

## Classics

⌘ Author's Choice

### Human Cytochrome P450s: The Work of Frederick Peter Guengerich

**Purification and Characterization of the Human Liver Cytochromes P-450 Involved in Debrisoquine 4-Hydroxylation and Phenacetin O-Deethylation, Two Prototypes for Genetic Polymorphism in Oxidative Drug Metabolism**

(Distlerath, L. M., Reilly, P. E. B., Martin, M. V., Davis, G. G., Wilkinson, G. R., and Guengerich, F. P. (1985) *J. Biol. Chem.* 260, 9057–9067)

**Human Liver Microsomal Cytochrome P-450 Mephenytoin 4-Hydroxylase, a Prototype of Genetic Polymorphism in Oxidative Drug Metabolism. Purification and Characterization of Two Similar Forms Involved in the Reaction**

(Shimada, T., Misono, K. S., and Guengerich, F. P. (1986) *J. Biol. Chem.* 261, 909–921)

**Characterization of Rat and Human Liver Microsomal Cytochrome P-450 Forms Involved in Nifedipine Oxidation, a Prototype for Genetic Polymorphism in Oxidative Drug Metabolism**

(Guengerich, F. P., Martin, M. V., Beaune, P. H., Kremers, P., Wolff, T., and Waxman, D. J. (1986) *J. Biol. Chem.* 261, 5051–5060)

In the 1980s, the group of Frederick Peter Guengerich at Vanderbilt University published three papers in the *Journal of Biological Chemistry* that had a major impact on the pharmaceutical industry and the field of biochemical research on cytochrome P450s. The three papers described the purification and characterization of four cytochrome P450s that metabolized specific drugs in the human liver. “Fred Guengerich was really the pioneer in understanding human P450s,” states Allan Conney at Rutgers University. The isolation of human cytochrome P450s by the Guengerich group introduced a way for the pharmaceutical industry to test drugs for human toxicity before they are developed and released into the market.

Cytochrome P450s are heme-containing enzymes that function mainly in the liver but are also present in other organs. Their job is to oxidize drugs, toxic chemicals, and endogenous molecules such as steroids. Seventy-five percent of the enzymes that break down drugs in the human body are cytochrome P450s. Five cytochrome P450s carry out 90% of the drug breakdown. Four of these five cytochrome P450s were described in this trio of papers.

Guengerich entered the world of cytochrome P450s as a postdoctoral fellow in Minor J. Coon's laboratory at University of Michigan in 1973. “I've never gotten out of the business since,” remarks Guengerich. “When I got into the game, there were some people who thought



Fred Guengerich of Vanderbilt University.

there was only one form of cytochrome P450 in an animal. Nobody really knew anything about P450s in humans.”

Researchers in the 1970s, like those in Conney’s group, started to separate multiple forms of cytochrome P450 from animals, such as rats and rabbits, demonstrating that, on the contrary, an animal could have different types of cytochrome P450. When he established his own laboratory at Vanderbilt University in 1975, Guengerich decided to work on rat cytochrome P450s. By 1982, Guengerich’s laboratory had purified nine different rat cytochrome P450s.

In the early 1980s, approximately 40% of drug candidates failed on the market because their pharmacokinetic properties were poorly appreciated in humans. “The whole area of drug metabolism in people was really pretty mysterious at that time,” says Guengerich. The pharmaceutical industry was testing drugs in animals prior to market release, but when people took the drugs, there were often some nasty surprises.

It was becoming increasingly clear to Guengerich that the human versions had to be different from animal cytochrome P450s and warranted their own study. He also knew about a clinical pharmacologist named Robert Smith at St. Mary’s Hospital Medical School in London who, along with other volunteers, had swallowed 40 mg of a hypertension drug called debrisoquine in 1975. Although the other volunteers experienced no side effects, Smith’s blood pressure dropped precipitously and remained that way for two days. Later, Smith repeated the experiment in families and identified ones whose members were unable to properly process the drug. “This may not sound like a big deal, but it really struck me. Basically, [Smith] found that some people were missing the gene related to the metabolism of a particular drug,” says Guengerich. “This meant there was a single P450 that was dominant in the metabolism of a single drug.”

So in the early 1980s, Guengerich’s group began to focus on purifying the human cytochrome P450 that was responsible for metabolizing debrisoquine. However, obtaining good quality human livers was a major stumbling block, and the Guengerich group struggled with livers obtained from autopsies. Then came a stroke of luck.

The wife of one of Guengerich’s postdoctoral fellows, Phil Wang, was a nurse at a local Nashville hospital. One day, Luke Skelley of the Nashville Regional Organ Procurement Agency was visiting the hospital administrators, and Wang’s wife mentioned to him that her husband needed good quality human livers for his research. Skelley contacted Guengerich for a collaboration. Anytime the agency found itself stranded with a donor liver for which they could not locate a recipient in time, the agency would offer the organ to the laboratory for research purposes.

With the collaboration in place, the research took off. Invariably, the call to collect the waiting liver “came in the middle of the night,” says Guengerich. “I took my turn with the rest of the people in the laboratory with being on call, cutting the liver up when it came in, and putting it away in the freezer.”

Linda Distlerath, a postdoctoral fellow, took the lead on the first JBC paper that described the cytochrome P450 that metabolized debrisoquine. The work was “very laborious for a number of reasons,” recounts Guengerich. “All the chromatography had to be done in the presence of detergents. The detergents had to be removed from each fraction before we could assay for the catalytic activity. We had to use a gas chromatography-mass spectrometry assay for testing catalytic activity. But somehow, Linda did it, and she found this protein, which we called P450<sub>DB</sub> because it metabolizes debrisoquine.”

Subsequently, P450<sub>DB</sub> became known as P4502D6. The same paper described another P450 that metabolized phenacetin, an analgesic that is not used these days since the discovery that it is carcinogenic in rodents.

Using the same experimental procedures of chromatographic separation, gel electrophoresis, amino acid composition analysis, immuno-inhibition studies, and steady-state kinetic assays, Tsutomu Shimada and others from the Guengerich laboratory went on to purify two cytochrome P450s that metabolize the anticonvulsant mephenytoin. The group also isolated the cytochrome P450 for the vasodilator nifedipine. Guengerich did the bulk of the work for that project, so he was first author on the paper.

The hard and tedious isolations carried by the Guengerich laboratory were “to a much greater degree of purity than many of the earlier attempts,” explains Conney. “Human liver is

much harder to work with than rat liver because there are smaller amounts of P450s, and you're not treating the humans with inducers to increase the levels of specific P450s."

The laborious experimental procedures done by the Guengerich laboratory to purify the cytochrome P450s are no longer done, note Guengerich and Conney, thanks to recombinant DNA technology. Researchers can just express the cytochrome P450 of their choice in bacterial or eukaryotic cell culture and use the enzyme in an assay.

Now, there are 57 known human cytochrome P450s, "and a lot of that work was done by Guengerich," says Conney. With cytochrome P450s isolated in test tubes, "you can much more critically understand the importance of these different catalytic activities," he adds.

With the mephenytoin work, Guengerich says that later research showed that the cytochrome P450s his group had isolated were only part of the story. "We thought at that time that we had the mephenytoin hydroxylase. Later work by others would show that another enzyme did that, and we were only having a little bit of activity in our fractions." As for P450<sub>NF</sub>, which metabolizes nifedipine, now known as P4503A4, "we had no idea that this one would actually wind up being the main player and work on half the drugs on the market," states Guengerich.

It also turns out P4503A4 and the cytochrome P450 that metabolizes phenacetin, P4501A2, play a major role in the bioactivation of carcinogens. "These are major areas of interest in the field of cancer research and molecular epidemiology," says Guengerich. He explains there is a possibility that the amount of some of these enzymes a person has will determine whether or not he or she is predisposed to cancer when exposed to an environmental carcinogen.

Guengerich says his body of work clearly illustrates how fundamental research can influence applied research. "I've been very fortunate that I've been able to find a niche where I can do fundamental biochemistry," he says. "I consult a fair amount in the pharmaceutical industry, and it's neat to see some of the things we did actually have a major impact on how people develop drugs."

DOI 10.1074/jbc.O112.000003

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*Rajendrani Mukhopadhyay (ASBMB's Senior Science Writer) wrote the introduction.*