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Functional Expression of Yeast Artificial Chromosome–Human Multidrug Resistance Genes in Mouse Cells

Hitoshi Kusaba,¹ Kimitoshi Kohno,¹ Keizo Asakuno,¹
 Michihiko Kuwano,¹ Katsuzumi Okumura,² Eric D. Green,³
 David Schlessinger,⁴ and Morimasa Wada^{1,5}

¹Department of Biochemistry, Kyushu University School of Medicine, Maidashi, Fukuoka 812-82, Japan;

²Faculty of Bioresources, Mie University, Tsu, Mie 514, Japan; ³National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland 20892; ⁴Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

Multidrug resistance (MDR) genes, which are ATP-binding cassette family genes, encode the cell surface glycoprotein, P-glycoprotein, which functions as an energy-dependent drug efflux pump. Two relevant human genes, *PGY1* and *PGY3*, are located on human chromosome 7, and three relevant mouse genes, *mdr1a*, *mdr1b*, and *mdr2*, are located on mouse chromosome 5. An LMDI cell line was established after the transfer of a 580-kb yeast artificial chromosome (YAC) clone carrying the human MDR locus into mouse L cells; the cell line was shown to have stably integrated YAC DNA in an apparent intact form. Using LMDI cells as the parental cell line, five vincristine-resistant sublines, designated LMDI-V50, LMDI-V100, LMDI-V200, LMDI-V500, and LMDI-V1000, were isolated by exposure to increasing concentrations of the drug. LMDI-V50, LMDI-V100, LMDI-V200, LMDI-V500, and LMDI-V1000 showed 3-, 7-, 13-, 45-, and 110-fold higher resistance to the cytotoxic effects of vincristine, respectively, than their parental counterpart, LMDI. Immunofluorescence, Western blot, and Northern blot analyses revealed that the human *PGY1* gene or its product was overexpressed, accompanied by gene amplification. The human *PGY3* gene was also overexpressed in the LMDI-V20, LMDI-V100, and LMDI-V1000 cell lines. Southern blot and fluorescence in situ hybridization (FISH) analyses demonstrated that although essentially the entire YAC DNA was integrated in mouse genome and amplified, the endogenous mouse *mdr* genes were not amplified in these drug-resistant cell lines. Similar results were obtained by the analyses of vincristine-resistant cell lines isolated from four independent subclones of LMDI cells. Thus, in contrast to their mouse counterparts, the integrated human MDR genes retained susceptibility to both gene activation and amplification, during the selection of drug-resistant mouse cell lines. The possibility that transferred YACs may retain regulatory properties observed in the cells of origin, and may have a chromatin structure that favors augmented expression, is discussed.

The acquisition of multidrug resistance (MDR) in vitro is commonly associated with the increased expression of the cellular surface protein, P-glycoprotein, which functions as an energy-dependent drug efflux pump, resulting in a decrease of intracellular drug concentration (Pastan and Gottesman 1987; Bradley et al. 1988; van der

Blik and Borst 1989). Mammalian P-glycoproteins are encoded by small families of the linked genes, of which there are two known members in humans (*PGY1* and *PGY3* which are also called *MDR1* and *MDR3*, respectively), (Roninson et al. 1984; Ueda et al. 1987; Chin et al. 1989; van der Blik and Borst 1989; Lincke et al. 1990), and three members each in mice (*mdr1a*, *mdr1b*, and *mdr2*) (Gros et al. 1986a,b; Devault and Gros 1990) and hamsters (*pgp1*, *pgp2*, and *pgp3*) (Endicott et al. 1991). The transfection of cDNA derived from *PGY1*, *mdr1a*, and *mdr1b* confer mul-

⁵Corresponding author.
 E-MAIL wada@biochem1.med.kyushu-u.ac.jp; FAX 81-92-632-4198.

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tidrug resistance, whereas *PGY3* and *mdr2* cDNA does not confer this resistance (Gros et al. 1986a; Ueda et al. 1987; van der Bliek et al. 1988; Hsu et al. 1989; Devault and Gros 1990; Schinkel et al. 1991). The functional role of each class of MDR genes has also been studied in vivo. Mice defective in the *mdr2* gene cannot secrete phospholipids into bile (Smit et al. 1993), whereas mice defective in *mdr1a* have increased sensitivity to drugs and an impaired blood-brain barrier (Schinkel et al. 1994). *PGY1* and *PGY3* have been mapped to human chromosome 7 (Fojo et al. 1986; Chin et al. 1989), and three rodent counterparts have been localized on mouse chromosome 5 (Hsu et al. 1989).

The entire human MDR locus covers ~230 kb, and the human *PGY1* and *PGY3* genes are closely linked, separated by only 34 kb of intergenic DNA region and transcribed in the same direction (Chin et al. 1989; Lincke et al. 1991; Matsuda et al. 1993). The human *PGY1* gene is expressed in various tissues, especially in the epithelia of excretory organs, whereas the *PGY3* gene is expressed only in the liver (Chen et al. 1986; van der Bliek et al. 1986). In many drug-resistant cultured cell lines, a high level of MDR gene expression is associated with amplification of the human MDR locus (Riordan et al. 1985; van der Bliek et al. 1988). However, in vitro and in vivo studies in rodent and human models have demonstrated that the expression of the MDR gene can also be enhanced by a number of exogenous stimuli, including anticancer agents, heat shock, hepatectomy, carcinogens, arsenite, sodium butyrate, and retinoic acid (Fairchild et al. 1987; Thorgeirsson et al. 1987; Bates et al. 1989; Mickley et al. 1989; Chin et al. 1990; Marino et al. 1990). Utilizing a construct containing the human *PGY1* promoter fused to the chloramphenicol acetyltransferase (CAT) gene, we have demonstrated, in transient expression assays, that anticancer agents such as vincristine and daunomycin can activate the human *PGY1* promoter (Kohnno et al. 1989). In addition, we have isolated human KB cancer cell lines, stably transfected with a CAT reporter gene driven by the human *PGY1* promoter (Kohnno et al. 1990). Using these cell lines, we have recently demonstrated that serum starvation, heat shock, anticancer agents, and ultraviolet light irradiation can activate the human *PGY1* gene promoter (Kohnno et al. 1992; Miyazaki et al. 1992; Tanimura et al. 1992; Uchiumi et al. 1993a,b; Asakuno et al. 1994). These observations suggest that the human *PGY1* gene

could be inducible by environmental stress (Kohnno et al. 1994). The increase in *PGY1* promoter-driven CAT activity in response to these exogenous stimuli is mediated through rapid transcriptional activation (Uchiumi et al. 1993a). This activation, in turn, appears to be mediated through nuclear *trans*-acting factors (Kohnno et al. 1992; Uchiumi et al. 1993a; Asakuno et al. 1994; Kohnno et al. 1994). Our recent study has demonstrated that expression of the *PGY1* promoter-driven CAT is increased along with expression of the endogenous *PGY1* gene in human cancer cells during the early steps of selection by drugs but is decreased with the onset of *PGY1* gene amplification (Kohnno et al. 1994). During the selection of resistant phenotypes by drugs, there appears to be a switch from transcriptional activation to increased expression from an amplified number of human *PGY1* gene copies (Kohnno et al. 1994; see also Discussion).

How is the expression of the entire MDR locus, including the *PGY1* and *PGY3* genes, modulated during the acquisition of a MDR phenotype? One route to analyze the function and regulation of the entire human MDR locus could be provided if the entire large DNA segment containing this locus could be transferred into other mammalian cells. Recently, transfer of loci this large has been accomplished using yeast artificial chromosome (YAC)-mediated transfections (D'Urso et al. 1990; Gnirke et al. 1991; Huxley et al. 1991; Strauss and Jaenisch 1992). It has been shown that YAC can be transferred from yeast to mouse cells without major or detectable rearrangement and integrated into mouse chromosome by either of polyethylene glycol (PEG)-mediated spheroplast fusion (Pachnis et al. 1990; Pavan et al. 1990) or lipofection (Gnirke et al. 1991). Previously, we have documented a neomycin-resistant cassette introduced into YAC. The modified YACs were transferred into human cells by a PEG-mediated fusion method (Wada et al. 1994b). Also, we have isolated YAC clones containing the *PGY1* gene after screening a YAC library prepared from total human DNA using primer pairs of the promoter region as a probe (Matsuda et al. 1993). In this study we introduced a modified YAC clone containing the entire human MDR locus into mouse L cells and established a series of vincristine-resistant cell lines. The expression of individual members of both human and mouse MDR gene families was then studied to assess the comparative regulation of all the genes in the same cohort of cells.

RESULTS

Establishment of Mouse Cell Lines Carrying YAC-MDR DNA

To permit the selection of stable transformants,

the neomycin (*neo*) gene was retrofitted to the right arm by introduction of the pRV1 plasmid (Fig. 1) for selection after the transfer of YAC DNA into the mouse cell (Srivastava and Schless-

inger 1991). The pRV1 plasmid carries the *URA3* gene disrupted by *LYS2* and *neo* genes, and *LYS2*-*neo* genes are then expected to be inserted at the right arm by homologous recombination after introduction into yeast carrying the YAC clone (Fig. 1A). Modification was confirmed by Southern blot analysis, as shown in Figure 1B. *SalI* digestion of YAC DNA generated 3-kb fragments from the right arm of the YAC-MDR 167 (167), but a 13-kb fragment was generated by equivalent restriction digestion of the recombinant YAC-MDR167-*neo* (167-*neo*) (see Fig. 1A).

To obtain stable transformants, 10^8 yeast cells containing modified YAC were fused to 2×10^6 mouse L cells by PEG-mediated spheroplast fusion in three separate experiments (Wada et al. 1994b). Finally, one stable *neo*-resistant clone, LMD1, was isolated and expanded for further analysis. Transfer and integration of the YAC into mouse genome were assessed initially by pulsed-field gel electrophoresis (PFGE) and Southern blot analysis using right arm, left arm, and *neo* fragments as probes (data not shown). The DNA sequence information derived from the *PGY1* and *PGY3* genes and both insert ends of YAC-MDR167 allowed the development of PCR assays specific for four sequence-tagged sites (STSs; Green 1993). These PCR assays were used to analyze DNA purified from LMD1 cells; four STSs were detected in LMD1 cells and human placenta DNA but not in L cells (Fig. 2). We used labeled YAC-MDR167 DNA to perform an in situ hybridization study, and hybridization fluorescence signal was observed in LMD1

Figure 1 Integration of the *neo* gene into YAC carrying the human MDR region. (A) Schematic representation of integration of *neo* gene. (Top) MDR region and right arm of original YAC. *SalI* restriction site is shown; (middle) *LYS2* and the *neo* gene have been inserted into the *URA3* gene; (bottom) part of the modified YAC. The size of each region is indicated in kilobases. (B) Southern blot analysis of the modified YAC. DNA was extracted from each yeast clone carrying original YAC (167) or modified YAC (167-*neo*), and PFGE (left) or conventional gel electrophoresis after digestion by *SalI* (right) was performed. DNA was transferred onto a nylon membrane and hybridized with right arm (Rt. arm) or *neo* probe as indicated.

Figure 2 Identification of STSs on DNA prepared from LMD1 cells by PCR analysis. (A) PCR assays specific for four STSs derived from *PGY1*, (B) *PGY3*, (C) left end, and (D) right end were used to analyze DNA from human placenta, LMD1, and mouse L cell line. The sequences of each STS are described in Methods. The expected electrophoretic positions of the PCR products are indicated by arrowheads.

cells (Fig. 3A,C). The in situ hybridization results were consistent with the results obtained by PCR assays. Two copies of the YAC clones were found to be integrated into different mouse chromosomes.

The coordinate amplification of both right and left vector arms in stepwise manner in the drug-resistant derivatives of LMD1 (see Fig. 9, below) also suggest that whole YAC DNA has been integrated into same chromosomal position in an apparent intact form.

Drug resistance to an anticancer agent, vincristine, is often associated with increased cellular levels of P-glycoprotein, which is encoded by the human *PGY1* gene (Akiyama et al. 1985; Kohno et al. 1988; Kohno et al. 1994). We analyzed drug sensitivity to vincristine by carrying out a colony-forming assay. However, LMD1 cells showed only a 1.5-fold resistance to vincristine compared with that shown by L cells (data not shown). A series of vincristine-resistant cell lines was isolated by exposing the LMD1 cells to an increasing concentration of the drug; these lines were des-

ignated LMD1-V50, LMD1-V100, LMD1-V200, LMD1-V500, and LMD1-V1000. Dose-response curves demonstrated that these cell lines showed 3-, 7-, 13-, 45-, and 110-fold higher resistance to vincristine than their parental counterpart, LMD1 (Table 1). These drug-resistant cell lines also showed cross-resistance to other agents, including colchicine, adriamycin, and actinomycin D, suggesting that the sublines possessed a MDR phenotype (data not shown).

Expression of Human and Mouse MDR Genes in Vincristine-resistant Cell Lines

Cell-surface human P-glycoprotein was specifically recognized by the monoclonal antibody MRK16 developed against the P-glycoprotein

Figure 3 Detection of YAC DNA in a mouse chromosome of clones LMD1 and LMD1-V1000 by in situ hybridization. FISH images of a representative metaphase spread (A,B) and interphase nucleus (C,D) from LMD1 (A,C) and LMD1-V1000 (B,D), respectively. Biotinylated total DNA from yeast carrying YAC (167) was hybridized onto slides from each cell line and detected with FITC-avidine, and the slides were counterstained by DAPI. Images were captured by a cooled CCD camera system and directly printed by Fuji Pictography 3000 from a Macintosh computer.

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Table 1. Expression of endogenous and introduced genes during selection by vincristine resistance in mouse cells

Cell lines	IC ₅₀ (ng/ml)	Relative resistance ^a	Human <i>PGY1</i> genes ^b		Endogenous mouse genes ^c			
			mRNA levels	copy number	<i>mdr1a</i> mRNA levels	<i>mdr1b</i> mRNA levels	<i>mdr1a</i> copy number	<i>mdr1b</i> copy number
L	20	0.7	—	—	1	N.D.	1	N.D.
LV50	60	2	—	—	3	N.D.	1	N.D.
LMD1	30	1	1	1	1	1	1	1
LMD1-V50	53	2	3	4	1	1	1	1
LMD1-V100	93	3	8	5	1	1	1	1
LMD1-V200	260	9	12	10	1	1	1	1
LMD1-V500	500	17	23	15	1	1	1	1
LMD1-V1000	1333	44	31	45	1	1	1	1
LMD1-V100A	100	3	5	9	1	N.D.	1	N.D.
B	150	5	10	12	1	N.D.	1	N.D.
C	120	4	7	8	1	N.D.	1	N.D.
D	200	7	8	16	1	N.D.	1	N.D.
LMD1-V500A	310	10	12	25	1	N.D.	1	N.D.
B	360	12	11	14	1	N.D.	1	N.D.
C	300	10	9	11	1	N.D.	1	N.D.
D	300	10	9	15	1	N.D.	1	N.D.

^aRelative resistance was determined from dose-response curves to vincristine by colony formation assays when IC₅₀ of LMD1 was normalized as 1.0. IC₅₀ was defined as the dose that decreased the surviving fraction to 50% of the initial fraction.

^bCellular mRNA levels and copy number of the introduced human *PGY1* gene were determined by Northern blot analysis (Fig. 6A) and Southern blot analysis (Fig. 9A); the copy number of LMD1 cells was taken to be 1.0. The relative values were the average of two independent assays, and the two analyses showed 20% variation from the average.

^cCellular mRNA levels of mouse *mdr1a* were determined by Northern blot analysis (Fig. 6B); those of LMD1 were normalized as 1.0. Cellular mRNA levels of mouse *mdr1b* could not be determined by Northern blot analysis and were determined from RT-PCR assay (Fig. 7C). Copy numbers of *mdr1a* and *mdr1b* were determined from Southern blot analysis (data not shown).

(Hamada and Tsuruo 1986). No overexpression of mouse P-glycoprotein was detected by MRK16 in the MDR mouse LV50 cells derived from L cells (data not shown). The expression of human P-glycoprotein was increased on the cell surfaces of the mouse vincristine-resistant cell lines, but no increased expression of the P-glycoprotein was observed in the parental LMD1 cells (Fig. 4). Cellular levels of P-glycoprotein in these cell lines were also analyzed by Western blot analysis with an MC6-4 polyclonal antibody, which recognizes both mouse and human P-glycoprotein. Again, cellular levels of human P-glycoprotein were correlated with increased drug resistance (Fig. 5). Also, mouse P-glycoprotein was expressed in LV500 cells but not in parental L cells (Fig. 5).

We examined whether the human P-glycoprotein overexpression was caused by enhanced expression of the human *PGY1*. Northern blot analysis showed that the human *MDR1* gene

mRNA was overexpressed in the series of drug-resistant cell lines (Fig. 6A). Cellular *PGY1* mRNA levels correlated well with the results of both Western blot and the immunofluorescence study and the degree of drug resistance. Relative RNA levels, quantified by determining the radioactivity of the autoradiographs, are shown in Table 1. The expression of the *PGY3* gene, located close to the *PGY1* gene (Lincke et al. 1991; Matsuda et al. 1993), was also examined. *PGY3* gene mRNA was not detected in the parental LMD1 cell by RT-PCR analysis but was detected by RT-PCR in the drug-resistant cell lines (Fig. 7A). Expression of the *PGY3* gene thus appeared to be enhanced in LMD1-V50, LMD1-V100, and LMD1-V1000 cells.

Northern blot analysis was carried out on the same filter to determine whether endogenous *mdr1a* and *mdr1b* genes were expressed in these cell lines. The mRNA levels of *mdr1a* were similar in LMD1, LMD1-V100, and LMD1-V1000 cells

Figure 4 Expression of P-glycoprotein in LMD1-V50, LMD1-V100, LMD1-V200, LMD1-V500, and LMD1-V1000 cells. Immunofluorescence staining of P-glycoprotein with specific monoclonal antibody against human P-glycoprotein showed stepwise expression of cell-surface P-glycoprotein in LMD1-V50 (B), LMD1-V100 (C), LMD1-V200 (D), LMD1-V500 (E), and LMD1-V1000 (F) cells, but not in the drug-sensitive parental LMD1 cells (A). Bar, 50 μ m.

while the MDR mouse LV50 cells showed increased *mdr1a* mRNA levels compared with the parental L cells (Fig. 6B). Cellular *mdr1a* mRNA levels were thus not increased in vincristine-resistant cell lines in comparison with their parental counterpart LMD1 cells. We could not detect any apparent expression of other mouse *mdr1b* genes by Northern analysis. Using RT-PCR analysis, mRNA levels of both *mdr1a* and *mdr1b* genes were similar in LMD1 and derivative drug-resistant cell lines, suggesting that the mouse genes were not overexpressed (Fig. 7B,C). Reproducible results were obtained for four independent resistant sublines (Table 1). Cellular *PGY1* mRNA levels again correlated well with the degree of drug resistance (Fig. 8).

Amplification of Human MDR Gene in Vincristine-resistant Cell Lines

Increased P-glycoprotein expression in MDR cell

lines is caused in part by gene amplification (Roninson et al. 1986; Shen et al. 1986; van der Bleik et al. 1988). We analyzed the amplification of both the introduced and the endogenous genes. Human MDR genes introduced in L cells were predominantly amplified during drug selection (Fig. 9): The copy number of the human *PGY1* gene was 4, 5, 10, 15, and 45 in LMD1-V50, LMD1-V100, LMD1-V200, LMD1-V500, and LMD1-V1000 cells, respectively, when that of LMD1 was taken to be 1.0. Amplification of the human MDR locus was also confirmed in interphase spread of LMD1-V1000 cells by fluorescence in situ hybridization (FISH), as shown in Figure 3D. Amplified signals were observed in the interphase nucleus with less condensed chromosomes (Fig. 3C,D). Amplification was not clear in metaphase spread possibly because the signal was already strong even in LMD1 which is usual case with YAC DNA as a probe (Fig. 3A,B). In contrast with human *PGY1* gene, endogenous *mdr1* genes were not amplified (Table 1). Both right and left vector arms were also observed to be amplified in stepwise manner (Fig. 9).

DISCUSSION

Several lines of evidence have indicated that any gene can be transferred into mammalian cells in YACs (D'Urso et al. 1990; Gnirke et al. 1991; Huxley et al. 1991; Strauss and Jaenisch 1992). The generation of transgenic mouse lines carrying large fragments of DNA in YACs has been reported (Peterson et al. 1993; Schedl et al. 1993; Strauss et al. 1993) and transgenes are expressed at a level comparable to that of the correspond-

Figure 5 Western blot analysis of total protein extracted from LMD1, LMD1-V50, LMD1-V100, LMD1-V500, and LMD1-V1000 cell lines. Total protein from each cell line was separated by SDS-PAGE and blotted as described in Methods. P-glycoprotein was then detected by indirect immuno staining. The positions of mouse and human P-glycoprotein are indicated by arrowheads.

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Figure 6 Northern blot analysis of RNA extracted from LMD1, LMD1-V50, LMD1-V100, LMD1-V200, LMD1-V500, and LMD1-V1000 cell lines. Total RNA from each cell line was size fractionated on a 1% formaldehyde agarose gel, transferred onto a nylon membrane, and hybridized to human *PGY1*-specific probe (A) and mouse *mdr1a*-specific probes (B). (Bottom) Ethidium bromide staining of the RNA gel prior to transfer; 28S, 18S, *PGY1*, and *mdr1a* RNA are indicated. As controls, we analyzed an MDR cell line, VJ-300, derived from human cancer KB cells, and an MDR cell line, LV50, derived from mouse L cells.

ing endogenous genes. Furthermore, expression of the transgenes is tissue specific and developmentally correct (Peterson et al. 1993; Strauss et al. 1993). Ling and colleagues first detected plasma membrane P-glycoprotein after the successful DNA-mediated transfer of MDR (Debenham et al. 1982). Our present findings demonstrate that the MDR genomic region introduced in YACs also retains a genomic structure that responds to induction by gene amplification and enhanced expression of a functional protein. Also, we have extended the approach to examine the comparative expression of the different human genes and the endogenous mouse genes in the same cells.

During the selection of a series of drug-

resistant cell lines of LMD1 cells carrying the 580-kb DNA segment containing both the human *PGY1* and *PGY3* locus, the introduced genes *PGY1* and *PGY3* were amplified and their copy number was increased in parallel with cellular increases of drug-resistance levels (Table 1). Human P-glycoprotein was observed on the cell surfaces of the drug-resistant derivatives of LMD1 cells, indicating that the introduced human *PGY1* gene could produce a functional gene product. The human *PGY3* gene generally shows tissue-specific expression, especially in liver (van der Bliek et al. 1988). However, this gene was detected by RT-PCR analysis in the drug-resistant cell lines, indicating that its expression is probably attributable to both gene amplification and the accessibility to the basal transcription factors, very likely without the appearance of liver-specific transcription factors during drug selection.

More dramatic is the differential expression of human MDR genes compared with that of mouse. The expression of the *mdr1a* genes, which are thought to be involved in the acquirement of the MDR phenotype in mouse cells, was compared. The mouse vincristine-resistant cell lines derived from the L cells, LV50, overexpressed the *mdr1a* and *mdr1b* genes as it did their human analogs, suggesting the induction of expression of the mouse genes after exposure to drugs. However, in vincristine-resistant derivatives of L cells carrying human MDR-YAC, no overexpression and no amplification of the mouse genes were found. From these results, the human and mouse genes may respond differentially to two fundamental modes of drug resistance: one, a "first-line" response, based on transcriptional activation; the other, usually observed at higher levels of drugs, based on gene amplification. Previous indications of such a two-phase mechanism came, for example, from our study of a series of a human cancer KB cell line in which a stably integrated CAT gene fused to the *PGY1* promoter was established, and a series of vincristine-resistant cell lines (Kohno et al. 1994) was isolated. Expression of the CAT gene increased along with that of the endogenous human *PGY1* gene in the earlier steps of the selection, and amplification of the *PGY1* gene followed when relative drug resistance to vincristine is increased to >10-fold, suggesting that *PGY1* gene activation occurs before gene amplification in this system (Kohno et al. 1994). In a study whose results are relevant here, Shen and colleagues (1986) have also reported that transcriptional activation pre-

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Figure 7 Analysis of expression of several MDR by RT-PCR. Total RNA was extracted from each cell line and RT-PCR analysis was performed, as described in Methods, using primer pairs specific to PGY3 (A), *mdr1a* (B), or *mdr1b* (C). Arrowheads indicate positions of expected sizes of PCR products.

cedes amplification of the *PGY1* gene during the selection of increasingly drug-resistant cell lines.

Detectable levels of human *PGY1* gene expression were observed in LMD1 parental cells by RT-PCR, suggesting that the human MDR genes introduced in LMD1 cells are integrated in the mouse genome in an active chromatin structure. There is then no detailed mechanism in a temporal or causative relation of transcriptional activation and gene amplification, but some features have been defined.

Regarding the regulation of gene expression, the steady-state mRNA levels of the human *PGY1* gene were predominantly high compared with endogenous mouse counterparts in the drug-resistant cell lines. Consistent with these results, we have demonstrated previously that the human *PGY1* promoter was active in mouse NIH-3T3 cells that were stably transfected with a human *PGY1* promoter-driven reporter gene (Tanimura et al. 1992). The failure to augment gene expression, however, apparently does not result from a lack of appropriate transcription factors, because human and mouse genes are in the same nucleus and cytoplasm in these experiments. It might have been expected that if anything, the endogenous mouse genes would be better served by autologous cellular factors. Again, preferential

amplification of the introduced human MDR region might also involve features of chromatin structure.

As for amplification, if high levels of transcription were somehow required, the lesser transcriptional response of the mouse genes could explain their relative failure to develop high copy number at higher levels of drugs. Raymond and Gros (1990) have shown that consensus sequence elements identified as TATA, GGCGGG, and CCAAT at positions -27, -47, and -83, respectively, of the 5' end of the mouse *mdr1* gene confer basal promoter activity, and also sequences upstream of position -141 up or down-regulate the basal level of reporter gene expression in a cell-type-specific manner. The sequences responsible for the basal promoter and the cell-type-specific expression of the mouse *mdr1a* gene appear to differ considerably from those of the human *PGY1* gene (Ueda et al. 1987; Kohno

et al. 1990). It is possible that the factors involved in *PGY1* gene activation are present in mouse cells and that these factors activate the mouse counterparts less, because promoter sequences of the mouse *mdr1a* gene differ from those of the

Figure 8 Correlation between expression of human *PGY1* gene and degree of drug resistance. Relative expression of human *PGY1* in each cell lines are plotted against IC_{50} of corresponding cell lines. Data from Table 1 were used.

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Figure 9 Southern blot analysis of genomic DNA. DNA was extracted from VJ300, LMD1, LMD1-V50, LMD1-V100, LMD1-V200, LMD1-V500, and LMD1-V1000 cell lines and digested with *Hind*III. The digested DNA was then electrophoresed, transferred onto a nylon membrane, and hybridized with PGY1, right arm, left arm, and β -actin probes. Positions of fragments hybridized with the probes (arrowheads) are indicated. A band is observed in every lane at the same intensity below the position of the left arm, which is the result of nonspecific hybridization.

human *PGY1* gene. Alternatively or in addition, there could be several more possible mechanisms generating the difference of gene expression and amplification between introduced human *PGY1* gene and endogenous mouse *mdr* genes. First, it has been reported that the transcription and amplification can be affected by the position where the introduced genes have been integrated (Wahl et al. 1984; Carroll et al. 1987). The MDR-YAC clone may be integrated into some unstable region of the mouse genome of LMD1 cells. Second, the YAC DNA integrated into the mouse genome is intrinsically susceptible to gene expression or amplification because it is accompanied by yeast genomic DNA that is intrinsically unstable in mouse cell lines. Although we did not determine the location of yeast genomic DNA introduced, it is reported that YAC DNA introduced is accompanied by yeast genomic DNA (Featherstone and Huxley 1993; Nonet and Wahl 1993). YAC DNA introduced into mammalian cells by fusion is intrinsically unstable and frequently

amplified because these processes, which have the ability to replicate temporally proceed through an extrachromosomal state (Featherstone and Huxley 1993; Nonet and Wahl 1993). On the other hand, the fusion lines with integrated YAC appear to be fairly stable (Featherstone and Huxley 1993; Nonet and Wahl 1993). Consistent with this observation is the fact that a few more neo-resistant clones were isolated in our experiment and these clones were unstable and reverted to be neo-sensitive clones during cultivation. We then decided to use one stable fusion line, LMD1, which has only two copies of introduced YAC DNA for further analysis. Isolation and analyses of several more fusion lines using a YAC with the mouse genomic DNA to be introduced as control material would clarify these possibilities, but this experiment remains to be studied further.

METHODS

YAC Clone

The YAC clone, yWSS167 (YAC-MDR167), used in this study was isolated as described previously (Brownstein et al. 1989; Green and Olson 1990; Matsuda et al. 1993), from the YAC library of the Center for Genetics in Medicine at Washington University School of Medicine (Fig. 1). It is 580 kb and contains about 300 kb of *MDR* gene region and ~300 kb of 7p region (Torigoe et al., this issue).

Cell Lines and Cell Culture

Human epidermoid cancer KB cells were grown as described previously (Kohno et al. 1988, 1994). Mouse L cells were grown as described previously (Kohno et al. 1988). LV50, established after the L cells were continuously exposed to 50 ng/ml vincristine (Nacalai Tesque, Inc., Kyoto, Japan). LMD1 was isolated from L cells after the PEG-mediated spheroplast fusion of yeast containing 580 kb of YAC-MDR 167-neomycin clones that had been isolated and reconstructed with a selectable marker (*neo* gene) (Pavan et al. 1990; Wada et al. 1994b). A series of vincristine-resistant sublines LMD1-V50, LMD1-V100, LMD1-V200, LMD1-V500, and LMD1-V1000 were then isolated by increasing the concentration of vincristine in the selection media to 50, 100, 200, 500, and 1000 ng/ml, as described previously (Kohno et al. 1994). Four independent resistant clones were isolated from LMD1 cells and were designated LMD1-V100A, LMD1-V500A, LMD1-V100B, LMD1-V500B, LMD1-V100C, LMD1-V500C, LMD1-V100D, and LMD1-V500D.

Synthetic Oligonucleotides

Several primer pairs were prepared for PCR and RT-PCR analysis. YAC end clones were isolated by the bubble-PCR

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method and sequenced to develop STSs (Green 1993). The sequences of oligonucleotides were human *PGY1*, AACGGAAGCCAGAACATTCC and AGGCTTCCTGTG-GCAAAGAG; human *PGY3*, AGTGGTGTTCAGAATGG and CTGTAGCAAAAGTTCATAA; left end of insert of YAC-MDR167, CCGTTTAAATTCAGCTC and ATCTTCT-TAATGTGGGTC; right end of insert of YAC-MDR167, ATTTTGTATCTTCTCCCC and CTTCGCTTATCAA-GAATC; *mdr1a*, GTAATGCCAGTGCTAGAGGC and TAAT-GTGTGCGTGTGTGTGC; and *mdr1b*, AGTGGCTCT-TGAAGCCG and CTCTCAAACCTCCATCACC.

Cell Survival Assay by Colony Formation

Cell survival was determined by plating 300 cells in 60-mm dishes, as described previously (Kohn et al. 1988, 1994). The dose which decreased the surviving fraction to 10% of the initial fraction was defined as the LD₉₀. Relative drug resistance was determined by dividing the LD₉₀ of vincristine for the resistant clones by the LD₉₀ of LMD1 cells.

DNA and RNA Isolation

High molecular weight DNA was isolated from each cell line, as described previously (Sambrook et al. 1989). RNA was isolated by a single-step method with acid buffer containing 8 M guanidium thiocyanate-chloroform, as previously described (Chomczynski and Sacchi 1987).

Southern and Northern Blot Analyses

Genomic DNA digested with *EcoRI* was separated on a 0.8% agarose gel and transferred to a nylon filter. Total cellular RNA was separated on a 1% agarose gel containing 2.2 M formaldehyde, prior to transfer to a nylon filter. DNA and RNA were cross-linked to the filter by UV irradiation. A ³²P-labeled DNA probe was prepared by the random priming method (Feinberg and Vogelstein 1983). The *PGY1* cDNA fragment was purified from pMDR105 (kindly provided by Dr. M.M. Gottesman, National Cancer Institute, Bethesda, MD). The PCR product of mouse *mdr1a* was cloned in pGEM-T vector (Promega Co., Madison, WI) and the insert was purified from this clone to prepare the *mdr1a*-specific probe. The *PvuII-EcoRI* and *EcoRI-PvuII* fragments of the pBR322 plasmid DNA were used as probes for the left and right arms of the YAC vector, respectively. Although the *EcoRI-PvuII* fragment contains 375 bp from the left arm of pYAC4 and hybridizes to the left arm weakly, this band can be easily distinguished from the right arm by size. Gene amplification and mRNA levels of the human *PGY1* and endogenous mouse *mdr1a* genes were determined with a Bioimaging Analyzer (BAS 2000; Fuji Film Co., Tokyo, Japan) (Kohn et al. 1994).

PCR and RT-PCR Analyses

cDNA was prepared with 1 µg of total RNA and 2.5 µM random primer (Takara), using mouse mammary tumor virus (MMTV) reverse transcriptase. An amount of cDNA equivalent to 100 ng of total RNA or 100 ng of genomic

DNA was mixed with 1 µM primer pairs and 1 unit of *Taq* DNA polymerase (Amersham Life Science, Buckinghamshire, England). PCR was carried out in 5-µl aliquots in a DNA Thermal Cycler (Astec Co., Fukuoka, Japan). An initial 5-min denaturation at 95°C was followed by 35 cycles of PCR. Each cycle included 1 min of denaturation at 95°C, followed by 2 min of primer annealing at 60°C, and 2 min of polymerization at 72°C. The last cycle of PCR included an additional 10-min extension at 72°C. Each reaction was analyzed by electrophoresis on a 5% polyacrylamide gel, and PCR products were detected by ethidium bromide staining after gel electrophoresis.

Western Blot and Indirect Immunofluorescence of P-glycoprotein

Whole-cell extracts from each cell line were run in 7.5% SDS-polyacrylamide gel electrophoresis and electroblotted onto a nitrocellulose filter in 25 mM Tris-HCl (pH 8.3), 192 mM glycine, and 20% methanol for 4 hr at 20 V. The filter was blocked with 2.5 % nonfat milk in Tris-buffered saline for 1 hr at room temperature and then incubated for 1 hr with 40 µg/ml of anti-C antibody prepared against the P-glycoprotein. The filter was washed four times with Tris-buffered saline, incubated with horseradish peroxidase-linked secondary antibody, and then developed according to the manufacturer's specification (ECL western blotting detection reagents; Amersham Life Science, Buckinghamshire, England). Antibody MC6-4 was kindly provided by S. Akiyama (Kagoshima University School of Medicine). Subconfluent cells on coverslips were fixed in 1% paraformaldehyde/PBS for 30 min at 4°C. Then they were washed with PBS and incubated for 30 min at 4°C with 10 µg/ml of MRK16 (a monoclonal anti-human P-glycoprotein antibody, which was donated by T. Tsuruo) (Tokyo University, Japan) (Hamada and Tsuruo 1986). The cells were then washed and incubated for an additional 30 min at 4°C with 1:100 diluted rhodamine-conjugated anti-mouse IgG. After a second wash with PBS, the cells were mounted on slides and observed using a Nikon fluorescence microscopy, with a Bio-Rad MRC 600 laser scanning confocal imaging system (Kohn et al. 1994).

Fluorescence in situ Hybridization

Probe labeling and in situ hybridization were done as described elsewhere (Wada et al. 1994a). Briefly, 2 µg of total yeast DNA containing each YAC clone was labeled by nick translation with biotin-16-dUTP (Boehringer Mannheim). Approximately 200 ng of labeled probes, 2 µg of total yeast DNA (without YAC), 2 µg of human COT-1 DNA (GIBCO BRL, Gaithersburg, MD), and 4 µg of salmon sperm DNA were ethanol-precipitated together and redissolved in 10 µl of hybridization mixture [50% (vol/vol) formamide, 2 × SSC, 10% dextran sulfate]. (All of the DNA was digested previously with DNase I to yield fragments of <500 bp.) After denaturation at 75°C for 5 min, the probes were pre-annealed at 37°C for 20 min to block signals derived from repetitive sequences and hybridized onto metaphase chromosomes. The nuclei were prepared from each cell line by a conventional method. Following overnight incubation, subsequent post-hybridization washes, and blocking with

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bovine serum albumin, probes were detected with fluorescein isothiocyanate (FITC)-conjugated avidin (Boehringer Mannheim). Chromosomes and nuclei were counterstained with 0.2 mg/ml of 4', 6-diamidino-2-phenylindole (DAPI, Sigma). Fluorescence signals were imaged using a Zeiss Axioskop epifluorescence microscope equipped with a cooled charge coupled device (CCD) camera (Photometrics, PXL 1400). Image acquisition was performed on a Macintosh Quadra 840 AV computer with the software program pseudocolored and merged using Adobe Photoshop 2.5J (Adobe Systems Inc.). DAPI and FITC images were shown in blue and green, respectively. The merged images of FITC and DAPI were directly printed by Fuji Pictography 3000 from a Macintosh computer.

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REFERENCES

- Akiyama, S.-I., A. Fojo, J.A. Hanover, I. Pastan, and M.M. Gottesman. 1985. Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. *Somatic Cell Mol. Genet.* **11**: 117–126.
- Asakuno, K., K. Kohno, T. Uchiumi, T. Kubo, S. Sato, M. Isono, and M. Kuwano. 1994. Involvement of a DNA binding protein, MDR-NF1/YB-1, in human *MDR1* gene expression by actinomycin D. *Biochem. Biophys. Res. Commun.* **199**: 1428–1435.
- Bates, S.E., L.A. Mickley, Y.N. Chen, N. Richert, J. Rudick, J.L. Biedler, and A.T. Fojo. 1989. Expression of a drug resistance gene in human neuroblastoma cell lines: Modulation by retinoic acid-induced differentiation. *Mol. Cell. Biol.* **9**: 4337–4344.
- Bradley, G., P.F. Juranka, and V. Ling. 1988. Mechanism of multidrug resistance. *Biochim. Biophys. Acta.* **948**: 87–128.
- Brownstein, B.H., G.A. Silverman, R.D. Little, D.T. Burke, S.J. Korsmeyer, D. Schlessinger, and M.V. Olson. 1989. Isolation of single-copy human genes from a library of yeast artificial chromosome clones. *Science* **244**: 1348–1351.
- Carroll, S.M., P. Gaudray, M.L. DeRose, J.F. Emery, J.L. Meinkoth, M. Subler, D.D. Von Hoff, and G.M. Wahl. 1987. Characterization of an episome produced in hamster cells that amplify a transfected CAD gene at high frequency: Functional evidence for a mammalian replication origin. *Mol. Cell. Biol.* **7**: 1740–1750.
- Chen, C.-J., J.E. Chin, K. Ueda, D. Clark, I. Pastan, M.M. Gottesman, and I.B. Roninson. 1986. Internal duplication and homology with bacterial transport proteins in the *mdr1* (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* **47**: 381–389.
- Chin, J.E., R. Soffir, K.E. Noonan, K. Choi, and I.B. Roninson. 1989. Structure and expression of the human *MDR* (P-glycoprotein) gene family. *Mol. Cell. Biol.* **9**: 3808–3820.
- Chin, K.V., S. Tanaka, G. Darlington, I. Pastan, and M.M. Gottesman. 1990. Heat shock and arsenite increase expression of the multidrug resistance (*MDR1*) gene in human renal carcinoma cells. *J. Biol. Chem.* **265**: 221–226.
- Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156–159.
- Debenham, P.G., N. Kartner, L. Siminovitich, J.R. Riordan, and V. Ling. 1982. DNA-mediated transfer of multiple drug resistance and plasma membrane glycoprotein expression. *Mol. Cell. Biol.* **2**: 881–889.
- Devault, A. and P. Gros. 1990. Two members of the mouse *mdr* gene family confer multidrug resistance with overlapping but distinct drug specificities. *Mol. Cell. Biol.* **10**: 1652–1663.
- D'Urso, M., I. Zucchi, A. Ciccodicola, G. Palmieri, F.E. Abidi, and D. Schlessinger. 1990. Human glucose-6-phosphate dehydrogenase gene carried on a yeast artificial chromosome encodes active enzyme in monkey cells. *Genomics* **7**: 531–534.
- Endicott, J.A., F. Sarangi, and V. Ling. 1991. Complete cDNA sequences encoding the Chinese hamster P-glycoprotein gene family. *J. DNA Seq. Map.* **2**: 89–101.
- Fairchild, C.R., S.P. Ivy, T. Rushmore, G. Lee, P. Koo, M.E. Goldsmith, C.E. Myers, E. Farber, and K.H. Cowan. 1987. Carcinogen-induced *mdr* overexpression is associated with xenobiotic resistance in rat preneoplastic liver nodules and hepatocellular carcinomas. *Proc. Natl. Acad. Sci.* **84**: 7701–7705.
- Featherstone, T. and C. Huxley. 1993. Extrachromosomal maintenance and amplification of yeast artificial chromosome DNA in mouse cells. *Genomics* **17**: 267–278.
- Feinberg, A.P. and B.A. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6–13.
- Fojo, A., B. Lebo, N. Shimizu, J. Chin, I. Roninson, G. Merlino, M. Gottesman, and I. Pastan. 1986. Localization

KUSABA ET AL.

- of multidrug resistance-associated DNA sequences to human chromosome 7. *Somatic Cell Mol. Genet.* **12**: 415–420.
- Gnirke, A. T. Barnes, D. Patterson, D. Schild, T. Featherstone, and M.V. Olson. 1991. Cloning and in vivo expression of the human GART gene using yeast artificial chromosomes. *EMBO J.* **10**: 1629–1634.
- Green, E.D. 1993. Physical mapping of human chromosomes: Generation of chromosome-specific sequence-tagged sites (STSs). In *Methods in molecular genetics* (ed. K.W. Adolph), pp. 192–210. Academic Press, San Diego, CA.
- Green, E.D. and M.V. Olson. 1990. Systematic screening of yeast artificial-chromosome libraries by use of the polymerase chain reaction. *Proc. Natl. Acad. Sci.* **87**: 1213–1217.
- Gros, P., Y. Ben-Neriah, J. Croop, and D. Housman. 1986a. Isolation and expression of complementary DNA that confers multidrug resistance. *Nature* **323**: 728–731.
- Gros, P., J. Croop, and D. Housman. 1986b. Mammalian multidrug resistance gene: Complete cDNA sequence indicates strong homology to bacterial transport proteins. *Cell* **47**: 371–380.
- Hamada, H. and T. Tsuruo. 1986. Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumor cells as revealed by monoclonal antibodies. *Proc. Natl. Acad. Sci.* **83**: 7785–7789.
- Hsu, S.I., L. Lothstein, and S.B. Horwitz. 1989. Differential overexpression of three *mdr* gene family members in multidrug-resistant J774.2 mouse cells. *J. Biol. Chem.* **264**: 12053–12062.
- Huxley, C., Y. Hagino, D. Schlessinger, and M. Olson. 1991. The human HPRT gene on a yeast artificial chromosome is functional when transferred to mouse cells by cell fusion. *Genomics* **9**: 742–750.
- Kohno, K., J. Kikuchi, S. Sato, H. Takano, Y. Saburi, K. Asoh, and M. Kuwano. 1988. Vincristine-resistant human cancer KB cell line and increased expression of multidrug-resistant gene. *Jpn. J. Cancer Res.* **79**: 1238–1246.
- Kohno, K., S.-I. Sato, H. Takano, K. Matsuo, and M. Kuwano. 1989. The direct activation of human multidrug resistance gene (*MDR1*) by anticancer agents. *Biochem. Biophys. Res. Commun.* **165**: 1415–1421.
- Kohno, K., S. Sato, T. Uchiyumi, H. Takano, S. Kato, and M. Kuwano. 1990. Tissue-specific enhancer of the human multidrug-resistance (*MDR1*) gene. *J. Biol. Chem.* **265**: 19690–19696.
- Kohno, K., S. Sato, T. Uchiyumi, H. Takano, H. Tanimura, M. Miyazaki, K. Matsuo, K. Hidaka, and M. Kuwano. 1992. Activation of the human multidrug resistance (*MDR1*) gene promoter in response to inhibitors of DNA topoisomerases. *Int. J. Oncol.* **1**: 73–77.
- Kohno, K., H. Tanimura, Y. Nakayama, S. Sato, Y. Makino, M. Wada, A.T. Fojo, and M. Kuwano. 1994. Cellular control of human multidrug resistance 1 (*MDR1*) gene expression in the absence and presence of gene amplification in human cancer cells. *J. Biol. Chem.* **269**: 20503–20508.
- Lincke, C.R., A. van der Bliek, G.J. Schuurhuis, T. van der Velde-Koerts, J.J.M. Smit, and P. Borst. 1990. The multidrug resistance phenotype of human BRO melanoma cells transfected with a wild-type human *mdr1* cDNA. *Cancer Res.* **50**: 1779–1785.
- Lincke, C.R., J.J.M. Smit, T. van der Velde-Koerts, and P. Borst. 1991. Structure of the human *MDR3* gene and physical mapping of the human *MDR* locus. *J. Biol. Chem.* **266**: 5303–5310.
- Marino, P.A., M.M. Gottesman, and I. Pastan. 1990. Regulation of the multidrug resistance gene in regenerating rat liver. *Cell Growth Differ.* **2**: 57–62.
- Matsuda, T., S. Sato, M. Wada, K. Kohno, E. Green, D. Schlessinger, and M. Kuwano. 1993. The isolation of human multidrug resistance 1 and 3 genes from a library of yeast artificial chromosome clones. In *The mechanism and new approach on drug resistance of cancer cells*. (ed. T. Miyazaki, F. Takaku, and K. Sakurada), pp. 29–32. Elsevier, Amsterdam, The Netherlands.
- Mickley, L.A., S.E. Bates, N.D. Richert, S. Currier, S. Tanaka, F. Foss, N. Rosen, and A.T. Fojo. 1989. Modulation of the expression of a multidrug resistance gene (*mdr-1*/P-glycoprotein) by differentiating agents. *J. Biol. Chem.* **264**: 18031–18040.
- Miyazaki, M., K. Kohno, T. Uchiyumi, H. Tanimura, K. Matsuo, M. Nasu, and M. Kuwano. 1992. Activation of human multidrug resistance-1 gene promoter in response to heat shock stress. *Biochem. Biophys. Res. Commun.* **187**: 677–684.
- Nonet, G.H. and G.M. Wahl. 1993. Introduction of YACs containing a putative mammalian replication origin into mammalian cells can generate structures that replicate autonomously. *Somatic Cell. Mol. Genet.* **19**: 171–192.
- Pachnis, V., L. Pevny, R. Rothstein, and F. Costantini. 1990. Transfer of a yeast artificial chromosome carrying human DNA from *Saccharomyces cerevisiae* into mammalian cells. *Proc. Natl. Acad. Sci.* **87**: 5109–5113.
- Pastan, I. and M. Gottesman. 1987. Multiple-drug resistance in human cancer. *N. Engl. J. Med.* **316**: 1388–1393.
- Pavan, W.J., P. Hieter, and R.H. Reeves. 1990. Modification and transfer into an embryonal carcinoma cell line of a 360-kilobase human-derived yeast artificial chromosome. *Mol. Cell. Biol.* **10**: 4163–4169.

YAC–HUMAN MDR GENE EXPRESSION

- Peterson, K.R., C.H. Clegg, C. Huxley, B.M. Josephson, H.S. Haugen, T. Furukawa, and G. Stamatoyannopoulos. 1993. Transgenic mice containing a 248-kb yeast artificial chromosome carrying the human β -globin locus display proper developmental control of human globin genes. *Proc. Natl. Acad. Sci.* **90**: 7593–7597.
- Raymond, M. and P. Gros. 1990. Cell-specific activity of *cis*-acting regulatory elements in the promoter of the mouse multidrug resistance gene *mdr1*. *Mol. Cell. Biol.* **10**: 6036–6040.
- Riordan, J.R., K. Deuchars, N. Kartner, N. Alon, J. Trent, and V. Ling. 1985. Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. *Nature* **316**: 817–819.
- Roninson, I.B., H.T. Abelson, D.E. Housman, N. Howell, and A. Varshavsky. 1984. Amplification of specific DNA sequences correlates with multi-drug resistance in Chinese hamster cells. *Nature* **309**: 626–628.
- Roninson, I.B., J.E. Chin, K. Choi, P. Gros, D.E. Housman, A. Fojo, D-W. Shen, M.M. Gottesman, and I. Pastan. 1986. Isolation of human *mdr* DNA sequences amplified in multidrug-resistant KB carcinoma cells. *Proc. Natl. Acad. Sci.* **83**: 4538–4542.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Schedl, A., L. Montoliu, G. Kelsey, and G. Schütz. 1993. A yeast artificial chromosome covering the tyrosinase gene confers copy number-dependent expression in transgenic mice. *Nature* **362**: 258–261.
- Schinkel, A.H., M.E.M. Roelofs, and P. Borst. 1991. Characterization of the human *MDR3* P-glycoprotein and its recognition by P-glycoprotein-specific monoclonal antibodies. *Cancer Res.* **51**: 2628–2635.
- Schinkel, A.H., J.J.M. Smit, O. van Tellingen, J.H. Beijnen, E. Wagenaar, L. van Deemter, C.A.A.M. Mol, M.A. van der Valk, E.C. Robanus-Maandag, H.P.J. te Riele, A.J.M. Berns, and P. Borst. 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.
- Shen, D.-W., A.T. Fojo, J.E. Chin, I.B. Roninson, N. Richert, I. Pastan, and M.M. Gottesman. 1986. Human multidrug-resistant cell lines: Increased *mdr1* expression can precede gene amplification. *Science* **232**: 643–645.
- Smit, J.J.M., A.H. Schinkel, R.P.J. Oude Elferink, A.K. Groen, E. Wagenaar, L. van Deemter, C.A.A.M. Mol, R. Ottenhoff, N.M.T. van der Lugt, M.A. van Room, M.A. van der Valk, G.J.A. Offerhaus, A.J.M. Berns, and P. Borst. 1993. Homozygous disruption of the murine *mdr2* P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* **75**: 451–462.
- Srivastava, A.K. and D. Schlessinger. 1991. Vectors for inserting selectable markers in vector arms and human DNA inserts of yeast artificial chromosomes (YACs). *Gene* **103**: 53–59.
- Strauss, W.M. and R. Jaenisch. 1992. Molecular complementation of a collagen mutation in mammalian cells using yeast artificial chromosomes. *EMBO J.* **11**: 417–422.
- Strauss, W., J. Dausman, C. Beard, C. Johnson, J.B. Lawrence, and R. Jaenisch. 1993. Germ line transmission of a yeast artificial chromosome spanning the murine alpha 1 (I) collagen locus. *Science* **259**: 1904–1907.
- Tanimura, H., K. Kohno, S. Sato, T. Uchiumi, M. Muyazaki, M. Kobayashi, and M. Kuwano. 1992. The human multidrug resistance 1 (*MDR1*) gene promoter has an element that responds to serum starvation. *Biochem. Biophys. Res. Commun.* **183**: 917–924.
- Thorgeirsson, S.S., B.E. Huber, S. Sorrell, A. Fojo, I. Pastan, and M.M. Gottesman. 1987. Expression of the multidrug-resistant gene in hepatocarcinogenesis and regenerating rat liver. *Science* **236**: 1120–1122.
- Torigoe, K., S. Sato, H. Kusaba, K. Kohno, M. Kowano, K. Okumura, E.D. Green, L.-C. Tsui, S.W. Scherer, D. Schlessinger, and M. Wada. 1995. A YAC-based contig of 1.5 Mb spanning the human multidrug resistance gene region and delineating the amplification unit in two human multidrug resistant cell lines. *Genome Res.* (this issue).
- Uchiumi, T., K. Kohno, H. Tanimura, K. Matsuo, S. Sato, K. Asakuno, H. Abe, and M. Kuwano. 1993a. Activation of the human multidrug resistance (*MDR1*) gene promoter in response to environmental stress. I. Anticancer agents and ultraviolet light irradiation. In *The mechanism and new approach on drug-resistance of cancer cells* (ed. T. Miyazaki, F. Takaku, and K. Sakurada), pp. 71–75, Elsevier, Amsterdam, The Netherlands.
- Uchiumi, T., K. Kohno, H. Tanimura, K. Matsuo, S. Sato, Y. Uchida, and M. Kuwano. 1993b. Enhanced expression of the human multidrug resistance 1 gene in response to UV light irradiation. *Cell Growth & Differ.* **4**: 147–157.
- Ueda, K., I. Pastan, and M.M. Gottesman. 1987. Isolation and sequence of the promoter region of the human multidrug-resistance (P-glycoprotein) gene. *J. Biol. Chem.* **262**: 17432–17436.
- van der Bliek, A.M. and P. Borst. 1989. Multidrug resistance. *Adv. Cancer Res.* **52**: 165–203.
- van der Bliek, A.M., T. van der Velde-Koerts, V. Ling, and P. Borst. 1986. Overexpression and amplification of five genes in a multidrug-resistant Chinese hamster ovary cell line. *Mol. Cell. Biol.* **6**: 1671–1678.
- van der Bliek, A.M., F. Baas, T. van der Velde-Koerts, J.L. Biedler, M.B. Meyers, R.F. Ozols, T.C. Hamilton, H. Joenje, and P. Borst. 1988. Genes amplified and

KUSABA ET AL.

overexpressed in human multidrug resistant cell lines. *Cancer Res.* **48**: 5927–5932.

Wada, M., K. Abe, K. Okumura, H. Taguchi, K. Kohno, F. Imamoto, D. Schlessinger, and M. Kuwano. 1994a. Chimeric YACs were generated at unreduced rates in conditions that suppress coligation. *Nucleic Acids Res.* **22**: 1651–1654.

Wada, M., Y. Ihara, M. Tatsuka, H. Mitsui, K. Kohno, M. Kuwano, and D. Schlessinger. 1994b. HPRT yeast artificial chromosome transfer into human cells by four methods and an involvement of homologous recombination. *Biochem. Biophys. Res. Commun.* **200**: 1693–1700.

Wahl, G.M., B.R. de Saint Vincent, and M.L. DeRose. 1984. Effect of chromosomal position on amplification of transfected genes in animal cells. *Nature.* **307**: 516–520.

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H Kusaba, K Kohno, K Asakuno, et al.

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References

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