BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **221,** 129–132 (1996)
ARTICLE NOS. 0539

Transgenic Yeast Expressing Human Cytochrome P450s Can Serve as a Tool in Studies of the Mechanisms of Their Induction by Various Effectors

Irina E. Kovaleva, Eugene Yu. Krynetskii, and Valentin N. Luzikov

A. N. Belozersky Institute of Physico-Chemical Biology, M. V. Lomonosov Moscow State University, Moscow 119899, Russia

Received January 17, 1996

Transgenic *Saccharomyces cerevisiae* yeast strains were constructed which express *CYP2D6* and *CYP3A4* genes under control of an artificial promoter. When added to the growth medium, sparteine, a substrate for CYP2D6, was shown to increase the content of this cytochrome P450 isoform in yeast cells. No such increase was observed when a proteinase-deficient yeast mutant was used as a parent strain. Nifedipine, a substrate for CYP3A4, failed to affect the level of *CYP3A4* expression even in wild yeast cells. These results suggest that expression of *CYP2D6* in human liver can at least partially be controlled post-transcriptionally by its inductors while for *CYP3A4* such a mechanism is hardly possible.

The cytochrome P450 gene superfamily includes over 150 isoforms responsible for metabolism of a variety of substrates. The multiplicity of cytochrome P450s is consistent with the diversity in the mechanisms regulating their intracellular contents. The cytochrome P450s responsible for xenobiotic metabolism are usually induced by their substrates which seem to be effective at different stages of gene expression: (i) gene transcription, (ii) mRNA processing, (iii) mRNA stabilization, (iv) mRNA translation, and (v) enzyme stabilization (see for review [1]). According to current knowledge, the most commonly encountered mechanisms involve gene transcription and mRNA stabilization, though enzyme stabilization is of importance too. Unfortunately, differentiation of all the above mechanisms is still conjectual because of the lack of adequate experimental approaches. Obviously, one possible way to circumvent this problem includes uncoupling gene transcription from other stages of cytochrome P450 expression.

In this paper we advance transgenic yeast strains, expressing exogenous (human) cytochrome P450s under control of foreign promoters, as a model system allowing one to elucidate at least whether an inductor is effective at the post-transcriptional stages (iii) to (v) of cytochrome P450 gene expression.

MATERIALS AND METHODS

Saccharomyces cerevisiae yeast, strains Y1153 (αMAT leu4 ura3 STA1 INH1 Chi^S) and 2805 (αMAT pep4::3 prb1-δ can1 GAL2 his3δ ura3-52), were used in this work. The former was from the collection of the Institute of Genetics and Selection of Industrial Microorganisms (Moscow, Russia) and the latter was a generous gift of Dr. Sang-Ki Rhee (GERI, Korea). The conditions of yeast growth were as in [2, 3]. The yeasts were cultivated with the inductors for the acid phosphatase promoter and the GAL10-CYC1 promoter.

Construction of yeast strains expressing *CYP2D6* gene under control of the acid phosphatase promoter and of the GAL10-CYC1 promoter was described earlier [2, 4]. Expression of *CYP3A4* gene in yeast cells was performed as in [3].

Yeast microsomes were isolated according to [5]. The content of cytochrome P450s in the microsomal fraction was determined from their difference spectra (CO reduced against reduced), the extinction coefficient of cytochrome P450 at 450 nm being taken equal to 91 $mM^{-1}cm^{-1}$ [6].

RESULTS AND DISCUSSION

It has earlier been shown [2, 3, 4, 7] that human liver CYP2D6 and CYP3A4 synthesized in yeast are located in the endoplasmic reticulum membranes. The foreign proteins showed the immuno-

0006-291X/96 $18.00

reactivity and spectral properties of the intact enzymes. They were found to be catalytically active towards their specific substrates both in intact cells and in isolated microsomal fraction.

Expression CYP2D6 in Wild Yeast Cells and in a Proteinase-Deficient Mutant

cDNA for CYP2D6 inserted into a pYeDP (1/8–2) plasmid was expressed under control of the GAL10-CYC1 promoter in the yeast Y1153 strain and in the proteinase-deficient 2085 mutant. The content of CYP2D6 in the microsomal fraction isolated from yeast cells at the late exponential phase was 200–230 and 60–100 nmoles per mg of microsomal protein for the 2805 and Y1153 strains, respectively. These results are likely to suggest that cytochrome P450IID6 suffers degradation under the action of vacuolar proteinases in the Y1153 yeast.

Effect of Sparteine on the Level of CYP2D6 Expression in Yeast Cells

Figure 1 shows the difference spectra of yeast cells (the 20B12 strain) expressing *CYP2D6* in the pYP plasmid [2] under control of the acid PH05 promoter. The yeast culture was grown either with or without sparteine (100 μg/ml), a substrate for CYP2D6. The spectra had peaks at 450 nm, which is inherent in the native cytochrome P450s. It is evident from the values of the peaks that the content of CYP2D6 was ca. 20% higher for the culture grown in the presence of spectrine.

Similar results were obtained when the content of CYP2D6 was measured in the microsomal fractions of yeast cells expressing *CYP2D6* in the pYeDP plasmid. In those experiments the yeast culture was supplemented with sparteine (1 mM) after a 12-h growth and incubated further for 20 h. In the control experiments no sparteine was added. The microsomal fractions were isolated afterwards and CYP2D6 specific content in these fractions was measured from the difference spectra. For the Y1153 strain this value was again ca. 20% higher when the growth medium contained sparteine (Fig. 2). No such effect of sparteine was observed for the 2085 proteinase-deficient mutant.

Expression of CYP3A4 in Yeast Cells with or without Nifedipine

The *CYP3A4* gene inserted into the pYeDP plasmid was expressed in the Y1153 and 2085 yeast strains. The specific content of CYP3A4 in the microsomal fraction was higher by a factor of 3 for the proteinase-deficient 2805 strain thus suggesting the involvement of vacuolar proteinases in control over the steady-state level of this foreign protein in yeast cells.

As in the previous experiments, the Y1153 strain was cultured either with or without of a substrate for CYP3A4, namely nifedipine (200 μm). The microsomal fractions were isolated and the specific CYP3A4 content was measured from the difference spectra of these fractions. The values obtained proved to be almost the same in both cases thus testifying that nifedipine has no effect on the level of CYP3A4 expression in yeast cells.

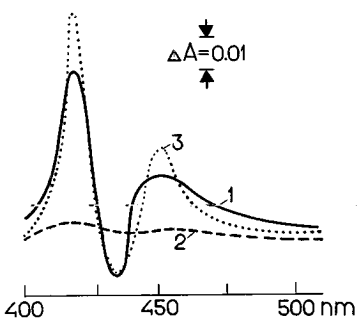

FIG. 1. Reduced CO difference spectra of transformed yeast cells expressing *CYP2D6.* 1, cells grown with sparteine; 2, cells grown without sparteine; 3, base line.

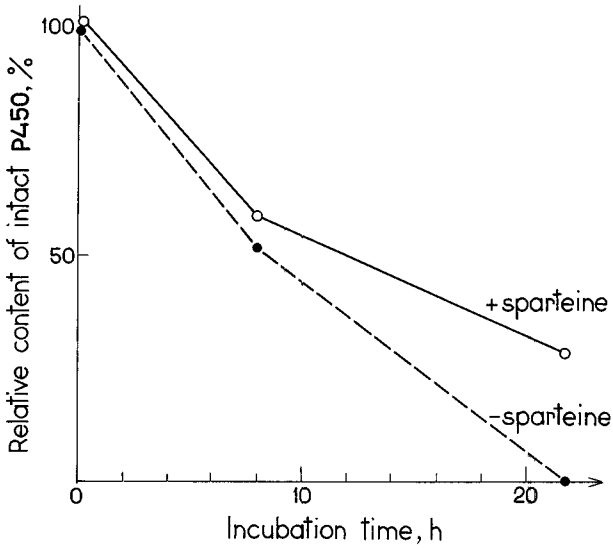

FIG. 2. Reduced CO difference spectra of the microsomal fraction from transformed yeast cells expressing *CYP2D6*. Solid line, cells grown with sparteine; dotted line, cells, grown without sparteine. In both cases protein concentration was 1 mg per ml.

Protective Effect of Sparteine on the Degradation of CYP2D6 in Isolated Yeast Microsomes

The microsomal fraction was isolated from yeast cells (strain 2805) expressing the *CYP2D6* gene. The microsomes were washed with a potassium buffer (pH 7.4) and stored in liquid nitrogen. The aliquotes of the microsomal suspension (1 mg of protein per ml) were incubated in the same buffer at 37°C for different intervals and the difference spectra were recorded thereafter. The peak at 450 nm decreased whereas the peak at 420 nm increased during such incubation thus pointing to the denaturation of CYP2D6. Addition of sparteine to the incubation medium hindered the denaturation to some extent. The protective effect averaged 20–30 % after a 24-h incubation which was long enough for almost complete denaturation in the absence of the substrate (Fig. 3). No stabilization of CYP3A4 was observed when nifedipine was added to the microsomes isolated from transgenic yeast and incubated under the same conditions.

These data show a potential capacity of sparteine to stabilize the native CYP2D6 structure which in turn might be indicative of a post-translational mechanism of sparteine stimulatory effect on CYP2D6 expression.

By and large, this work demonstrates that yeasts expressing cDNAs for exogenous cytochrome P450s can indeed be used as model systems for selective studies of post-transcriptional effects of various cytochrome P450 effectors. At this stage we have confined ourselves to establishing the fact that this approach is applicable to CYP2D6. It has been shown that the content of this particular isoenzyme is to some extent regulated post-transcriptionally [8]. As to correct recognizing of individual post-transcriptional mechanisms (e. g., mRNA stabilization, stimulation of translation and enzyme stabilization) additional experiments in the framework of general approach are required. Further elaboration of the approach calls for a new object (other cytochrome P450s) and for a new effector which would show more pronounced post-transcriptional effects.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Fundamental Research (grant No. 04-6841 to V.N.L.) and jointly by the International Science Foundation with Russian Government (grant No. JAZ100 to V.N.L.).

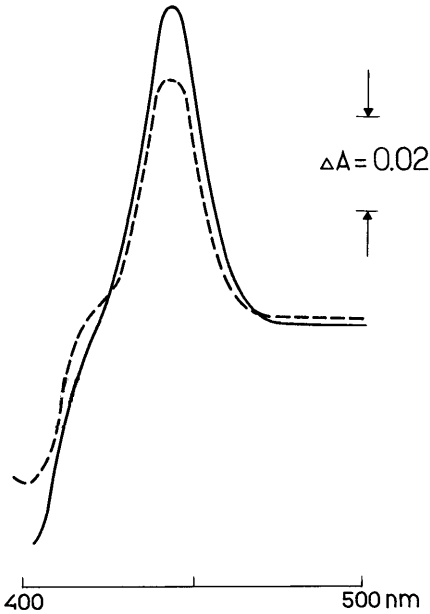

FIG. 3. Effect of sparteine on thermal denaturation of CYP2D6 in the microsomal fraction isolated from transformed yeast.

REFERENCES

1. Porter, T. D., and Coon, M. J. (1991) *J. Biol. Chem.* **266,** 13469–13472.
2. Krynetskii, E. Yu., Kovaleva, I. E., El'darov, M. A., and Luzikov, V. N. (1993) *Mol. Biol.* [Engl. transl.] **27**(2)**,** 677–680.
3. Krynetskii, E. Yu., Kovaleva, I. E., and Luzikov, V. N. (1994) *Mol. Biol.* [Engl. transl.] **28,** 63–66.
4. Krynetsky, E. Yu., Drutsa, V. L., Kovaleva, I. E., Luzikov, V. N., and Uvarov, V. Yu. (1993) *FEBS Letters* **336,** 87–89.
5. Pompon, D. (1988) *Eur. J. Biochem.* **117,** 285–293.
6. Omura, T., and Sato, R. (1964) *J. Biol. Chem.* **239,** 2370–2378.
7. Krynetskii, E. Yu., Kovaleva, I. E., and Luzikov, V. N. (1994) *Biochemistry (Moscow)* **59,** 203–206.
8. Treluyer, J.-M., Jacoz-Aigrain, E., Alvarez, F., and Cresteil, T. (1991) *Eur. J. Biochem.* **202,** 583–588.