J. Pharm. Pharmacol. 1998, 50: 567–574
Received February 13, 1997

Use of Transgenic Animals in Understanding Molecular Mechanisms of Toxicity*

C. ROLAND WOLF AND COLIN J. HENDERSON

Imperial Cancer Research Fund, Molecular Pharmacology Unit, Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK

Abstract

Understanding molecular mechanisms of chemical toxicity and the potential risks of drugs to man is a pivotal part of the drug development process. With the dramatic increase in the number of new chemical entities arising from high throughput screening, there is an urgent need to develop systems for the rapid evaluation of potential drugs so that those agents which are most likely to be free of adverse effects can be identified at the earliest possible stage in drug development. The complex mechanisms of action of chemical toxins has made it extremely difficult to evaluate the precise toxic mechanism and also the relative role of specific genes in either potentiating or ameliorating the toxic effect. This problem can be addressed by the application of genetic strategies. Such strategies can exploit strain differences in susceptibility to specific toxic agents or, with the rapidly developing technologies, can exploit the use of transgenic animals where specific genes can be manipulated and subsequent effects on chemical toxicity evaluated.

Transgenic animals can be exploited in a variety of ways to understand mechanisms of chemical toxicity. For example, a human gene encoding a drug metabolizing enzyme can be directly introduced and the effects on toxic response evaluated. Alternatively, specific genes can be deleted from the mouse genome and the consequences on toxicological response determined. Many toxic chemical agents modulate patterns of gene expression within target cells. This can be used to screen for responses to different types of toxic insult. In such experiments the promotor of a stress-regulated gene can be ligated to a suitable reporter gene, such as *lacZ*, or green fluorescent protein, and inserted into the genome of an appropriate test species. On administration of a chemical agent, cells which are sensitive to the toxic effects of that chemical will express the reporter, which can then be identified using an appropriate assay system. This latter strategy provides the potential for screening a large number of compounds rapidly for their potential toxic effects and also provides information on tissue and cellular specificity. Experiments using transgenic animals can be complex, and care must be taken to ensure that the results are not affected by background activities within the species being used. For example, the introduction of a specific human cytochrome P450 gene may have no effect on the metabolic disposition of a drug or toxin because of the background activity within the mouse. As the toxicity of a chemical agent is determined by a wide range of different factors including drug uptake, metabolism, detoxification and repair, differences between man and the species being used could potentially generate a toxic response in the animal model whereas no toxicity may be observed in man.

In spite of these confounding factors, the application of transgenic animals to toxicological issues has enormous potential for speeding up the drug discovery process and will undoubtedly become part of this process in the future.

Correspondence: C. J. Henderson, Imperial Cancer Research Fund, Molecular Pharmacology Unit, Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK. E-Mail: m.rooney@icrf.icnet.uk

*Presented at the British Pharmaceutical Conference, Scarborough, September 15–18, 1997.

The potential of transgenic technology to investigate the biological role and regulation of drug metabolizing enzymes has yet to be fully realized. Mammalian, yeast, insect and bacterial systems may provide in-vitro models for assessing the role

Table 1. Potential uses of transgenic systems.

Manipulation	Uses
Promotor	Gene regulation Toxicity screens
Introduction of new gene	Gene function
Gene inactivation (knockout)	Constitutive functions Stress response Role in toxicity and carcinogenesis (carcinogenicity tests)
Mutator mice	Toxicity screens Mutation tests
Reporter mice	Mutation tests Toxicity screens Promotor regulation
Transgene introduction	Metabolic activation and detoxification Pathways of toxicity Toxic metabolite migration
Gene deletion	Pathways of toxicity Role of specific genes in toxic response Endogenous function
Gene replacement	Role of alleles in toxic and carcinogenic response

of cytochrome P450 in drug metabolism and chemical carcinogenesis, but may not necessarily reflect the in-vivo situation, since other factors such as pharmacokinetic parameters, are important. In-vivo models provide the opportunity to study the biological effects of gene expression under physiologically relevant conditions, and allow the inclusion of pharmacotoxicological endpoints.

Despite intensive study, the endogenous role of the majority of drug metabolizing enzymes, i.e. P450 and glutathione *S*-transferase (GST), have yet to be elucidated. Transgenic animals constitute a powerful tool to unravel the physiological functions of these enzymes. Using this approach it is possible, for example, to replace an endogenous gene with the corresponding human sequence, allowing the study of a human drug metabolizing enzyme in a heterologous system. Alternatively, replacement of an endogenous coding sequence with a mutant gene, or indeed removal or inactivation of the gene, as in knock-out mice, allows investigation of the role of this gene and its products in normal homeostasis or in toxicological responses.

Table 1 outlines the possible uses of different transgenic systems, while Table 2 summarizes the effects of gene deletions in the field of drug metabolism to date. Table 3 lists potential problems with transgenic experiments and suggests possible solutions.

One transgenic approach has been the development of a regulatable promotor system, in which gene expression is tightly controlled, either in-vitro, for example in tissue culture, or in-vivo in transgenic animals. Expression of a protein under the control of an inducible promotor allows the assessment of the effects of a single gene product in an otherwise normal animal at the appropriate developmental time. The promotors of heat shock proteins (Schweinfest et al 1988), metallothionein (Hu & Davidson 1990) or steroid-responsive genes (Ko et al 1989) have been employed in this regard, but have suffered from 'leakiness' i.e. detectable levels of expression in the uninduced state, and relatively poor fold-increases in expression after induction. We have used the promotor of the CYP1A1 gene, which is not only not constitutively expressed, but also highly inducible by compounds such as polycyclic aromatic hydrocarbons and polychlorinated hydrocarbons (Gelboin 1980) to create a construct in which the rat CYP1A1 promotor and 5′-flanking regions are cloned upstream of the *lacZ* reporter gene. In mouse lines derived from this construct, transgene expression was found to be completely absent in untreated animals. Treatment with 3-methylcholanthrene resulted in an extremely substantial increase (>1000-fold) in expression in a variety of tissues, including liver, adrenal, kidney and intestine, and at lower levels in

Table 2. Effect of gene deletions on drug metabolism.

Gene knockout	Phenotype	Reference
Ah receptor	Hepatic defects	Schimdt et al (1996)
	Hepatic defects	Fernandez-Salguero et al (1995; 1996)
	Immune system deficiencies Resistance to dioxin toxicity	
ARNT	Abnormal angiogenesis	Maltepe (1997)
CYP1A2	Neonatal lethality Respiratory distress	Pineau et al (1995)
	Deficient drug metabolism	Liang et al (1996)
CYP2E1	Acetaminophen hepatotoxicity	Lee et al (1996)
PPARα	Abolition of peroxisome	Lee et al (1995); Gonzalez (1997)

Table 3. Potential problems and solutions with transgenic experiments.

Problem	Solution
Background activity due to structural homology from same gene family or overlapping substrate specificities between gene families	Delete background
Species differences in gene regulation	Express protein under homologous promotor
Species differences in other enzymes or toxicological response	Exploit sex/strain differences e.g. AhR deficiency, female DA rat
	Exploit differences in kinetic properties or susceptibility to inhibitors
	Use different species e.g. *Drosophila*

other tissues such as spleen, lung, pancreas and reproductive organs (Campbell et al 1996). Moreover, the pattern of transgene expression paralleled the reported expression profile for CYP1A1 protein, supporting the concept that the CYP1A1 promotor can drive expression of heterologous genes in a tightly regulated manner, and providing a model system to establish the identity of the factors involved in the regulation of this gene.

Another in-vivo drug metabolism model developed in *Drosophila melanogaster* (Jowett et al 1991) has proved an extremely valuable model for genotoxicity studies. The ease of *Drosophila* genetics and the availability of several biological endpoints makes this organism extremely attractive in transgenic terms. In our studies the rat CYP2B1 gene was cloned into a vector containing a transposable P element, which are very mobile in the *Drosophila* genome and readily integrate into chromosomes. The vector also contained a phenotypic marker for red eye colour, allowing for simple identification of transgenics. The larval *Drosophila* promotor (LSP1) directed the expression of CYP2B1 to the fat body of the larvae, as verified by immunoblotting, and the catalytic properties of the expressed protein toward various alkoxy-resorufin analogues were similar to the values found for the purified enzyme, showing that the P450 reductase coupled well with the mammalian P450. Using a somatic mutation and recombination test (SMART), it was found that CYP2B1 expression led to a more than tenfold increase in genotoxicity of cyclophosphamide when compared with control. In a similar approach, canine CYP1A1 has been expressed in *Drosophila* under the control of a heat shock promotor, and its role in the toxicity of 7,12 dimethylbenz[a]anthracene (DMBA) studied in larvae (Komori et al 1993), using premature larval mortality as the biological endpoint. It was found that DMBA toxicity was increased after induction of the heterologous CYP1A1. These examples demonstrate that *Drosophila* has a valuable role to play in the investigation of cytochrome P450 and chemical mutagenesis. However, drug metabolism in insects and mammals is rather distinct and this may limit comparisons between the two species.

The use of transgenic animals to elucidate the physiological roles of mammalian proteins is increasing steadily, and several techniques for gene transfer into animals have been developed (Kollias & Grosveld 1992; Boyd & Samid 1993). For example, the gene of interest may be introduced by microinjection into the germ line of mice. However, as integration of the transgene is random, positional effects of expression and effects on other genes have to be taken into account. In another technique, pluripotent embryonal stem cells are transfected with the transgene together with a selection marker. Cells which have taken up the transgene are introduced into blastocysts which are implanted into foster mice, and identification of chimerae can be made by the mosaic hair colour pattern. Those chimerae in which germ line transmission of the transgene occurs can then be bred to generate mice homozygous for the transgene. A transgene may also be introduced into somatic cells in-vitro by retroviral transfer for example. Cells with the heterologous gene are then implanted into the adult organism where they produce the protein encoded by the foreign gene (Valerio 1992).

We have recently established transgenic mice which specifically express the human GSTA1 in the epidermal cells of the mouse skin using the epidermal-specific keratin VI promotor (Simula et al 1993). Transgene expression was confirmed by Western blotting and enzymatically by measuring the α-class specific GST isomerase activity towards androsten 3,17-dione. The skin was chosen as the site of expression of the enzyme because not only is it an easily accessible target tissue, both for the presentation of chemicals and the assessment of toxicological endpoints, but also GSTA1 is not expressed in mouse skin, providing an ideal background onto which to introduce the transgene

(Raza et al 1991). Finally, with respect to chemically-induced skin tumour formation, the mouse skin is a well characterized tissue (Brown & Balmain 1995). Skin painting experiments should allow the direct evaluation of the role of this and other transgenes in chemical carcinogenesis. Pilot skin painting experiments are now being conducted to investigate the role of the GST in carcinogen-induced DNA adduct and tumour formation.

The difficulties encountered when using xenobiotics to induce P450 in-vitro make transgenic animals an extremely valuable resource in the study of the mechanisms underlying the inductive process. Transgenic mice have been generated containing various lengths of the 5′-flanking region of CYP2B2 as well as the entire coding and 3′-noncoding region of this gene (Ramsden et al 1993). Constructs containing 800 base pairs of the promotor region were constitutively expressed in mouse liver and kidney at high levels, but this construct was not inducible by phenobarbital. The inclusion of an additional 19 kilobases of 5′-flanking region led to a very low basal, but phenobarbital-inducible, hepatic expression of the transgene, analogous to the expression pattern observed for CYP2B2 in rat liver. It appears that previously identified elements 50–100 base pairs upstream from the transcription start site of CYP2B genes, are on their own insufficient to mediate phenobarbital induction, although gene-positional effects may have influenced induction of the CYP2B transgenes in this study. The regulatory regions of CYP1A1 have also been studied in transgenic animals, using a chloramphenicol acetyl transferase (CAT) reporter construct containing 2·6 kilobases of 5′-flanking DNA (Jones et al 1991). The expression pattern of the reporter was found to be similar to the pattern of CYP1A1 expression in-vivo.

The physiological and pharmacotoxicological functions of drug metabolizing enzymes may also be studied using gene deletion or knock-outs. The aryl hydrocarbon receptor (AhR) mediates the regulation of cytochromes P450 in-vivo, and has also been implicated in dioxin-mediated teratogenesis, apoptosis and immunosuppression. The AhR gene was inactivated in embryonic stem cells using a positive–negative selection strategy (Fernandez-Salguero et al 1995), with a positive selection marker (neo) interrupting the AhR gene in the targeting vector and the negative selection marker, herpes simplex virus-thymidine kinase (HSV-TK), situated several kilobases upstream of the AhR gene segment. After homologous recombination, the neo selection marker is retained and the HSV-TK marker lost, so that correctly-targeted embryonal stem cells will survive in a medium containing G-418 and gancyclovir, whereas cells in which non-homologous recombination has taken place should be killed by gancyclovir due to the expression of the HSV-TK. The inactivation of the AhR yielded pleomorphic symptoms, a high proportion of the animals dying of indeterminate cause in the perinatal period. Treatment with dioxin induced hepatic expression of CYP1A1 and UGT1*6 in $AhR^{+/+}$ animals but not in $AhR^{-/-}$ mice, demonstrating that a functional AhR is required for induction in the liver. Histological examination of tissues isolated from the mice with the inactivated AhR revealed pronounced liver fibrosis and a highly reduced liver–body weight ratio. The AhR may also play an important role in the development and the maintenance of the immune system, since young $AhR^{-/-}$ mice displayed a decreased population of splenic lymphocytes. The mechanisms of this effect are at present unclear.

A second $AhR^{-/-}$ mouse line, generated by replacing exon 2, the region of the gene encoding the basic/helix-loop-helix domain essential for receptor dimerization and DNA binding (Schmidt et al 1996), with a neomycin resistance gene under the control of an exogenous promotor, displayed no overt phenotypical changes from their wild-type counterparts, although the null mice did exhibit a slower growth rate and thus decreased body weight in the postnatal period. Detailed analysis of these mice revealed that the liver was significantly smaller (approx. 25%) than in their wild-type litter mates, and the organ was visibly different in the first two weeks of life, being pale in colour and of a spongy texture, although these features had disappeared by a month after birth. A metabolic defect in hepatocyte function was suggested as the underlying cause for these observations, indicating a temporal requirement for AhR expression. By about six weeks, the majority of the $AhR^{-/-}$ mice began to suffer from portal fibrosis and hypercellularity, while approximately 50% of these animals also displayed congestive splenomegaly, which may have been a result of decreased liver blood flow. This study could not distinguish whether the phenotypic changes noted in these AhR mice were as a direct result of defective liver growth and maturation, or whether the mice were more susceptible to an unidentified environmental agent as a consequence of the gene deletion. It is interesting to note the differing phenotypes observed from these two studies, for which no explanation is immediately apparent; genetic background, previously cited in other cases where deletion of the same gene by two groups has led to differing phenotypic consequences, is unlikely to be the cause in this case, since both mouse lines were on a

129 × C57BL/6 background. Equally, the possibility of partial inactivation, a 'leaky' deletion, or formation of a new gene product at the targeted allele, seems unlikely since both groups showed the absence of functional AhR protein by a lack of an inductive response of CYP1A1 or CYP1A2 to treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and in the case of Schmidt et al (1996) by an absence of immunoreactive AhR protein on Western blots.

CYP1A2 is a highly conserved mammalian P450, involved in the oxidative metabolism of xenobiotics, including polycyclic aromatic hydrocarbons, which are also capable of inducing the expression of this enzyme. Two groups have recently established mouse lines in which CYP1A2 has been deleted by homologous recombination in embryonal stem cells. Pineau et al (1995) inserted a neomycin resistance gene into the first coding exon of the CYP1A2 gene, and although heterozygote animals appeared normal, homozygous mutant mice died within 1 h of birth displaying severe respiratory distress. The penetrance of this phenotype was incomplete, with 3% of animals surviving to adulthood and appearing phenotypically normal. The null mice which died had decreased expression of surfactant apoprotein in type II alveolar cells and a reduced gallbladder size. Liang et al (1996) created CYP1A2 knock-out mouse by replacing part of exon 2, and all of exons 3 to 5, with the hypoxanthine phosphoribosyltransferase gene. In contrast to the previous study, homozygous null mutants were completely viable and appeared phenotypically indistinguishable from their heterozygote or wild-type litter mates. Further study of these animals revealed a role for CYP1A2 in zoxazolamine metabolism, with CYP1A2 null mice displaying a ninefold increase in paralysis time with this muscle relaxant. Several possible reasons for the phenotypic discrepancies were suggested; either the genetic background, which was C57BL/6 in their report, and CF-1 or Swiss Black in the first, or the presence of a viral or respiratory pathogen in a genetically susceptible host. The possibility also remains that the answer lie in the different approaches used to create the targeted allele.

CYP2E1 is the major P450 responsible for the metabolism of ethanol, and it also carries out the metabolism of a wide range of xenobiotics, including acetaminophen, carbon tetrachloride, pyrazole and dimethylnitrosamine, as well as endogenous chemicals such as acetone and arachidonic acid. Lee et al (1996) generated a CYP2E1 null mouse by replacing part of intron 1, exon 2 and part of intron 2 with the bacterial neomycin resistance gene under the control of the phosphoglycerate kinase-1 promotor. Mice homozygous null for CYP2E1 were found to be phenotypically indistinguishable from wild-type mice, but were found to exhibit increased resistance to acetaminophen toxicity, such that at 400 mg kg^{-1} acetaminophen, 50% of wild-type mice died compared with none in the null group. Histological examination of liver tissue and estimation of serum bilirubin, creatinine and alkaline phosphatase confirmed that acetaminophen hepatotoxicity was mediated by CYP2E1, although it cannot be the only enzyme involved, since at higher doses of acetaminophen (600 mg kg^{-1}) toxicity also began to appear in the null mice.

We have recently generated a mouse line which is nulled for both murine GST-pi genes, P1 and P2 (Henderson et al, unpublished). GST-pi expression is greatly elevated in rat pre-neoplastic hepatic foci (Morimura et al 1993; Kora et al 1996), and in drug-resistant cell lines (Cowan et al 1986; Black & Wolf 1991; Wareing et al 1993), and has also been found to be altered in a variety of human tumours, such as ovarian (Green et al 1993; Hamada et al 1994), colon (Mulder et al 1995), lung (Hida et al 1994; Bai et al 1996), bladder (Singh et al 1994), gastric (Schipper et al 1996) and testicular (Katagiri et al 1993). In many cases, GST-pi expression is positively correlated with malignant transformation and resistance to chemotherapeutic drugs, and inversely correlated with patient survival. Interestingly however, there are also reports that patient survival is greater with tumours which stain positive for GST-pi, for example in renal carcinoma (Grignon et al 1994). It has also been reported that in human prostatic carcinoma, expression of GST-pi is completely absent, whereas in normal prostatic and benign hyperplastic tissue, GST-pi expression is unaltered (Lee et al 1994), probably as a result of hypermethylation of the GST-pi promotor. These findings were confirmed in a subsequent study, although GST-pi expression was found to correlate with the presence of basal cells, a phenotype which is absent in prostatic carcinoma, leading to the suggestion that GST-pi may be more involved in the process of epithelial differentiation than malignant transformation (Cookson et al 1997).

Cloning and characterization of the murine GSTP1 and P2 genes has been reported (Bammler et al 1994), and we have developed mouse lines in which both genes have been deleted, or in which only GSTP1 is missing (Henderson et al, unpublished). In the mouse liver, GSTP1 is expressed at much higher levels than GSTP2, and displays far greater catalytic activity towards a range of substrates. In addition, the expression of GSTP1 is sexually differentiated in the liver, levels in male

mice approximately an order of magnitude higher than that in females (Bammler 1995).

Initial results with the double knock-out (GSTP1/P2) mouse line suggest that GST-pi is a non-essential gene, since the mice displayed no phenotypic changes or reproductive difficulties, with litter sizes and sex distribution no different from their wild-type counterparts. However, treatment of wild-type mice with acetaminophen resulted in a sexual differentiation of the hepatotoxic effects of this compound, as evidenced by histological examination of the liver, and estimation of serum levels of enzymes considered diagnostic for hepatocellular damage, such that male mice were much more sensitive than females. When the GSTP1/P2 null male mice were similarly treated, they exhibited increased resistance to the hepatotoxic effects of the drug, essentially abolishing the sexual differentiation observed in the wild-type mice. In man and mice, acetaminophen is activated to a hepatotoxic metabolite, *N*-acetyl-*p*-benzoquinonimine (NABQI), by the cytochrome P450 monooxygenase system, principally CYP2E1, although CYP3A4 and CYP1A2 may also play a role (Patten et al 1993; Thummel et al 1993; Casley et al 1997). The sexual dimorphism in renal toxicity observed in mice (Hu et al 1993) has been attributed to the sexually differentiated expression of CYP2E1 in this organ (Henderson et al 1990). However, in the liver, CYP2E1 expression is not sexually differentiated, either in the wild-type or GSTP1/P2 knock-out mice (Henderson, unpublished observation). NABQI is detoxified by conjugation with glutathione, either spontaneously, or by enzymatic means, with the major GST involved being GST-pi, in both rats and man (Coles et al 1988). Although one might expect that a GSTP1/P2$^{(-/-)}$ mouse would exhibit increased sensitivity to the effects of acetaminophen, having a decreased ability to conjugate and detoxify the hepatotoxic metabolite NABQI, in fact, as outlined above, the converse appears to be the case, thus rendering unclear the mechanism of involvement of GST-pi in acetaminophen metabolism.

In mice and rats, peroxisome proliferators cause a pleiotropic response in the livers of both species, the animals exhibiting cellular hypertrophy and hyperplasia, an increased number and size of peroxisomes, and increased transcription of enzymes involved in the β-oxidation of fatty acids. These coordinate events may represent an adaptive response in the process of lipid homeostasis; the mechanism is still unclear, but these events are indicative of the importance of peroxisome proliferation in the regulation of fatty acid metabolism. A peroxisome proliferator-activated receptor (PPARα), a member of the steroid receptor superfamily was first cloned from mice, and a further five isoforms have been described, with homologues being identified in a number of species. The identification of a peroxisome proliferator response element in the promotor region of target genes lends strength to the suggestion that PPARs act as transcription factors, although direct interaction of peroxisome proliferators with PPAR remains to be demonstrated. However, on the basis of structural similarities, it is assumed that a ligand-binding domain exists within PPAR. Lee et al (1995) produced a PPARα knockout mouse by replacing an 83 base-pair segment of exon 8, within the putative ligand-binding domain, with a neomycin resistance gene. The resultant homozygous null mice were apparently phenotypically normal; however, when challenged with peroxisome proliferators, these mice failed to display the associated pleiotropic response, leading to the conclusion that PPARα is an essential requirement for the action of these chemicals and the mediation of the effects of peroxisome proliferators (Gonzalez 1997).

The above data demonstrate the power of transgenic animals in understanding both mechanisms of chemical toxicity as well as identifying the enzymes involved in mediating toxic response. A cautionary note is that a large number of transgenic animals are becoming available, particularly where genes involved in the carcinogenic process have been either deleted or mutant forms introduced. Such models, which are hypersensitive to tumourigenic agents, are now being used by regulatory agencies as part of the drug registration process. Such applications, in addition to being highly controversial, run a serious risk of confounding the process of risk assessment. This is because pathways of carcinogenesis in animals may be significantly different to those in man; for example, p53 mutations are often not observed in laboratory animals. Also, if sufficient genes are modified, animals with an extremely high rate of spontaneous tumour incidence can be generated, making any additional effects in response induced by a chemical agent almost meaningless. A further example can be found in mice carrying the mutant *ras* gene in the skin, which develop tumours simply as a consequence of wounding. There is a serious risk that the introduction of such tests will put back developments in estimating the risks of chemical agents to man by many years.

The use of transgenic mice is opening new avenues for the study of mechanisms of chemical toxicity, gene regulation and carcinogenesis. The application of these approaches should lead to a much greater understanding of potential risks

associated with exposure to specific chemical agents and will provide novel strategies for the rapid assessment of toxicity as part of the pre-clinical drug development process.

References

Bai, F., Nakanishi, Y., Kawasaki, M., Takayama, K., Yatsunami, J., Pei, X. H., Tsuruta, N., Wakamatsu, K., Hara, N. (1996) Immunohistochemical expression of glutathione *S*-transferase-Pi can predict chemotherapy response in patients with nonsmall cell lung carcinoma. Cancer 78: 416–421

Bammler, T. K., Driessen, H., Finnstrom, N., Wolf, C. R. (1995) Amino acid differences at positions 10, 11 and 104 explain the profound catalytic differences between two murine pi-class glutathione *S*-transferases. Biochemistry 34: 9000–9008

Bammler, T. K., Smith, C. A., Wolf, C. R. (1994) Isolation and characterization of two mouse Pi-class glutathione *S*-transferase genes. Biochem. J. 298: 385–390

Black, S. M., Wolf, C. R. (1991) The role of glutathione-dependent enzymes in drug resistance. Pharmacol. Ther. 51: 139–154

Boyd, A. L., Samid, D. (1993) Molecular biology of transgenic animals. J. Animal Sci. 71: 1–9

Brown, K., Balmain, A. (1995) Transgenic mice and squamous multistage skin carcinogenesis. Cancer Metastasis Rev. 14: 113–124

Campbell, S. J., Carlotti, F., Hall, P. A., Clark, A. J., Wolf, C. R. (1996) Regulation of the CYP1A1 promotor in transgenic mice: an exquisitively sensitive on-off system for cell-specific gene regulation. J. Cell Sci. 109: 2619–2625

Casley, W., Menzies, J., Mousseau, N., Girard, M., Moon, T., Whitehouse, L. (1997) Increased basal expression of hepatic Cyp1a1 and Cyp1a2 genes in inbred mice selcted for susceptibility to acetaminophen hepatoxicity. Pharmacogenetics 7: 283–293

Coles, B., Wilson, I., Wardman, P., Hinson, J. A., Nelson, S. D., Ketterer, B. (1988) The spontaneous and enzymatic reaction of *N*-acetyl-*p*-benzoquinonimine with glutathione: a stopped-flow kinetic study. Arch. Biochem. Biophys. 264: 253–260

Cookson, M. S., Reuter, V. E., Linkov, I., Fair, W. R. (1997) Glutathione *S*-transferase pi (GST-pi) class expression by immunohistochemistry in benign and malignant prostate tissue. J. Urol. 157: 673–676

Cowan, K. H., Batist, G., Tulpule, A., Sinha, B. K., Myers, C. E. (1986) Similar biochemical changes associated with multidrug resistance in human breast cancer cells and carcinogen-induced resistance to xenobiotics in rats. Proc. Natl Acad. Sci. USA 83: 9328–9332

Fernandez-Salguero, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M., Gonzalez, F. J. (1996) Aryl-hydrocarbon receptor-deficient mice are resistant to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin-induced toxicity. Toxicol. Appl. Pharmacol. 140: 173–179

Fernandez-Salguero, P., Pineau, T., Hilbert, D. M., McPhail, T., Lee, S. S. T., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., Gonzalez, F. J. (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin binding Ah receptor. Science 268: 722–726

Gelboin, H. V. (1980) Benzo[a]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed function oxidases. Physiol. Rev. 60: 1107–1166

Gonzalez, F. (1997) Recent update on the PPARa-null mouse. Biochimie 79: 139–144

Green, J. A., Robertson, L. J., Clark, A. H. (1993) Glutathione *S*-transferase expression in benign and malignant ovarian tumours. Br. J. Cancer 68: 235–239

Grignon, D. J., Abdel Malak, M., Mertens, W. C., Sakr, W. A., Shepherd, R. R. (1994) Glutathione *S*-transferase expression in renal cell carcinoma: a new marker of differentiation. Mod. Pathol. 7: 186–189

Hamada, S., Kamada, M., Furumoto, H., Hirao, T., Aono, T. (1994) Expression of glutathione *S*-transferase-pi in human ovarian cancer as an indicator of resistance to chemotherapy. Gynecol. Oncol. 52: 313–319

Henderson, C. J., Scott, A. R., Yang, C. S., Wolf, C. R. (1990) Testosterone-mediated regulation of mouse renal cytochrome P-450 isoenzymes. Biochem. J. 266: 675–681

Hida, T., Kuwabara, M., Ariyoshi, Y., Takahashi, T., Sugiura, T., Hosoda, K., Niitsu, Y., Ueda, R. (1994) Serum glutathione *S*-transferase-pi level as a tumor marker for non-small cell lung cancer. Potential predictive value in chemotherapeutic response. Cancer 73: 1377–1382

Hu, M. C. T., Davidson, N. (1990) A combination of the derepression of the lac operator-repressor system with positive induction by glucocorticoid and metal ions provides a high-level-inducible gene expression system based on the human metallothionein IIA promotor. Mol. Cell. Biol. 10: 6141–6151

Hu, J. J., Lee, M. J., Vapiwala, M., Reuhl, K., Thomas, P. E., Yang, C. S. (1993) Sex-related differences in mouse renal metabolism and toxicity of acetaminophen. Toxicol. Appl. Pharmacol. 122: 16–26

Jones, S. N., Jones, P. G., Ibarguen, H., Caskey, C. T., Craigen, W. J. (1991) Induction of the CYP1A1 dioxin-responsive enhancer in transgenic mice. Nucleic Acids Res. 19: 6547–6551

Jowett, T., Wajidi, M. F. F., Oxtoby, E, Wolf, C. R. (1991) Mammalian genes expressed in *Drosophila*: a transgenic model for the study of mechanisms of chemical mutagenesis and metabolism. EMBO J. 10: 1075–1081

Katagiri, A., Tomita, Y., Nishiyama, T., Kimura, M., Sato, S. (1993) Immunohistochemical detection of P-glycoprotein and GSTP1-1 in testis cancer. Br. J. Cancer 68: 125–129

Ko, M. S. H., Sugiyama, N., Takano, T. (1989) An auto-inducible vector conferring high glucocorticoid inducibility upon stable transformant cells. Gene 84: 383–389

Kollias, G., Grosveld, F. (1992) The Study of Gene Regulation in Transgenic Mice. Academic Press, London

Komori, M., Kitamura, R., Fukuta, H., Inoue, H., Baba, H., Yoshikawa, K., Kamataki, T. (1993) Transgenic drosophila carrying mammalian cytochrome-P-4501A1 - an application to toxicology testing. Carcinogenesis 14: 1683–1688

Kora, T., Sugimoto, M., Ito, K. (1996) Cellular distribution of glutathione *S*-transferase P gene expression during rat hepatocarcinogenesis by diethylnitrosamine. Int. Hepatol. Commun. 5: 266–273

Lee, W. H., Morton, R. A., Epstein, J. I., Brooks, J. D., Campbell, P. A., Bova, G. S., Hsieh, W. S., Isaacs, W. B., Nelson, W. G. (1994) Cytidine methylation of regulatory sequences near the pi-class glutathione *S*-transferase gene accompanies human prostatic carcinogenesis. Proc. Natl Acad. Sci. USA 91: 11733–11737

Lee, S. S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez Salguero, P. M., Westphal, H., Gonzalez, F. J. (1995) Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol. Cell. Biol. 15: 3012–3022

Lee, S. S., Buters, J. T., Pineau, T., Fernandez Salguero, P., Gonzalez, F. J. (1996) Role of CYP2E1 in the hepatotoxicity of acetaminophen. J. Biol. Chem. 271: 12063–12067

Liang, H. C., Li, H., McKinnon, R. A., Duffy, J. J., Potter, S. S., Puga, A., Nebert, D. W. (1996) Cyp1a2$^{(-/-)}$ null mutant mice develop normally but show deficient drug metabolism. Proc. Natl Acad. Sci. USA 93: 1671–1676

Maltepe, E., Schmidt, J. V., Baunoch, D., Bradfield, C. A., Simon, M. C. (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature 386: 403–407

Morimura, S., Suzuki, T., Hochi, S., Yuki, A., Nomura, K., Kitagawa, T., Nagatsu, I., Imagawa, M., Muramatsu, M. (1993) Trans-activation of glutathione transferase P gene during chemical hepatocarcinogenesis of the rat. Proc. Natl Acad. Sci. USA 90: 2065–2068

Mulder, T. P., Verspaget, H. W., Sier, C. F., Roelofs, H. M., Ganesh, S., Griffioen, G., Peters, W. H. (1995) Glutathione *S*-transferase pi in colorectal tumors is predictive for overall survival. Cancer Res. 55: 2696–2702

Patten, C. J., Thomas, P. E., Guy, R. L., Lee, M., Gonzalez, F. J., Guengerich, F. P., Yang, C. S. (1993) Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chem. Res. Toxicol. 6: 511–518

Pineau, T., Fernandez-Salguero, P., Lee, S. S. T., McPhail, T., Ward, J. M., Gonzalez, F. J. (1995) Neonatal lethality associated with respiratory distress in mice lacking cytochrome P450 1A2. Proc. Natl Acad. Sci. USA 92: 5134–5138

Ramsden, R., Sommer, K. M., Omiecinski, C. J. (1993) Phenobarbital induction and tissue-specific expression of the rat cyp2b2 gene in transgenic mice. J. Biol. Chem. 268: 21722–21726

Raza, H., Awasthi, Y. C., Zaim, M. T., Eckert, R. L., Mukhtar, H. (1991) Glutathione *S*-transferases in human and rodent skin: multiple forms and species-specific expression. J. Invest. Dermatol. 96: 463–467

Schipper, D., Verspaget, H., Mulder, T., Sier, C., Ganesh, S., Roelofs, H., Peters, W. (1996) Correlation of glutathione *S*-transferases with overall survival in patients with gastric carcinoma. Int. J. Oncol. 9: 357–363

Schmidt, J. V., Su, G. H., Reddy, J. K., Simon, M. C., Bradfield, C. A. (1996) Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proc. Natl Acad. Sci. USA 93: 6731–6736

Schweinfest, C. W., Jorcyk, C. L., Fujiwara, S., Papas, T. S. (1988) A heat shock-inducible eukaryotic expression vector. Gene 71: 207–210

Simula, T. P., Crichton, M. B., Black, S. M., Pemble, S., Bligh, H. F. J., Beggs, J. D., Wolf, C. R. (1993) Heterologous expression of drug-metabolizing-enzymes in cellular and whole animal-models. Toxicology 82: 3–20

Singh, S. V., Xu, B. H., Tkalcevic, G. T., Gupta, V., Roberts, B., Ruiz, P. (1994) Glutathione-linked detoxification pathway in normal and malignant human bladder tissue. Cancer Lett. 77: 15–24

Thummel, K. E., Lee, C. A., Kunze, K. L., Nelson, S. D., Slattery, J. T. (1993) Oxidation of acetaminophen to *N*-acetyl-*p*-aminobenzoquinone imine by human CYP3A4. Biochem. Pharmacol. 45: 1563–1569

Valerio, D. (1992) Retrovirus Vectors for Gene Therapy Procedures. Academic Press, London

Wareing, C. J., Black, S. M., Hayes, J. D., Wolf, C. R. (1993) Increased levels of alpha-class and pi-class glutathione *S*-transferases in cell lines resistant to 1-chloro-2,4-dinitrobenzene. Eur. J. Biochem. 217: 671–676