Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4737–4741, June 1990
Cell Biology

Functional expression of murine multidrug resistance in *Xenopus laevis* oocytes

(mRNA expression/P glycoprotein/multidrug transporter)

GONZALO CASTILLO*, JUAN C. VERA*, CHIA-PING HUANG YANG†, SUSAN BAND HORWITZ†, AND ORA M. ROSEN*

*Program in Molecular Biology, Memorial Sloan-Kettering Cancer Research Center, New York, NY 10021; and †Departments of Molecular Pharmacology and Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

Contributed by Ora M. Rosen, March 23, 1990

ABSTRACT The development of multidrug resistance (MDR) is associated with the overproduction of a plasma membrane glycoprotein, P glycoprotein. Here we report the functional expression of a member of the murine mdr family of proteins and show that *Xenopus* oocytes injected with RNA encoding the mouse *mdr*1b P glycoprotein develop a MDR-like phenotype. Immunological analysis indicated that oocytes injected with the *mdr*1b RNA synthesized a protein with the size and immunological characteristics of the mouse *mdr*1b P glycoprotein. These oocytes exhibited a decreased accumulation of [^{3}H]vinblastine and showed an increased capacity to extrude the drug compared to control oocytes not expressing the P glycoprotein. In addition, competition experiments indicated that verapamil, vincristine, daunomycin, and quinidine, but not colchicine, can overcome the rapid drug efflux conferred by the expression of the mouse P glycoprotein.

Cultured mammalian cells selected for resistance to a single drug often develop simultaneous resistance to a structurally diverse group of drugs, a phenomenon referred to as multidrug resistance (MDR) (1–4). Recent studies indicate that this increased resistance is associated with the overproduction of a family of high molecular weight membrane glycoproteins, the P glycoproteins.

A number of cDNA clones encoding a family of structurally related P glycoproteins have been isolated from resistant cell lines obtained by stepwise drug selection protocols (5–9). These include cDNA clones for three rodent and two human mdr genes (9–11). Sequence analysis of these clones indicates that the corresponding proteins are composed of two homologous halves, each encoding six predicted transmembrane domains and two putative ATP binding domains (5–8).

The MDR phenotype in resistant cells is characterized by a decreased intracellular drug accumulation as compared to sensitive cells. This decreased intracellular accumulation is associated with an increased drug efflux from these cells in a process that appears to be ATP-dependent (12–14). These and additional observations (15–20) have led to the hypothesis that the P glycoprotein acts as an energy-dependent drug-efflux pump and functions directly as a multidrug transporter in MDR cells. In fact, cDNA and genomic transfection experiments have provided evidence indicating that increased expression of the human, hamster, and mouse P glycoprotein can mediate the MDR phenotype (21–29). Interestingly, these studies have shown that the transfection of a single member of the P glycoprotein gene family results in the transfer of a variable MDR phenotype to the recipient drug-sensitive cells. In addition, the levels of resistance attained in cDNA transfected cells expressing high levels of P glycoprotein are in no manner similar to those observed in cell lines transfected with genomic DNA from MDR cells or to cells lines selected by stepwise selection in the presence of increasing concentration of drugs (28, 29).

We report herein the functional expression of a member of the mouse family of mdr genes (*mdr*1b) in *Xenopus laevis* oocytes and present evidence supporting the notion of an active participation of the P glycoprotein in the MDR phenotype. The results presented in this communication indicate that oocytes expressing the mouse *mdr*1b protein exhibit a decreased accumulation of vinblastine and extrude the drug at a rate several times higher than that of control oocytes not expressing the P glycoprotein.

MATERIALS AND METHODS

Materials. Vinblastine sulfate, daunomycin, actinomycin D, vincristine, colchicine, and quinidine were obtained from Sigma. [^{3}H]Vinblastine sulfate (specific activity, 10.5 Ci/mmol; 1 Ci = 37 GBq) was obtained from Amersham.

Plasmids. The cDNA encoding the mouse *mdr*1b P glycoprotein was provided by Stephen Hsu (9) and was subcloned into the *Eco*RI site of the plasmid pBluescript ks+ (Stratagene).

***In Vitro* Transcription.** The plasmid containing the mdr1b cDNA was linearized with *Hin*dIII and used for *in vitro* transcription and capping (30, 31).

Oocyte Preparation and RNA Injection. *X. laevis* oocytes were obtained from ovaries from mature frogs by treatment with collagenase, injected with RNA, and incubated for 2 days at 18°C in Barth medium (30, 31).

[^{3}H]Vinblastine Accumulation. Oocytes (5–20 per group) were incubated for up to 120 min in 1 ml of Barth medium containing 2 μCi of [^{3}H]vinblastine plus different concentrations of unlabeled vinblastine. Uptake was terminated by washing the oocytes with ice-cold Barth medium. The oocytes were dissolved in 0.5 ml of 10 mM Tris·HCl (pH 7.8) containing 1% SDS, and the associated radioactivity was assayed by liquid scintillation spectroscopy. In studies on the effect of other drugs on the accumulation of [^{3}H]vinblastine, the respective drugs were present during the initial incubation period at the concentrations indicated in the figure legends.

[^{3}H]Vinblastine Efflux. Oocytes were incubated for 60 min at room temperature in Barth medium containing 100 μM [^{3}H]vinblastine. Under these conditions, injected and control oocytes achieved similar intracellular concentrations of [^{3}H]vinblastine. Efflux of vinblastine was initiated by three rapid washes in Barth medium (this step can be carried out in <30 sec) and then the oocytes were maintained in a great excess of Barth medium (usually 250 ml). Groups of 5–20 oocytes were taken at specified times and processed for liquid scintillation spectroscopy. Results are expressed as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: MDR, multidrug resistance.

percent of the total initial radioactivity present in oocytes after the 60-min incubation period.

Immunoblotting. Forty-eight hours after injection of water or *in vitro* synthesized RNA, groups of 100 oocytes were homogenized in 500 μl of 50 mM Tris·HCl, pH 7.8/5 mM EDTA containing 20 μg (each) of aprotinin, leupeptin, and soybean trypsin inhibitor per ml. After centrifugation at 800 $\times$ *g* for 10 min, the supernatant was subjected to a second round of centrifugation at 20,000 $\times$ *g* for 15 min. The 20,000 $\times$ *g* pellet was resuspended in electrophoresis sample buffer (32) and fractionated by SDS/polyacrylamide gel electrophoresis (32). Proteins were transferred electrophoretically to nitrocellulose membranes (33) and probed with the antipeptide antibody *mdr* (Ab-1) (Oncogene Science, Manhasset, NY). This is a rabbit, affinity-purified polyclonal antibody raised against the peptide Cys-Ala-Leu-Asp-Thr-Glu-Ser-Glu-Lys-Val-Val-Gln-Glu-Ala-Leu-Asp-Lys-Ala-Arg-Glu-Gly from the C-terminal region of P glycoprotein. As controls, plasma membrane-enriched fractions were prepared (34) from the murine cell lines J7.C1-100 (selected by resistance to colchicine) and J774.2 (the nonresistant sibling cell line) (9). The blots were also probed with an antipeptide antibody raised against a peptide from the linker region of the mouse mdr1b P glycoprotein (9). The detection system consisted of ^{125}I-labeled protein A.

RESULTS

Fig. 1 shows an immunoblot of oocytes microinjected with RNA generated by *in vitro* transcription of the cloned mouse *mdr*1b cDNA. Two days after the injection, total membranes were prepared, subjected to SDS/polyacrylamide gel electrophoresis, and probed with an affinity-purified antipeptide antibody directed against the C-terminal cytoplasmic region of P glycoprotein (see *Materials and Methods*). The antibody recognized a protein band that migrates with an apparent size of 140–170 kDa (Fig. 1, lane 2), which is not present in uninjected oocytes (Fig. 1, lane 1). For controls, we used membranes prepared from a cell line, J7.C1-100, that overexpresses the *mdr*1b protein. The antibody detected the mature 140-kDa P glycoprotein in this cell line (Fig. 1, lane 4), but it did not react with the respective protein in J774.2 cells (Fig. 1, lane 3), a cell line not expressing the MDR phenotype (9). Equivalent results were obtained when the immunoblotting experiments were repeated using an antipeptide antibody raised against a peptide from the linker region of the mdr1b protein (9) or the monoclonal antibody C219 (35) (data not shown).

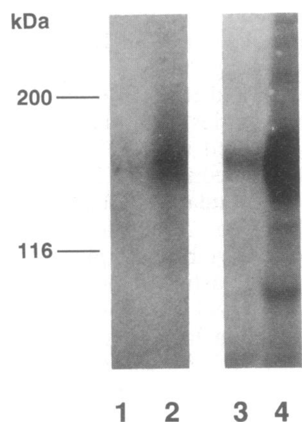

FIG. 1. Expression of the mouse *mdr*1b P glycoprotein in *Xenopus* oocytes. *(Left)* Oocytes were injected with 25 ng of *in vitro* synthesized RNA encoding the mouse *mdr*1b protein and were incubated for 2 days in Barth medium. Oocytes were solubilized and membranes were purified by differential centrifugation. Membranes corresponding to 20 oocytes (about 100 μg of protein) were solubilized in loading buffer and submitted to polyacrylamide gel electrophoresis in the presence of SDS. After transfer to nitrocellulose, the membranes were probed with the antipeptide antibody *mdr* (Ab-1). Lane 1, oocytes injected with water; lane 2, oocytes injected with RNA for MDR. *(Right)* As controls, membranes from the drug-resistant cell line J7.C1-100 (lane 4) and the drug-sensitive cell line J774.2 (lane 3) were also probed with the *mdr* (Ab-1) antipeptide antibody. About 30 μg of membrane protein was loaded in each lane. Positions of molecular mass markers are shown on the left.

Previous studies have demonstrated that cells overexpressing the P glycoprotein exhibit reduced accumulation of a wide variety of chemotherapeutic drugs (1–4, 12–14, 17, 19, 28, 29, 34). To determine the functional effects of expressing the *mdr*1b protein in *Xenopus* oocytes, we measured accumulation of radioactive vinblastine 2 days after injecting the RNA. In this experiment, groups of oocytes were incubated in the presence of different concentrations of labeled vinblastine and accumulation of the drug was monitored for up to 60 min. As shown in Fig. 2, the accumulation of vinblastine was markedly decreased in oocytes injected with RNA encoding the *mdr*1b protein, as compared with oocytes injected with water. As expected, increasing the concentration of the drug in the incubation medium produced a corresponding increase in the accumulation of vinblastine into uninjected oocytes, an observation related to the fact that this drug appears to diffuse passively through the cell plasma membrane. In fact, at each drug concentration tested, the amount of drug taken up by the oocytes after a 60-min incubation period corresponds to that expected if we assume an almost complete equilibrium between the internal and external concentrations of the drug (see Fig. 2). On the other hand, oocytes injected with RNA encoding the *mdr*1b protein showed a very low level of drug accumulation, and this uptake was not sensitive to changes in the concentration of vinblastine in the range of 0.3–1.0 μM (data not shown). At 1 μM vinblastine, injected oocytes accumulated 20-fold less drug than control oocytes. The difference in the uptake of vinblastine was no longer seen when injected and control oocytes were incubated in the presence of 100 μM vinblastine (data not shown).

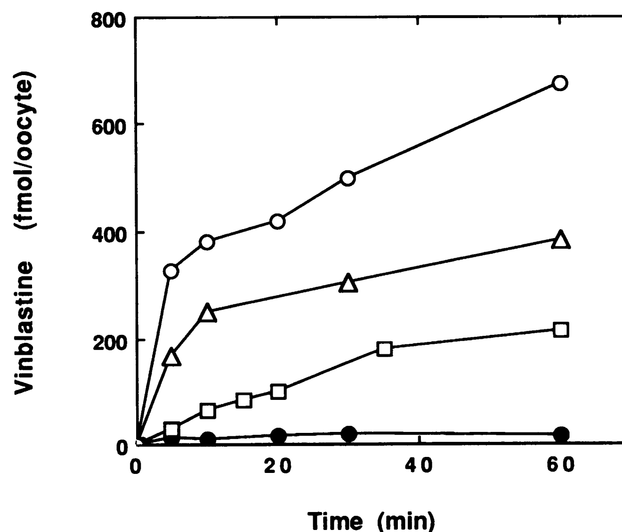

FIG. 2. Accumulation of vinblastine by oocytes expressing the mouse *mdr*1b protein. Oocytes were injected with 25 ng of RNA encoding the mouse *mdr*1b protein and vinblastine accumulation was measured 2 days after the injection (●). Controls (○, △, □) were injected with water. Oocytes were incubated in Barth medium containing 0.3 (□), 0.6 (△), or 1.0 (○, ●) μM [^{3}H]vinblastine for the indicated periods of time. The radioactivity associated with the oocytes was determined after washing the oocytes. Data represent the mean of two to three groups of 10–20 oocytes each.

In cells expressing P glycoprotein, the MDR phenotype is sensitive to, and can be totally reverted by, the Ca^{2+} channel blocker verapamil and related drugs (1–4, 19, 36). The reversal of resistance by verapamil is accompanied by a corresponding increase in the accumulation of drugs by the resistant cells. Therefore, it was decided to test whether verapamil affected the accumulation of vinblastine by oocytes expressing the *mdr*1b protein. As can be seen in Fig. 3, verapamil produced a dramatic effect in the accumulation of vinblastine by injected oocytes but did not affect significantly the accumulation of the drug in control cells (see also Fig. 4). In the presence of 100 μM verapamil, the accumulation of vinblastine by injected oocytes is similar to that seen in the respective controls (see Figs. 3 and 4).

Another fundamental property of cells expressing the MDR phenotype is that they show resistance to a series of chemically unrelated drugs (1–4). In addition, the accumulation of a given drug by a resistant cell line can be affected by the presence of other drugs. The results shown in Fig. 4 indicate that this is also true in the case of oocytes expressing the *mdr*1b protein. The drugs verapamil, daunomycin, and quinidine have a dramatic effect on the accumulation of vinblastine by injected oocytes. A significant effect was also observed when the uptake experiments were carried out in the presence of vincristine. Most interestingly, considering the fact that the cDNA encoding the *mdr*1b protein was isolated from cells selected by resistance to colchicine (9), the presence of 100 μM colchicine did not appear to affect the accumulation of vinblastine by the oocytes expressing the *mdr*1b protein. As expected, the presence of the different drugs mentioned above did not significantly affect the accumulation of vinblastine by control oocytes (see Fig. 4).

The diminished accumulation of drugs in resistant cells is believed to be the result of enhanced drug efflux with active participation of the P glycoprotein, and the reversion of the resistant phenotype by verapamil and related drugs appears to be mediated through the direct inhibition of the efflux rather than activation of drug uptake (1–4). To address this point in oocytes expressing the *mdr*1b protein, we measured efflux of vinblastine from oocytes loaded with the drug and studied the effect of verapamil on the efflux (Fig. 5). Vinblastine was released rapidly from oocytes expressing the *mdr*1b protein (50% of the drug was released in <10 min) but was released very slowly from control oocytes (about 10% of the drug was released in <10 min, with a maximum release of 20% in 60 min). The release of vinblastine by injected oocytes was completely inhibited by the presence of 100 μM verapamil in the incubation medium at levels equivalent to those observed with control oocytes (see Fig. 5). A partial degree of inhibition was observed when these experiments were carried out in the presence of 20 μM verapamil (data not shown). On the other hand, verapamil did not appear to affect

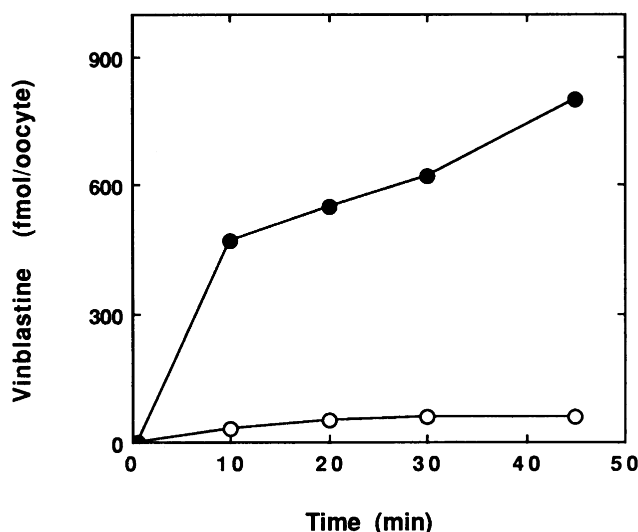

FIG. 3. Effect of verapamil on the accumulation of vinblastine by oocytes expressing the *mdr*1b protein. Oocytes were injected with 25 ng of RNA encoding the mouse *mdr*1b protein and incubated for 2 days in Barth medium. Accumulation of 1 μM [³H]vinblastine was measured in the absence (○) or the presence (●) of 100 μM verapamil. Data represent the mean of two to four groups of 5–10 oocytes each.

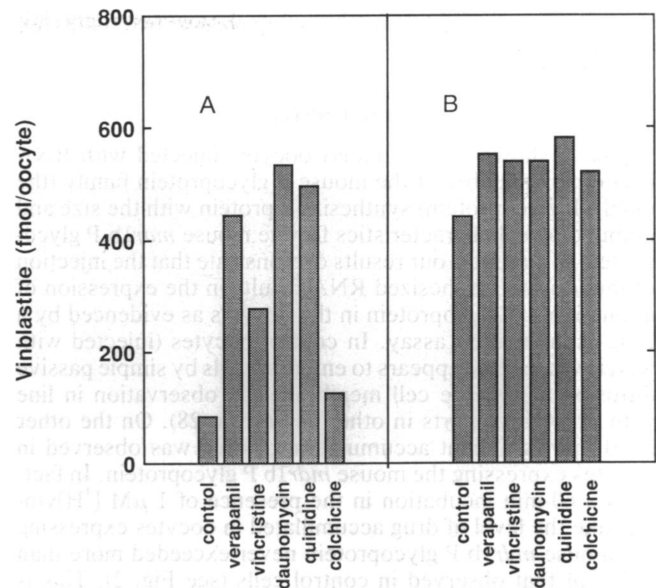

FIG. 4. Effect of different drugs on the accumulation of vinblastine by oocytes expressing the *mdr*1b protein. Oocytes were injected with RNA encoding the mouse *mdr*1b protein (*A*) and incubated for 2 days in Barth medium. Controls (*B*) were injected with water. Oocytes were incubated for 60 min in Barth medium containing 1 μM [³H]vinblastine (controls) or [³H]vinblastine plus 100 μM of the indicated drugs. Data represent the mean of two to six groups of 10 oocytes each.

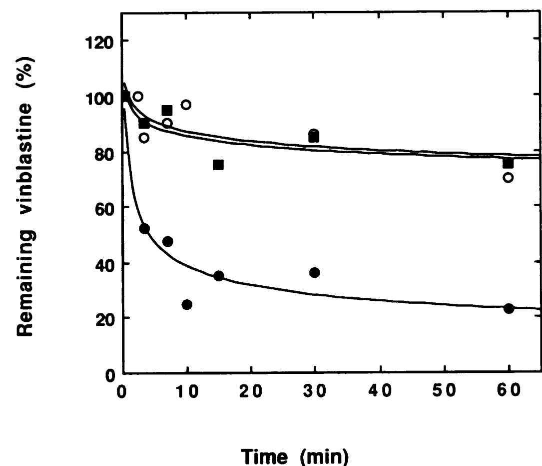

FIG. 5. Efflux of vinblastine from oocytes expressing the *mdr*1b protein. Oocytes were injected with 25 ng of RNA encoding the mouse *mdr*1b protein (●, ■) and incubated in Barth medium for 2 days. Controls (○) were injected with water. Oocytes were incubated in Barth medium containing 100 μM [³H]vinblastine for 60 min, washed, and incubated for the indicated times in Barth medium lacking vinblastine before measuring the amount of [³H]vinblastine remaining in the oocytes. This last incubation was carried out in the absence (○, ●) or the presence (■) of 100 μM verapamil. Data correspond to the mean of two to four groups of 5–10 oocytes each.

the release of vinblastine by control oocytes (data not shown).

DISCUSSION

We have shown that *X. laevis* oocytes injected with RNA encoding a member of the mouse P glycoprotein family (the *mdr*1b P glycoprotein) synthesize a protein with the size and immunological characteristics for the mouse *mdr*1b P glycoprotein. Moreover, our results demonstrate that the injection of the *in vitro* synthesized RNA results in the expression of a functional P glycoprotein in the oocytes as evidenced by a drug-accumulation assay. In control oocytes (injected with water) vinblastine appears to enter the cells by simple passive diffusion across the cell membrane, an observation in line with previous reports in other cell types (28). On the other hand, no significant accumulation of drug was observed in oocytes expressing the mouse *mdr*1b P glycoprotein. In fact, after a 60-min incubation in the presence of 1 μM [^{3}H]vinblastine the level of drug accumulated in oocytes expressing the mouse *mdr*1b P glycoprotein never exceeded more than 5–7% of that observed in control cells (see Fig. 2). This is interesting in comparison with reports that indicate differences from <2- (29) to at most 4-fold (28) in the levels of accumulation of drugs in mammalian cells made resistant through cDNA transfection, compared to untransfected cells. In fact, only highly resistant cells selected through the use of stepwise selection protocols show a large decrease, 5- to 15-fold, in the intracellular accumulation of drug when challenged (36, 37). One possible explanation for this apparent discrepancy is that the large decrease in drug accumulation observed in oocytes injected with the RNA encoding the mouse *mdr*1b P glycoprotein is due to the expression of large amounts of the P glycoprotein. However, the immunoblot analysis shown in Fig. 1 appears to suggest that this is not the case. Although it is hard to draw any conclusion on the quantitative aspects of these kinds of experiments, it is evident that oocytes express much less P glycoprotein than an equivalent preparation of membranes from a highly resistant murine cell line. This conclusion is based on the fact that the antibody used in these studies, *mdr* (Ab-1), was raised against a synthetic peptide representing a sequence present in the C-terminal region of P glycoprotein. In addition, identical results in terms of degree of reactivity were observed using a second antibody (9) raised against a synthetic peptide represented in the linker region of the mouse *mdr*1b P glycoprotein.

Given the characteristics of the experimental approach, which includes the injection of an *in vitro* synthesized RNA and a 2-day incubation period, it is easily concluded that the new functional characteristics expressed by the injected oocytes (in terms of drug accumulation) are the product of the expression of the mouse *mdr*1b P glycoprotein. This conclusion is reinforced by the competition experiments that indicate that verapamil, vincristine, daunomycin, and quinidine can overcome the resistance to drug accumulation conferred by the expression of the mouse *mdr*1b P glycoprotein into oocytes, indicating that the injected oocytes have developed a true MDR phenotype. A rather unexpected observation is the fact that high concentrations of colchicine did not affect the accumulation of vinblastine in injected oocytes (see Fig. 4), considering that the *mdr*1b cDNA was isolated from a cell line selected for resistance to colchicine (9). A similar phenomenon has been observed in studies of the effect of colchicine on the binding of vinblastine to cell membranes obtained from resistant cell lines selected for resistance to colchicine (16, 38), raising the prospect of the existence of different binding sites for different drugs.

Our results also show that expression of the mouse *mdr*1b P glycoprotein into *Xenopus* oocytes significantly increases the capacity of these cells to extrude preloaded vinblastine. The initial velocity of extrusion is 10- to 20-fold greater in oocytes injected with RNA than controls injected with water and probably accounts for the observed differences in the intracellular accumulation of drug observed in the uptake experiments. As expected, high concentrations of verapamil effectively inhibited the efflux of vinblastine from previously loaded cells, a prediction derived from the proposed role of the P glycoprotein as a drug-extrusion pump. This is also in line with the observation that no differences were observed in the accumulation of drug by oocytes injected with RNA, and the respective controls injected with water, when the uptake experiments were carried out in the presence of an elevated concentration of vinblastine (100 μM).

In conclusion, our results indicate that the mouse *mdr*1b P glycoprotein exhibits functional properties that fit its proposed role as a drug-extrusion pump and that its sole expression can confer a MDR phenotype in otherwise sensitive cells. Although mammalian P glycoproteins have been expressed in cDNA transfected cells, some of the results obtained studying those transfected cells are difficult to interpret due to the experimental protocol involved. One important point is that after the transfection step the cells must undergo at least two rounds of selection in the presence of drugs to be isolated as transfectants (24–29). This is related to the low level of resistance shown by the transfected cells. Although clear evidence for the expression of the transfected P glycoprotein has been presented, the selection protocol makes it difficult to establish the presence of the P glycoprotein as the only factor involved in the acquired MDR phenotype. In addition, the selection protocol makes it difficult to obtain cells expressing mutant proteins with a diminished efficiency as a drug-extrusion pump (27, 29). The *Xenopus* oocyte system offers the opportunity to address the study of the functional–structural properties of the mammalian P glycoprotein using an extremely simple experimental approach. The results described in this communication present the advantages offered by this system and provide unequivocal evidence that the sole expression of the mouse *mdr*1b P glycoprotein is enough to confer the MDR phenotype.

We thank Stephen Hsu for providing us with the mouse *mdr*1b cDNA. This work was supported by National Institutes of Health Grant DK 35158 and Grant NP 6225 from the American Cancer Society to O.M.R.

1. Gottesman, M. & Pastan, I. (1989) *J. Biol. Chem.* **263**, 12163–12166.
2. Juranka, P., Zastawny, R. & Ling, V. (1989) *FASEB J.* **3**, 2583–2592.
3. Endicott, J. & Ling, V. (1989) *Annu. Rev. Biochem.* **58**, 137–171.
4. van der Bliek, A. & Borst, P. (1989) *Adv. Cancer Res.* **52**, 165–202.
5. Gros, P., Croop, J. & Housman, D. (1986) *Cell* **47**, 371–380.
6. Gros, P., Raymond, M., Bell, J. & Housman, D. (1988) *Mol. Cell. Biol.* **8**, 2770–2778.
7. Chen, C., Chin, J., Ueda, K., Clark, D., Pastan, I., Gottesman, M. & Roninson, I. (1986) *Cell* **47**, 381–389.
8. Gerlach, J., Endicot, J., Juranka, P., Henderson, G., Sarangi, F., Deuchars, K. & Ling, V. (1986) *Nature (London)* **324**, 485–489.
9. Hsu, S., Lothstein, L. & Horwitz, S. (1989) *J. Biol. Chem.* **264**, 12053–12062.
10. Ng, W., Sarangi, F., Zastawny, R., Veinot-Drebot, L. & Ling, V. (1989) *Mol. Cell. Biol.* **9**, 1224–1232.
11. Chin, J., Soffir, R., Noonan, K., Choi, K. & Roninson, I. (1989) *Mol. Cell. Biol.* **9**, 3808–3820.
12. Dano, K. (1973) *Biochim. Biophys. Acta* **323**, 446–483.
13. Inaba, M., Kobayashi, H., Sakurai, Y. & Johnson, R. (1979) *Cancer Res.* **39**, 2200–2203.

14. Fojo, A., Akiyama, S., Gottesman, M. & Pastan, I. (1985) *Cancer Res.* **45**, 3002–3007.
15. Cornwell, M., Safa, A., Felsted, R., Gottesman, M. & Pastan, I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3847–3850.
16. Cornwell, M., Pastan, I. & Gottesman, M. (1987) *J. Biol. Chem.* **262**, 2166–2170.
17. Hamada, H. & Tsuruo, T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7785–7789.
18. Hamada, H. & Tsuruo, T. (1988) *J. Biol. Chem.* **25**, 1454–1458.
19. Willingham, M., Cornwell, M., Cardarelli, C., Gottesman, M. & Pastan, I. (1986) *Cancer Res.* **46**, 5941–5946.
20. Horio, M., Gottesman, M. & Pastan, I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3580–3584.
21. Debenham, P., Kartner, N., Siminovitch, L., Riordan, J. & Ling, V. (1982) *Mol. Cell. Biol.* **2**, 881–889.
22. Gros, P., Neriah, Y., Croop, J. & Housman, D. (1986) *Nature (London)* **323**, 728–731.
23. Gros, P., Fallows, D., Croop, J. & Housman, D. (1986) *Mol. Cell. Biol.* **6**, 3785–3790.
24. Ueda, K., Cardarelli, C., Gottesman, M. & Pastan, I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3004–3008.
25. Deuchars, K., Du, R.-P., Naik, M., Evernden-Porelle, D., Kartner, N., van der Bliek, A. & Ling, V. (1987) *Mol. Cell. Biol.* **7**, 718–724.
26. Choi, K., Chen, C., Kriegler, M. & Roninson, I. (1988) *Cell* **53**, 519–529.
27. Currier, S., Ueda, K., Willingham, M., Pastan, I. & Gottesman, M. (1989) *J. Biol. Chem.* **264**, 14376–14381.
28. Hammond, J., Johnstone, R. & Gros, P. (1989) *Cancer Res.* **49**, 3867–3871.
29. Azzaria, M., Schurr, E. & Gros, P. (1989) *Mol. Cell. Biol.* **9**, 5289–5297.
30. Vera, J. C. & Rosen, O. M. (1989) *Mol. Cell. Biol.* **9**, 4187–4195.
31. Vera, J. C. & Rosen, O. M. (1990) *Mol. Cell. Biol.* **10**, 743–751.
32. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
33. Rivas, C., Vera, J. C. & Maccioni, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6092–6096.
34. Roy, S. & Horwitz, S. (1985) *Cancer Res.* **45**, 3856–3863.
35. Kartner, N., Evernden-Porelle, D., Bradley, G. & Ling, V. (1985) *Nature (London)* **316**, 820–823.
36. Tsuruo, T., Iida, H., Tsukagoshi, T. & Sakurai, Y. (1981) *Cancer Res.* **41**, 1967–1972.
37. Meyers, M., Rittmann-Grauer, L., O'Brien, J. & Safa, A. (1989) *Cancer Res.* **49**, 3209–3214.
38. Cornwell, M., Gottesman, M. & Pastan, I. (1986) **261**, 7921–7928.