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STABILIZATION OF CYP3A4 mRNA BY CO-EXPRESSION OF CYTOCHROME B_5 IN *E. COLI.*

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Human cytochrome P450 (CYP or P450) 3A4 (CYP3A4) is the most abundant among P450s in human liver. We previously reported that the expression of CYP3A4 in membranes prepared from *E. coli* coexpressed the bicistronic construct of CYP3A4 and NADPH-P450 reductase with cytochrome b_5 (b5) was showed 20~60% higher than that in membranes from *E. coli* expressed only the bicistronic construct with culturing longer times (48-72h). This result might indicate that the coexpression of b5 resulted in the stabilization of P450 protein or P450 mRNA. To study the effect of b5 on the elevation of expression of CYP3A4 in protein level, we determined the protein-protein interaction in *E. coli* membranes by immunoprecipitation. CYP3A4, NADPH-P450 reductase and b5 were interacted in *E. coil* membrane judging by co-immunoprecipitation of all of them with each antibody. Subsequently, to observe the inhibition of degradation of CYP3A4 by the coexpression of b5, we isolated the 20S proteasome from *E. coli* DH5α cells and incubated it with *E. coli* membranes expressed P450 3A4bc alone or CYP3A4bc with b_5 under various reaction conditions. The degradation of CYP3A4 by 20S proteasome was not significantly affected by the coexpression of b5. To elucidate the effect of b_5 on the transcription of CYP3A4, the amount of CYP3A4 mRNA in the *E. coli* coexpressed 3A4bc with b_5 cultured for various times were compared to that in the *E. coli* expressed 3A4bc alone using the RT-PCR. The level of CYP3A4 mRNA in *E. coli* coexpressed b5 was increased in a culture-time dependent manner. And the halflife of CYP3A4 mRNA was also increased by co-expression of b5 in the experiment of mRNA decay analysis with transcriptional inhibitor, ripampicin.

Taken together these results, we conclude that the higher levels of CYP3A4 in the membranes obtained from *E. coli* coexpressed CYP3A4bc with b5 might result in the stabilization of mRNA of CYP3A4 by b5.