【S-7】

Biphasic Effects of the Flavonoids Quercetin and Naringenin on the Metabolic Activation of 2-Amino-3,5-dimethylimidazo[4,5-F]quinoline by *Salmonella Typhimurium* TA1538 Coexpressing Human Cytochrome P450 1A2, NADPH-Cytochrome P450 Reductase, and Cytochrome b_5

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Quercetin and naringenin are representative flavonoids that not only exert antiestrogenic, cholesterol-lowering and antioxidant activities but also can modulate the metabolism of many xenobiotics. The activity of the specific form(s) of CYP450 is likely to be a major determinant of susceptibility to chemically induced carcinogenesis between which varies among between individuals due to different dietary habits as well as genetic characteristics. People consume cooked meat or fish together with various vegetables containing substantial amounts of quercetin and naringenin that can modify the enzyme activity of CYP1A2 to stimulate or to inhibit the mutagenic activities of HCAs.

Heterocyclic amines (HCAs) produced by cooking meat products at high temperatures are promutagens that are activated by cytochrome P450 (CYP) 1A2. Using a newly developed *Salmonella typhimurium* TA1538/1A2bc-b5 strain, we tested the effect of quercetin and naringenin on the mutagenicity of 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ). TA1538/1A2bc-b5 bears two plasmids, one expressing human CYP1A2 and NADPH-P450 reductase (NPR), and the other plasmid which expresses human cytochrome b5 (cyp b5). TA1538/1A2bc-b5 cells showed high activities of 7-ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin O-demethylase (MROD) associated with CYP1A2 and are very sensitive to mutagenesis induced by several HCAs. MeIQ was found to be the strongest mutagen among the HCAs tested in this system. Mutagenicity of MeIQ was enhanced 50% and 42% by quercetin at 0.1 and 1 mM, respectively, but suppressed 82% and 96% at 50mM and 100mM. Naringenin also increased the MeIQ-induced mutation about 37% and 22% at 0.1 and 1 mM, but suppressed it 32% and 63% at 50mM and 100mM concentrations,

respectively, in TA 1538/1A2bc-b5 cells. Thus, they stimulated the MeIQ induced mutation at low concentrations, but strongly suppressed it at high concentrations. This biphasic effect of flavonoids was due to the stimulation or the inhibition of CYP1A2 activity in a dose-dependent manner judging by the activities of EROD or MROD in the *Salmonella* cells.

Collectively, it is likely that the biphasic effects of quercetin and naringenin on the MeIQ-induced mutagenesis in *S typhimurium* TA1538/CYP1A2bc-b5 were due to their differential modification of the CYP1A2 activity in these cells.

Biphasic effects of the flavonoids on the MeIQ metabolic activation in CYP1A2 genetically engineered *Salmonella typhimurium* TA1538

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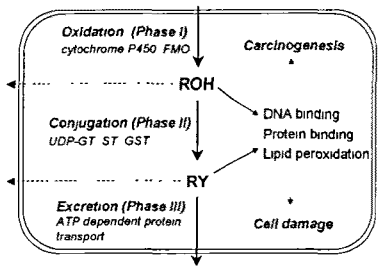
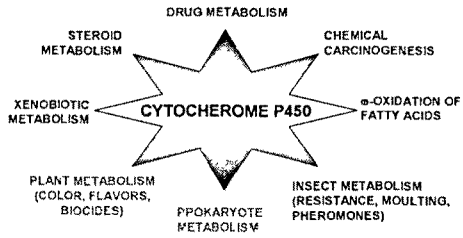
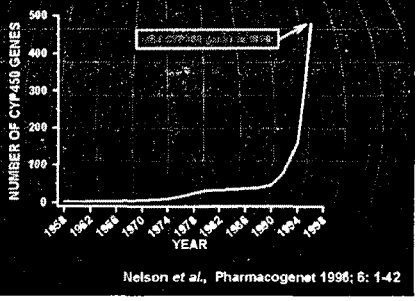
Xenobiotics Biotransformation



Many Roles of Cytochrome P450 in Metabolism



Discovery of CYP450 Genes



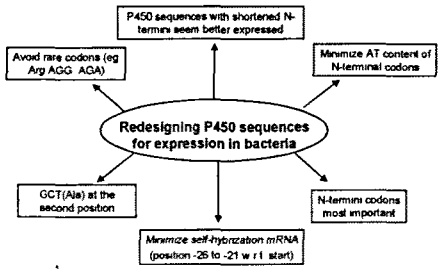
Human CYP450 Families

Isoform	Substrates
CYP1, CYP2, CYP3	*drugs , xenobiotics*
CYP4, CYP5, CYP8	fatty acids, prostaglandins, thromboxanes
CYP7, CYP11, CYP17 CYP19, CYP20, CYP21 CYP24, CYP27, CYP39	steroid hormones

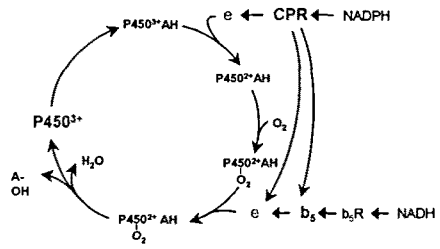
Systems for the heterologous expression of cytochrome P450s

- ❒ Bacteria (Escherichia coli, Salmonella typimurium etc.)
- ❒ Yeast (Saccharomycetes cerevisiae, Schizosaccaromyces pombe)
- ❒ Insect cells
- ❒ Mammalian cells

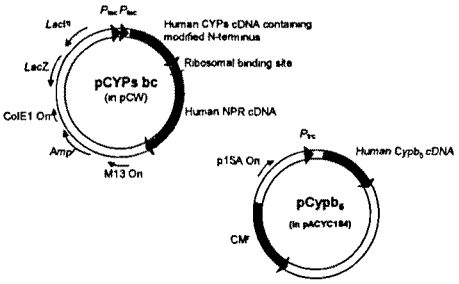
Scheme for redesigning cytochrome P450 sequences for expression in *E. coli*



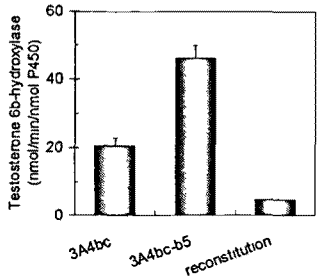
The catalytic cycle of cytochrome P450



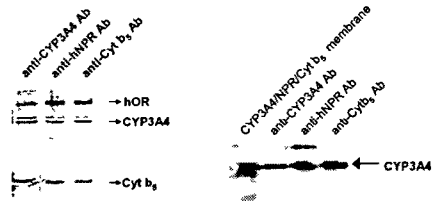
Structure of plasmids used for the bacterial expression



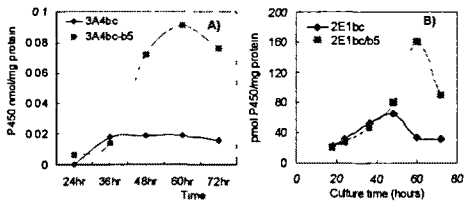
Testosterone 6β-hydroxylase activity in various CYP3A4 reaction systems



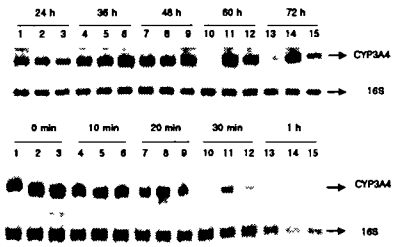
Identification of protein interactions in *E. coli* membrane by immunoprecipitation assay



Culture time dependent-CYP expression levels in membrane fraction between CYP/CPR and CYP/CPR/cyt b_5



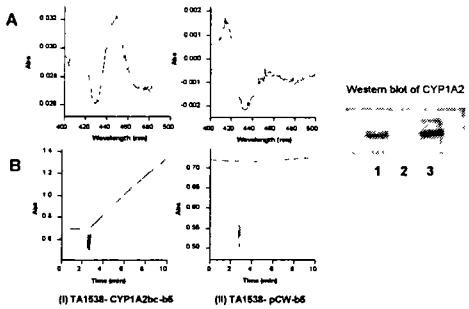
Effect of cytochrome b_5 on the CYP3A4 transcription and mRNA decay in E. coli



Salmonella typhimurium strains and plasmids established in the present study

	characteristics
Plasmids	
pCW	CYP cDNA expression vector, pBR322 derivative, *Amp*^{r}
p1A2bc	pCW derivative bearing human CYP1A2 and NPR, *Amp*^{r}
pACYC184	Use for human cyp b5 expression, *Cm*^{r}, *Tc*^{r}
pCyp b5	pACYC184 derivative bearing human cyp b5, *Cm*^{r}
Strains	
LB5000	*metA22, metE551, trpC2, ilv-452 H1-b2-e,n,x, fla-66, rpsL120, xyl-404, leu, hsdL6 hsdSA29, hsdSB*
TA1538	*hisD3052, uvrB, rfa*
TA1538/ pCW-pACYC184	TA1538 harboring pCW and pACYC184, *Amp*^{r}, *Cm*^{r}, *Tc*^{r}
TA1538/pCW-b5	TA1538 harboring pCW and pCyp b5, *Amp*^{r}, *Cm*^{r}
TA1538/1A2bc-b5	TA 1538 harboring pCYP1A2bc and pCyp b5, *Amp*^{r}, *Cm*^{r}

CYP CO difference spectra and hNPR activities in membrane fractions prepared from *Salmonella* strains

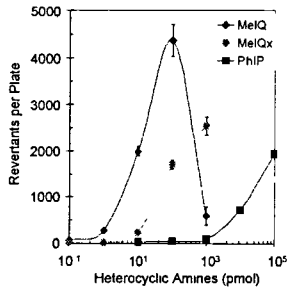


CYP1A2 contents and catalytic activities of CYP1A2 and hCPR in membrane fractions and whole cells of *Salmonella* strains

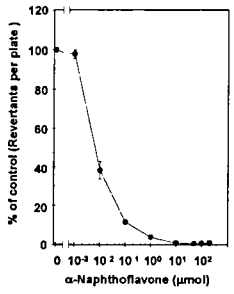
	CYP1A2	hCPR	EROD	MROD	EROD	MROD
	(pmol/mg protein)	(nmol cyt c reduced/mg protein)	(nmol product/ min/ nmol of P450)		(pmol product/min/ ml culture)	
TA1538/pCW-pACYC184	0	0 03 ± 0 01	< 0 02	< 0 02	< 0 02	< 0 02
TA1538/1A2bc-b5	151 ± 23	6 82 ± 1 31	0 99 ± 0 01	1 47 ± 0 11	50 4 ± 7 8	68 6 ± 1 5

- CYP1A2 and hNPR in *Salmonella* strains were expressed at 25 °C for 24 h CYP1A2 content was determined by Fe^{2+}-CO versus Fe^{2+} difference spectra
- Values are presented as mean ± SD (n=3)

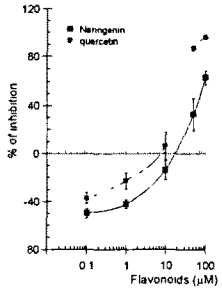
Sensitivity of genetically engineered TA 1538/1A2bc-b5 cells to HCAs.



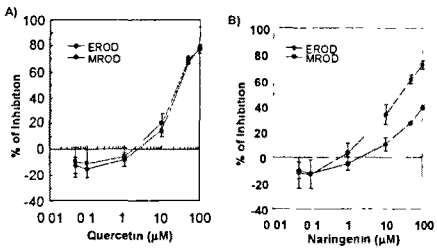
Inhibition of mutagenic activation of MeIQ by α-naphthoflavone in TA1538/1A2bc-b5 cells



Concentration-dependent biphasic effects of quercetin and naringenin on the activation of MeIQ in TA1538/1A2bc-b5



Inhibition of MROD and EROD by quercetin or naringenin in TA1538/1A2bc-b5 cells



CONCLUSIONS

- The biphasic effects of quercetin and naringenin on the MeIQ-induced mutagenesis in *S. typhimurium* TA1538/ CYP1A2bc-b5 were due to their differential modification of the CYP1A2 activity in these cells.

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Thank you