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Symposium Overview: Characterization of Xenobiotic Metabolizing Enzyme Function Using Heterologous Expression Systems

Alan J. Townsend,* Kinsley K. Kiningham,† Daret St. Clair,† Thomas R. Tephly,‡ Charles S. Morrow,* and F. Peter Guengerich§

*Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157; †Graduate Center for Toxicology, University of Kentucky, Lexington, Kentucky 40506; ‡Dept. of Pharmacology, University of Iowa, Iowa City, Iowa 52240; and §Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

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Genetically modified cell lines can be very useful models for assessing the toxicologic effects of modulation of expression of individual gene products in comparison to their isogenic parental control cell lines. This symposium begins with an overview of general issues related to development and utilization of model systems created by transfection of cell lines to induce elevated expression of metabolic enzymes of toxicologic relevance. Selected studies that illustrate the heterologous expression rationale and various approaches to transgenic-cell model construction are represented. Results to date with cells engineered to express specific transfected genes are discussed, with emphasis on the effects of expression of selected phase I or phase II enzymes on cellular sensitivity to several toxic end-points. The individual sections highlight the utility of these model cell lines for examining the role of enzyme catalysis and function in metabolism of biologically active xenobiotic or endobiotic compounds of interest in toxicology. Both activating and detoxifying enzymes are discussed, with principal emphasis on the latter. This symposium includes talks on transfected cells that express aldehyde dehydrogenases, superoxide dismutase, UDP-glycosyltransferases, glutathione transferases, and cytochrome P450 isozymes. In addition to the general toxicologic utility and advantages of these genetically engineered cell lines, this overview emphasizes their particular contributions to the insights obtained to date with the specific model cell lines.

Key Words: heterologous expression; transgenic cell models; aldehyde dehydrogenase; superoxide dismutase; UDP-glycosyltransferase; glutathione transferase; cytochrome P-450; cell culture; cytotoxicity; genotoxicity.

Introduction to Transgenic Modeling (A. J. Townsend)

DNA-mediated gene transfer was first discovered in experiments with bacteria (Avery, 1944). Later studies demonstrated oncogenic transformation of nonmalignant mammalian cells following transfection with DNA from cells that had previously been transformed, either by an oncogenic virus (Hill and Hillova, 1972) or by exposure to chemical carcinogens (Coo- per, 1982; Shih, 1979). Cell transfection techniques are now commonly used experimentally in a wide range of disciplines. The purpose of this symposium is to present and discuss some of the ways that this technology can be advantageously applied to studies on the toxicologic effects of overexpression of enzymes involved in xenobiotic metabolism.

Gene transfer technology has progressed from the crude genomic DNA-mediated transfection used to identify oncogenes to more refined techniques that employ viral or plasmid expression vectors with only the essential sequence elements needed for expression. In addition to the classic single gene transfer, it is now possible to sequentially transfect or to co-express from a single vector more than one gene in the same cell line, thus allowing, for example, reconstruction of up to several steps in a metabolic pathway. Technical aspects of vector design and cell transfection strategies and methods are beyond the scope of this symposium, and have been detailed elsewhere (Kriegler, 1991; Murray, 1991). However, a few comments regarding key aspects of model construction and application are warranted.

The choice of expression vector is a critical one, with the main decisions being between viral infection vs. non-viral transfection systems, and transient expression vs. stable integration into the cellular genome. Viral vectors are useful when it is essential to obtain expression in a high proportion of cells in the target cell line. Similar advantages can be attained by transient transfection with an autonomously replicating or “episomal” plasmid vector. These approaches can yield very high levels of expression, particularly when coupled with strong promoter/enhancer sequences and a drug resistance gene as selectable marker for amplification. Disadvantages of some viral vectors include production difficulties, requirement for helper viruses or packaging cell lines, and restricted range of
host cell lines with appropriate receptors or other compatibility issues.

Stable integration of the vector into cellular DNA is useful when it is advantageous to maintain consistent expression levels over a long period. For this purpose, however, plasmid vectors typically have a low frequency (\(<10^{-3}\)) of stable integration into the cellular genome. Hence it is necessary to select for the presence of a cointegrated marker gene that confers resistance to cytotoxic drugs such as G-418 or hygromycin, and to have a good screening assay to identify the positive expressing clonal transfectant lines. Some viral vectors are also useful for stable transduction, but it may be more difficult to obtain a high copy number and high expression levels per stable clonal transfectant line than with a plasmid vector. There may also be instances in which it is useful to express a particular gene product in non-mammalian cells, for example to produce a protein for purification or for metabolic or enzymologic studies with crude preparations.Vectors for use in bacteria, yeast, or insect cells are commercially available, and some of these systems can yield exceptionally high expression levels.

A key advantage of the heterologous expression approach derives from the ability to compare cells that express the gene(s) of interest to control cells that are otherwise genetically and phenotypically identical. Thus, the control cells are typically transfected with a control vector, either having no cDNA insert or with the cDNA in reverse orientation (antisense), and subsequently carried through the same steps as those applied to cells transfected with the expression vector. This approach works well for experiments involving transient expression after parallel transfections of the parent cell line with both vectors. However, for stable transfection, in which stable transfectants are isolated as clonal cell lines, the presumption of an isogenic background can be confounded by microheterogeneity within the parent cell line. This problem can be circumvented, initially, by transfecting a recently cloned isolate of the target cell line, and later by including several clonal control and producer lines in the experimental design, preferably with a range of expression levels. Following isolation of stable clonal transfectant cell lines, it is also advisable to compare key parameters, such as population doubling times and levels of critical cofactors, that are required for proper functioning of the transfected gene product. Since population heterogeneity may develop in any cell line over time, it is important to monitor expression when cell lines are cultured continuously over long periods, and to periodically replace all stock cultures from aliquots cryogenically preserved from the early isolates.

The convenience of having cultured cells for analysis of cellular cytotoxicity, as well as for easy and inexpensive access to subcellular components and metabolites, are obvious advantages of the cellular approach to the study of individual gene function in toxicology. However, there are also disadvantages. First, cultured cell lines are immortal and grow rapidly, and thus they differ from most cells in normal mammalian tissues. Second, the parent cell line may not express other genes that may play an important role in the function of a particular gene. For example, Dr. Morrow showed that protection against the cytotoxic effects of 4-nitroquinoline-1-oxide by expression of human GSTP1 was only observed when the gene was co-expressed with a transmembrane protein that transports the GSH conjugate produced by GSTP1 out of the cell (described below). Finally, it is important to bear in mind that major differences exist between the pharmacodynamics of toxin exposure in cultured cells as contrasted with the exposure conditions and cellular environment in vivo. One example is that the volume of the extracellular medium typically exceeds the volume of the cells by at least 100-fold in culture, whereas it is much less in most tissues. Thus if toxin exposure is long-term or continuous, adequate metabolic clearance from the medium may be difficult. This concern may be dealt with by testing the efficacy of the expressed gene over variable exposure intervals, as well as over a range of concentrations and with cell lines expressing different levels of the gene of interest. Exploring the effects of expression over the full range of these variables may also yield additional insight into the mechanistic events that determine the functional roles of the gene products being studied.

**Heterologous Expression in V79 Cells of Aldehyde Dehydrogenase-3 (ALDH-3) Confers Protection in Vitro against Toxicity of Lipid Aldehydes Associated with Lipid Peroxidation (Alan J. Townsend)**

Aldehyde dehydrogenases comprise a family of redox-active isozymes that catalyze oxidation of aldehydes to carboxylic acids (Lindahl, 1992; Sladek, 1989). The well-known major acetaldehyde-metabolizing ALDH-2 is mitochondrial, while ALDH-3, which catalyzes oxidation of many of the principal lipid aldehyde products of lipid peroxidation, is cytosolic. A second acetaldehyde-oxidizing isozyme is ALDH-1, also expressed in the cytosol. We have previously developed stably transfected MCF-7 (human breast carcinoma) and V79 (hamster lung fibroblast) cells via plasmid-based transfection that express a range of activities of human ALDH-1 or ALDH-3. These cell lines were utilized to demonstrate the functional ability of both the human cytosolic ALDH-1 and ALDH-3 isozymes to confer potent resistance to oxazaphosphorine (OAP) anticancer drugs, including Mafosfamide, 4-hydroperoxycyclophosphamide, and 4-hydroperoxyifosfamide (Bunting, 1994; Bunting and Townsend, 1996a; Bunting and Townsend, 1996b). The OAP drugs, among which cyclophosphamide is the most widely used, are metabolized to yield bidentate DNA crosslinking agents by way of an aldehyde intermediate, which can be detoxified via oxidation by either isozyme. During the course of these studies, we showed that the level of resistance was highly correlated with the amount of ALDH expressed, and we discovered that the efficacy of ALDH-mediated OAP resistance was also influenced by cel-
lular glutathione pools (Bunting and Townsend, 1998). This suggested that ALDH-mediated and GSH-dependent cellular resistance mechanisms may function cooperatively in protection against reactive aldehydes.

More recently, we have shown that expression of hALDH-3 also confers resistance in V79 cells to the lethal or growth inhibitory effects of a range of lipid aldehydes that can be produced under conditions of cellular redox imbalance. Some of these aldehydes, such as 4-hydroxynonenal (HNE), have been implicated as major contributors to cellular toxicity that occurs as a result of lipid peroxidation (Esterbauer, 1991). The degree of resistance to growth inhibition conferred by hALDH-3 expression was variable but correlated (R, 0.80) with the ability of the aldehyde to serve as substrate for ALDH-3 (Fig. 1). While exposure to HNE caused strong (greater than 95%) inhibition of DNA synthesis in control (empty vector-transfected) cells, this inhibition was prevented in cells expressing hALDH-3, but not hALDH-1 (details to be published elsewhere). The potent 91% depletion of cellular glutathione (GSH) by 10 μM HNE in the control line was also attenuated to only 21% depletion in V79/hALDH-3 transfectants, and similar results were also obtained with several other lipid aldehydes. Thus, as described above for the hALDH-3-mediated resistance to OAP drug toxicity, the ALDH and GSH detoxification pathways also appear to interact cooperatively in protection against lipid aldehyde toxicity. These studies have clearly demonstrated the ability of hALDH-3 to protect at the cellular level against the cytotoxic effects of lipid aldehydes, and the utility of transgenic cell modeling for these purposes.

The Role and Mechanism of Mitochondrial Superoxide Dismutase in the Defense against Oxidative Stress Induced by Xenobiotic and Endobiotic Sources (Kinsley K. Kiningham)

Reactive oxygen species (ROS) can be generated by ionizing radiation, chemotherapeutic drugs, environmental xenobiotics, or by aerobic metabolic reactions including cellular respiration. Manganese superoxide dismutase (MnSOD) is an essential antioxidant enzyme, which catalyzes the conversion of superoxide radical to hydrogen peroxide and molecular oxygen within the mitochondrial matrix. Numerous tumor types (spontaneous, virally-induced, chemically-induced, etc.) have reduced levels of MnSOD compared to their normal counterparts (Oberley and Buettner, 1979; Sun, 1990). A cellular imbalance in the antioxidant status and/or ROS is thought to contribute to a pro-oxidant environment in cancer (Cerutti, 1985). We hypothesize that a defect in the mitochondrial antioxidant system plays a major role in the development of cancer.

Utilizing stable-transfection techniques, we have overexpressed human MnSOD in various “normal” and cancer cell lines, which can be used in vitro or in vivo to further elucidate the role of MnSOD in cancer development. First, the involvement of MnSOD in the process of malignant transformation was examined. In mouse embryonic fibroblasts (10T1/2), overexpression of MnSOD protected cells from radiation, but not 3-methylcholanthrene-induced neoplastic transformation, suggesting a specific suppression by MnSOD for ROS generating agents (St. Clair, 1992). Furthermore, MnSOD enhanced differentiation of the C3H10T1/2 cell line upon treatment with 5-azacytidine, leading us to hypothesize that MnSOD may promote differentiation by modulation of the intracellular redox status, which leads to alterations in expression of differentiating genes (St. Clair, 1994).

Subsequently, we have shown in a mouse fibrosarcoma cell line (FSa-II) that cells transfected with MnSOD cDNA were capable of forming tumors in syngeneic mice but that their metastatic capabilities were diminished (Safford, 1994). In vitro experiments revealed that MnSOD overexpressing fibrosarcoma cells were resistant to radiation-induced cytotoxicity under aerobic conditions; whereas the survival curves for the MnSOD-transfected and control cells were identical when these tumor cells were irradiated in vivo under hypoxic conditions (Urano, 1995). Furthermore, a reduced radiation dose was required to control one-half of the irradiated tumors formed by
the MnSOD-transfected cells, and the number of tumor cells required to make a tumor in half of the mice was markedly increased in the MnSOD-transfectants, suggesting reduced tumorigenicity of these cells (St. Clair, 1997; Urano, 1995). In addition, we have shown that overexpression of MnSOD in the murine fibrosarcoma cell line selectively modulates the activity of specific redox-sensitive transcription factors (Kiningham and St. Clair, 1997). A decrease in DNA-binding activity as well as transcriptional activity of the jun-associated transcription factors, activator protein 1 (AP-1) and cyclic AMP-responsive-element binding protein (CREB), decreased as the level of MnSOD activity increased. Furthermore, expression of an AP-1 target gene, bcl-xL, was decreased in MnSOD-transfected cell lines. This suggests that overexpression of MnSOD may exert its tumor-suppressor activity in part by modulating the activity of protooncogenes.

These cell models allow for great flexibility in studying not only MnSOD mechanisms of tumor suppression, but also the role of ROS and mitochondria in essential cellular processes such as cell death (apoptosis). Currently we are using these transgenic cell models to further our knowledge in this area. We have shown that overexpression of MnSOD protects against cell death mediated by oxidative stress in either mitochondrial or extramitochondrial compartments. Murine fibrosarcoma cells cultured under alkaline conditions were found to exhibit an increase in ROS and specific lipid-peroxidation products, which was accompanied by mitochondrial damage, DNA fragmentation and cell death. Overexpression of MnSOD reduced the levels of intracellular ROS and calcium, maintained the mitochondrial membrane potential and prevented apoptotic cell death (Majima, 1998). Taken together as depicted in Figure 2, our findings suggest that cellular mitochondrial antioxidant status plays a critical role in the destiny of a cell under oxidative stress.

Use of Transfected Eukaryotic Cell Lines to Assess the Function of UDP-Glucuronosyltransferases in Toxicologic Studies (Thomas R. Tephly)

The glucuronidation of xenobiotics and endobiotics is catalyzed by UDP-glucuronosyltransferases UGTs), a multigene family of proteins which resides in the endoplasmic reticulum of cells from a number of organs in mammals. The separation of active UGTs from hepatic microsomal membrane preparations has been extremely difficult due to their lability and sensitivity to most detergents. Although our laboratory has purified and characterized a number of such proteins, which were able to mediate the glucuronidation of endobiotics and xenobiotics, it was difficult to harvest enough protein to conduct extensive substrate specificity studies or to determine the complete primary amino acid sequence of any of these enzymes. Molecular cloning and stable expression in eukaryotic cells has allowed this.

Transient expression of UGT cDNAs in COS cells and stable expression in V79 cells have been used effectively in the laboratories of Dr. Burchell (Dundee) (Burchell, 1994), Dr. Owens (NIH) (Ritter, 1990) and Dr. Mackenzie (Adelaide) (Mackenzie, 1993) to provide for an understanding of the role of various UGTs in the glucuronidation of endobiotics such as bilirubin and steroids and xenobiotics such as phenols and polycyclic hydrocarbon metabolites. Our laboratory has also cloned and expressed a number of UGTs and have used human embryonic kidney (HK) 293 cells to obtain stable expression (King, 1996). In addition, we have used several different separatory systems to study the role of certain UGTs in the glucuronidation of opioids and non-opioid tertiary amines. We have shown that human and rat UGTs–1A1 (considered “bilirubin UGTs”) catalyze the glucuronidation of buprenorphine (an oripavine opioid) as efficiently as bilirubin. In addition, human UGT1A3 and the newly identified UGT1A8 also catalyze the glucuronidation of opioids, although much less efficiently than UG1A1. UGT2B1 in rats and polymorphic UGT2B7 in humans are extremely reactive with both morphinan and oripavine opioids.

Studies using stably expressed UGTB7 have shown that UGTB7 is a very important human UGT. It catalyzes a wide variety of substrates and is the only UGT that catalyzes the formation of both morphine 3- and 6-glucuronide. Morphine 6-glucuronide is a metabolite of morphine that may be as much as 50 times more potent than morphine. Recently we have shown the presence of UGT2B7 in the human brain, where the generation of the 6-glucuronide may be important in the action of morphine as an analgesic.

The formation of quaternary ammonium glucuronides is an important and relatively unique human metabolic reaction. Agents such as antihistamines, antidepressants, and antipsychotics are known to be eliminated primarily as quaternary ammonium glucuronides. UGT1A4 and UGT1A3 expressed in RK293 cells catalyze these reactions; UGTs that are about 93%
similar in their primary amino acid sequences. However, UGT1A4 is many-fold more active than UGT1A3 in carrying out this reaction, and is also very active in catalyzing the N-glucuronidation of carcinogenic primary amines (e.g. 4-aminobiphenyl, benzidine). Surprisingly, this isoform also reacts at very high efficiency with plant sterols such as the sapogenins, tigogenin, hecogenin, and diosgenin, and with progestins leading to the elimination of pregnanediol glucuronide. HK 293 cells have also been useful in studies of transient transfection whereby rapid measurement of mutated forms of UGTs can be assessed for activity. Such studies are useful for the determination of active-site regions and binding of substances with UGTs that have high identity but different substrate specificities, as well as UGTs that have low amino acid sequence similarity but which have similar substrate specificities.

Role of Combined Expression of Glutathione S-Transferases and Multidrug Resistance Protein in Cellular Resistance to Toxic Electrophiles
(Charles S. Morrow)

Our laboratory has been concerned with the functions of phase II xenobiotic-conjugating enzymes and phase III xenobiotic efflux proteins in the detoxification of both antineoplastic drugs and genotoxic carcinogens. We have been particularly interested in the role of the cytosolic isozymes of glutathione S-transferase (GST) in cellular resistance to drug and carcinogen exposure. Many antineoplastic drugs and electrophilic carcinogens are GST substrates. However, increased expression of various GST isozymes by stable transfection into MCF7 cells has generally failed to confer consistent and significant resistance to the anticancer drugs or complete protection from the toxicities of the carcinogens studied (Fields, 1994; Leyland, 1991; Moscow, 1989; Townsend, 1992). Several explanations for this failure have been suggested (Tew, 1994). We have proposed that many of the glutathione-xenobiotic conjugates may themselves be toxic. Moreover, most of these conjugates are more water soluble and therefore less permeable to the cell membrane than the parent xenobiotic toxin. Thus, in the absence of a suitable glutathione-conjugate export system, increased expression of GST may result in only partial detoxification while facilitating the intracellular accumulation of these conjugates. With the identification of the membrane-associated multidrug resistance protein (MRP) as an important glutathione conjugate efflux transporter (Jedlitschky, 1994; Leier, 1994; Muller, 1994), we hypothesized that MRP and GSTs may act in synergy to confer cellular resistance to some anticancer drugs and to some carcinogens and mutagens (Morrow, 1998a). A schematic representation of this proposed interaction is depicted in Figure 3.

We have developed a series of parental and derivative MCF7 breast carcinoma cell lines that express: (1) neither MRP nor GST; (2) MRP or GST singly; or (3) MRP and GST in combination. We have used these cellular tools to explore the relationship of various isozymes of GST and MRP in the detoxification of a model carcinogen, 4-nitroquinoline-1-oxide (NQO) and a nitrogen mustard anticancer drug, chlorambucil.

NQO is a favorable model toxin to test our hypothesis, because it forms a stable conjugate with glutathione in GST-catalyzed reactions. Moreover, with human GST P1-1, the catalytic efficiency and rate enhancement of this conjugation reaction with glutathione is quite good (Aceto, 1990; Stanley and Benson, 1988). Previous studies indicated that while GST P1-1 could afford significant, but incomplete protection of cells from DNA adduct formation by NQO, GST P1-1 conferred no protection from NQO cytotoxicity in MCF7 cells (Fields, 1994). In the present studies we find that increased expression of MRP alone confers modest resistance to both DNA adduct formation and cytotoxicity. However, combined expression of GST P1-1 and MRP confers nearly complete protection from DNA adduct formation and high level (10 to 40-fold) resistance to NQO cytotoxicity in MCF7 cells. Additionally, in GST P1-1-expressing cells, co-expression of MRP profoundly increases the rate of efflux of the glutathione conjugate of NQO (4-glutathionyl-quinoline 1-oxide, QO-SG), while simultaneously reducing its intracellular accumulation. These data indicate that combined expression of MRP and GST P1-1 confer high-level resistance to both the geno- and cytoxicities of NQO. The MRP component of this resistance is associated with MRP-mediated efflux of QO-SG. The resistance may be partially attributable to reduced intracellular levels of QO-SG and other metabolites that may also be substrates of MRP-dependent transport (Morrow, 1999a).

The bifunctional alkylating agent, chlorambucil, can form glutathione conjugates both spontaneously and in GST-catalyzed reactions. Members of the alpha class cytosolic GSTs, including human GST A1-1, are suggested to participate in chlorambucil detoxification by catalysis of monoglutathionyl chlorambucil formation (Ciaccio, 1991; Meyer, 1992). However, the monogluthathionyl derivative retains one of its reactive chloroethyl groups and thus is likely to remain significantly toxic. It has also been proposed that GST A1-1 may provide some protection from chlorambucil toxicity by binding its monogluthathionyl derivative to the enzyme active site with

![FIG. 3. Model of GST/MRP detoxification synergy. This diagram shows the drug or xenobiotic conjugated with GSH after entering the cell, at which point it becomes a substrate for export from the cell by the MRP transmembrane conjugate efflux pump. The efflux is by active transport at the expense of ATP utilization. Details of the model are discussed in the text.](image-url)
Expression of Human Cytochrome P450 and Glutathione Transferases in Bacteria (F. P. Guengerich)

The first successful efforts with heterologous mammalian cytochrome P450 expression involved yeast and later COS-1 monkey kidney cells. Problems in expressing mammalian P450s in bacteria were solved by the use of more efficient vector systems, adjustment of temperature and other growth conditions, and slight modification of 5' sequences. The vector pCW provided useful results (Barnes, 1991), and we have done all subsequent expression studies with this system. Most of the purified enzymes can be obtained in relatively large amounts at low cost and have been utilized in a number of ways. The proteins can be used in biophysical studies that require large amounts of protein (e.g., rapid reaction kinetics, crystallization trials, determination of oxidation-reduction potentials). The recombinant proteins can be used to elicit antibodies, which can be used as reagents for a variety of purposes. The P450s, when combined with NADPH-P450 reductase, show characteristic catalytic activities. It is now possible to prepare such reconstituted systems and store them frozen for future use (Shaw, 1997). A particular advantage of the high yields of P450s from bacterial systems is the use in generating oxidation products for identification through mass and NMR spectrometry.

Bacterial expression of human P450s has also been utilized in the development of systems for genotoxicity analysis. One approach is to express the P450 in the usual *Escherichia coli* system and then examine genotoxicity in an independent bacterial system, e.g., the *umu* test or the Ames test itself. Alternatively, it is possible to express the P450 in a bacterial test system, and this has been done in the case of human P450 1A2 expression in *Salmonella typhimurium* TA1538. Recently, systems have been developed for the simultaneous expression of both P450 and NADPH-P450 reductase in bacteria using a bicistronic version of the basic pCW vector (Fig. 4). Approximately equal amounts of the P450 and the reductase are produced from the plasmid, driven by a tandem tac promoter element upstream of the P450 cDNA insert. Each of the two messages is positioned just downstream of separate Shindelgarno ribosome recognition sequences (SD#1 and SD#2). Approximately equal amounts of the P450 and the reductase are produced during translation of the message.

Another recent use of the bicistronic expression vectors is the development of a new tester system for mutagenicity assays (Josephy, 1998). This approach is similar to the Ames reversion test but uses *E. coli* and lactose selection. The bicistronic vector system was shown to be more active with 2-aminoanthracene than the present system devoid of the expressed enzymes but fortified with the classic Aroclor 1254-induced rat post mitochondrial supernatant (Josephy, 1998).

Work with mammalian glutathione (GSH) transferase expression has been more technically straightforward, because this is a soluble, cytosolic protein. The resulting proteins have been crystallized and also utilized extensively in site-directed mutagenesis work. In our own laboratory, we successfully expressed rat GSH transferase 5–5 in *S. typhimurium* TA1535 and demonstrated that such functional expression led to the

![FIG. 4. Major sequence elements of the bicistronic bacterial expression vector for P450 and its associated reductase. A single bicistronic mRNA is produced from the plasmid, driven by a tandem tac promoter element upstream of the P450 cDNA insert. Each of the two messages is positioned just downstream of separate Shine-Delgarno ribosome recognition sequences (SD#1 and SD#2). Approximately equal amounts of the P450 and the reductase are produced during translation of the message.](image-url)
mutagenicity of dihalomethanes and dihaloethanes (Thier, 1993). The same results were obtained with our orthologous theta-class GSH transferase (T1). These GSH transferases could also be expressed from the vector containing the umu response element in the S. typhimurium-based test system (Oda, 1996). Interestingly, several bifunctional alkylating agents show considerably enhanced bacterial mutagenicity in the presence of theta-class GSH transferases but decreased umu genotoxicity after such expression (Shimada, 1996).

REFERENCES


