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Differential Regulation of P-Glycoprotein Genes in Primary Rat Hepatocytes by Collagen Sandwich and Drugs

Chow H. Lee*

Chemistry Program, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada

Abstract P-glycoprotein (Pgp) is a small family of plasma membrane proteins, which are capable of transporting substrates across cell membranes. Class I and II Pgp are able to transport drugs and have been shown to mediate multidrug resistance (MDR). Class III Pgp is a long chain phospholipid transporter and does not mediate MDR. The regulation of all three Pgp genes is still poorly understood. For instance, it is not clear if the three Pgp genes are co-regulated or differentially regulated by external stimuli. This study examined the effect of drugs and collagen sandwich system on expression and transcription of all the three Pgp genes in primary rat hepatocytes. Consistent with previous findings, dramatic overexpression (25-fold) of Class II Pgp mRNA was seen, upon culturing of hepatocytes onto a single layered collagen gel. Hepatocytes sandwiched between two layers of collagen gel exhibited decreased (4.5-fold) Class II Pgp mRNA expression as compared to the single layer system. Treatment of hepatocytes cultured on the single layer collagen system with cytoskeletal disrupting (cytochalasin D, colchicine) but not cytoskeletal stabilizing (phalloidin, taxol) drugs, suppressed Class II Pgp expression. In all cases, no change in Class II Pgp transcription was observed as demonstrated by nuclear run-on studies. This suggests that collagen configuration and drugs affect Class II Pgp mRNA expression predominantly through post-transcriptional mechanisms. In contrast, parallel increases in mRNA expression and transcription of Class I Pgp gene were observed upon culturing of hepatocytes, in the collagen sandwich system, and treatment with some drugs (cytochalasin D, colchicine, and phalloidin). This suggests that Class I Pgp gene is regulated primarily via transcriptional mechanisms by these stimuli. On the other hand, Class III Pgp gene appears to be post-transcriptionally co-regulated with Class II Pgp gene by treatment with the drugs, while collagen configuration affected both transcription and post-transcription of Class III Pgp gene. Finally, dose-dependent studies using cycloheximide provided further evidence that the two MDR-associated genes are not co-regulated. This study has implications for future studies on the molecular mechanisms of Pgp gene regulation. J. Cell. Biochem. 86: 12–20, 2002. © 2002 Wiley-Liss, Inc.

Key words: Pgp; collagen sandwich; drugs; hepatocytes

P-glycoprotein (Pgp) is a member of the superfamily of eukaryotic and prokaryotic ATP-binding cassette transporter proteins [Klein et al., 1999] and is thought to mediate multidrug resistance (MDR) by pumping out various structurally and functionally unrelated drugs [Endicott and Ling, 1989]. Pgp genes are comprised of three family members in rodents, termed Class I, II, and III (Table I). The Class I Pgp (Pgp) is predominantly found in epithelial and endothelial cells, and it functions as an efflux pump to excrete xenobiotics [Schinkel, 1997]. Similarly, the Class II Pgp (Pgp2) is found in many normal tissues and can function as a drug efflux pump [Schinkel, 1997]. The Class III Pgp (Pgp3), which is not associated with MDR and is the most abundant Pgp species in some normal tissues, has been shown to be necessary for phospholipid transport across the hepatic bile canalicular membrane [Schinkel, 1997].

Pgp are overexpressed in many types of human cancers, and in some cases this correlates with poor response to chemotherapy [Ling, 1997]. Such observations have prompted intense studies into the expression of Pgp genes. Treatment of cells with chemotherapeutic drugs

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*Correspondence to: Chow H. Lee, Chemistry Program, University of Northern BC, 3333 University Way, Prince George, BC V2N 4Z9, Canada. E-mail: leec@unbc.ca.

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TABLE I. Classification of P-Glycoprotein Isoforms

	P-glycoprotein isoforms class		
	I	II	III
Human	mdr1	—	mdr3/mdr2
Hamster	pgp1	pgp2	pgp3
Mouse	mdr3/mdr1a	mdr1/mdr1b	mdr2
Rat	pgp1/mdr1a	pgp2/mdr1b	pgp3/mdr2

[Kohno et al., 1989; Chin et al., 1990a; Fardel et al., 1997; Furuya et al., 1997], UV light [Uchiumi et al., 1993], heat shock [Chin et al., 1990b], hormones [Altuvia et al., 1993; Zhao et al., 1993; Schuetz et al., 1995a], extracellular matrix proteins [Schuetz and Schuetz, 1993; Tatsuta et al., 1994], reactive oxygen species [Ziemann et al., 1999], and cytokine [Hirsch-Ernst et al., 1998] has resulted in increased levels of Pgp mRNA. In most if not all of these studies, only the expression and regulation of one or two Pgp isoforms were examined. Thus, there is a gap in our understanding on how all three Pgp genes are expressed and regulated by various factors.

Various animal liver models and primary cultured hepatocytes have been extensively used in the study of Pgp gene expression. By employing these models, direct comparison and contrast between in vivo and in vitro, in addition to studies on physiological and pathological conditions, were possible. For instance, significant increases in the Class I and II Pgp mRNAs have been demonstrated in various liver carcinogenesis models [Nakatsukasa et al., 1992; Teeter et al., 1993; Fardel et al., 1994] and liver regeneration [Nakatsukasa et al., 1992, 1993]. Significant changes in Pgp expression in primary hepatocytes have also been observed upon culturing [Lee et al., 1993] and treatment with carcinogens or drugs [Gant et al., 1991, 1992; Schuetz et al., 1995a; Hill et al., 1996]. However, in most if not all cases, the expression and regulation of all three Pgp genes were not studied. Thus, it remains unknown if these related genes are co-regulated or respond differently to different stimuli.

Hepatocytes cultured between two layers of collagen gel to form a 'sandwich configuration' have been shown to exhibit gene expression patterns and other phenotypes similar to those in vivo [Dunn et al., 1992; Otsu et al., 2000; Annaert et al., 2001]. The expression and transcription of liver-specific genes were found to be restored to levels close to in vivo. In contrast, expression of the so-called ubiquitously-expressed genes, such as β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were decreased with no change in transcriptional activities [Otsu et al., 2000]. The sandwich hepatocyte system thus provides an opportunity to explore how the expression and regulation of the different Pgp isoforms may differ.

This study reports an examination of the effects of collagen sandwich configuration and drugs on all three Pgp mRNA expression and transcription in primary rat hepatocytes. Results obtained showed that the Class I Pgp is predominantly via transcriptional mechanism by collagen configuration and drugs, while the Class II Pgp is co-regulated post-transcriptionally with cytoskeletal genes. In contrast, the Class III Pgp appears to be regulated by both mechanisms.

MATERIALS AND METHODS

Animals and Chemicals

Male Fischer rats, weighing about 200 g, given free access to laboratory chow and water, were used for hepatocyte preparation in all experiments. Williams' Medium E (WME) was obtained from GIBCO-BRL (Toronto, Canada), Swim's S-77 medium and antibiotics were from Sigma (St. Louis, MO). LHSA medium was prepared as previously described [Oliver et al., 1978]. Collagenase was from Boehringer Mannheim GmbH (Penzberg, Germany). All other chemicals were from Sigma.

Hepatocyte Isolation and Culture

Hepatocytes were isolated under sterile conditions by a two-step collagenase perfusion procedure as described by Whiting and Edwards [1979]. Low-centrifugal speeds (50*g*) were used during washing of cells with LHSA medium to minimize the contamination of hepatocytes with smaller non-parenchymal cells. The washed hepatocyte pellet was resuspended in warm Williams' medium E containing penicillin (100 U/ml) and streptomycin sulfate (100 μg/ml). This is called standard culture medium. Collagen gels were prepared by distributing 1 ml of collagen gel solution (one part 10 × Williams' medium E and nine parts collagen solution at 3 mg/ml) evenly over a 100-mm tissue culture dish and incubated at 37°C for at least 1 h before

use. This is termed dried collagen dishes or single layer collagen. Cell suspension in standard culture medium was routinely plated at a density of about 3.5×10^4 cells/cm^2 onto 100-mm culture dishes. Cells were allowed to attach onto the dishes for 4 h at 37°C in a humidified atmosphere of 5% CO_2 in air. The medium was then changed to fresh standard culture medium and thereafter with daily medium changes. For the sandwich system, an additional 1-ml collagen gel solution was distributed over the cells after 1 day of culture at 37°C and 5% CO_2. Culture medium was first removed and care was taken to ensure that second layer of collagen was evenly spread over the entire dish. Thirty minutes of incubation at 37°C were allowed for gelation and attachment of the second layer before medium was replaced.

Preparation of Type I Collagen

Type I collagen was prepared from Fischer rat tail tendons, essentially as described by Michalopoulos and Pitot [1975]. Usually, seven or eight tails were prepared at one time. Tendons were allowed to dry in a flow cabinet for about 60 min before being sterilized under UV light for 20 h. Concentrated collagen stock (3 mg/ml) was prepared by stirring sterile fibers overnight at room temperature in 0.1% acetic acid. A diluted collagen solution (0.3 mg collagen fibrils/ml 0.1% acetic acid) was used to coat dishes (see above).

Total RNA Isolation and Slot Blot Analysis

Cells were harvested from cultures in solution D (4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) and total RNA extracted as previously described [Chomczynski and Sacchi, 1986]. Concentrations of total RNA were determined spectrometrically, and 10 μg were applied onto Hybond-N$^+$ filter (Amersham Pharmacia Biotech, UK) using a slot blot apparatus. Filters were baked for 2 h at 80°C and prehybridized in 50% deionized formamide, 5 × SSPE (0.9 M NaCl, 0.05 M sodium phosphate, pH 7.7, 0.005 M EDTA), 5 × Denhardt's, and 2% SDS for 4 h at 42°C. Hybridization was performed overnight at the same temperature in 50% deionized formamide, 5 × SSPE, 10% dextran sulphate, 1 × Denhardt's, 2% SDS, and $> 2 \times 10^6$ cpm/ml DNA probe. Filters were then subjected to two 20 min washes at room temperature in 2 × SSC and 0.1% SDS, followed by one 20 min wash at 42°C in 1 × SSC and 0.1% SDS. Filters were then finally washed at 60°C in 0.5 × SSC, 0.1% SDS, and 0.1 × SSC, 0.1% SDS with 20 min each. Autoradiography was carried out using a Computing Densitometer (Molecular Dynamics, CA).

Nuclear Run-On Analysis

Isolation of nuclei was carried out in a cold room (4°C). Approximately 4.8×10^8 cells were homogenized in 0.34 M sucrose buffer (0.34 M sucrose; 5 mM Tris, pH 8; 5 mM $MgCl_2$; 1 mM PMSF; 1 mM DTT) and the homogenate centrifuged at 2,500 rpm at 4°C for 10 min. The crude nuclear pellet was further fractionated by resuspension in 2 M sucrose buffer and centrifugated at 21,000 rpm at 4°C for 40 min. The nuclear pellet was resuspended in nuclear storage buffer (50 mM Tris-Cl, pH 8.3; 40% glycerol; 5 mM $MgCl_2$; 0.1 mM EDTA) and centrifuged for 5 min at 4°C at 1,000 rpm. The final nuclear pellet, containing approximately 4.0×10^8 nuclei from 4.8×10^8 cells, was resuspended in 150 μl nuclear storage buffer and froze immediately in liquid nitrogen. Thawed nuclei were resuspended in 200 μl of complete reaction buffer containing 10 mM Tris-Cl (pH 8.0), 5 mM $MgCl_2$, 0.3 M KCl, 1 mM ATP, 1 mM CTP, 1 mM UTP (Promega Biotech, Madison, WI), and 30 μl of [α^{32}P]GTP (10 mCi/ml; 3,000 Ci/mmol). The mixture was incubated at 30°C for 30 min, and newly transcribed RNA was isolated as previously described [Lee et al., 1995]. Filters containing 0.5 μg/slot of isolated and gel purified cDNA or genomic fragment were hybridized with equal counts of radioactivity (at least 1×10^6 cpm/ml) from different samples and blots washed according to previously described protocol [Lee et al., 1995]. Blots were quantified using a Computing Densitometer (Molecular Dynamics, CA). Genomic clones, which span 3′ untranslated regions of Rat Class I, II, and III Pgp genes [Deuchars et al., 1992], were used as DNA probes. These sequences have been shown to be gene-specific and do not pose cross-hybridization problems [Deuchars et al., 1992]. Plasmid vector pUC9 containing unrelated DNA sequences was used as control for a non-specific background signal.

RESULTS

Analysis of Pgp mRNA Expression and Transcription in Single Gel and Sandwich Systems

The hepatocytes sandwich system [Dunn et al., 1992] was used to explore the possible

differential expression and regulation of Pgp genes. In agreement with previous findings [Lee et al., 1993], freshly isolated hepatocytes cultured on collagen-coated dishes exhibited significant increase in Pgp1 (about 2.5-fold) and Pgp2 (about 25-fold) mRNA expression with time in culture (4 vs. 48 h single layer; Fig. 1). On the other hand, both Pgp3 (about 7-fold) and Cx32 (about 6-fold) mRNA levels were decreased [Lee et al., 1993; Fig. 1]. After 48 h in culture, hepatocytes cultured under the sandwich system expressed approximately 4.5-fold lower amount of Pgp2 mRNA as compared to its single layer counterparts (Fig. 1). Differences in Pgp1 and Pgp3 mRNA levels (1.5-fold lower in the sandwich system) between the sandwich and single layer systems were modest (Fig. 1). In contrast, Cx32 mRNA levels were approximately 3-fold higher in the sandwich system (Fig. 1).

To determine if changes in transcription are responsible for the changes in steady-state mRNA levels of these genes, nuclear run-on assays were performed. Consistent with previous findings [Lee et al., 1995], the transcription rates of Pgp2 in hepatocytes were found to be unaffected with time in culture (Table II). The sandwich system also had no effect on the transcription rate of Pgp2 (Table II). These

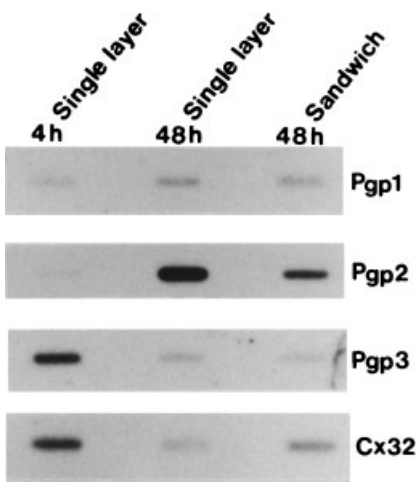

Fig. 1. Pgp and Cx32 mRNA levels of the single gel and the sandwich systems as measured by slot blot hybridization. Hepatocytes were isolated and cultured in the single gel layer and sandwich system as described in Materials and Methods. Ten micrograms of total RNA isolated from 4 h (single layer) and 48 h (both systems) were subjected to slot blot analysis. Blots were hybridized with ^{32}P-labeled Pgp genes specific and Cx32 DNA probes and exposed overnight before autoradiography was performed.

TABLE II. Pgp Transcriptional Activities of the Single Layer and the Sandwich Systems

	Time in culture (h)		
	4	48	48
Genes	Single layer	Single layer	Sandwich
Pgp1	1.0	2.1 ± 0.13	1.2 ± 0.13
Pgp2	1.0	1.1 ± 0.15	0.9 ± 0.20
Pgp3	1.0	0.3 ± 0.11	0.6 ± 0.20
Cx32	1.0	0.2 ± 0.05	0.9 ± 0.11
GAPDH	1.0	0.9 ± 0.13	1.1 ± 0.12

Transcriptional activities (expressed as specific activity/number of nuclei) were determined by nuclear run-on assays as previously described [Lee et al., 1995]. After subtracting from pUC9, quantified values (48-h single layer and sandwich) were normalized to values at 4-h single layer. Results shown were from four different experiments using separate cell preparations.

results suggest that post-transcriptional control is predominantly responsible for the significant changes in Pgp2 mRNA levels with time in culture and upon layering of a second layer of collagen. Pgp1 transcription increased about 2-fold with time in culture, and the sandwich system suppressed this increase (Table II). Both Pgp3 and Cx32 transcriptional rates were found to decrease about 3–4-fold with time in culture but higher rates of transcription were observed in the sandwich system as compared to the single layer system (2-fold for Pgp3 and 3-fold for Cx32, Table II). Like Pgp2, the transcription rate of a housekeeping gene, GAPDH, remained unchanged under all conditions.

Effects of Cytoskeletal Disrupting and Stabilizing Drugs on Pgp mRNA Expression and Transcription

Cytoskeletal disrupting, but not cytoskeletal stabilizing drugs, had been previously shown to significantly decrease Pgp2 mRNA expression in cultured hepatocytes [Lee et al., 1995]. Thus, it was of interest to determine whether similar effects can be seen with Pgp1 and Pgp3 mRNA expression. Figure 2 shows slot blot analysis of all the three Pgp genes upon treatment with cytoskeletal disrupting and stabilizing drugs for 20 h. In agreement with previous report, [Lee et al., 1995], both cytochalasin D and colchicine significantly decreased Pgp2 mRNA levels as compared to the controls (Fig. 2). In contrast, phalloidin, which stabilizes microfilaments and taxol, which stabilizes microtubules, had no effect on Pgp2 mRNA levels (Fig. 2). β-lumicolchicine, an isomer of colchicine, which lacks tubulin-binding properties, also had no effect on Pgp2 mRNA expression (Fig. 2). The

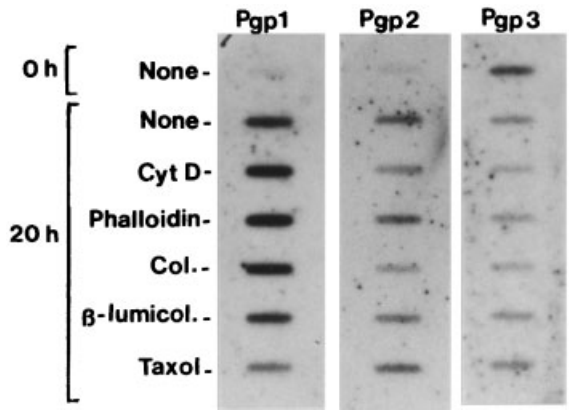

Fig. 2. Effects of cytoskeletal disrupting and stabilizing drugs on Pgp genes expression. Freshly isolated hepatocytes were allowed to attach to collagen-coated dishes for 4 h. Some cultures were harvested for total RNA (indicated as 0 h), while others were changed to fresh medium containing no addition (none), 1.6 μM cytochalasin D, 1.6 μM phalloidin, 1.5 μM colchicine, 1.5 μM β-lumicolchicine, or 1.5 μM taxol. After a further 20-h incubation, cultures were harvested for total RNA. Each slot has 10 μg total RNA analyzed by slot blot as described in Materials and Methods.

effects of these drugs on Pgp3 mRNA levels were very similar to that observed for Pgp2 mRNA, in that cytoskeletal disrupting drugs decreased its expression while cytoskeletal stabilizing drugs had no effect (Fig. 2). In contrast, these drugs had different effects on Pgp1 mRNA levels. As shown in Figure 2, cytochalasin D, phalloidin, and colchicine caused approximately 2-fold induction of Pgp1 mRNA levels as compared to the control. β-lumicolchicine had no significant effect while taxol decreased Pgp1 mRNA levels by approximately 4-fold (Fig. 2).

To determine if there are parallel changes in transcription, nuclear run-on assays were performed. Results from four different nuclear run-on experiments are summarized in Table III. In repeated studies, there was no indication that these drugs had any significant effect on Pgp2 transcription. GAPDH transcription used as control, was also unaffected by treatment with these drugs. On the other hand, cytochalasin D, phalloidin, and colchicine clearly induced Pgp1 transcription by about 1.6-fold (Table III). β-lumicolchicine had no significant effect, while taxol decreased Pgp1 transcription by about 2-fold.

Effect of Cycloheximide on Pgp mRNA Expression in Cultured Hepatocytes

The protein synthesis inhibitor, cycloheximide, has been shown to induce Pgp2 mRNA levels in cultured hepatocytes, and there has been controversy as to how cycloheximide exerted this effect [Gant et al., 1992; Schuetz et al., 1995b]. In an attempt to determine if Pgp genes are co-regulated by the drug and to help resolve the controversy, various concentrations of cycloheximide were tested. Results in Figure 3 shows that cycloheximide dose-dependently induced Pgp2 mRNA levels, with maximal effect at 3 μg/ml (6-fold). Cycloheximide was also found to induce Pgp3 (3-fold) and α-tubulin (2-fold) mRNA levels, with maximal effect at 0.4 μg/ml. In contrast, at all concentrations tested, the protein inhibitor does not significantly affect both Pgp1 and GAPDH mRNA expression (Fig. 3). The effect of cycloheximide on transcription of all the three Pgp genes was also examined. Treatment of cultured hepatocytes with the protein synthesis inhibitor for 20 h had no effect on Pgp1 transcription, while induction of Pgp2 (4-fold) gene was seen (data not shown). A 2.5-fold induction of Pgp3 transcription was observed upon treatment with cycloheximide (data not shown).

TABLE III. Transcriptional Activities of Pgp Genes Following Treatment With Drugs

Time in culture and treatment	Genes		
	Pgp1	Pgp2	GAPDH
0 h			
None	1.0	1.0	1.0
20 h			
None	2.5 ± 0.2	1.1 ± 0.12	0.9 ± 0.11
Cyt D	4.2 ± 0.12	0.9 ± 0.13	0.9 ± 0.12
Phalloidin	3.8 ± 0.3	0.9 ± 0.10	1.1 ± 0.20
Colchicine	4.1 ± 0.13	1.2 ± 0.20	1.1 ± 0.15
β-lumicolchicine	2.1 ± 0.20	0.8 ± 0.30	1.0 ± 0.13
Taxol	1.1 ± 0.20	0.9 ± 0.21	1.2 ± 0.30

Transcriptional activities (expressed as specific activity/number of nuclei) were determined by nuclear run-on assays as previously described [Lee et al., 1995]. After subtracting from pUC9, quantified values were normalized to control values (0 h with no treatment). Results shown were from four different experiments using separate cell preparations.

DISCUSSION

Previous studies have shown that Pgp isoforms are functionally distinct [Devault and Gros, 1990], and their genes may be regulated differently [Croop et al., 1989]. To date, relatively few studies have focused on understanding how the three Pgp genes respond differently to different stimuli or factors. Based on the knowledge that (i) Pgp gene expression can be

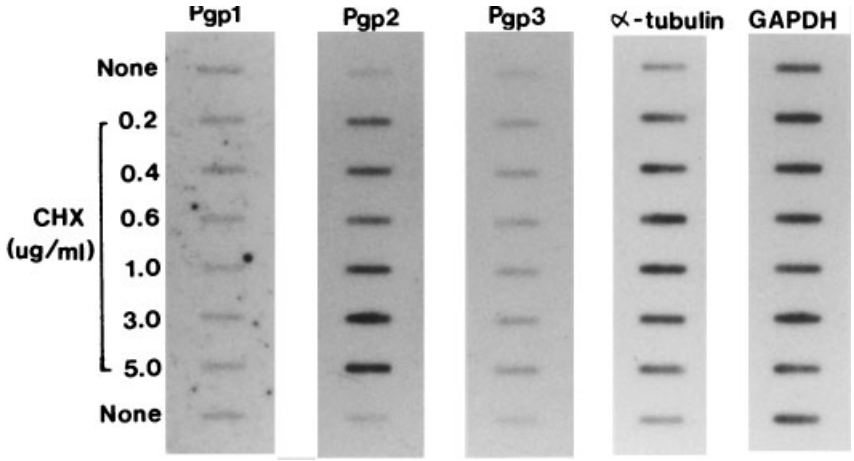

Fig. 3. Effects of cycloheximide on Pgp gene expression in cultured hepatocytes. Hepatocytes were isolated and cultured as described in Materials and Methods. After 4 h for cell attachment, cultures were replaced with fresh standard culture medium containing no addition (none) or different concentrations of cycloheximide as shown. After a further 20-h incubation, cultures were harvested for total RNA. Ten micrograms total RNA from each sample was analyzed by slot blot as described in Materials and Methods.

modulated by extracellular matrix proteins; (ii) collagen configuration can influence gene expression in cultured hepatocytes; and (iii) some drugs can affect Pgp gene expression, the present study was conducted. This study examined the effects of hepatocyte sandwich configuration, cytoskeletal disrupting/stabilizing drugs, and protein synthesis inhibitor on expression and transcription of all three Pgp genes in primary rat hepatocytes.

Upon culturing of hepatocytes, the expression of Pgp2 mRNA increased approximately 25-fold (4 vs. 48 h, Fig. 1). However, there was no change in transcriptional activity of Pgp2 gene (Table II), suggesting that post-transcriptional control is the predominant mechanism responsible for the dramatic overexpression of Pgp2 mRNA. This is in accordance with the previous finding that mRNA stability is primarily responsible for overexpression of Pgp2 transcript in hepatocytes [Lee et al., 1995]. Hepatocytes cultured for 48 h in the sandwich system exhibited significantly lower levels of Pgp2 mRNA (Fig. 1), but there was no parallel decrease in transcription (Table II). This suggests that the two-layered collagen configuration induces destabilization of Pgp2 mRNA, which is consistent with the recent report of destabilization of β-actin mRNA in hepatocytes upon culturing in the sandwich system [Otsu et al., 2000]. This is in good agreement with the proposition that Pgp2 and cytoskeletal genes are regulated by similar post-transcriptional mechanism, in cultured hepatocytes [Lee et al., 1993, 1995]. On the other hand, Pgp1 mRNA appeared to be controlled predominantly through transcriptional mechanisms, both during culturing and upon layering of another collagen gel. Parallel increases in Pgp1 mRNA levels (Fig. 1) and transcription (Table II) were observed as hepatocytes progressed from 4 to 48 h in culture. Similarly, a 2-fold decrease in both Pgp1 mRNA levels and transcription were seen in hepatocytes cultured in the sandwich system as compared to the single layer system. There was approximately 7-fold decrease in Pgp3 mRNA levels in hepatocytes progressing from 4 to 48 h in culture (Fig. 1). This was accompanied by only a 3-fold decrease in Pgp3 transcription (Table II), suggesting that both transcriptional and post-transcriptional mechanisms contributed to the changes in steady-state Pgp3 mRNA levels. Interestingly, upon layering between two layers of collagen, 48-h cultured hepatocytes exhibited 2-fold decrease in Pgp3 mRNA levels as compared to those cultured on a single layer of collagen (Fig. 1). Surprisingly, this was accompanied by a 2-fold increase in Pgp3 transcription. This suggests that the 2-fold increase in transcription was insufficient to account for the decrease in the steady-state Pgp3 mRNA levels and that the sandwich system must have caused destabilization of Pgp3 mRNA.

In an effort to further understand the differential regulation of Pgp genes, the effects of cytoskeletal disrupting/stabilizing drugs were studied. Consistent with earlier findings [Lee et al., 1995], it was found that cytoskeletal disrupting drugs (cytochalasin D and colchicine) but not cytoskeletal stabilizing drugs (phalloidin, taxol), suppressed Pgp2 mRNA expression in cultured hepatocytes (Fig. 2). Cytochalasin D and colchicine had no effect on Pgp2 transcription, suggesting that these drugs

suppressed Pgp2 mRNA levels via induction of Pgp2 mRNA destabilization mechanism. This is in accordance with earlier findings of rapid degradation of Pgp2 mRNA upon treatment with cytochalasin D [Lee et al., 1995]. In contrast, cytochalasin D, colchicine, and phalloidin were found to induce Pgp1 mRNA expression (Fig. 2). An almost parallel increase in Pgp1 transcription by these drugs but not by the inactive β-lumicolchicine (Table III) suggests that transcriptional mechanism via perturbation of cytoskeleton is predominantly involved. The exact mechanism for this is currently unclear, although this result is somewhat unexpected since in other cell type, disruption of cytoskeleton has been shown to activate p53, which may, in turn, downregulate Pgp1 transcription. The effects of cytoskeletal disrupting/stabilizing drugs on Pgp3 gene expression are very similar to that observed for Pgp2 gene. Only treatment with cytochalasin D and colchicine suppressed Pgp3 mRNA levels (Fig. 2), and this was not accompanied by changes in Pgp3 transcription (Table III). Thus, like Pgp2 mRNA, Pgp3 mRNA appears to be susceptible to mRNA degradation mechanism, which involves cytoskeletal elements.

So the questions remain: (i) what is the molecular mechanism of transcription which controls Pgp1, Pgp3, albumin, and other liver-specific genes expression and (ii) what is the mRNA stability mechanism which controls Pgp2, Pgp3, and other ubiquitously-expressed genes in cultured hepatocytes? The extracellular matrix has been shown to modulate liver transcription factors, including HNF-3α [Dipersio et al., 1991]. HNF-3α, in turn, has been reported to regulate the transcription of tyrosine aminotransferase [Unterman et al., 1995], albumin [Zaret et al., 1988], and aldolase [Raymondjean et al., 1991]. HNF-3α thus represents a possible candidate for the transcriptional activation/repression of Pgp1 and Pgp3 genes in cultured hepatocytes. Similarly, the molecular mechanism of mRNA stability governing Pgp2, Pgp3, and the other ubiquitously-expressed (e.g., tubulin, actin) genes in cultured hepatocytes is not clear. Whether changes in amount of ribonucleases, RNA-binding protein(s), or both are involved, remain unknown.

This is the first study to show that treatment of cultured hepatocytes with cycloheximide for 20 h had no effect on Pgp1 mRNA expression and transcription. This is in contrast to the effect of cycloheximide on Pgp1 mRNA expression and transcription in liver cells in vivo [Lee, 2001]. Reasons for such differences are not known but may reflect differences in transcriptional control between cultured hepatocytes and liver cells in vivo [Clayton and Darnell, 1983]. In agreement with previous reports, cycloheximide significantly induced Pgp2 mRNA expression and transcription in cultured hepatocytes [Gant et al., 1992; Lee et al., 1995; Hill et al., 1996] and in liver in vivo [Lee, 2001]. While the effect of cycloheximide on Pgp2 gene appears to involve both transcriptional [Gant et al., 1992; Lee et al., 1995] and post-transcriptional [Schuetz et al., 1995b; this study] mechanisms, the induction of Pgp3 mRNA expression by the protein synthesis inhibitor apparently involved predominantly transcriptional mechanisms. The exact mechanism whereby cycloheximide affects Pgp gene expression in cultured hepatocytes is not clear. An involvement of transcriptional repressor and direct effect on signaling pathways has been suggested. These possibilities could be distinguished by using additional protein synthesis inhibitors, which has completely different modes of action. Although the results with cycloheximide reported here have not shed lights on the molecular mechanisms governing Pgp gene expression and regulation, it has provided further evidence that the two MDR-associated genes, Pgp1 and Pgp2, are not regulated in the same manner.

In summary, this study has provided direct evidence that the three Pgp genes are differentially regulated. Pgp1 gene is predominantly regulated via transcriptional mechanisms by collagen configuration and some drugs, whereas Pgp2 gene is regulated predominantly via post-transcriptional mechanism. In contrast, Pgp3 gene can be regulated by both mechanisms. The study with cycloheximide lends further support for the evidence that Pgp1 and Pgp2 genes are not co-regulated. These results have implications for future studies on the molecular mechanisms of Pgp gene regulation with relevance to humans.

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