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# Identification of a multidrug resistance-like system in *Tetrahymena pyriformis*: evidence for a new detoxication mechanism in freshwater ciliates

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**Abstract The freshwater ciliate *Tetrahymena pyriformis* is an ubiquitous organism that is present in all aquatic ecosystems. This protozoan showed a clear resistance against some polycyclic aromatic hydrocarbons which can be attributed to an efflux pump probably of the multidrug resistance (MDR) type. Immunocytochemical detection showed a positive stain of ciliate cells using the monoclonal antibodies 4E3, raised against P-glycoprotein (P-gp). The kinetics of P-gp expression were studied for control cultures and cultures treated with 15 μM benzo(a)pyrene. Western blot analysis using the Ab1, anti-P-gp polyclonal antibodies indicates the presence of two bands of 66 and 96 kDa of which the intensity increased with time in benzo(a)pyrene-treated ciliates. Uptake experiments with target compounds for the MDR pump, namely adriamycin, rhodamine 123 and two polycyclic aromatic hydrocarbons, benzo(a)pyrene and 7,12-dimethylbenzanthracene, were carried out by flow cytometry, in the presence or absence of cyclosporin (an inhibitor of the multidrug resistant pump). The data indicate that the accumulation of these compounds by ciliate cells is significantly enhanced in the presence of cyclosporin. This suggests that *Tetrahymena* is provided with a P-gp-like system that is functionally active in a way similar to that of the mammalian P-gp.**

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*Key words:* Multidrug resistance pump; P-glycoprotein; Flow cytometry; Polycyclic aromatic hydrocarbon; Adriamycin; Rhodamine 123; *Tetrahymena pyriformis* GL

## 1. Introduction

Protozoan ciliates are unicellular eukaryotic organisms that are present in all aquatic ecosystems. The freshwater ciliate *Tetrahymena pyriformis* is an organism of choice in cytotoxicity studies [18]. Its short generation time and the fact that it can be grown in axenical culture are especially advantageous for studying the action of xenobiotics on several generations of cells. This ciliate has been used to determine the effects of different xenobiotics including insecticides [9], herbicides [21], mycotoxins [5] or drugs such as antibiotics [1].

In spite of its general sensitivity to toxic agents, *T. pyriformis* does not show any significant sign of cytotoxicity in the presence of polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BP), 3-methylcholanthrene (3MC), benzanthracene (BA) and 7,12-dimethylbenzanthracene (DMBA) [2]. The latter are highly toxic ubiquitous environmental contaminants and have been used as representative compounds to investigate the mode of action of PAHs, in particular cytochrome P450 induction [4]. The PAH uptake, studied in *T. pyriformis* by flow cytometry (FCM) in our laboratory, shows immediate accumulation followed by rapid elimination of these products by the cells. Results of gas chromatography-mass spectrometry analysis indicate that PAH elimination is not linked to biotransformation but to rapid efflux of these molecules by the cells [2].

Multidrug resistance (MDR) refers to patterns of resistance that develop in tumor cells against a wide range of natural product-derived anti-cancer agents. P-glycoprotein (P-gp), a transmembrane efflux pump encoded by the MDR gene ($MDR_1$), transports various lipophilic drugs out of cells that initially enter the cells by passive diffusion [19]. BP and 3MC are known as substrates for the MDR efflux pump in a hepatocyte culture [7]. The presence of the MDR efflux pump or MDR efflux pump-like has already been demonstrated in freshwater and marine organisms such as mussels and sponges [14], in the parasitic protozoans *Leishmania donovani* [11] and *Plasmodium falciparum* [13] and even in bacterial cells [10].

The activity of the MDR pump can be modulated by molecules such as verapamil and cyclosporin which act as competitive inhibitors [17,20]. Data obtained in our laboratory on BP uptake in *T. pyriformis* show that, in the presence of verapamil, BP is accumulated in larger amounts in cells. With cyclosporin, the accumulation of this PAH is several times greater than under control conditions [2]. These results suggest that the resistance of *T. pyriformis* against PAH cytotoxicity may be attributed to the rapid efflux of these compounds from the cells via an efflux pump probably of the MDR type.

The purpose of this study was to provide further evidence for the existence of a P-gp-like system in *T. pyriformis*. In this work, immunocytochemical detection shows a positive stain of ciliate cells and Western blot analysis indicates the presence of two polypeptides. Uptake experiments with two target compounds for the MDR pump, adriamycin (ADR) and rhodamine 123 (Rhod), and two PAHs, BP and DMBA, indicate that these compounds are clearly more accumulated by ciliate cells in the presence of cyclosporin, a well-known inhibitor of the MDR pump. Overall, these results strongly suggest that *T. pyriformis* is provided with a P-gp-like system that is functionally active in a way similar to the mammalian P-gp.

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## 2. Materials and methods

### 2.1. Materials

BP and DMBA were purchased from Aldrich Chemical (Milwaukee, WI, USA). Proteose peptone and yeast extract were purchased from Difco Laboratories (Detroit, MI, USA). Cyclosporin was purchased from Sandoz Canada (Veterinary Teaching Hospital of Ontario, Veterinary College, Guelph, Ont., Canada). 4E3 monoclonal antibodies were purchased from Signet Laboratories (Dedham, MA, USA). Ab1 anti-P-gp mouse polyclonal antibodies were purchased from CIE Oncongene science (Horby, Ont., Canada). Anti-rat F(ab′) monoclonal antibodies were purchased from Cedar Lane (Ont., Canada). Rhod, ADR, anti-mouse IgG monoclonal antibodies and other chemicals were purchased from Sigma Chemical (St. Louis, MO, USA).

### 2.2. T. pyriformis culture

*T. pyriformis* GL, a micronucleate strain in the exponential growth phase, was used. Ciliate culturing was carried out axenically at 28°C in PPYS medium (7.5 g/l proteose peptone, 7.5 g/l yeast extract, salt solution) [1].

### 2.3. Xenobiotic exposure

BP and DMBA were dissolved in dimethyl formamide (DMF). ADR and Rhod were dissolved in dimethylsulfoxide (DMSO). The final concentrations of DMF and DMSO in the culture were 0.2 and 0.5%, respectively. The cells were exposed to concentrations of 1 μM BP, DMBA and Rhod and 30 μM ADR.

### 2.4. Uptake of xenobiotics by FCM

BP, DMBA, Rhod and ADR are fluorescent molecules. Their uptake by *T. pyriformis* was measured by FCM using a FACS Vantage flow cytometer (Becton Dickinson) according to the technique described by Bamdad et al. [2]. PAHs were excited at 351–364 nm with an Enterprise ion argon laser (COHERENT), which had an output power of 50 mW. The fluorescence intensities were obtained using a 405 ± 10 nm bandpass filter. The emission wavelengths for ADR and Rhod were 660 and 514 nm, respectively. For each cytometric parameter investigated (Rhod, ADR and PAH fluorescence), 10 000 events (cells) were analyzed per condition and fluorescence measurements were on the logarithmic scale. The mean fluorescence for any given population was provided by Lysis II instrument software and expressed in fluorescence units (FU). Each experiment was performed at least three times.

### 2.5. Immunocytochemistry

15 ml of ciliate culture in the exponential growth phase (about 20 000 cells/ml) was concentrated (centrifugation at 3000 × *g* for 5 min). The pellet was fixed for 5 min in 4% paraformaldehyde in phosphate-buffered saline (PBS) pH = 7.2, at room temperature. After three washes in PBS, the cells were laid on slides. Next, after washing in PBS, the cells were incubated for 60 min in a blocking solution (PBS pH = 7.4, 11.5 mM $NaH_2PO_4$, 99.2 mM NaCl, 2.1 mM $MgCl_2$, 1.05 g/l casein, 30 g/l bovine serum albumin, 100 ml fetal bovine serum). After three washes in 0.07% Tween-PBS, the cells were incubated for 1 h at room temperature in humidified boxes with the mouse monoclonal antibodies 4E3 or with control isotype IgG2a anti-rat antibody at 5 μg/ml. After washing three times in 0.07% Tween-PBS, the slides were incubated for 1 h at room temperature in anti-mouse IgG alkaline phosphatase-conjugated goat anti-mouse secondary antibody diluted 1:250. The slides were then washed three times in 0.07% Tween-PBS and the positive reaction was developed by placing the samples for 45 min in a solution composed of 200 μg/ml 5-bromo-4-chloro-3-indolyl phosphate, 200 μg/ml nitro blue tetrazolium, 100 mM NaCl, 5 mM $MgCl_2$ in 100 mM Tris buffer pH = 9.5.

### 2.6. Western blot analysis

Control cells and cells treated with 15 μM BP for 10, 60 or 120 min were centrifuged (3000 rpm, 10 min at 4°C) and the pellet was recovered in 4 ml of buffer solution (0.8 M sucrose, 5 mM Tris-HCl, 5 mM $MgCl_2$, pH = 7.2). After centrifugation (3000 rpm, 5 min at 4°C), the supernatant was removed and the pellet was treated with 300 μl of 0.4 M sucrose buffer, pH = 7.2, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 5 μM leupeptin, 1 mM EGTA, 1 mM EDTA). The cells were then homogenized with a potter tissue homogenizer at 4°C. Cell extracts were sonicated (2 × 10 s at 4°C) and diluted 1:4 in sample buffer composed of 47.5% (v/v) deionized water, 12.5% 0.5 mM Tris-HCl pH 6.8, 10% glycerol, 20% SDS (10% w/v), 5% β-mercaptoethanol and 5% bromophenol blue (1% w/v) to a final protein concentration of 0.5 mg/ml. The samples were sonicated (4 × 10 s at 4°C) and used for immunodetection of P-gp by Western blot analysis according to the technique described by Beaulieu et al. [3] and using the anti-P-gp polyclonal antibodies Ab1 (diluted 1:2000).

## 3. Results

### 3.1. Immunodetection of P-gp in Tetrahymena

Immunodetection of P-gp was first carried out in *T. pyriformis* by immunocytochemistry. Immunocytochemical detection of P-gp was performed on cells fixed with 4% parafor-

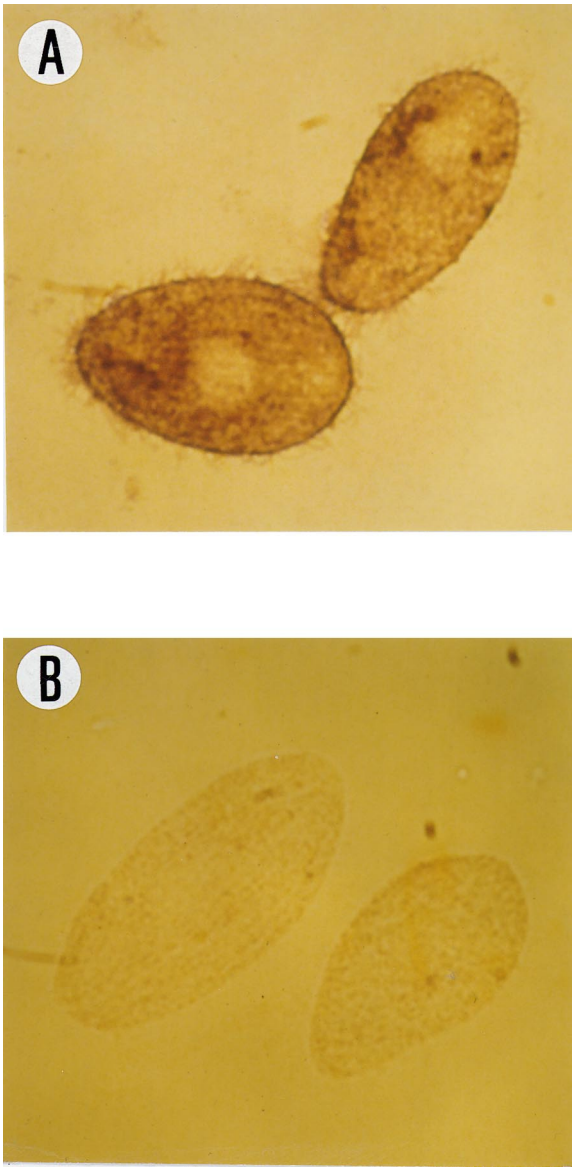


Fig. 1. Immunocytochemical detection of P-gp in *T. pyriformis* GL. (A) The ciliate cells were fixed with 4% paraformaldehyde and then stained with the mouse monoclonal anti-P-gp antibodies 4E3 at 5 μg/ml. The immunocomplexes were incubated for 1 h at room temperature in anti-mouse IgG alkaline phosphatase-conjugated goat anti-mouse secondary antibody diluted 1:250. (B) Control anti-mouse IgG alkaline phosphatase-conjugated goat anti-mouse secondary antibody diluted 1:250. Magnification, ×40.

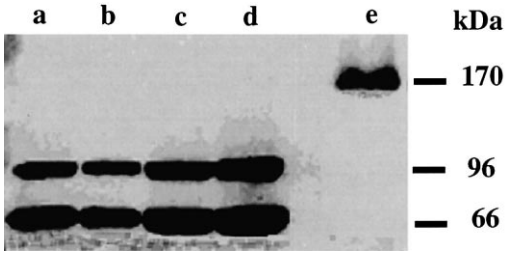


Fig. 2. Western blot analysis of P-gp in *T. pyriformis* GL. The ciliate cultures were treated with 15 μM BP for 10, 60 and 120 min and then processed as described in Section 2. The polyclonal anti-P-gp antibodies Ab1 were used for this detection. (a) Control culture, (b) cultures treated with BP for 10 min, (c) 60 min, (d) 120 min, (e) kidney brush border membranes used as positive control.

maldehyde, using the mouse monoclonal antibodies anti-P-gp 4E3. In parallel, controls for isotype IgG2a and for secondary antibodies were performed using the anti-rat F(ab′) monoclonal antibodies and the anti-mouse IgG alkaline phosphatase-conjugated antibodies, respectively. The results showed a positive stain with the anti-P-gp 4E3 (Fig. 1A), in contrast to what was seen in the controls (Fig. 1B).

Immunodetection of P-gp was then carried out by Western blot analysis on a *T. pyriformis* control culture and cultures treated with 15 μM BP for 10, 60 and 120 min. The polyclonal anti-P-gp Ab1 was used for this detection. The results are presented in Fig. 2. They show the presence of two proteins with apparent molecular masses of 66 and 96 kDa, detected both in controls (a) and treated ciliates (b–d). The stain intensity of these two bands seems to be less pronounced in the control cultures (a) and in the cells treated with BP for 10 min (b) than in samples exposed to BP for 60 (c) and 120 min (d).

### *3.2. Uptake of Rhod, ADR, DMBA and BP by T. pyriformis*

In this series of experiments, the uptake of Rhod and ADR, two well-known target molecules for P-gp, and the uptake of the two PAHs BP and DMBA was studied in *T. pyriformis*.

The kinetics of Rhod uptake were studied under different conditions over a 3 h period in ciliate culture (Fig. 3). The uptake of 1 μM Rhod led to a rapid increase in intracellular fluorescence (40.5 FU at 30 min) that decreased slightly afterwards (27.8 FU at 120 min). Rhod uptake (1 μM) studied in the presence of 1 μM BP (added in ciliate culture at the same time) showed a greater accumulation of Rhod in cells with 52.4 FU at 30 min. Addition of 5 μM cyclosporin (an inhibitor of the P-gp pump) 5 min before addition of Rhod led to a much more rapid Rhod accumulation in the cells (130.6 FU at 15 min). This rate then decreased (56.9 FU) at 120 min and remained stable. In parallel, addition of 5 μM cyclosporin to

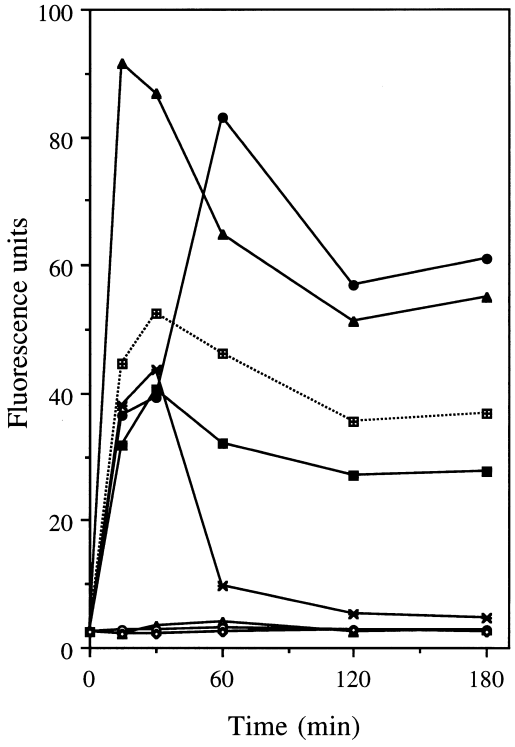


Fig. 3. FCM analysis of Rhod-induced fluorescence in the presence of cyclosporin in *T. pyriformis* GL cultures. Time course of mean cellular fluorescence of control cells and of cells exposed to 1 μM Rhod and 5 μM cyclosporin (cyclo). Data from one experiment are representative of three similar experiments. –○–, 0.5% DMSO control; –△–, 5 μM cyclo; –■–, 1 μM Rhod; –◇–, 1 μM BP; –×–, 1 μM Rhod and 1 μM BP added at the same time; –●–, 1 μM Rhod during 30 min and then 5 μM cyclo added; –▲–, 5 μM cyclo during 5 min and then 1 μM Rhod added; –×–, 1 μM Rhod during 40 min and cells were transferred in free Rhod medium.

ciliate cultures 30 min after treatment with Rhod (1 μM) again produced a clear accumulation of Rhod in the cells (82.9 FU at 60 min). Finally, Rhod uptake studied in a ciliate culture treated with 1 μM Rhod for 40 min (43.6 FU at 30 min) and then transferred to culture medium without Rhod showed a rapid and clear decrease in Rhod from the cells (9.9, 5.2 and 4.7 FU at 60, 120 and 180 min, respectively).

With 30 μM ADR (Table 1), intracellular fluorescence increased rapidly over the first 60 min (17.6 FU) and then remained relatively stable (17.4 FU at 180 min). In the presence of 5 μM cyclosporin added to the culture 5 min before ADR, ADR accumulation increased clearly and rapidly with a maximum of 46.1 FU at 60 min.

Table 1
FCM analysis of ADR, DMBA and BP-induced fluorescence in the presence of cyclosporin in *T. pyriformis*

| Time, min | Controls | | | ADR | | DMBA | | BP | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.5% DMSO | 0.2% DMF | 5 μM cyclo | 30 μM ADR | ADR/cyclo | 1 μM DMBA | DMBA/cyclo | 1 μM BP | BP/cyclo at 60 min |
| 0 | 5 | 2.7 | 6 | 4.3 | 4.3 | 4.3 | 4.3 | 2.7 | 2 |
| 30 | 5.6 | 2.6 | 6.4 | 14.7 | 39.3 | 39.3 | 54. | 259.4 | 210.3 |
| 60 | 5 | 3.3 | 6.4 | 17.6 | 46.1 | 19.6 | 329.3 | 126 | 125 |
| 120 | 4.4 | 2.7 | 7.5 | 14.3 | 35.2 | 9.9 | 552.5 | 90.7 | 1409.4 |
| 180 | 5 | 2.6 | 7.2 | 17.4 | 35.1 | 14 | 280.4 | 76.9 | 831 |

Time course of mean cellular fluorescence of controls cells: 0.5% DMSO, 0.2% DMF and 5 mM cyclosporin (cyclo). Cells exposed to 30 μM ADR, 1 μM DMBA or 1 μM BP. Cells exposed at the same time to 5 μM cyclosporin and 30 μM ADR (ADR/cyclo) or 5 μM cyclosporin and 1 μM DMBA (DMBA/cyclo). Cells exposed to 1 μM BP and to 5 μM cyclosporin after 60 min of treatment to BP (BP/cyclo at 60 min). Data from one experiment are representatives of three similar experiments.

The results on DMBA accumulation (Table 1) showed an increase in intracellular fluorescence with 1 μM of this PAH, with a maximum of 39.33 FU at 30 min. This rate decreased and reached the control values at 120 min (9.9 FU). In the presence of 5 μM cyclosporin added 5 min before DMBA, the PAH was accumulated very rapidly in ciliate cells with a maximum of 552.5 FU at 120 min. This rate decreased to 280.4 FU at 180 min.

BP uptake followed in ciliate culture (Table 1) showed that this PAH at 1 μM was accumulated rapidly by the cells (259.4 FU at 30 min) but its cellular level decreased progressively thereafter (76.9 FU at 180 min). Moreover, addition of 5 μM cyclosporin, 60 min after BP addition, caused an extensive increase in BP fluorescence in cells with 125.8 FU at 60 min (before cyclosporin addition) and 1409 FU at 120 min (after cyclosporin addition).

## 4. Discussion

Many aquatic organisms thrive and reproduce in polluted waters. This fact indicates that they are well-equipped with defense systems against many toxic xenobiotics simultaneously present in the environment. According to Kurelec [14], the biochemical mechanism underlying such 'multixenobiotic' resistance in freshwater and marine mussels, in several marine sponges and in freshwater fish is similar to the mechanism of MDR found in tumor cells that have become refractory to treatment, a phenomenon that occurs with a variety of chemotherapeutic agents. P-gp, a transmembrane efflux pump for anti-tumor drugs, transports lipophilic molecules out of cells that initially enter the cells by passive diffusion [19].

The freshwater ciliate *T. pyriformis* is a ubiquitous unicellular eukaryotic organism that shows resistance against PAH toxicity (BP, 3MC, BA and DMBA). Previous studies carried out in our laboratory showed that this resistance is linked to a rapid efflux of these compounds by the cells. The efflux mechanism could be attributed to the presence of an efflux pump of the P-gp type since in the presence of P-gp inhibitors, PAH accumulation was enhanced [2]. The present study provides further evidence supporting the existence of a P-gp-like system in *Tetrahymena*.

Immunodetection methods were applied to confirm the presence of a P-gp-like system in *T. pyriformis*. The results of immunocytochemistry showed clear positive staining of the cells. The mouth organelles are labelled in accordance with their high ciliary density. The labelling of the cell body periphery may be related to the presence of two membrane systems: the plasma membrane, in continuity with the ciliary membrane system, and that of underlying alveolar sacs. Western blot analysis revealed two immunoreactive bands corresponding to proteins of 66 and 96 kDa. Moreover, exposure of the cells to BP led to enhanced expression of the proteins, as judged from the increase in the intensity of the bands in samples from cultures incubated with this compound for 60 and 120 min. It is worth noting that the time course of cellular accumulation of BP, as studied by FCM in *T. pyriformis* [2], is correlated with the P-gp expression results. Indeed, BP accumulated rapidly in ciliate cells within about 20 min, it was then eliminated efficiently by the cells and after 60 min of exposure, the amount of BP in the cells had stabilized at very low levels.

Usually, the MDR (in human) or mdr (in non-human species) phenotype in mammalian cells involves increased expression of an approximately 170 kDa plasma membrane P-gp [19]. The blood brain barrier also expresses a P-gp with a molecular weight of 190 kDa [3]. In a marine sponge, a 150 kDa drug-binding glycoprotein was described [14] and in other sponge species and in mussels, a 170 kDa protein was found [14]. In P388 murine leukemia cells, a mini P-gp with a molecular weight of approximately 65 kDa was identified [12]. In prokaryotic cells, a striking homology was detected between P-gp and the HlyB protein, a 66 kDa *Escherichia coli* membrane protein required for export of hemolysin. P-gp can be seen as a tandem duplication of the HlyB protein [10]. The presence of immunoreactive proteins observed by Western blotting suggests that *T. pyriformis* expresses two polypeptides (66 and 96 kDa) that share epitopes with the mammalian P-gp but that are smaller than the latter.

In order to support the evidence obtained from immunodetection, the presence of a P-gp-like system acting as a multidrug transporter in *T. pyriformis* was verified through uptake studies. The transport of molecules that are well-known targets for the P-gp pump, namely Rhod and ADR, was measured by FCM. This methodology allows for the accumulation of fluorescent substrates by cells to be followed on a real time basis. FCM has been widely used for studying MDR [15]. It is a novel approach for examining the response of unicellular organisms such as *Tetrahymena* when they are exposed to environmental contaminants [2]. Rhod is a model molecule, frequently used along with FCM for P-gp efflux studies [8,16]. Rhod intracellular levels were clearly more important in the presence of cyclosporin, whether the inhibitor was added prior to Rhod or later during the course of the experiment, providing additional support for the existence of a P-gp-like mechanism. Moreover, under conditions where Rhod and BP were added in a cell culture at the same time, Rhod accumulation was greater. Since BP is a known target for P-gp, it can therefore compete with Rhod for the efflux pump. This may explain the larger accumulation of Rhod under these conditions. The cytotoxic drug ADR, an antracyclin highly associated with resistance mechanisms, has the advantage of being fluorescent [6,15]. The uptake of ADR followed in *T. pyriformis* showed a rapid cellular accumulation of this product reaching levels that remained stable. In the presence of cyclosporin, a classical inhibitor of P-gp, ADR accumulation was clearly enhanced. Finally, the DMBA and BP transport study in *T. pyriformis* showed a rapid uptake and efflux of these molecules by the cells. However, adding cyclosporin at the beginning or during the course of the experiment clearly increased the rate of DMBA or BP accumulation, again suggesting a role for a P-gp-like system.

The uptake experiments that we carried out in *T. pyriformis* using different compounds always demonstrated a rapid influx of these molecules into cells, while efflux profiles varied according to each molecule. With ADR and Rhod, after the initial phase of accumulation, there was a stabilization of the cellular fluorescence, indicating that the rate of influx and that of efflux were in equilibrium. In the case of PAHs, a clear and rapid efflux of the compounds was detected with the ciliate. The PAH efflux remained effective over time, allowing the cells to eliminate most of the toxic compound [2]. The fact that *T. pyriformis* is a ubiquitous organism in freshwater and the fact that PAHs are a major source of pollution in aquatic ecosystems may explain the particular action of a

P-gp-like system against PAHs in *Tetrahymena*. This ciliate probably developed a new way of detoxication by this cell membrane resistance mechanism. The molecular characterization of an MDR type system in *Tetrahymena* deserves further investigation.

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## References

[1] Bamdad, M., David, L. and Grolière, C.A. (1995) Appl. Microbiol. Biotechnol. 44, 206–209.
[2] Bamdad, M., Reader, S., Grolière, C.A., Bohatier, J. and Denizeau, F. (1997) Cytometry 28, 170–175.
[3] Beaulieu, E., Demeule, M., Pouliot, J.F., Averill-Bates, D.A., Murphy, G.F. and Beliveau, R. (1995) Biochim. Biophys. Acta 1233, 27–32.
[4] Bhagavatula, M., Shuo, C., Donghui, L. and Randerath, K. (1993) Carcinogenesis 14, 879–886.
[5] Bijl, J.P., Rousseau, D.M., Dive, D.G. and Van Peteghem, G.H. (1988) J. Assn. Off. Anal. Chem. 71, 282–285.
[6] Claudio, J.A. and Emerman, J.T. (1996) Breast Cancer Res. Treat. 41, 111–122.
[7] Fardel, O., Lecueur, V., Corlu, A. and Guilluzo, A. (1996) Biochem. Pharmacol. 51, 1427–1436.
[8] Feller, N., Kuiper, C.M., Lankelma, J., Ruhdal, J.K., Scherper, R.J., Pinedo, H.M. and Broxterman, H.J. (1995) Br. J. Cancer 72, 543–549.
[9] Fritzsche, U. and Cleffmann, G. (1987) Nat. Wiss. 74, 191–192.
[10] Gerlach, J.H., Endicott, J.A., Juranka, P.F., Henderson, G., Sarangi, F., Deuchars, K.L. and Ling, V. (1986) Nature 324, 485–489.
[11] Henderson, D.M., Sifri, C.D., Rodgers, M., Wirth, D.F., Hendrickson, N. and Ulman, B. (1992) Mol. Cell. Biol. 12, 2855–2865.
[12] Kawai, K., Kusano, I., Ido, M., Sakurai, M., Shiraishi, T. and Yatani, R. (1994) Biochem. Biophys. Res. Commun. 198, 804–810.
[13] Krogstad, D.J., Gluzman, I.Y., Herwald, B.L., Schlesinger, P.H. and Wellems, T.E. (1992) Biochem. Pharmacol. 43, 57–62.
[14] Kurelec, B. (1992) Crit. Rev. Toxicol. 22, 23–43.
[15] Léonce, S. and Burbridge, M. (1993) Biol. Cell 78, 63–68.
[16] List, A.F. (1996) Leukemia 10, 937–942.
[17] Muller, C., Bailly, J.D., Jaffrezou, J.P., Goubin, F. and Laurent, G. (1994) Bull. Cancer 81, 386–391.
[18] Nilsson, J.R. (1989) Eur. J. Protistol. 25, 2–25.
[19] Robert, J. (1994) Bull. Cancer 81, 400–408.
[20] Ross, D.D., Doyle, L.A., Yang, W., Tong, Y. and Cornblatt, B. (1995) Biochem. Pharmacol. 50, 1673–1683.
[21] Traunspurger, W., Schafer, H. and Remede, A. (1996) Chemosphere 33, 1129–1141.