
	ABCCs	**MRPs**	No	Consider	Consider	Consider
Uptake	**SLC22A6**	**OAT1**	Yes	Yes	Consider	If >25% active renal secretion
	SLC22A8	**OAT3**	Yes	Yes	Consider	If >25% active renal secretion
	SLCO1B1	**OATP1B1**	Yes	Yes	If >25% clearance is hepatic	If >25% clearance is hepatic or biliary
	SLCO1B3	**OATP1B3**	Yes	Yes	If > 25% clearance is hepatic	If >25% clearance is hepatic or biliary
	SLC22A1	**OCT1**	Consider	No	Consider	No
	SLC22A2	**OCT2**	Yes	Yes	Consider	If >25% active renal secretion
	SLC47A1	**MATE1**	Consider	Consider	Consider	Consider
	SLC47A2	**MATE2K**	Consider	Consider	Consider	Consider

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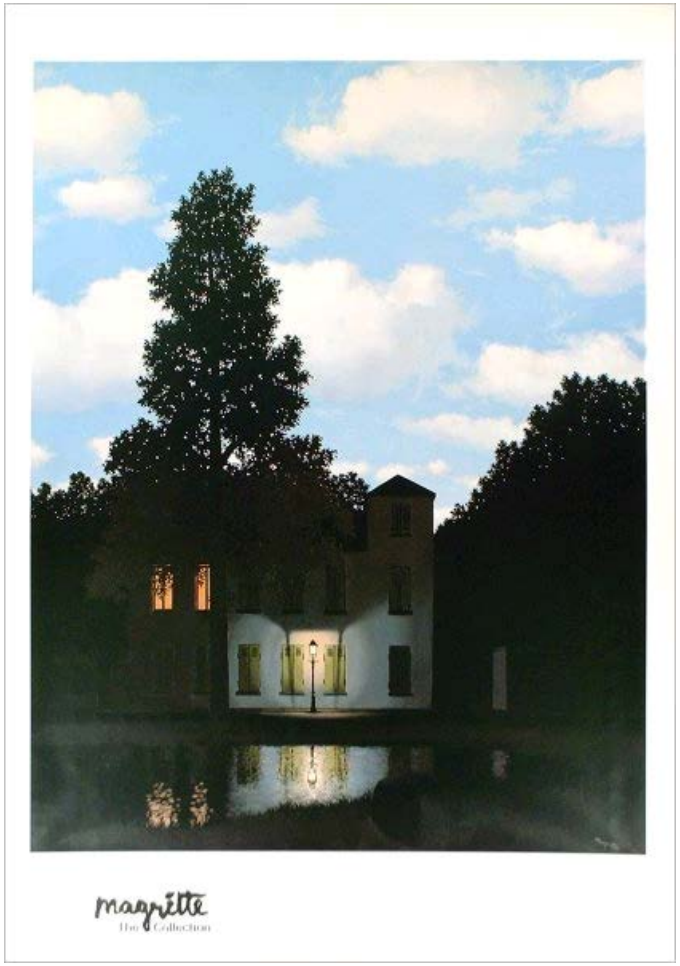
http://dx.doi.org/10.3389/fphar.2014.00248

A still uncertain future for EPI ....



But still a lot of work ahead ….

The abundance of transporters in eukaryotic cells

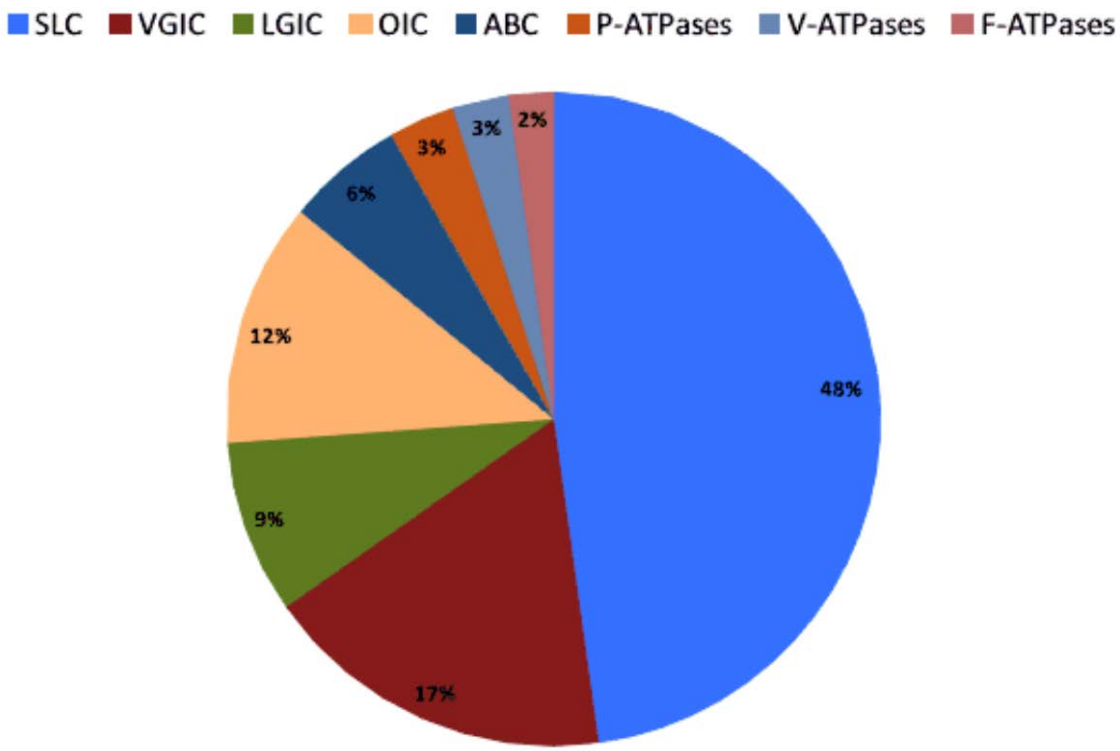


Fig. 2. Pie chart depicting the proportion of genes encoding transporter-related proteins (total number: 826).

SLC = solute carrier;
VGIC = voltage gated ion channels
LGIC = ligand gated ion channels
OIC = other ion channels (e.g. aquaporins, connexins);
ABC = ABC transporters;
P-ATPases = P-type ATPases
V-ATPases = V-type ATPases
F-ATPases = F-type ATPases.

Hediger et al. Molecular Aspects of Medicine 34 (2013) 95–107