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# Quantification and cellular localization of expression in human skin of genes encoding flavin-containing monooxygenases and cytochromes P450

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**Abstract**

The expression, in adult human skin, of genes encoding flavin-containing monooxygenases (FMOs) 1, 3, 4, and 5 and cytochromes P450 (CYPs) 2A6, 2B6, and 3A4 was determined by RNase protection. Each FMO and CYP exhibits inter-individual variation in expression in this organ. Of the individuals analysed, all contained CYP2B6 mRNA in their skin, 90% contained FMO5 mRNA and about half contained mRNAs encoding FMOs 1, 3, and 4, and CYPs 2A6 and 3A4. The amount of each of the FMO and CYP mRNAs in skin is much lower than in the organ in which it is most highly expressed, namely the kidney (for FMO1) and the liver (for the others). In contrast to the latter organs, in the skin FMO mRNAs are present in amounts similar to, or greater than, CYP mRNAs. Only the mRNA encoding CYP2B6 decreased in abundance in skin with increasing age of the individual. All of the mRNAs were substantially less abundant in cultures of keratinocytes than in samples of skin from which the cells were derived. In contrast, an immortalized human keratinocyte cell line, HaCaT, expressed FMO3, FMO5, and CYP2B6 mRNAs in amounts that fall within the range detected in the whole skin samples analysed. FMO1, CYP2A6, and CYP3A4 mRNAs were not detected in HaCaT cells, whereas FMO4 expression was markedly increased in this cell line compared to whole skin. *In situ* hybridization showed that the expression of each of the FMOs and CYPs analysed was localized to the epidermis, sebaceous glands and hair follicles. 

*Keywords:* Flavin-containing monooxygenases; Cytochrome P450; Skin; Human; Keratinocyte; In vitro

## 1. Introduction

Flavin-containing monooxygenases (FMOs) and cytochromes P450 (CYPs) play an essential role in the detoxification of a wide range of foreign compounds [1–4]. Substrates for FMOs are structurally diverse and contain, as their site of oxidation, a soft nucleophilic atom such as nitrogen, sulphur, phosphorous, or selenium [2]. They include therapeutic drugs [5–9]; pesticides, such as organophosphates and carbamates [10–12]; and dietary-derived compounds, including trimethylamine, a breakdown product of choline and lecithin [13]. A defect in the FMO-mediated *N*-oxidation of trimethylamine is the cause of the inherited disorder trimethylaminuria, or fish-odour syndrome [14–16].

Six *FMO* genes, designated *FMO1–FMO6*, have been identified in humans [17; http://www.sanger.ac.uk/HGP/Chr1/]. Each gene exhibits a distinct tissue-specific pattern of expression [18,19] and each FMO has a different substrate preference [20–22]. The proteins are located in the membranes of the endoplasmic reticulum and are present in greatest abundance in tissues such as liver, lung, and kidney [17–19]. Loss-of-function mutations of *FMO3* constitute the genetic basis of trimethylaminuria [23]. The human *FMO2* gene encodes a truncated, non-functional protein [24]. Other mammals, including non-human primates, do not carry the truncation mutation [24,25] and hence express functional FMO2 protein.

CYP substrates include numerous therapeutic drugs and

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*Abbreviations:* FMO, flavin-containing monooxygenase; CYP, cytochrome P450; KGM, keratinocyte growth medium; DMEM, Dulbecco's modified Eagle's medium.

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other xenobiotics, and endogenous compounds such as steroid hormones and fatty acids [3]. Some chemicals are substrates for both FMOs and CYPs. However, in such cases, reactions catalyzed by the two types of enzymes may produce different classes of chemical, as is the case with the substrate nicotine [26], or different isomers, for example, in the case of the organophosphate phorate, mouse liver FMO produces (−)-phorate and Cyp2b9 (+)-phorate [27].

To date fourteen mammalian CYP families have been identified [28; http://drnelson.utmem.edu/nelsonhomepage.html]. Of these, the CYP 1, 2, 3, and 4 families are involved in the metabolism of foreign compounds. Each CYP exhibits distinct tissue-specific patterns of expression and, unlike FMOs, several CYPs are induced on exposure to xenobiotics [3,4].

There is increasing realization of the importance of FMOs and CYPs in extra-hepatic metabolism. However, nothing is known concerning the expression of specific FMO forms in human skin and reports of specific CYP forms in human skin are limited. Cosmetics, drugs, household products, and agrochemicals are just some examples of the types of compounds to which the skin is exposed. The skin possesses both Phase I and Phase II detoxification enzymes, including CYPs, glutathione transferases, sulphotransferases, and epoxide hydrolases [29–31]. It therefore has the capacity to detoxify harmful chemicals [32–34] and hence provides a protective metabolic as well as physical barrier between our internal organs and the environment.

In this study, we have quantified the expression of four members of the *FMO* gene family, *FMOs 1*, *3*, *4*, and *5*, in the skin of different individuals and compared the results with those obtained for *CYPs 2A6*, *2B6*, and *3A4*. Skin models are being developed for biotransformation and other physiological studies, and it is important to ascertain how closely they represent the situation in human skin. We have therefore determined the expression of these *FMOs* and *CYPs* in two such models, primary keratinocyte cultures derived from human skin and an immortalized human keratinocyte cell line, HaCaT [35].

## 2. Materials and methods

### 2.1. Cell culture

#### 2.1.1. Primary human epidermal keratinocytes

Skin samples, from breast reduction mammoplasties, were obtained from the Stephen Kirby Skin Bank, Queen Mary's University Hospital, Roehampton, UK. Skin was placed, epidermal side up, in a Petri dish and washed with phosphate-buffered saline (PBS) to remove debris and blood. Parallel cuts, 2–3 mm deep and 2–3 mm apart, were made in the tissue. The tissue was then placed, dermal side up, in a fresh Petri dish, covered with Liebovitz L-15 medium, containing Dispase (4 units/mL) (GIBCO BRL), kanamycin (10 μg/mL) (GIBCO BRL), gentamycin (10 μg/mL) (Sigma Chemical Co.) and Ciproxin™ (10 μg/mL) (Bayer), and incubated at room temperature for 24 hr. The epidermis was then removed from the dermis using fine watchmaker's forceps.

Keratinocytes were released by treatment of the epidermis with trypsin/EDTA (GIBCO BRL) at 37° for 3 min. The resultant cell suspension was centrifuged at 220 g for 3 min. The whole procedure was repeated four times. The five cell pellets were pooled and resuspended in KGM (Clonetics). The cells were plated in KGM containing 10% (v/v) foetal bovine serum and incubated at 37° in an atmosphere of 5% $CO_2$/95% air. After attachment of cells (approximately 24 hr), the plating medium was replaced with serum-free KGM. The culture medium was replaced every 3 days until keratinocytes reached 90% confluency, at which time they were harvested.

#### 2.1.2. HaCaT cells

The HaCaT cell line [35] was a gift from Prof. Norbert E. Fusenig (German Cancer Research Foundation). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) foetal bovine serum, penicillin (0.1 mg/mL), and streptomycin (0.025 μg/mL). All tissue culture reagents were from Sigma Chemical Co. Cells were harvested at 70–80% confluency.

### 2.2. Isolation of RNA

Adult human liver, lung, and kidney samples, used as positive controls, were obtained as described previously [18]. Total RNA was isolated by the guanidinium thiocyanate/LiCl method [36]. Total RNA from human skin samples was isolated using the guanidinium thiocyanate/guanidine HCl method [37], whereas total RNA from primary human keratinocytes and HaCaT cells was isolated with the Ultraspec™ RNA isolation kit (Biotecx Laboratories Inc.). In each case, the extraction method used was chosen to allow optimal isolation of RNA from the particular source material. RNA was resuspended in sterile water and stored at −80°. RNA concentration was determined from absorbance at 260 nm.

### 2.3. RNase protection assays

Plasmids for the production of antisense RNA probes for FMO1 (p8A1-6), FMO3 (pBSform2/6) and FMO4 (p2A1L-2) were as described previously [18]. For FMO5, p1C1/1b, a recombinant plasmid that contained an insert of approximately 600 bp encoding the 5′ end of the corresponding mRNA, isolated from a human placenta cDNA library [17], was digested with *Nsi*I and *Hin*dIII to release a 226-bp coding region fragment. This fragment was gel-purified and ligated to pBS that had been digested with *Pst*I and *Hin*dIII to form the RNase protection plasmid p1C1/1/C. Subsequently, 10 μg of p1C1/1/C was linearized by incubation with *Sma*I. After digestion was complete, the reaction mix was treated with proteinase K and SDS, as described previously [38], extracted once with phenol–

chloroform (1:1, v/v), and ethanol-precipitated. Plasmids for the transcription of CYP2A6, CYP2B6, and CYP3A4 antisense probes were as described previously [39].

Radiolabelled antisense RNA probes were synthesized from the above plasmids using [$\alpha$-$^{32}$P]CTP (800 Ci/mmol, Amersham) and purified as described previously [38,40]. RNase protection assays, based on the method of Myers *et al*. [41], were performed essentially as described [38,40]. Each hybridization mix contained, in a total volume of 30 $\mu$L, up to 30 $\mu$g of sample RNA, $1 \times 10^5$ cpm of the appropriate $^{32}$P-labelled probe and, where necessary, sufficient calf liver tRNA to bring the final concentration of RNA to 30 $\mu$g. For some analyses, two probes were hybridized simultaneously to the same sample of RNA. To ensure linearity of dose–response, each assay was done using two different amounts of sample RNA, each in duplicate. After digestion of unhybridized probe with RNase cocktail (1/625 dilution) (Ambion Inc.), protected probe was electrophoresed through an 8M urea/6% polyacrylamide gel, visualized by autoradiography, and quantified by scanning densitometry. In the case of CYP2B6 mRNA, two protected fragments were consistently observed, both of which were quantified. A comparison of the autoradiographic signal derived from the protected species with a standard curve of undigested probe permitted quantification of the mRNA in terms of molecules/ng total RNA [42].

### 2.4. In situ hybridization

Antisense and sense RNA probes for *in situ* hybridization were generated as described above for RNase protection assays except that they were radiolabelled with [$\alpha$-$^{35}$S]UTP (1250 Ci/mmol, NEN Dupont). *In situ* hybridization, based on the method of Angerer *et al*. [43], was performed on wax-embedded full-thickness human skin. Each hybridization mix (0.48 M NaCl, 8 mM Tris–HCl, pH 7.5, 1.6 mM EDTA, pH 8, 0.8 X Denhardt's solution, 0.8 mg/mL of tRNA, 10% (w/v) dextran sulphate, 40% (v/v) deionized formamide) contained $1 \times 10^7$ cpm/mL of $^{35}$S-labelled probe. Hybridization was carried out overnight at 55°. To remove unhybridized probe sections were treated with RNase [43] and washed twice, for 15 min each, at 55° in 0.2 × SSC, 0.1% (v/v) 2-mercaptoethanol. Slides were coated with NTB-2 emulsion (Eastman Kodak), and placed at 4° for 4–6 weeks, after which they were developed. Sections were counterstained with haematoxylin/eosin. As a control, duplicate sections were hybridized with the corresponding sense RNA probe.

## 3. Results

### 3.1. Quantification of FMO and CYP mRNAs in human skin

The expression, in adult human skin, of four members of the *FMO* gene family, *FMOs* 1, 3, 4, and 5, and of members of three different *CYP* gene subfamilies, *CYP2A6*, *CYP2B6* and *CYP3A4*, was investigated by quantitative RNase protection assays. This allows comparisons to be made of the amounts of each FMO or CYP mRNA expressed within an individual and among different individuals. Fig. 1 shows representative autoradiograms of RNase protection assays of mRNAs encoding FMO1, FMO3, FMO4, and FMO5. Discrete bands of the expected size were obtained for the protected species of FMO1 (Fig. 1A), FMO4 (Fig. 1C), and FMO5 (Fig. 1B and C). In some samples, the protected species for FMO3 (Fig. 1B) is a doublet. This may reflect the presence of allelic variants in these individuals. The amount of each FMO mRNA detected is given in Table 1. Owing to limited amounts of tissue available, it was not possible to quantify all of the mRNA species in the same sample.

FMO1 mRNA was analysed in samples of skin from nine individuals. Of these, four contained no detectable FMO1 mRNA. The other five all expressed the mRNA, in amounts ranging from 9 to 163 molecules/ng total RNA, an inter-individual variation of about 18-fold. In comparison, the content of FMO1 mRNA in the kidney, the organ in which it is most highly expressed in humans, ranged from 80 to 850 molecules/ng total RNA (this study and [18]).

The mRNA encoding FMO3 was detected in five of nine individuals analysed (Table 1). The content of the mRNA ranged from 45 to 111 molecules/ng total RNA, an inter-individual variation of less than 2.5-fold. In comparison, in the liver, its major site of expression, the content of the mRNA ranged from 360 to 5250 molecules/ng RNA (this study and [18]).

Four of nine individuals analysed expressed FMO4 mRNA, in amounts ranging from 2–9 molecules/ng total RNA, an inter-individual variation of about 4.5-fold. In all but one of the individuals, the FMO4 mRNA is the least abundant of the FMO mRNAs analysed. The content of the mRNA in liver is between 20 and 45 molecules/ng RNA (this study and [18]).

FMO5 mRNA was present in seven of eight individuals analysed, in amounts between 23 and 128 molecules/ng total RNA. The only sample in which this FMO mRNA was not detected was 58(19/9), a fifty-eight-year-old female who failed to express any FMO. The integrity of this RNA sample was confirmed by agarose gel electrophoresis (data not shown) and this individual did express CYP2B6 mRNA, albeit in low amounts (see Table 1). The inter-individual variation in the amount of FMO5 mRNA was 5.6-fold. In comparison FMO5 mRNA is present in the liver, its major site of expression, in amounts ranging from 500 to 5700 molecules/ng RNA.

Representative RNase protection assays of CYP2A6, CYP2B6, and CYP3A4 mRNAs are shown in Fig. 2. The contents of these mRNAs present in the skin of individuals analysed are shown in Table 1. Of the nine individuals analysed, five expressed CYP2A6 mRNA in amounts ranging from 4 to 106 molecules/ng total RNA, an inter-indi-

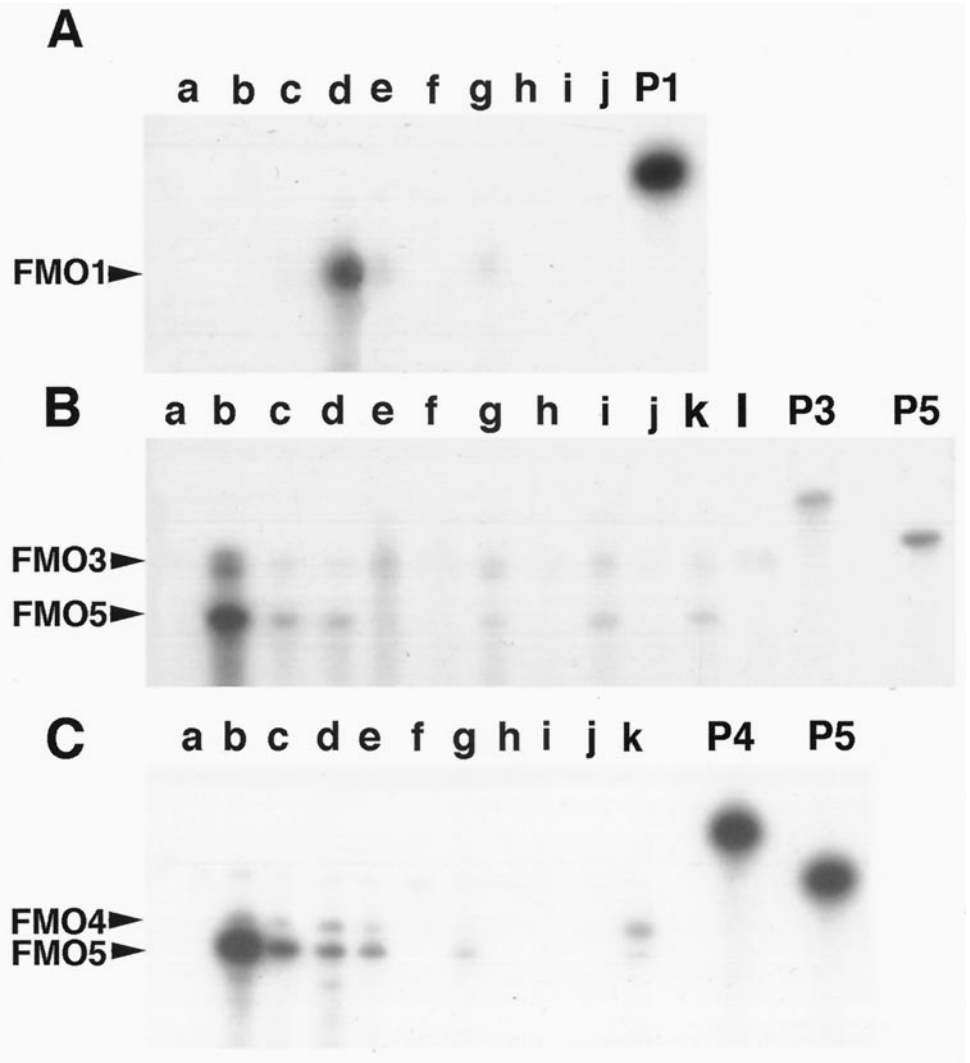


Fig. 1. Quantification of FMO mRNAs. (A) An antisense probe for FMO1 mRNA was hybridized to tRNA (a), or to total RNA isolated from liver (b), lung (c), kidney (d), skin (e, g, i) or primary keratinocyte cultures (f, h, j). RNA was from individuals 46(10/9) (e, f), 28(24/9) (g, h) or 58(19/9) (i, j). Undigested probe is shown in track P1. The arrowhead indicates the protected fragment. (B) Antisense probes for FMO3 and FMO5 mRNAs were hybridized, in the same sample, to tRNA (a), or to total RNA isolated from liver (b), lung (c), kidney (d), skin (e, g, i, k), primary keratinocyte cultures (f, h, j) or HaCaT cells (l). RNA was from individuals 31(2/8) (e, f), 502 (g, h), 24(7/11) (i, j) or 24(21/11) (k). Undigested probes are shown in tracks P3 (FMO3) and P5 (FMO5). Arrowheads indicate the protected fragments. (C) Antisense probes for FMO4 and FMO5 mRNAs were hybridized, in the same sample, to tRNA (a) or to total RNA isolated from liver (b), lung (c), kidney (d), skin (e, g, i), primary keratinocyte cultures (f, h, j) or HaCaT cells (k). RNA was from individuals 46(10/9) (e, f), 28(24/9) (g, h) or 58(19/9) (i, j). Undigested probes are shown in tracks P4 (FMO4) and P5 (FMO5). Arrowheads indicate the protected fragments. Quantities of RNA analysed were: liver (5 $\mu$g), lung (10 $\mu$g), skin (30 $\mu$g), primary keratinocyte cultures (30 $\mu$g), HaCaT cells (30 $\mu$g (A, C), 10 $\mu$g (B)), tRNA (30 $\mu$g).

vidual variation of 27-fold. The content of this mRNA in liver is between 10 and 3000 molecules/ng RNA (this study and [44]). The antisense probe used for the analysis of CYP2A6 mRNA is complementary to a region of the mRNA that differs in several positions from the corresponding region of CYP2A7 and CYP2A13 mRNAs [45] and thus would not detect the latter mRNAs.

All three individuals analysed contained CYP2B6 mRNA in amounts ranging from 1 to 20 molecules/ng total RNA, an inter-individual variation of 20-fold. This compares with a mRNA content of between 20 and 900 molecules/ng RNA in liver (this study and [44]).

Three of seven individuals analysed expressed CYP3A4 mRNA in amounts ranging from 47 to 189 molecules/ng total RNA, an inter-individual variation of about 4-fold. In comparison, the content of this mRNA in the liver was between 150 and 16,000 molecules/ng RNA (this study and [44]). The antisense probe for CYP3A4 mRNA was synthesized from a region of the cDNA (nucleotides 1519 to 1759) which contains numerous nucleotide differences from the corresponding region of sequences encoding CYP3A3, 3A5, and 3A7 [46–48], and thus will detect only CYP3A4 mRNA.

The content, in human skin, of none of the FMO and

Table 1
Quantification of FMO and CYP mRNAs in human skin

| Sample | Age of donor (yr) | FMO1 | FMO3 | FMO4 | FMO5 | CYP2A6 | CYP2B6 | CYP3A4 |
|---|---|---|---|---|---|---|---|---|
| 24(7/11) | 24 | N.D. | 87 | N.D. | 94 | N.D. | N.D. | N.D. |
| 24(21/11) | 24 | 34 | 90 | 7 | 101 | 21 | N.D. | N.D. |
| 28(9/8) | 28 | 0 | 45 | 6 | N.D. | 0 | N.D. | 0 |
| 28(24/9) | 28 | 20 | N.D. | 0 | 23 | 107 | 20 | 190 |
| 28(12/11) | 28 | N.D. | N.D. | N.D. | N.D. | 4 | N.D. | 0 |
| 29(1/10) | 29 | 0 | 0 | 0 | 26 | 0 | N.D. | N.D. |
| 31(2/8) | 31 | 41 | 111 | 2 | 101 | 0 | N.D. | N.D. |
| 46(10/9) | 46 | 164 | N.D. | 9 | 128 | N.D. | 8 | 0 |
| 47(24/9) | 47 | 9 | 0 | 0 | N.D. | 8 | N.D. | 47 |
| 58(19/9) | 58 | 0 | 0 | 0 | 0 | N.D. | 1 | 0 |
| 502(1) | Not known | N.D. | 80 | N.D. | 68 | 55 | N.D. | 69 |
| H | Not known | 0 | 0 | 0 | N.D. | 0 | N.D. | N.D. |

RNA amounts are given as molecules/ng total RNA; N.D. = not determined.
Samples are from mammoplasties, except for H, which is from an abdominoplasty.
Each sample is from a different individual.

CYP mRNAs analysed was correlated with that of any other. There was no correlation between the age of an individual and the content in skin of mRNA encoding FMO 1, 3, 4, or 5 or CYP2A6 or 3A4 (Table 1). However, the content of CYP2B6 mRNA in skin decreased as a function of age, with a correlation coefficient ($r$) of +1. Although this mRNA was quantified in only three individuals, the content of none of the other mRNAs analysed in these individuals (encoding FMO1, FMO4, FMO5, and CYP3A4) was correlated with age.

### *3.2. Quantification of FMO and CYP mRNAs in cultures of keratinocytes derived from human skin*

Keratinocyte cultures are used as a model experimental system for biotransformation studies in skin. However, little information is available concerning the expression in such cells of various FMOs and CYPs, and, consequently, it is not known how well these cells reflect the extent of *FMO* and *CYP* expression found in human skin. The values obtained for each FMO and CYP mRNA analysed in keratin-

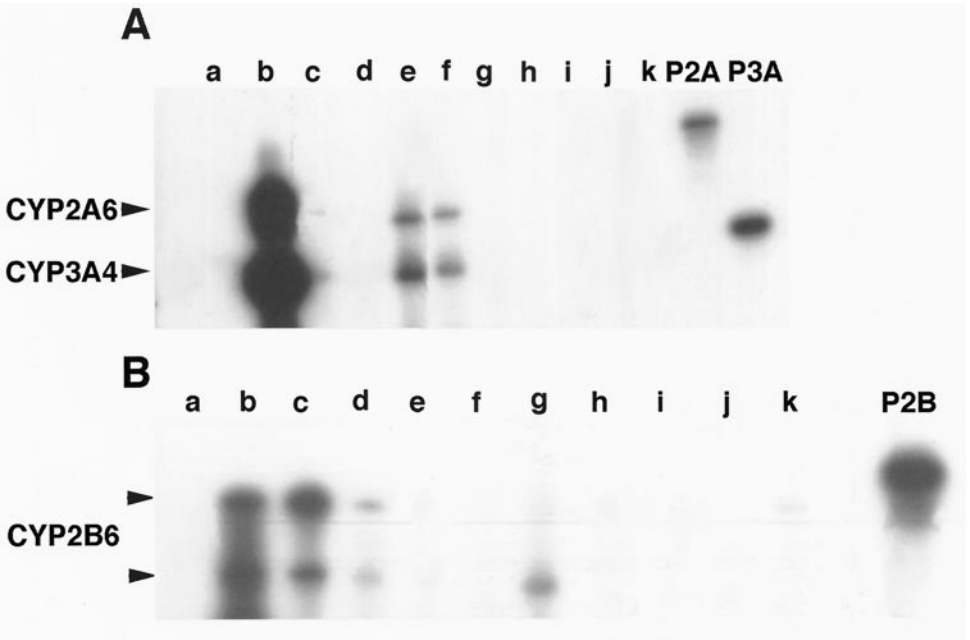


Fig. 2. Quantification of CYP mRNAs. (A) Antisense probes for CYP2A6 and CYP3A4 mRNAs were hybridized, in the same sample, to tRNA (a) or total RNA isolated from liver (b), skin (c, e, f, h), keratinocyte cultures (d, g, i, j) or HaCaT cells (k). RNA was from individuals 47(24/9) (c, d), 28(24/9) (e), 502 (f, g), 28(9/8) (h, i) or 44(4/11) (j). Undigested probes are shown in tracks P2A (CYP2A6) and P3A (CYP3A4). Arrowheads indicate the protected fragments. (B) An antisense probe for CYP2B6 mRNA was hybridized to tRNA (a) or to total RNA isolated from liver (b), lung (c), kidney (d), skin (e, g, i), primary keratinocyte cultures (f, h, j) or HaCaT cells (k). RNA was from individuals 46(10/9) (e, f), 28(24/9) (g, h) or 58(19/9) (i, j). Track P2B shows undigested probe. Arrowheads indicate protected fragments. Quantities of RNA analysed were: liver (5 $\mu$g), lung (10 $\mu$g), skin (30 $\mu$g), primary keratinocyte cultures (30 $\mu$g) and HaCaT cells (30 $\mu$g).

Table 2
Quantification of FMO and CYP mRNAs in keratinocyte cultures and HaCaT cells

| Sample | Age of donor (yr) | FMO1 | FMO3 | FMO4 | FMO5 | CYP2A6 | CYP2B6 | CYP3A4 |
|---|---|---|---|---|---|---|---|---|
| 24(7/11) | 24 | N.D. | 22 | N.D. | 21 | 0 | N.D. | 178 |
| 28(9/8) | 28 | 0 | 0 | 4.7 | N.D. | 0 | N.D. | 0 |
| 28(24/9) | 28 | 0 | N.D. | 0 | 0 | 0 | 2.5 | 34 |
| 29(1/10) | 29 | 0 | 0 | 0 | N.D. | 0 | N.D. | N.D. |
| 31(2/8) | 31 | 0 | 17.5 | 1.6 | 16 | 0 | N.D. | N.D. |
| 46(10/9) | 46 | 0 | 0 | 0 | 0 | N.D. | 6 | 0 |
| 44(4/11) | 44 | N.D. | N.D. | N.D. | N.D. | 0 | N.D. | 0 |
| 47(24/9) | 47 | 0 | 0 | 0 | 0 | 0 | N.D. | 0 |
| 58(19/9) | 58 | 0 | 0 | 0 | 0 | N.D. | 0 | 0 |
| 502(1) | Not known | N.D. | 13 | N.D. | 10 | 0 | N.D. | 0 |
| H | Not known | 0 | 0 | 0 | 17.5 | 0 | N.D. | N.D. |
| HaCaT cells | | 0 | 30 | 28 | 16 | 0 | 15 | 0 |

RNA amounts are given as molecules/ng total RNA; N.D. = not determined.
Samples are from mammoplasties, except for H, which is from an abdominoplasty.

ocyte cultures are shown in Table 2. Cultures of keratinocytes derived from eight individuals all failed to express *FMO1*, even those prepared from individuals, e.g. 46(10/9), who had a relatively high content of FMO1 mRNA in their skin (Tables 1 and 2). A comparison of the results in Tables 1 and 2 indicates that the contents of mRNAs encoding FMOs 3, 4 and 5, and CYPs 2B6 and 3A4 is 75 to 100% lower in cultured keratinocytes than in the skin samples from which the cells were derived. The only exceptions were FMO4 mRNA, in individuals 28(9/8) and 31(2/8), which was decreased 15 and 25%, respectively, and CYP2B6 mRNA, in individual 46(10/9), which was decreased by 25%. No FMO1 or CYP2A6 mRNAs were detected in any of the nine keratinocyte cultures analysed.

The immortalized keratinocyte cell line HaCaT was also analysed for its ability to express FMOs and CYPs (Table 2). The amounts of mRNAs encoding FMO3, FMO5 and CYP2B6 in this cell line are more similar to those in human skin than were those present in keratinocyte cultures. However, the content of FMO4 mRNA in HaCaT cells is at least 3-fold that in any of the human skin samples analysed (Tables 1 and 2). The mRNAs encoding FMO1 and CYP2A6, which were not detected in any of the keratinocyte cultures, were also absent from HaCaT cells, as was CYP3A4 mRNA (Table 2).

### *3.3. Cellular localization of FMO and CYP mRNAs in human skin*

The cellular localization of the various FMO and CYP mRNAs was determined by *in situ* hybridization of fixed sections of human skin. The antisense RNA probes used for these experiments were the same as those used for RNase protection assays, except that they were labelled with $^{35}S$ instead of $^{32}P$. As controls, the corresponding sense probes were hybridized to human skin sections. Messenger RNAs encoding FMOs 1, 3, 4, and 5 are localized in the epidermis, sebaceous glands and hair follicles (Fig. 3). CYP2A6, CYP2B6, and CYP3A4 mRNAs also are localized within the epidermis and sebaceous glands (Fig. 4). The localization of the FMO and CYP mRNAs in the epidermis is uniform and not restricted to particular regions, i.e., the stratum spinosum, the stratum granulosum or the stratum germinativum. In sebaceous glands, each of the FMO and CYP mRNAs is restricted to the sweat producing cells.

## 4. Discussion

Previous studies (reviewed in [30]) have detected FMO in human skin, but did not identify which forms of the enzyme were expressed, nor whether there was any inter-individual variation in expression. *FMOs 1*, *3*, *4*, and *5* displayed inter-individual variation in their expression in skin, with *FMO1* showing the greatest variation and *FMO3* the least. Some individuals failed to express one or more *FMOs*. This variation may be due, in part, to normal hormonal fluctuations. All but one of the skin samples analysed were from females, and FMO3 is known to be down-regulated during menstruation [49], whereas FMO5 is up-regulated by progesterone [50]. Inter-individual variations in the expression of *FMO3* and *FMO5* are greater in liver than in skin. We found variations of 14.5- and 11-fold for *FMO3* and *FMO5*, respectively, in liver. This compares well with the results of Overby *et al.* [51], who observed inter-individual variations of about 9- and 10-fold, respectively, for FMO3 and FMO5. In contrast to Overby *et al.* [51], we found that, in liver, the expression of *FMO3* was not always greater than that of *FMO5*.

The three CYP mRNAs analysed also showed inter-individual variation in their content in human skin. CYP3A4 exhibited the least variation (4-fold), whereas CYP2B6 and CYP2A6 varied 20- and 27-fold, respectively. The extent of this variation is much less in skin than in liver

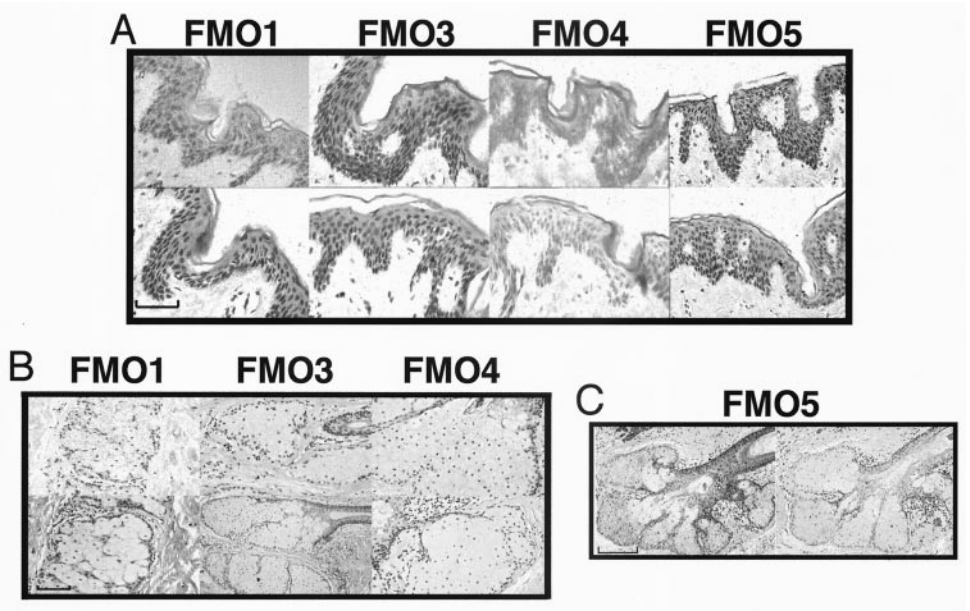


Fig. 3. *In situ* hybridization analyses of FMO mRNAs in human skin. Sections from individual 24(21/11) were incubated with $^{35}$S-labelled antisense or sense FMO1, 3, 4, and 5 probes, as indicated. (A) Epidermis. Upper panel, antisense; lower panel, sense. (B) Sebaceous gland. Upper panel, antisense; lower panel, sense. (C) Sebaceous gland and hair follicle. Left-hand panel, antisense; right-hand panel, sense. Scale bar represents 50 $\mu$m.

(this study and [44]). Unlike *FMOs*, *CYP* expression is known to be regulated in liver by a variety of foreign chemicals. The variations in *CYP* gene expression in the skin could be due, in part, to topical or oral exposure to different chemicals.

Of the mRNAs analysed only that encoding CYP2B6 decreased in abundance in human skin as a function of age. In a study of age-related changes in CYP activities in the liver, Tanaka [52] showed that CYP3A4-associated metabolic activities decrease with age. However, the activity of CYP2B6 was not assessed by Tanaka.

FMOs and CYPs share a number of substrates in common. One of these is the organophosphorus pesticide phorate [12,53]. In mouse liver, CYPs are responsible for 68–85% of phorate sulfoxidation. In contrast, in mouse skin, FMOs account for 66–69% of this activity [53]. So, in this species, FMOs play a more important role than CYPs in the metabolism of phorate in the skin. This may be due to differences in the relative abundance of FMO and CYP isoforms in mouse skin compared to the liver. Our results show that, in contrast to the situation in human liver, in human skin mRNAs encoding FMOs 1, 3 and 5 are as abundant as those encoding CYPs 2A6 and 3A4. Therefore, in humans, the relative contribution to xenobiotic metabolism of FMOs compared to CYPs may be greater in skin than in the liver.

Differences between individuals in the expression patterns or catalytic activities of detoxification enzymes such as the FMOs and CYPs can be due to a number of factors. These include polymorphic variation or mutation in the genes encoding these proteins; exposure to foreign chemicals that induce or repress certain CYPs; or physiological substances such as hormones. Inter-individual variations in the expression of *FMOs* and *CYPs* in the skin may explain some of the adverse reactions seen on exposure to topically applied chemicals or other compounds to which the skin (or body) is exposed.

The expression of genes encoding FMOs 1, 3, 4, and 5 and CYPs 2A6, 2B6, and 3A4 is co-localized in the epidermis, sebaceous glands and hair follicles, and the corresponding proteins are therefore optimally situated to detoxify xenobiotics to which the skin is exposed. Using rat polyclonal antibodies raised against CYP1A1/A2 and CYP2B1/B2, Pendlington *et al*. [54] showed the presence of CYPs in mouse, rat and human epidermis and sebaceous glands. Weak immunoreactivity, in human sebaceous glands, was detected using an antibody to a CYP called P450hA7 [55]. However, no CYP activity was detected in human skin, [54]. The authors suggested this might be due to the lack of availability of fresh tissue for their studies.

We have shown that both CYP and FMO mRNAs are poorly maintained in keratinocyte culture. This may be a function of the culture conditions, or a consequence of the disruption of the cellular structure of the skin. The release of keratinocytes from whole skin requires harsh trypsinization and this may lead to a decrease in abundance of the FMO and CYP mRNAs investigated. In our experience, skin from donors under the age of 25 years is easier to disaggregate. Skin from young individuals is also easier to homogenize when preparing RNA. The age of the donor did not, however, correlate with loss of FMO and CYP mRNAs in cultured keratinocytes. Two CYP-related activities, ethoxycoumarin-*O*-deethylation and ethoxyresorufin-*O*-deethylation, have been detected in human keratinocyte cultures

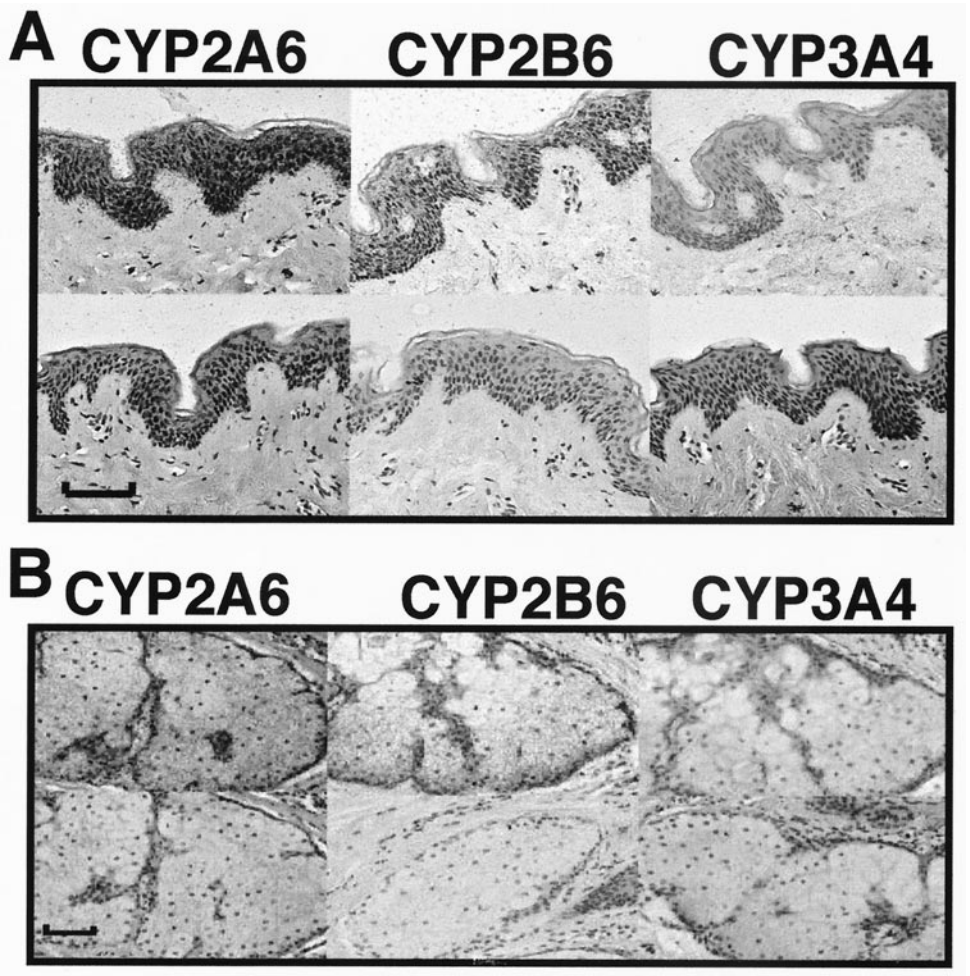


Fig. 4. *In situ* hybridization analyses of CYP mRNAs in human skin. Sections from individual 24(21/11) were incubated with $^{35}$S-labelled antisense (upper panel) or sense (lower panel) probes for CYP2A6, CYP2B6, or CYP3A4, as indicated. (A) Epidermis. (B) Sebaceous gland. Scale bar represents 20 $\mu$m.

[56]. However, neither the age of the donor nor the number of individuals investigated was reported.

In contrast to primary keratinocyte cultures, the immortalized keratinocyte cell line, HaCaT, expresses a number of FMO and CYP mRNAs in amounts similar to those found in intact human skin. In addition, the cell line is easier to culture than primary keratinocytes and is far more consistent with respect to CYP and FMO mRNA content. HaCaT cells may therefore provide a better experimental system for biotransformation studies than cultures of human keratinocytes. However, the content of FMO4 mRNA in HaCaT cells is greater than in whole skin. This may be due to the effect of a constituent of the medium in which the cells are grown or a function of the immortalized state of these cells.

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