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Increased sterigmatocystin-induced mutation frequency in *Saccharomyces cerevisiae* expressing cytochrome P450 CYP2B1

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Currently almost 25% of the world population will develop cancer. The DNA damage associated with exposure to environmental chemicals is likely to play a major role in this process [1]. Consequently, rapid and reliable tests to determine the carcinogenic/mutagenic potential of chemicals are required. The large majority of chemical carcinogens are only mutagenic when a suitable metabolic activation system is present [2]. Almost invariably these activation systems comprise hepatic subcellular fractions containing cytochrome P450 isoenzymes [3–6]. This is a limitation in the various predictive tests that are in use since short-lived or highly reactive species will be formed outside the cell and may, therefore, not interact with the DNA to elicit a detectable response [7]. There is currently a need for new mutation tests with additional properties including endogenous activation [8]. This can be achieved by the expression of P450 isoenzymes in suitable recipient cells using the growing number of cDNAs now available. *Saccharomyces cerevisiae* was identified as a suitable organism for such studies due to its ease of manipulation and the presence of cytochrome P450 reductase which is required for a functional monooxygenase activity. The rat CYP2B1 (P450IIB1, the major phenobarbital (PB*)-inducible cytochrome P450 form) cDNA was expressed using a 2 μM-based yeast expression vector, pMA56 [9] and mutation assays carried out to establish whether the potent mycotoxin STC was activated to mutagenic products by this enzyme.

Materials and Methods

The details regarding the subcloning of the rat CYP2B1 cDNA have been outlined elsewhere [10]. The yeast strain used in these experiments, KY118 (α, trpΔ1, ade2-101°, ura3-52, his3-200, lys2-801^{am}) was chosen because of its extremely low (almost undetectable) P450 levels. The lithium acetate method [11] was used to introduce the expression vector into the yeast strain.

Protein levels were determined using the folin phenol reagent [12] and cytochrome P450 from the reduced carbon monoxide difference spectrum [13]. The O-dealkylation of benzyloxyresorufin was determined as described previously [10, 14].

Western blots were carried out [15–16] using polyclonal antisera to either CYP2B1 or CYP2C6 proteins. Mutation assays were carried out using L-canavanine resistance as a determinant of mutation frequency [10, 17–20].

Results and Discussion

The carcinogenic mycotoxin STC is produced by fungal species of the *Aspergillus, Bipolaris* and *Penicillium* genera [21]. This compound has been shown to induce hepatocellular carcinomas after oral or intraperitoneal administration as well as squamous cell carcinomas after repeated application to the skin [22]. Although the STC-producing fungi are widespread the health hazard to humans remains unclear as the presence of STC in foodstuffs has been found only rarely despite extensive surveillance programmes [23].

STC is of interest as a model compound in studies of the mechanism of carcinogenesis due to its structural homology with aflatoxin B_1. Although the DNA lesion produced by STC has been isolated and characterized [24] little information is available on the metabolic activation of STC. However, it has been demonstrated that mutation rates are increased by the use of PB-induced microsomes. Thus, this study was carried out to determine if CYP2B1 (the major rat PB-inducible cytochrome form) is involved in the activation of this compound.

The levels of CYP2B1 expression following transfection of yeast with pMA56 carrying the CYP2B1 insert are shown in Fig. 1. Densitometric analysis of the Western blots indicated that CYP2B1 represented between 0.1–0.2% of total yeast protein. This protein was not found in the control strain (56 par). When probed with antisera specific to CYP2C6 no cross-reacting bands were observed in either strain indicating that the CYP2B1 antisera is specific for

* Abbreviations: PB, phenobarbital; STC, sterigmatocystin.

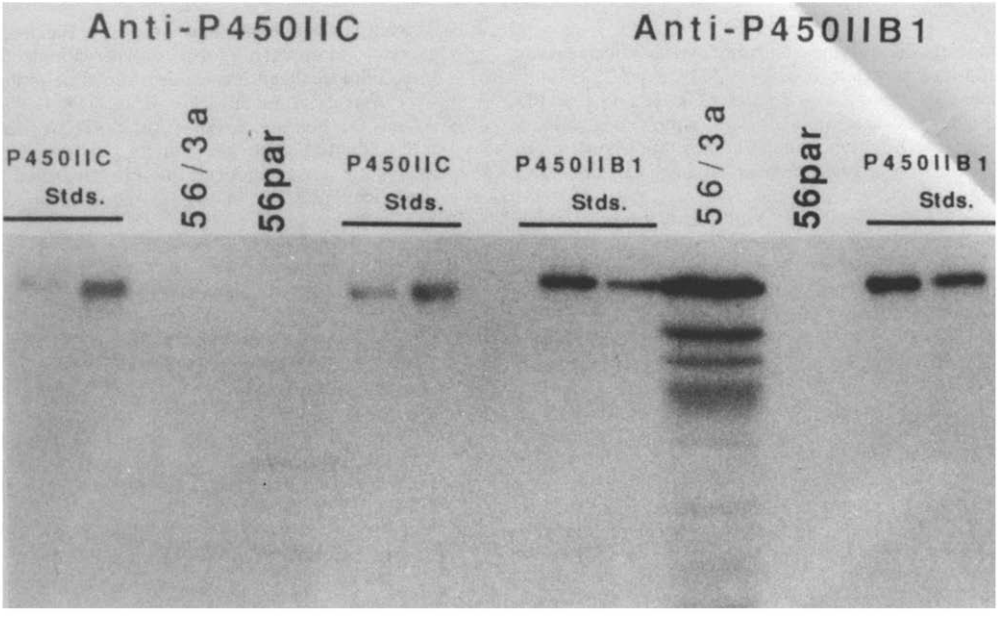

Fig. 1. Expression of CYP2B1 protein in *S. cerevisiae*. Cells were transfected with p56/3a (expressing rat CYP2B1 protein) and p56par (control vector with no cDNA insert) and Western blot analysis was carried out on whole cell extracts (50 μg protein) as detailed in Materials and Methods. Antibodies raised against the rat CYP2B1 (P450IIB1) or CYP2C6 proteins were used to identify the protein expressed in KY118 and to test the specificity of the recognition, respectively.

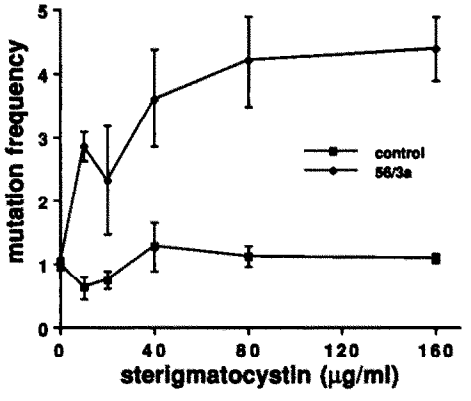

Fig. 2. Mutation frequency in *S. cerevisiae* strains exposed to STC. The mutation frequency of each strain was calculated with increasing concentrations of STC (up to 160 μg/mL) by selection of resistance to L-canavanine [10]. The strain producing CYP2B1 (56/3a) showed an increase of up to 4-fold (at 160 μg/mL). The control strain (56 par) showed no such dose-related increase in mutation frequency. When repeated three times the same results were achieved.

the recombinant protein. Further studies using SDS–PAGE and Western blotting localized the protein to the microsomal fraction of the yeast. Activity measurements using the CYP2B1 substrate benzyloxyresorufin gave a value of 0.16 nmol/min/mg microsomal protein demonstrating that the expressed protein was catalytically active. The control strain exhibited no activity to this substrate.

When exposed to STC (Fig. 2) a dose-dependent increase in mutation frequency up to 4-fold was observed in yeast containing CYP2B1; no increase was observed in the control cells. These results demonstrate that CYP2B1 converts STC to its mutagenic (STC-epoxide) form and is probably the enzyme predominantly responsible for its activation in phenobarbital-induced microsomal fractions. This is interesting in view of the reports that a member of the CYP3A gene family is the major enzyme catalysing this reaction in human liver [25].

These studies also exemplify the potential of yeast systems for establishing the role of mammalian drug-metabolizing enzymes in the metabolism of chemical carcinogens and demonstrate that the expression of cytochrome P450 isoenzymes in *S. cerevisiae* represents an attractive short term test system that does not require the addition of exogenous factors to elicit a response. The sensitivity of this system could be improved significantly by generating yeast strains with new selectable markers and more sensitive endpoints to establish mutation rates. Work to optimize the co-expression of mammalian P450 reductase and P450s is also in progress [26].

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