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Functional expression and activity screening of all human cytochrome P450 enzymes in fission yeast

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Here, a complete set of recombinant fission yeast strains that coexpress each of the 57 human cytochrome P450 (CYP) enzymes together with their natural human electron transfer partner(s) was cloned. This strain collection was tested with two luminogenic probe substrates, and 31 human CYPs (including the orphan enzymes CYP2A7, CYP4A22 and CYP20A1) were found to metabolize at least one of these. Since other substrates are known for the remaining enzymes, all human CYPs are now shown to be active. Interestingly, CYP5A1 was found for the first time to work on a substrate other than prostaglandin H_2, and, moreover, to catalyze an aliphatic hydroxylation reaction that consumes molecular oxygen. Also, the ability of CYP11A1 to catalyze an aryl hydroxylation is another unexpected result.

Keywords: cytochrome P450; fission yeast; functional expression; gene library; *Homo sapiens*; pharmacology

Cytochrome P450 enzymes (CYPs or P450s) are a large family of hemoproteins present in all biological kingdoms, but not in all organisms. They can act on a huge variety of compounds and although they are most famous for catalyzing monooxygenase reactions, many members of the family also show additional activities [1]. While some anaerobes and microaerophiles lack CYPs, they are found in aerobic organisms in numbers ranging from two in the fission yeast *Schizosaccharomyces pombe* to several hundred in many plants [2]. In humans, there are 57 CYP genes predicted to code for functional enzymes. However, for three of these (the so-called orphans CYP2A7, CYP4A22, and CYP20A1) no substrates have been described so far, and for a range of others only very few substrates are known [3]. On the other hand, many human CYPs are well characterized, and a number of them were demonstrated to be responsible for the majority of Phase I reactions in human drug metabolism; among their substrates are not only active pharmaceutical ingredients (APIs) but also food-derived chemicals, environmental toxins, and carcinogens. In addition, human CYPs are involved in the biosynthesis, metabolism, and degradation of many endogenous compounds, such as steroids and fatty acids [3]. CYP5A1 (thromboxane A2 synthase, TBXAS1) and CYP8A1 (prostacyclin synthase, PTGIS) are special in that they catalyse isomerase reactions of prostaglandin H_2 that do not require molecular oxygen. Since a number of CYPs show a dramatic influence on drug clearance, their activity is an important consideration in drug development and the determination of the CYP reactivity profile for a small molecule is important for predicting its metabolic fate in both test animals and patients. In addition, several CYPs are drug targets, and some CYP inhibitors are already on the market, while others are being tested in clinical trials. Typically, CYPs involved in drug metabolism tend to be more promiscuous than those acting on endogenous compounds, but the

Abbreviations
Adx, adrenodoxin; APIs, active pharmaceutical ingredients; EMM, Edinburgh Minimal Medium; LDR, luciferin detection reagent.

separation between those two groups is not as stringent as was once thought [4].

All human CYPs are membrane-bound proteins that are typically located either on the cytoplasmic side of the endoplasmic reticulum (50 CYPs) or on the matrix side of the inner mitochondrial membrane (seven); however, they are also found in the nuclear envelope and on the plasma membrane. Strategies for working with such enzymes entail fundamental problems: one option is to subject the enzymes to significant mutagenesis in order to make them soluble; then, bacterial expression will allow for the enzyme to be purified and, subsequently, enable the determination of kinetic parameters and crystal structures. However, the influence of the mutagenesis on an enzyme's catalytic properties remains unknown. This appears to be especially true if one looks at very hydrophobic substrates, which is a common case with CYPs. The second option is to recombinantly express the wild type sequence of the enzyme (including its membrane anchor) and work with the membranes that contain it or even with whole cells. In this case artefacts caused by mutagenesis are not to be feared, but the options for an investigation of kinetic parameters are more limited. Both approaches have been used extensively, and not surprisingly, results may be inconsistent, as exemplified by the heterologous expression of CYP2S1 in *Saccharomyces cerevisiae* and *Escherichia coli* [5–7]. Still, our current understanding of the enzymes stems from a combination of the results obtained by both approaches [8]. In the past, we have successfully used fission yeast as an expression host for a number of human CYPs, as demonstrated by the first functional expression of human CYP4Z1 [9]. Therefore, it was the aim of the present study to create a complete library of recombinant fission yeast strains that express unmodified sequences of all 57 human CYPs together with their electron transfer partners, and to use this strain collection for the characterization of these enzymes.

Materials and methods

Materials

Luciferin-H, Luciferin-ME, and the NADPH regeneration system was from Promega (Madison, WI, USA). Triton-X100 was from Leagene (Beijing, China); glycerol was from Dingguo (Tianjin, China); DMSO was from Sigma (St. Louis, MO, USA). Tris-HCl was from AKZ-Biotech (Tianjin, China); potassium chloride, ammonium bicarbonate, potassium dihydrogen phosphate and potassium hydrogen phosphate were from Jiangtian Chemical (Tianjin, China); 1 X TBS buffer, 1 X PBS buffer were obtained from Corning (Manassas, VA, USA); ethyl acetate of analytical grade was from Yuanli Chemical (Tianjin, China); and white 96-well microtiter plates were from Nunc (Thermofisher scientific, Lagenselbold, Germany). All other chemicals and reagents used were of the highest grade available.

Microorganism and culture conditions

General DNA manipulation methods were performed using standard techniques [10] and the preparation of media and basic manipulation methods of *S. pombe* were carried out as described [11]. Briefly, fission yeast strains were cultivated at 30 °C in Edinburgh Minimal Medium (EMM) with supplements of 0.1 $g{\cdot}L^{-1}$ final concentration as required. Cell densities were determined using a Neubauer improved counting chamber. In all strains expression of the human CPR (NADPH-cytochrome P450 oxidoreductase) and CYPs is regulated by the thiamine-repressible nmt1 promoter of fission yeast [12]. Liquid cultures were kept shaking at 150 rpm. Solid medium was prepared by adding 2% agar (w/v) to the media prior to heat sterilization. Thiamine was used at a concentration of 5 μM throughout. For the cloning and propagation of yeast plasmids, the DH5α and TOP10 *E. coli* cells were cultured on the LB medium at 37 °C.

Construction of cytochrome P450 gene library

Fission yeast strain NCYC2036 with genotype *h- ura4-D.18* was used as a parental strain for the human CYP gene library construction. The integrative vector pCAD1 [12] is used for the expression of microsomal redox partner human NADPH-cytochrome P450 oxidoreductase (CPR) and mitochondrial redox partners adrenodoxin (Adx) and NADPH-adrenodoxin reductase (AdR); while the autosomally replicative vector pREP1 [13] was used for expression of human CYPs (Table 1). The cDNA sequences for human CPR, CYP2B6, 2C9, 2C19, 2D6, 2E1, 2J2, 2R1, 3A4, 3A5, 3A7, 3A43, 4Z1 were synthesized by Entelechon GmbH (Regensburg, Germany), and the remaining 45 CYPs, Adx, AdR were synthesized by General Biosystem (Anhui, China). All the cDNAs coding for the respective wild-type enzymes were ligated and cloned into NdeI/BamHI restriction sites in their respective vectors, and the correctness of all expression constructs were confirmed by automated sequencing. Transformation of fission yeast was carried out by lithium acetate method using freshly prepared or cryopreserved competent cells [12]. Strain CAD62 [12] was used as a background to transform all the 50 microsomal pREP1-CYP plasmids to yield strains that coexpress both CPR and one of the CYP isoforms. For the coexpression of seven mitochondrial CYPs, 2A peptide mediated linker system was used for the bicistronic expression of Adx and ADR genes in a single vector pCAD1. For this purpose, the AdR and Adx were interlinked via

Table 1. List of fission yeast strains used in this study.

Strain	Expressed protein(s)	Genotype	References
NCYC2036	None	*h- ura4-D.18*	[23]
CAD62	CPR	*h- ura4-D.18 leu1::pCAD1-CPR*	[12]
RAJ4	AdR, Adx	*h- ura4-D.18 leu1::pCAD1-AdR+Adx*	This study
RAJ27	CPR, CYP1A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP1A1*	This study
RAJ28	CPR, CYP1A2	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP1A2*	This study
RAJ29	CPR, CYP1B1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP1B1*	This study
RAJ122	CPR, CYP2A6	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2A6*	This study
RAJ127	CPR, CYP2A7	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2A7*	This study
RAJ132	CPR, CYP2A13	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2A13*	This study
CAD65	CPR, CYP2B6	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2B6*	[12]
RAJ142	CPR, CYP2C8	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2C8*	This study
CAD68	CPR, CYP2C9	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2C9*	[12]
RAJ147	CPR, CYP2C18	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2C18*	This study
CAD66	CPR, CYP2C19	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2C19*	[12]
CAD64	CPR, CYP2D6	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2D6*	[12]
RAJ157	CPR, CYP2E1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2E1*	This study
RAJ162	CPR, CYP2F1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2F1*	This study
PBT78	CPR, CYP2J2	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2J2*	This study
RAJ97	CPR, CYP2R1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2R1*	This study
RAJ172	CPR, CYP2S1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2S1*	This study
RAJ177	CPR, CYP2U1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2U1*	This study
RAJ182	CPR, CYP2W1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP2W1*	This study
CAD67	CPR, CYP3A4	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP3A4*	[12]
INA20	CPR, CYP3A5	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP3A5*	[24]
INA2	CPR, CYP3A7	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP3A7*	[25]
RAJ112	CPR, CYP3A43	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP3A43*	This study
RAJ187	CPR, CYP4A11	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4A11*	This study
RAJ192	CPR, CYP4A22	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4A22*	[22]
RAJ197	CPR, CYP4B1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4B1*	This study
RAJ202	CPR, CYP4F2	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4F2*	This study
RAJ207	CPR, CYP4F3	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4F3*	This study
RAJ212	CPR, CYP4F8	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4F8*	This study
RAJ217	CPR, CYP4F11	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4F11*	This study
RAJ222	CPR, CYP4F12	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4F12*	This study
RAJ227	CPR, CYP4F22	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4F22*	This study
RAJ232	CPR, CYP4V2	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4V2*	This study
RAJ237	CPR, CYP4X1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4X1*	This study
AZ3	CPR, CYP4Z1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4Z1*	[9]
RAJ242	CPR, CYP5A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP5A1*	This study
RAJ30	CPR, CYP7A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP7A1*	This study
RAJ31	CPR, CYP7B1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP7B1*	This study
RAJ32	CPR, CYP8A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP8A1*	This study
RAJ33	CPR, CYP8B1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP8B1*	[26]
RAJ78	AdR, Adx, CYP11A1	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP11A1*	This study
RAJ251	AdR, Adx, CYP11B1	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP11B1*	This study
RAJ256	AdR, Adx, CYP11B2	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP11B2*	This study
RAJ35	CPR, CYP17A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP17A1*	This study
RAJ36	CPR, CYP19A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP19A1*	This study
RAJ257	CPR, CYP20A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP20A1*	This study
RAJ37	CPR, CYP21A2	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP21A2*	This study
RAJ266	AdR, Adx, CYP24A1	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP24A1*	This study
RAJ267	CPR, CYP26A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP26A1*	This study
RAJ272	CPR, CYP26B1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP26B1*	This study
RAJ277	CPR, CYP26C1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP26C1*	This study
RAJ286	AdR, Adx, CYP27A1	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP27A1*	This study

Table 1. (Continued).

Strain	Expressed protein(s)	Genotype	References
RAJ291	AdR, Adx, CYP27B1	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP27B1*	This study
RAJ296	AdR, Adx, CYP27C1	*h- ura4-D.18 leu1:: pCAD1-AdR+Adx/pREP1-CYP27C1*	This study
RAJ297	CPR, CYP39A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP39A1*	This study
RAJ302	CPR, CYP46A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP4611*	This study
RAJ307	CPR, CYP51A1	*h- ura4-D.18 leu1:: pCAD1-CPR/pREP1-CYP51A1*	This study

T2A peptide sequence, and the stop codon was added only in the second cistron to generate a single RNA that expresses two distinct proteins. In addition, the Gly-Ser-Gly (GSG) linker is added between the AdR and 2A peptide sequence to improve the cleavage efficiency. The synthetic AdR+2A+Adx cDNA was cloned into pCAD1 and the resulting pCAD1-AdR+Adx plasmid was transformed into the NCYC2036 strain to yield strain RAJ4. To confirm the correct chromosomal integration of pCAD1-AdR+Adx construct into the *leu1* locus, the transformats were subjected to replica plating on EMM lacking leucine. The resultant strain RAJ4 was then used as a background strain to transform 7 mithochondrial pREP1-CYP plasmids to yield strains that coexpress both AdR+Adx and one of the CYP isoforms (Table 1). All the transformants were selected and confirmed based on auxotrophic selection (NCYC2036 - EMM+URA, strains harboring pCAD1 plasmid – EMM + LEU + THI and strains harboring both pCAD1 and pREP1 plasmids – EMM + THI) by replica-plating for three generations. For the cryopreservation of fission yeast based human CYP library, the permanent cultures were prepared in double concentrated yeast extract with additives (2X YEA; 6 g glucose, 1 g yeast extract, 20 mgof each lysine, histidine, adenine, uracil and leucine with 25% glycerol mixture) and stored at −80 °C.

Biotransformations

This was performed as described previously with slight modifications [14]. Briefly, fission yeast cells of the respective strains were cultured on EMM plates with 5 μM thiamine and supplements for 3 days at 30 °C. A single yeast colony was then transferred to a 10 mL liquid culture of EMM without thiamine and cultured at 30 °C, 230 rpm for 36 hours. As the recombinant expression is controlled by the strong endogenous *nmt1* promoter, all subsequent cultures were cultured in absence of thiamine to maintain the *nmt1* promoter in an active state. For each assay 5×10^7 cells (stationary growth phase) were transferred to 1.5 mL Eppendorf tubes, pelleted and incubated in 1 mL of 0.3% Triton-X100 in Tris-KCl buffer (200 mM KCl, 100 mM Tris-HCl pH 7.8) and incubated at room temperature for 60 minutes at 150 rpm for permeabilization. After three washes with 1 mL NH_4HCO_3 buffer (50 mM, pH 7.8) cells were resuspended in phosphate buffer (pH 7.4) and then the substrate and the NADPH regeneration system were added. The samples were incubated at 37 °C and 1000 rpm for 3 hours. For the luminescence measurements, the reaction mixture was centrifuged at 16 000 ***g*** for 1 min and the supernatant was transferred to white 96-well microtiter plates. To investigate the functional activity of human PAN CYPome, all 57 fission yeast strains were tested by enzyme-bag based biotransformation with the substrates Luciferin-H and Luciferin-ME at a final concentration of 100 μM. In addition, the fission yeast strains CAD62 and RAJ4 harboring only the redox donors without any CYP were used as a control.

Bioluminescence detection

This was performed as described previously with slight modifications [14]. Briefly, a concentrated CYP reaction mixture (containing fourfold concentrated substrate and potassium phosphate buffer) was added to the cell pellets after permeabilization and washing process. For inhibition assays, the CYP reaction mixture with the test inhibitors at indicated concentrations were pre-incubated at 37 °C for 10 minutes. CYP reactions were started by adding the twofold concentrated NADPH regeneration system. Samples were incubated for 3 hours at 37 °C and 1000 rpm. After centrifugation at 16 000 ***g*** for 1 min the supernatants were transferred to the white microtiter plates and an equal amount of reconstituted luciferin detection reagent (LDR) was added to each well. Plates were then incubated at room temperature for 20 minutes and the luminescence was recorded on a Magellan infinite 200Pro microplate reader (Tecan; Männedorf, Switzerland). For the Luciferin substrates screening the specific reaction conditions and substrates concentrations were as given in the instructions of the manufacturer (Promega). In all cases reaction parameters (reaction times and enzyme concentrations) were within the linear range. For inhibitor assays ketoconazole, 1-benzylimidazole, letrozole or HET0016 were added to a final concentration of 10 μM. All measurements were done at least three times in triplicates.

Statistical analysis

All data are presented as mean ± SD. Statistical significance was determined using a two-tailed t-test. Differences were considered significant if $P < 0.05$. Statistical analysis was done using GRAPHPAD PRISM 5. 01 (GraphPad Software, Inc., La Jolla, CA, USA).

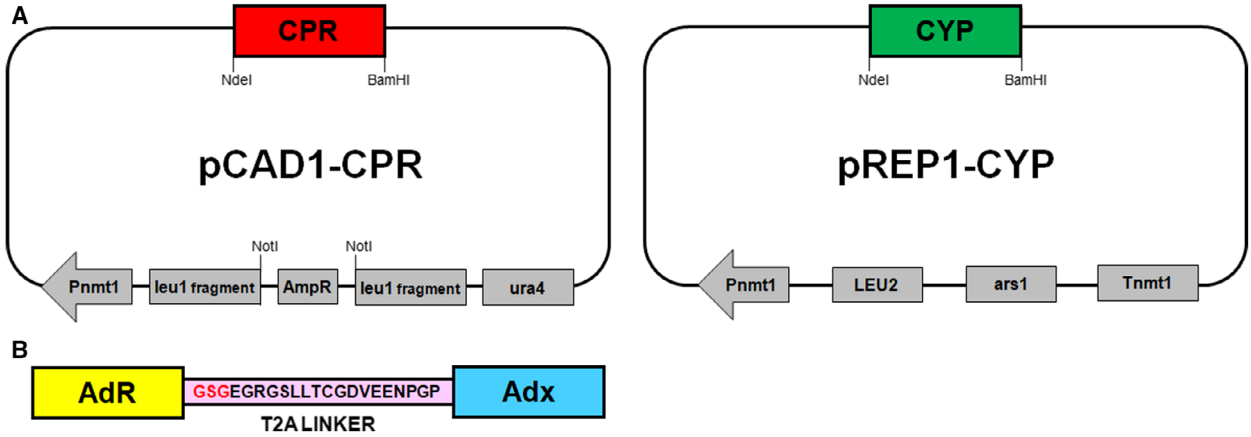

Fig. 1. (A) Plasmids for the co-expression of human CPR and CYP genes in fission yeast. (B) Strategy for the bicistronic expression of human AdR and Adx genes using a T2A linker.

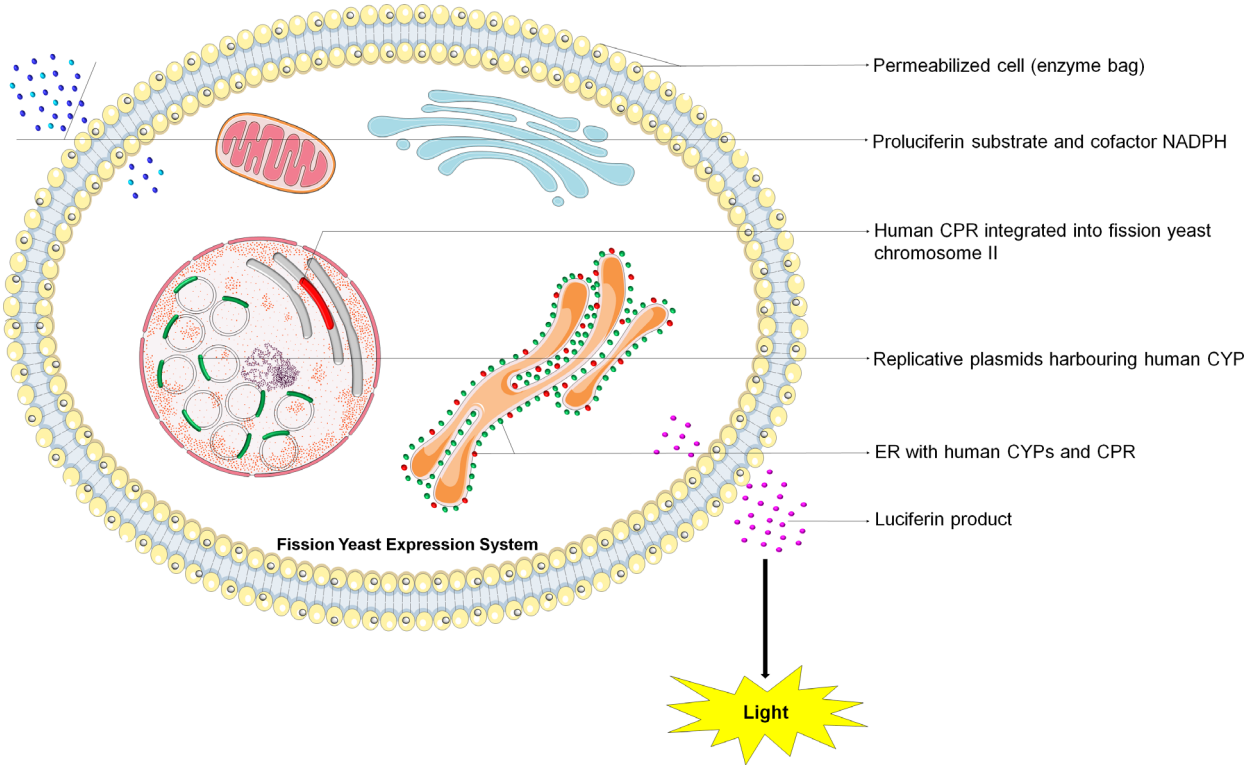

Fig. 2. Schematic representation of the recombinant fission yeast expression system and enzyme bag-catalyzed biotransformation of luminogenic substrates by human CYPs.

Results and Discussion

Construction of complete human CYPome

For the construction of a human pan-CYP library, synthetic DNAs coding for human CYPs were cloned into the replicating plasmid pREP1 (Fig. 1A); the resulting plasmids coding for 41 microsomal CYPs were then individually transformed into fission yeast strain CAD62 [12] to yield 41 new strains that coexpress one of these CYPs with human cytochrome P450 reductase (CPR or POR); nine such strains had already been described before (all strains are listed in Table 1). In order to have a mitochondrial electron

transfer system for the mitochondrial CYPs, a bicistronic expression construct was designed that encompasses synthetic DNAs coding for NADPH-dependent flavin reductase (adrenodoxin reductase, AdR), and the [2Fe-2S] ferredoxin (adrenodoxin, Adx), respectively. In this construct, the two DNAs are separated by a short sequence encoding the T2A peptide sequence (EGRGSLLTCGDVEENPGP) from the *Thosea asigna* virus to ensure efficient self-cleavage and sustained expression of the two proteins [15,16] (Fig. 1B). The AdR gene was positioned first, followed by the T2A linker and the Adx sequence (AdR-T2A-Adx); also, a Gly-Ser-Gly (GSG) linker was added between the AdR and the T2A peptide to add more flexibility and to enhance cleavage efficiency [17]. This construct was inserted into the fission yeast integrative plasmid pCAD1 [18] to yield pCAD1-AdR+Adx, which in turn was integrated into the *leu1* locus of fission yeast to generate the strain RAJ4. Next, the pREP1 plasmids harboring DNAs that code for the seven human mitochondrial CYPs (CYP11A1, CYP11B1, CYP11B2, CYP24A1, CYP27A1, CYP27B1, and CYP27C1) were individually transformed into RAJ4 to yield seven new strains that coexpress these CYPs with AdR and Adx. Thus, a complete set of strains that coexpress each one of the 57 human CYPs together with its natural human electron transfer partner(s) was created (Fig. 2).

Functional screening of the human CYPome

This new strain library was used in a functional screen to monitor the activity of all human CYPs towards the two luminogenic probe substrates Luciferin-H and Luciferin-ME (structures in Table S1); these proluciferins can be converted by CYPs to luciferin, which in turn produces light upon oxidation by luciferase [19]. Out of a number of commercially available proluciferins, the two compounds used in this study were selected because of two considerations: (a) It was desirable to compare a rather nonselective probe substrate (Luciferin-ME) with another one that is much

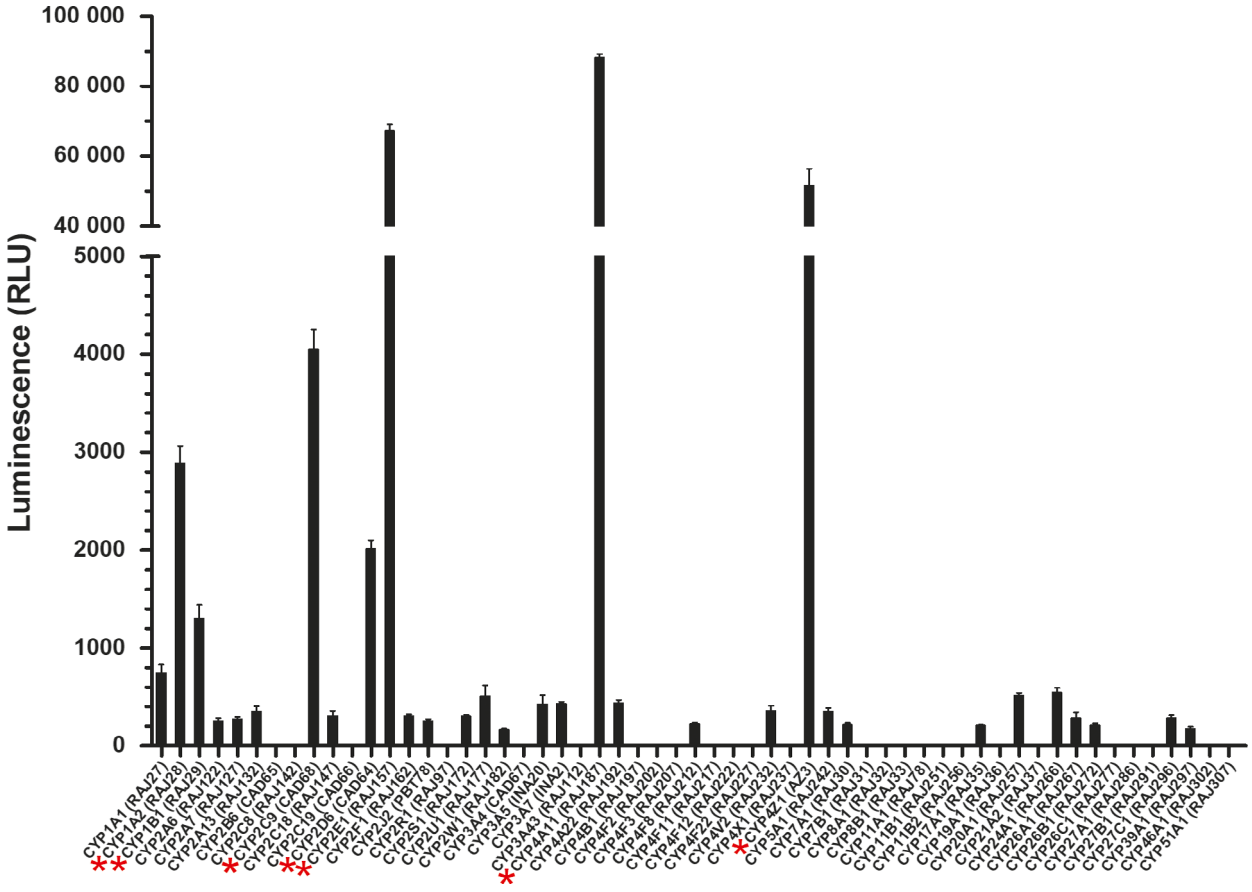

Fig. 3. Screening of human CYPs for activity towards Luciferin-ME**.** Activity assays were performed by enzyme-bag based biotransformation using recombinant fission yeast strains coexpressing both a human CYP and its redox partner(s) with Luciferin-ME as substrate. Activities are shown in relative light units (RLU). The most reactive CYPs are marked by red asterisks.

more specific for a single CYP (Luciferin-H as a selective CYP2C9 substrate). (b) It is known that CYP-dependent aliphatic and aryl hydroxylations do not follow the same reaction mechanism [20]. Thus, it was interesting to look at one aliphatic (using Luciferin-ME) and at one aryl (Luciferin-H) hydroxylation reaction, respectively. The biotransformation method employed makes use of permeabilized cells (enzyme bags) and has been established in our lab before [14] (Fig. 2). It was found that 31 human CYPs metabolized at least one of the two substrates, while the other 26 did not (or the activities observed were not statistically significantly different from the controls). More specifically, 20 CYPs hydroxylated Luciferin-ME only, two converted Luciferin-H only, and nine CYPs metabolized both substrates (Figs 3 and 4; Table S2).

Several conclusions can be drawn from these results: First of all, all three orphan CYPs (CYP2A7, CYP4A22, and CYP20A1) for which no substrate was known so far metabolized both probe substrates. CYP2A7 shows very high homology with CYP2A6 and would therefore likely display similar enzymatic properties; the activity data for both enzymes also point in this direction (Table S2). CYP4A22 shares an overall sequence identity of 96% with CYP4A11, which is highly expressed in the liver and kidney and metabolizes arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE), an important regulator of the vascular system [21]. The functional activity and substrate specificity of CYP4A22 are described in another publication in this issue [22]. CYP20A1 is intriguing because this gene is highly conserved from sea anemone and sponge to the human and CYP20 constitutes one of the original eleven P450 clans [2]; nevertheless, no activity of any CYP20 enzyme from any organism was reported so far. Also, CYP5A1 is shown here for the first time to work on a substrate other than prostaglandin H_2, and, moreover, to catalyze an aliphatic hydroxylation reaction that consumes molecular oxygen. In addition, the ability of

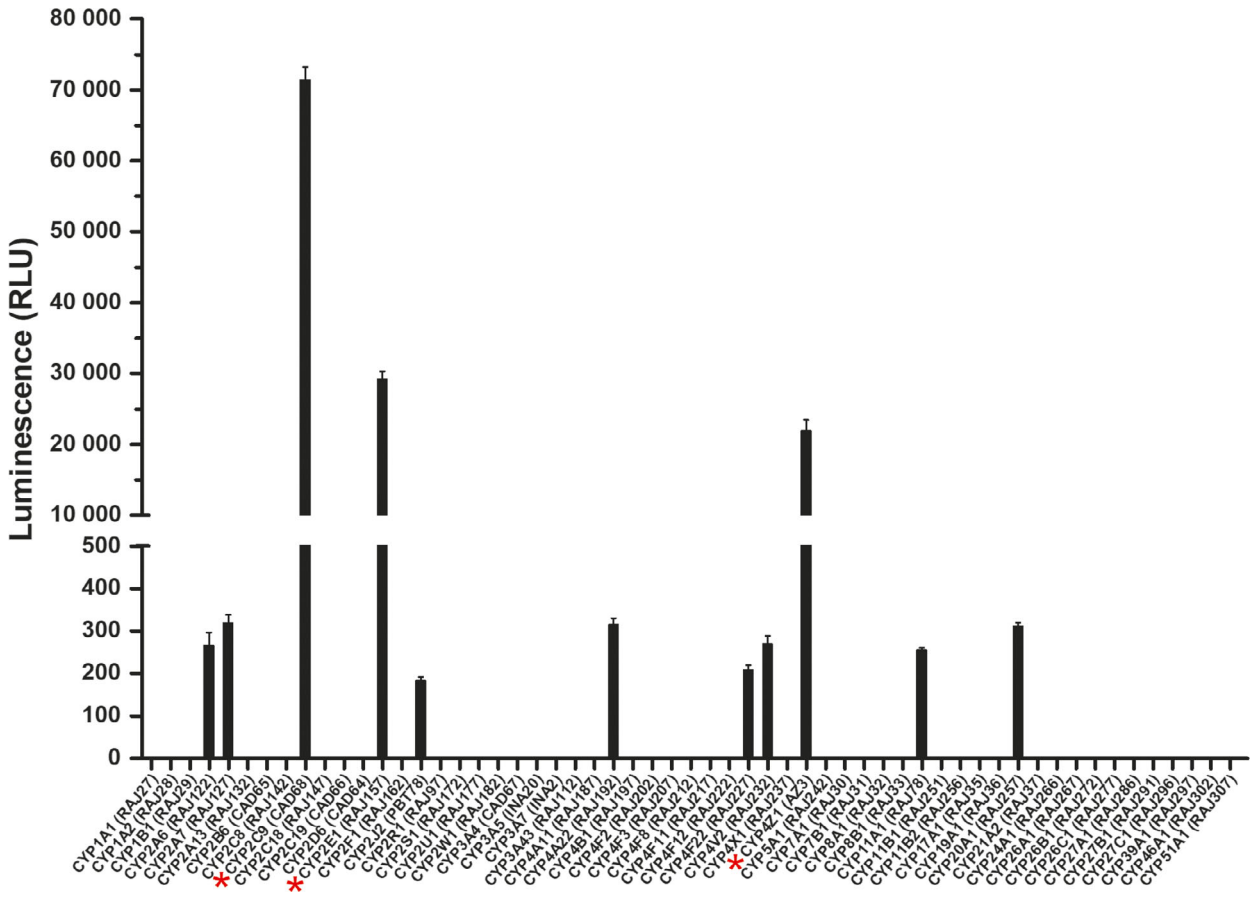

Fig. 4. Screening of human CYPs for activity towards Luciferin-H**.** Activity assays were performed by enzyme-bag based biotransformation using recombinant fission yeast strains coexpressing both a human CYP and its redox partner(s) with Luciferin-H as substrate. Activities are shown in relative light units (RLU). The most reactive CYPs are marked by red asterisks.

CYP11A1 (also known as P450scc, the side-chain cleaving enzyme that converts cholesterol into pregnenolone) to catalyze aryl hydroxylation of Luciferin-H represents another surprising result. Thus, there is now experimental evidence that all 57 human CYP genes code for active enzymes and for 56 of these enzymes at least one substrate for an aliphatic hydroxylation reaction is now known; the one exception is CYP8A1. By contrast, only a few human CYPs (including CYP2C9 and several members of the CYP1 and CYP3 families) have been described in the past to catalyze aryl hydroxylations; although our results presented in this study significantly expand that list (with CYP11A1, CYP20A1, and several members each of the CYP2 and CYP4 families), it still encompasses only a minor subset of all human CYPs. While we expect that more human CYPs will be shown in the future to catalyze this reaction type if dedicated efforts are made in this direction, it is still tempting to speculate that there is a mechanistic detail that prevents some human CYPs from performing aryl hydroxylations in principle. Clarifying this point is not only of interest for basic research but will also help in making better predictions for possible metabolism of drug candidates by CYPs.

Conclusion

We have for the first time demonstrated functional expression of all human CYPs (including the orphan enzymes CYP2A7, CYP4A22, and CYP20A1) within a single expression host and a comparison of their activities towards two probe substrates. A number of unexpected reactions were found for several enzymes, including CYP5A1 and CYP11A1. This strain collection is expected to facilitate the systematic investigation of these enzymes in the future.

Author contributions

MB and PD conceived and conceptualized the study. PD, LF, DW, SA, DM, SS, RAS, JL, and QL performed experiments. MB and PD wrote the manuscript. All authors have read and approved the manuscript.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Chemical structure of luminogenic CYP substrates used in this study.

Table S2. Catalytic activity of all human CYP enzymes towards two luminogenic substrates.