

# Multidrug Resistance and ABC Transporters in Parasitic Protozoa

Marc Ouellette\*, Danielle Légaré  
and Barbara Papadopoulos

Centre de Recherche en Infectiologie du Centre de Recherche du CHUL and Département de Microbiologie, Faculté de Médecine, Université Laval, 2705 boul. Laurier, Ste-Foy, Québec, G1V 4G2, Canada

## Abstract

**Drug resistance is an important problem in parasitic protozoa. We review here the role of ABC transporters in drug resistance in parasites. We have concentrated on gene and gene products for which there is a strong evidence for their role in resistance.**

## Introduction

Parasitic protozoa are responsible for some of the most devastating and prevalent diseases of humans and domestic animals. Protozoan parasites threaten the lives of nearly one third of the worldwide human population and also result in considerable losses of life and productivity of domesticated animals. Ideally, prevention would be the most efficient way to control parasitic protozoa but despite considerable efforts, there are no effective vaccines against any of the main parasites. For the moment, drugs are therefore the mainstays in our control of parasitic protozoan. However, the arsenal of antiprotozoal drugs is limited and this is exacerbated by the emergence of drug resistance. With effective vaccines not yet in sight and the development of new drugs proceeding slowly, the emergence of drug resistance in parasitic protozoa is therefore becoming a public health problem. In this manuscript, we will show that the field of drug resistance studies in parasitic protozoa is currently very active and that our understanding of drug resistance and multidrug resistance is progressing steadily. As we have already indicated (Borst and Ouellette, 1995), studies of resistance mechanisms can help in developing tools to recognize resistance; but can also point the way to more rational use of drugs and drug combinations to minimize or circumvent resistance; or finally to pinpoint intracellular targets for development of drugs not affected by the most common defenses.

In this review, we will concentrate on selective examples that we believe illustrate well our current understanding of drug resistance in parasitic protozoa mediated by membrane proteins. In particular, we will emphasize the role of ABC transporters in drug resistance. The genomes of a number of parasites are now underway

and numerous ABC transporters are being unraveled, although the function and localization of most of them are unknown. However, definitive proofs exist for some parasitic ABC transporters and drug resistance. Several mechanisms could account for increased activities of ABC transporters leading to drug resistance. This could be due to an increased amount of proteins due to gene amplification or overexpression; to point mutations in the structural gene; or if to be pumped out, the drug needs to be modified, possibly the rate of modification of the drug will determine the rate of transport. In addition to ABC transporters, other membrane proteins of parasites are involved in drug resistance. For example, down regulation of transport systems can lead to arsenical resistance in trypanosomes (Carter and Fairlamb, 1993; Maser *et al.*, 1999) or antifolate resistance in *Leishmania* (Kündig *et al.*, 1999).

## ABC Transporters

The ATP-binding cassette (ABC) proteins are ubiquitous and most of these proteins mediate transport across biological membranes (Higgins, 1992). The ATP-binding domains of the ABC proteins include the Walker A and B motifs and the "signature" or "C" motif just upstream of the Walker B site which distinguish members of the ABC superfamily from other ATP-binding proteins. The sequence conservation of the ABC domains has allowed the isolation of new ABC genes by hybridization, degenerated PCR, and by inspection of DNA sequence databases. The latter strategy is now the most efficient one and inventories and classification of ABC proteins have been made for several genomes (Decottignies and Goffeau, 1997; Klein *et al.*, 1999; Linton and Higgins, 1998; Quentin *et al.*, 1999; Saurin *et al.*, 1999; Taglicht and Michaelis, 1998; Tomii and Kanehisa, 1998). The family of ABC transporters is one of the largest families of proteins. In genomes whose sequence has been completed they represent a significant percentage of the whole coding regions with 29 genes in yeast (Decottignies and Goffeau, 1997) to more than 79 in *E. coli* (Blattner *et al.*, 1997). The human ABC transporters have been divided in at least seven subclasses (see <http://www.med.rug.nl/mdl/humanabc.htm>), while several more subclasses have been described for bacteria (<http://www-biology.ucsd.edu/~msaier/transport/titlepage2.html>). Not all ABC transporters have transmembrane domains and not all appear to be involved in drug resistance.

## Multidrug Resistance in Malaria Parasites

Chloroquine is the drug of choice for treating malaria, however resistance to chloroquine is widespread in every geographic region in which malaria is endemic. Chloroquine acts by inhibiting polymerization of the toxic heme that is released during hemoglobin degradation within the

\*For correspondence. Email [Marc.Ouellette@crchul.ulaval.ca](mailto:Marc.Ouellette@crchul.ulaval.ca);  
Tel. 418-654 2705; Fax. 418-654 2715.

digestive vacuole of the parasite (Sullivan *et al.*, 1996). The reduced efficacy of chloroquine and antifolate-based chemotherapy has led to increased use of mefloquine and artemisinin. In some part of the world, however, *Plasmodium falciparum*, the agent responsible for the most severe form of malaria, has become resistant to almost all antimalarial agents (Warhurst, 1999; White, 1998). Resistance to chloroquine shares several phenotypic features with multidrug resistance of mammalian tumor cell lines. In 1987, it was reported that chloroquine efflux is increased in resistant parasites (Krogstad *et al.*, 1987) while verapamil, the classical agent to reverse MDR in animal cells, is able to restore in part chloroquine sensitivity to resistant cells (Martin *et al.*, 1987). These observations led to the isolation of the ABC transporter *pfmdr1*, which is similar in structure and sequence to the mammalian P-glycoprotein (Foote *et al.*, 1989; Wilson *et al.*, 1989). The initial hypothesis that the *pfmdr1* gene product Pgh1 was the efflux pump that caused increased efflux of chloroquine has been under intensive scrutiny. This model was initially supported by the preferential association of chloroquine resistance with specific point mutations in *pfmdr1* (Foote *et al.*, 1990). This simple model was not supported, however, by the result of one genetic cross which indicated that the main resistance gene was on chromosome 7 on which *pfmdr1* is not located (Wellems *et al.*, 1991). The gene on chromosome 7 encodes for a membrane protein, PfCRT, localized to the parasite digestive vacuole (Fidock *et al.*, 2000), the site of action of chloroquine. Several mutations in PfCRT, in particular the K76T mutation, are linked to resistance and PfCRT, through a modulation of the pH of the digestive vacuole, may confer resistance by altering chloroquine transport or binding to heme (Fidock *et al.*, 2000).

Is Pgh1 involved in resistance and is it responsible for chloroquine efflux? Considerable debate has revolved around those issues but we can now comfortably answer yes to at least the first part of the question. Amplification of *pfmdr1* is observed in several isolates but when those are selected for higher chloroquine resistance *in vitro*, it results in deamplification of the *pfmdr1* gene. This deamplification is associated with an increase in mefloquine sensitivity (Barnes *et al.*, 1992). Conversely, *in vitro* selection for mefloquine resistance in *P. falciparum* led to *pfmdr1* amplification which is accompanied by increased sensitivity to chloroquine (Cowman *et al.*, 1994; Peel *et al.*, 1994). Two recent studies have shed considerable light on the role of *pfmdr1* in antimalarial resistance. By gene transfection it was demonstrated that mutations at residues 1034, 1042 and 1246 of Pgh1 can lead to quinine resistance in various cell backgrounds and are also involved in chloroquine resistance although the latter depends on the strain background (Reed *et al.*, 2000). This is a further indication that resistance to chloroquine is multifactorial but that Pgh1 is implicated. Interestingly, the presence of these mutations leads to mefloquine and halofantrine sensitivity (Reed *et al.*, 2000). The analysis of a genetic cross has also implicated mutations in the *pfmdr1* gene of *P. falciparum* with increased sensitivity to mefloquine and artemisinin (Duraisingh *et al.*, 2000). Thus, amplification of wild-type *pfmdr1* can lead to mefloquine resistance and chloroquine susceptibility while specific point mutations in

*pfmdr1* will lead to chloroquine resistance and to mefloquine sensitivity. Changes in *pfmdr1* can modulate resistance to a number of structurally unrelated drugs and is therefore part of a true MDR phenotype.

The mechanism by which Pgh1 confers resistance or sensitivity to drugs remains to be firmly established. Interestingly, it was shown by transfection experiments that *pfmdr1* status can modulate the transport of either chloroquine or mefloquine with wild-type alleles associated with increased chloroquine accumulation, hence increased sensitivity (Reed *et al.*, 2000). Interestingly, Pgh1, as the PfCRT, is localized to the membrane of the digestive vacuole (Cowman *et al.*, 1991). It remains to be established whether the modulation in transport is mediated directly by Pgh1 or by indirect means such as modulation in pH (Bray *et al.*, 1999; van Es *et al.*, 1994). It is possible that the resistance-linked mutations observed in PfCRT and Pgh1, which are correlated with a change in the pH of the digestive vacuole, are compensated by other mutations, some of which may be involved in the resistance phenotype. Indeed, several studies are suggesting that the *in vivo* drug response of the infected patient is not tightly linked to the main mutations in PfCRT and Pgh1.

In addition to *pfmdr1*, at least two other ABC transporters have been described in *P. falciparum*, *pfmdr2* (Rubio and Cowman, 1994; Zalis *et al.*, 1993) and GCN20 (Bozdech *et al.*, 1998) but neither of them appears to be involved in drug resistance.

### ABC Transporters in Leishmania

Pentavalent antimonial compounds are the drugs of choice for treating all form of leishmaniasis, a number of diseases ranging from self-healing cutaneous lesions to fatal visceral infection, caused by various species of the protozoan parasite *Leishmania*. Antimony resistant *Leishmania* are found with increased frequency throughout endemic areas, and in some part of India they can reach as much as 50% of the isolates (Faraut-Gambarelli *et al.*, 1997; Grogl *et al.*, 1992; Ibrahim *et al.*, 1994; Jackson *et al.*, 1990; Lira *et al.*, 1999; Sundar *et al.*, 1997). Our understanding of metal resistance in *Leishmania* is derived almost exclusively from *in vitro* work and it remains to be seen whether the mechanism found in lab strains will also occur in unresponsive strains isolated from patients.

The ABC transporter PGPA (Ouellette *et al.*, 1990) has been implicated in metal resistance by analysis of drug resistant mutants in which the PGPA gene was found to be amplified, and by gene transfection experiments (Callahan and Beverley, 1991; Detke *et al.*, 1989; Légaré *et al.*, 1997; Ouellette *et al.*, 1991; Papadopoulou *et al.*, 1994). PGPA is more closely related to the multidrug resistance protein (MRP) of mammalian cells than to the human P-glycoprotein (Légaré *et al.*, 1994). It was shown by transfection that in several genetic backgrounds PGPA was only able of conferring low level resistance to metals. This led to our suggestion that PGPA requires other factors for conferring resistance and by analogy to the MRP (Borst *et al.*, 1999) and the GS-X pumps (Ishikawa *et al.*, 1994), these other factors may be involved in thiol metabolism (Borst and Ouellette, 1995). This contention was reinforced by our discovery that trypanothione, constituted of two

glutathione molecules conjugated to spermidine and the major reduced thiol of *Leishmania*, was increased in all metal resistant *Leishmania* analyzed (Haimeur *et al.*, 2000; Légaré *et al.*, 1997; Mukhopadhyay *et al.*, 1996). The basis for this increase is understood: it is due to amplification of the *GSH1* gene (Grondin *et al.*, 1997) and overexpression of the *ODC* gene (Haimeur *et al.*, 1999) coding for  $\gamma$ -glutamylcysteine synthase and ornithine decarboxylase respectively, the two rate limiting steps in glutathione and spermidine biosynthesis. The co-transfection of *PGPA* and *GSH1* (or *ODC*) indicated that the two gene products act synergistically to confer high level resistance to metals (Grondin *et al.*, 1997) (Haimeur *et al.*, 1999). This synergistic interaction is observed, however, only into a specific genetic background and, by analogy to the GS-X pump mediated resistance mechanisms, we are proposing that *PGPA* metal-mediated resistance is due to a combination of higher levels of trypanothione, higher activity of a putative trypanothione-metal conjugase, and increased activity of the transporter. An increase in only one of these steps is not sufficient to observe high level resistance. Several evidences are supporting this model although proofs for increased activity of the conjugase are still required.

The mechanism by which *PGPA* confers resistance is becoming clearer. Transport experiments has linked *PGPA* with decreased uptake (Callahan *et al.*, 1994), increased efflux (Singh *et al.*, 1994) and without any marked effects on the steady-state accumulation of the metals (Papadopoulou *et al.*, 1994). Subcellular localisation experiments have recently been completed and indicated that *PGPA* is clearly located intracellularly and not in the plasma membrane suggesting that it may confers resistance by sequestering the metal-thiol conjugates into vesicles. Preliminary attempts using isolated vesicles enriched for *PGPA* have shown indeed that these vesicles can transport metal-thiol conjugates (Légaré *et al.*, in preparation). The nature of these vesicles are currently under investigation but resistance by sequestration of metal-thiol conjugates via ABC transporters have already been described in yeast (Ghosh *et al.*, 1999; Li *et al.*, 1997; Ortiz *et al.*, 1995; Tommasini *et al.*, 1996).

A common mechanism of resistance to metals in *Leishmania* is active efflux of the metal (Dey *et al.*, 1994) and studies of everted membrane vesicles have shown that this system also recognizes metal-thiol conjugates (Dey *et al.*, 1996). This ATP-dependent efflux system is not rate limiting however, and the rate of formation of the substrate may be the rate limiting step and hence govern the rate of efflux (Dey *et al.*, 1996). *PGPA* is part of a multigene family with at least 4 other members named *PGPB* to *PGPE* all similar to *MRP* (Légaré *et al.*, 1994). It is possible that one of these genes may correspond to this efflux system.

The sequence of the *Leishmania* genome is rapidly progressing and several other ABC transporters are being discovered. One for which considerable work has been done is the *Leishmania MDR1* gene (Henderson *et al.*, 1992). This gene, which is highly similar to the human P-glycoprotein, is amplified in several *Leishmania* species selected for a number of drugs part of the mammalian MDR spectrum and transfection of the *MDR1* gene indeed

bestow resistance to these drugs (Chiquero *et al.*, 1998; Chow *et al.*, 1993; Gueiros-Filho *et al.*, 1995; Katakura *et al.*, 1999). *Leishmania* is usually not in contact with these drugs but the *MDR1* gene product may provide resistance against other xenobiotics related to these drugs. The mechanism by which the *Leishmania MDR1* confers resistance is unclear although sequestration in vesicles close to the mitochondria is one favored mechanism (Chow and Volkman, 1998). The mechanism by which the *Leishmania MDR1* gene confers resistance appears therefore to differ considerably from the mechanism found in mammalian cells, which consists in active extrusion of the drug. The nucleotide binding site of the *Leishmania MDR1* has been studied in details and the binding of flavonoids to this sequence was shown to correlate with the efficiency of reversal of daunomycin resistance, suggesting a novel strategy to reverse resistance (Perez-Victoria *et al.*, 1999).

### ABC Transporters in Other Parasites

ABC transporters are without any doubt present in every parasitic protozoan. For a selected number of them, there is either definitive or strong circumstantial proof that they are involved in drug resistance. ABC transporters have been found in *Entamoeba histolytica*, a protozoan causing dysentery and liver abscesses. A P-glycoprotein homologue was found to be overexpressed in cells resistant to emetine, a second line drug against this parasite (Samuelson *et al.*, 1990). This P-glycoprotein is part of a large gene family with at least 4 members and two pseudogenes (Descoteaux *et al.*, 1995) and the ability of EhPgp1 to lead to emetine resistance in *E. histolytica* was confirmed by gene transfection experiments (Ghosh *et al.*, 1996).

Several ABC transporters have been described in *Trypanosoma brucei* (Maser and Kaminsky, 1998), one is highly similar to the *Leishmania PGPA* protein while another is related to the *Leishmania MDR1*. The expression of these genes appears not to be changed in drug resistant mutants, however (Maser and Kaminsky, 1998). A *Trypanosoma cruzi* ABC transporter, most likely the *Leishmania PGPA* homologue has also been characterized (Dallagiovanna *et al.*, 1996) although its role in resistance is unknown. It would be of interest to test by gene transfection if the *PGPA* genes of trypanosomes were able of conferring resistance to metals, since arsenicals, which are related to antimonials, are the drug of choice in the treatment of late stage African trypanosomiasis.

ABC transporters have been found in *Trichomonas vaginalis* (Johnson *et al.*, 1994), in *Cryptosporidium parvum* (Perkins *et al.*, 1999) and in a number of parasitic worms such as *Schistosoma* (Bosch *et al.*, 1994) and *Onchocerca volvulus* (Huang and Prichard, 1999) although the role of any of these proteins in drug resistance needs to be established. The situation seems to differ in the sheep nematode parasite *Haemonchus contortus* in which resistance to ivermectin and related drugs is an increasing problem. The expression of a P-glycoprotein from *H. contortus* was higher in ivermectin-selected than unselected strains (Xu *et al.*, 1998) and the multidrug resistance reversing agent verapamil increased the efficacy of ivermectin in resistant strains (Molento and Prichard,

1999). Ivermectin is a likely substrate for a P-glycoprotein since a disruption of the gene of a P-glycoprotein in mice, results in hypersensitivity to ivermectin (Schinkel *et al.*, 1994).

### Concluding Remarks

Drug resistance is an important problem in parasitic diseases, which is exacerbated by the limited number of drugs available. Studies on drug resistance can help in order to find strategies to increase the efficacy or the life span of the few drugs available. The isolation of genes involved in drug resistance can allow the development of tests to detect resistance rapidly which may reduce the use of drugs that would be otherwise useless and may limit the use of last resort drugs only when absolutely required while dealing with resistant parasites. Our understanding of drug resistance mechanisms can also suggest novel therapeutic strategies. For instance, in the PGPA mediated antimony resistance in *Leishmania*, thiols are important and we found *in vitro*, that when reducing thiol biosynthesis using specific inhibitors we can reduce PGPA mediated resistance (Haimeur *et al.*, 1999) (Légaré *et al.*, in preparation). A combination of thiol biosynthesis inhibitors and antimony was therefore shown to revert resistance in antimony resistant *Leishmania*. Combination therapy has been suggested as a strategy to delay antimalarial drug resistance (White, 1999). The dichotomous action of *pfmdr1* mutations on two categories of drugs (quinine and chloroquine on one side, and halofantrine, mefloquine and artemisinin on the other) may suggest that a combination with a member of each group of drugs could lead to a reversal of Pgh1 mediated resistance. Strategies and inhibitors to modulate the activity of efflux pumps are being developed and these could be useful in the treatment of parasitic diseases when a transport related mechanism, as the one described here, may be the main resistance mechanism.

### Acknowledgements

This work was supported in part by a group grant from the Canadian Institutes of Health Research (CIHR) to MOU and B.P. B.P. is an FRSC Scholar, and M.O. is an MRC Scientist.

### References

- Barnes, D.A., Foote, S.J., Galatis, D., Kemp, D.J. and Cowman, A.F. 1992. Selection for high-level chloroquine resistance results in deamplification of the *pfmdr1* gene and increased sensitivity to mefloquine in *Plasmodium falciparum*. *Embo J.* 11: 3067-3075.
- Blattner, F.R., Plunkett, G., 3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., and Shao, Y. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science.* 277: 1453-1474.
- Borst, P., Evers, R., Kool, M., and Wijnholds, J. 1999. The multidrug resistance protein family. *Biochim. Biophys. Acta.* 1461: 347-357.
- Borst, P., and Ouellette, M. 1995. New mechanisms of drug resistance in parasitic protozoa. *Ann. Rev. Microbiol.* 49: 427-460.
- Bosch, I.B., Wang, Z.X., Tao, L.F., and Shoemaker, C.B. 1994. Two *Schistosoma mansoni* cDNAs encoding ATP-binding cassette (ABC) family proteins. *Mol. Biochem. Parasitol.* 65: 351-356.
- Bozdech, Z., VanWye, J., Haldar, K., and Schurr, E. 1998. The human malaria parasite *Plasmodium falciparum* exports the ATP-binding cassette protein PFGCN20 to membrane structures in the host red blood cell. *Mol. Biochem. Parasitol.* 97: 81-95.
- Bray, P.G., Ward, S.A., and Ginsburg, H. 1999. Na<sup>+</sup>/H<sup>+</sup> antiporter, chloroquine uptake and drug resistance: inconsistencies in a newly

- proposed model. *Parasitol. Today.* 15: 360-363.
- Callahan, H.L., and Beverley, S.M. 1991. Heavy metal resistance: a new role for P-glycoproteins in *Leishmania*. *J. Biol. Chem.* 266: 18427-18430.
- Callahan, H.L., Roberts, W.L., Rainey, P.M., and Beverley, S.M. 1994. The PGPA gene of *Leishmania major* mediates antimony (SbIII) resistance by decreasing influx and not by increasing efflux. *Mol. Biochem. Parasitol.* 68: 145-149.
- Carter, N.S., and Fairlamb, A.H. 1993. Arsenical-resistant trypanosomes lack an unusual adenosine transporter [published erratum appears in *Nature* 361: 374]. *Nature.* 361: 173-176.
- Chiquero, M.J., Perez-Victoria, J.M., F, O.V., Gonzalez-Ros, J.M., del Moral, R.G., Ferragut, J.A., Castanys, S., and Gamarro, F. 1998. Altered drug membrane permeability in a multidrug-resistant *Leishmania tropica* line. *Biochem. Pharmacol.* 55: 131-139.
- Chow, L.M., Wong, A.K., Ullman, B., and Wirth, D.F. 1993. Cloning and functional analysis of an extrachromosomally amplified multidrug resistance-like gene in *Leishmania enriettii*. *Mol. Biochem. Parasitol.* 60: 195-208.
- Chow, L.M.C., and Volkman, S.K. 1998. Plasmodium and leishmania: the role of *mdr* genes in mediating drug resistance. *Exp. Parasitol.* 90: 135-141.
- Cowman, A.F., Galatis, D., and Thompson, J.K. 1994. Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the *pfmdr1* gene and cross-resistance to halofantrine and quinine. *Proc. Natl. Acad. Sci. USA.* 91: 1143-1147.
- Cowman, A.F., Karcz, S., Galatis, D., and Culvenor, J.G. 1991. A P-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole. *J. Cell Biol.* 113: 1033-1042.
- Dallagiovanna, B., Gamarro, F., and Castanys, S. 1996. Molecular characterization of a P-glycoprotein-related *tcpgp2* gene in *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* 75: 145-157.
- Decottignies, A., and Goffeau, A. 1997. Complete inventory of the yeast ABC proteins. *Nat. Genet.* 15: 137-145.
- Descoteaux, S., Ayala, P., Samuelson, J., and Orozco, E. 1995. Increase in mRNA of multiple Eh *pgp* genes encoding P-glycoprotein homologues in emetine-resistant *Entamoeba histolytica* parasites. *Gene.* 164: 179-184.
- Detke, S., Katakura, K., and Chang, K.P. 1989. DNA amplification in arsenite-resistant *Leishmania*. *Exp. Cell Res.* 180: 161-170.
- Dey, S., Ouellette, M., Lightbody, J., Papadopoulou, B., and Rosen, B.P. 1996. An ATP-dependent As(III)-glutathione transport system in membrane vesicles of *Leishmania tarentolae*. *Proc. Natl. Acad. Sci. USA.* 93: 2192-2197.
- Dey, S., Papadopoulou, B., Haimeur, A., Roy, G., Grondin, K., Dou, D., Rosen, B.P., and Ouellette, M. 1994. High level arsenite resistance in *Leishmania tarentolae* is mediated by an active extrusion system. *Mol. Biochem. Parasitol.* 67: 49-57.
- Duraisingh, M.T., Roper, C., Walliker, D., and Warhurst, D.C. 2000. Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the *pfmdr1* gene of *Plasmodium falciparum*. *Mol. Microbiol.* 36: 955-961.
- Faraut-Gambarelli, F., Piarroux, R., Deniau, M., Giusiano, B., Marty, P., Michel, G., Faugere, B., and Dumon, H. 1997. *In vitro* and *in vivo* resistance of *Leishmania infantum* to meglumine antimoniate: a study of 37 strains collected from patients with visceral leishmaniasis. *Antimicrob. Agents Chemother.* 41: 827-830.
- Fidock, D., Nomura, T., Talley, A., Cooper, R., Dzekunov, S., Ferdig, M., Ursos, L., Sidhu, A., Naudé, B., Deitsch, K., Su, X.-z., Wootton, J., Roepe, P., and Wellems, T. 2000. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol. Cell.* 6: 861-871.
- Foote, S.J., Kyle, D.E., Martin, R.K., Oduola, A.M., Forsyth, K., Kemp, D.J., and Cowman, A.F. 1990. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. *Nature.* 345: 255-258.
- Foote, S.J., Thompson, J.K., Cowman, A.F., and Kemp, D.J. 1989. Amplification of the multidrug resistance gene in some chloroquine-resistant isolates of *P. falciparum*. *Cell.* 57: 921-930.
- Ghosh, M., Shen, J., and Rosen, B.P. 1999. Pathways of As(III) detoxification in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* 96: 5001-5006.
- Ghosh, S.K., Lohia, A., Kumar, A., and Samuelson, J. 1996. Overexpression of P-glycoprotein gene 1 by transfected *Entamoeba histolytica* confers emetine-resistance. *Mol. Biochem. Parasitol.* 82: 257-260.
- Grogl, M., Thomason, T.N., and Franke, E.D. 1992. Drug resistance in leishmaniasis: its implication in systemic chemotherapy of cutaneous and mucocutaneous disease. *Am. J. Trop. Med. Hyg.* 47: 117-126.
- Grondin, K., Haimeur, A., Mukhopadhyay, R., Rosen, B.P., and Ouellette, M. 1997. Co-amplification of the gamma-glutamylcysteine synthetase gene *gsh1* and of the ABC transporter gene *pgpA* in arsenite-resistant *Leishmania tarentolae*. *Embo J.* 16: 3057-3065.

- Gueiros-Filho, F.J., Viola, J.P., Gomes, F.C., Farina, M., Lins, U., Bertho, A.L., Wirth, D.F., and Lopes, U.G. 1995. *Leishmania amazonensis*: multidrug resistance in vinblastine-resistant promastigotes is associated with rhodamine 123 efflux, DNA amplification, and RNA overexpression of a *Leishmania mdr1* gene. *Exp. Parasitol.* 81: 480-490.
- Haimeur, A., Brochu, C., Genest, P., Papadopoulou, B., and Ouellette, M. 2000. Amplification of the ABC transporter gene *PGPA* and increased trypanothione levels in potassium antimonyl tartrate (SbIII) resistant *Leishmania tarentolae*. *Mol. Biochem. Parasitol.* 108: 131-135.
- Haimeur, A., Guimond, C., Pilote, S., Mukhopadhyay, R., Rosen, B.P., Poulin, R., and Ouellette, M. 1999. Elevated levels of polyamines and trypanothione resulting from overexpression of the ornithine decarboxylase gene in arsenite-resistant *Leishmania*. *Mol. Microbiol.* 34: 726-735.
- Henderson, D.M., Sifri, C.D., Rodgers, M., Wirth, D.F., Hendrickson, N., and Ullman, B. 1992. Multidrug resistance in *Leishmania donovani* is conferred by amplification of a gene homologous to the mammalian *mdr1* gene. *Mol. Cell Biol.* 12: 2855-2865.
- Higgins, C.F. 1992. ABC transporters: from microorganisms to man. *Ann. Rev. Cell Biol.* 8: 67-113.
- Huang, Y.J., and Prichard, R.K. 1999. Identification and stage-specific expression of two putative P-glycoprotein coding genes in *Onchocerca volvulus*. *Mol. Biochem. Parasitol.* 102: 273-281.
- Ibrahim, M.E., Hag-Ali, M., el-Hassan, A.M., Theander, T.G., and Kharazmi, A. 1994. *Leishmania* resistant to sodium stibogluconate: drug-associated macrophage-dependent killing. *Parasitol. Res.* 80: 569-574.
- Ishikawa, T., Wright, C.D., and Ishizuka, H. 1994. GS-X pump is functionally overexpressed in cis-diamminedichloroplatinum (II)-resistant human leukemia HL-60 cells and down-regulated by cell differentiation. *J. Biol. Chem.* 269: 29085-29093.
- Jackson, J.E., Tally, J.D., Ellis, W.Y., Mebrahtu, Y.B., Lawyer, P.G., Were, J.B., Reed, S.G., Panisko, D.M., and Limmer, B.L. 1990. Quantitative *in vitro* drug potency and drug susceptibility evaluation of *Leishmania* ssp. from patients unresponsive to pentavalent antimony therapy. *Am. J. Trop. Med. Hyg.* 43: 464-480.
- Johnson, P.J., Schuck, B.L., and Delgado, M.G. 1994. Analysis of a single-domain P-glycoprotein-like gene in the early-diverging protist *Trichomonas vaginalis*. *Mol. Biochem. Parasitol.* 66: 127-137.
- Katakura, K., Iwanami, M., Ohtomo, H., Fujise, H., and Hashiguchi, Y. 1999. Structural and functional analysis of the LaMDR1 multidrug resistance gene in *Leishmania amazonensis*. *Biochem. Biophys. Res. Commun.* 255: 289-294.
- Klein, I., Sarkadi, B., and Varadi, A. 1999. An inventory of the human ABC proteins. *Biochim. Biophys. Acta.* 1461: 237-262.
- Krogstad, D.J., Gluzman, I.Y., Kyle, D.E., Oduola, A.M., Martin, S.K., Milhous, W.K., and Schlesinger, P.H. 1987. Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. *Science.* 238: 1283-1285.
- Kündig, C., Haimeur, A., Légaré, D., Papadopoulou, B., and Ouellette, M. 1999. Increased transport of pteridines compensates for mutations in the high affinity folate transporter and contributes to methotrexate resistance in the protozoan parasite *Leishmania tarentolae*. *Embo J.* 18: 2342-2351.
- Légaré, D., Hettema, E., and Ouellette, M. 1994. The P-glycoprotein-related gene family in *Leishmania*. *Mol. Biochem. Parasitol.* 68: 81-91.
- Légaré, D., Papadopoulou, B., Roy, G., Mukhopadhyay, R., Haimeur, A., Dey, S., Grondin, K., Brochu, C., Rosen, B.P., and Ouellette, M. 1997. Efflux systems and increased trypanothione levels in arsenite-resistant *Leishmania*. *Exp. Parasitol.* 87: 275-282.
- Li, Z.S., Lu, Y.P., Zhen, R.G., Szczypka, M., Thiele, D.J., and Rea, P.A. 1997. A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionato)cadmium. *Proc. Natl. Acad. Sci. USA.* 94: 42-47.
- Linton, K.J., and Higgins, C.F. 1998. The *Escherichia coli* ATP-binding cassette (ABC) proteins. *Mol. Microbiol.* 28: 5-13.
- Lira, R., Sundar, S., Makharia, A., Kenney, R., Gam, A., Saraiva, E., and Sacks, D. 1999. Evidence that the high incidence of treatment failures in Indian Kala- Azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. *J. Infect. Dis.* 180: 564-567.
- Martin, S.K., Oduola, A.M., and Milhous, W.K. 1987. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. *Science.* 235: 899-901.
- Maser, P., and Kaminsky, R. 1998. Identification of three ABC transporter genes in *Trypanosoma brucei* spp. *Parasitol. Res.* 84: 106-111.
- Maser, P., Sutterlin, C., Kralli, A., and Kaminsky, R. 1999. A nucleoside transporter from *Trypanosoma brucei* involved in drug resistance. *Science.* 285: 242-244.
- Molento, M.B., and Prichard, R.K. 1999. Effects of the multidrug-resistance-reversing agents verapamil and CL 347,099 on the efficacy of ivermectin or moxidectin against unselected and drug-selected strains of *Haemonchus contortus* in jirds (*Meriones unguiculatus*). *Parasitol. Res.* 85: 1007-1011.
- Mukhopadhyay, R., Dey, S., Xu, N., Gage, D., Lightbody, J., Ouellette, M., and Rosen, B.P. 1996. Trypanothione overproduction and resistance to antimonials and arsenicals in *Leishmania*. *Proc. Natl. Acad. Sci. USA.* 93: 10383-10387.
- Ortiz, D.F., Ruscitti, T., McCue, K.F., and Ow, D.W. 1995. Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. *J. Biol. Chem.* 270: 4721-4728.
- Ouellette, M., Fase-Fowler, F., and Borst, P. 1990. The amplified H circle of methotrexate-resistant *Leishmania tarentolae* contains a novel P-glycoprotein gene. *Embo J.* 9: 1027-1033.
- Ouellette, M., Hettema, E., Wust, D., Fase-Fowler, F., and Borst, P. 1991. Direct and inverted DNA repeats associated with P-glycoprotein gene amplification in drug resistant *Leishmania*. *Embo J.* 10: 1009-1016.
- Papadopoulou, B., Roy, G., Dey, S., Rosen, B.P., and Ouellette, M. 1994. Contribution of the *Leishmania* P-glycoprotein-related gene *ltpgpA* to oxyanion resistance. *J. Biol. Chem.* 269: 11980-11986.
- Peel, S.A., Bright, P., Yount, B., Handy, J., and Baric, R.S. 1994. A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (*pfmdr1*) of *Plasmodium falciparum* *in vitro*. *Am. J. Trop. Med. Hyg.* 51: 648-658.
- Perez-Victoria, J.M., Chiquero, M.J., Conseil, G., Dayan, G., Di Pietro, A., Barron, D., Castany, S., and Gamarro, F. 1999. Correlation between the affinity of flavonoids binding to the cytosolic site of *Leishmania tropica* multidrug transporter and their efficiency to revert parasite resistance to daunomycin. *Biochemistry.* 38: 1736-1743.
- Perkins, M.E., Riojas, Y.A., Wu, T.W., and Le Blancq, S.M. 1999. CpABC, a *Cryptosporidium parvum* ATP-binding cassette protein at the host-parasite boundary in intracellular stages. *Proc. Natl. Acad. Sci. USA.* 96: 5734-5739.
- Quentin, Y., Fichant, G., and Denizot, F. 1999. Inventory, assembly and analysis of *Bacillus subtilis* ABC transport systems. *J. Mol. Biol.* 287: 467-484.
- Reed, M.B., Saliba, K.J., Caruana, S.R., Kirk, K., and Cowman, A.F. 2000. Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. *Nature.* 403: 906-909.
- Rubio, J.P., and Cowman, A.F. 1994. *Plasmodium falciparum*: the *pfmdr2* protein is not overexpressed in chloroquine-resistant isolates of the malaria parasite. *Exp. Parasitol.* 79: 137-147.
- Samuelson, J., Ayala, P., Orozco, E., and Wirth, D. 1990. Emetine-resistant mutants of *Entamoeba histolytica* overexpress mRNAs for multidrug resistance. *Mol. Biochem. Parasitol.* 38: 281-290.
- Saurin, W., Hofnung, M., and Dassa, E. 1999. Getting in or out: early segregation between importers and exporters in the evolution of ATP-binding cassette (ABC) transporters. *J. Mol. Evol.* 48: 22-41.
- Schinkel, A.H., Smit, J.J., van Tellingen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P., and *et al.* 1994. Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell.* 77: 491-502.
- Singh, A.K., Liu, H.Y., and Lee, S.T. 1994. Atomic absorption spectrophotometric measurement of intracellular arsenite in arsenite-resistant *Leishmania*. *Mol. Biochem. Parasitol.* 66: 161-164.
- Sullivan, D.J., Jr., Gluzman, I.Y., Russell, D.G., and Goldberg, D.E. 1996. On the molecular mechanism of chloroquine's antimalarial action. *Proc. Natl. Acad. Sci. USA.* 93: 11865-11870.
- Sundar, S., Agrawal, N.K., Sinha, P.R., Horwitz, G.S., and Murray, H.W. 1997. Short-course, low-dose amphotericin B lipid complex therapy for visceral leishmaniasis unresponsive to antimony. *Ann. Intern. Med.* 127: 133-137.
- Taglicht, D., and Michaelis, S. 1998. *Saccharomyces cerevisiae* ABC proteins and their relevance to human health and disease. *Meth. Enzymol.* 292: 130-162.
- Tomii, K., and Kanehisa, M. 1998. A comparative analysis of ABC transporters in complete microbial genomes. *Genome Res.* 8: 1048-1059.
- Tommasini, R., Evers, R., Vogt, E., Mornet, C., Zaman, G.J., Schinkel, A.H., Borst, P., and Martinou, E. 1996. The human multidrug resistance-associated protein functionally complements the yeast cadmium resistance factor 1. *Proc. Natl. Acad. Sci. USA.* 93: 6743-6748.
- van Es, H.H., Karcz, S., Chu, F., Cowman, A.F., Vidal, S., Gros, P., and Schurr, E. 1994. Expression of the plasmodial *pfmdr1* gene in mammalian cells is associated with increased susceptibility to chloroquine. *Mol. Cell Biol.* 14: 2419-2428.
- Warhurst, D.C. 1999. Drug resistance in *Plasmodium falciparum* malaria. *Infection.* 27 Suppl 2, S55-58.
- Welles, T.E., Walker-Jonah, A., and Panton, L.J. 1991. Genetic mapping of the chloroquine-resistance locus on *Plasmodium falciparum* chromosome 7. *Proc. Natl. Acad. Sci. USA.* 88: 3382-3386.
- White, N.J. 1998. Drug resistance in malaria. *Br. Med. Bull.* 54: 703-15.
- White, N.J. 1999. Delaying antimalarial drug resistance with combination

- chemotherapy. *Parasitologia*. 41: 301-308.
- Wilson, C.M., Serrano, A.E., Wasley, A., Bogenschutz, M.P., Shankar, A.H., and Wirth, D.F. 1989. Amplification of a gene related to mammalian *mdr* genes in drug-resistant *Plasmodium falciparum*. *Science*. 244: 1184-1186.
- Xu, M., Molento, M., Blackhall, W., Ribeiro, P., Beech, R., and Prichard, R. 1998. Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Mol. Biochem. Parasitol.* 91: 327-335.
- Zalis, M.G., Wilson, C.M., Zhang, Y., and Wirth, D.F. 1993. Characterization of the *pfmdr2* gene for *Plasmodium falciparum* [published erratum appears in *Mol. Biochem. Parasitol.* 1994 63: 311]. *Mol. Biochem. Parasitol.* 62: 83-92.