THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 288, NO. 24, pp. 17063–17064, June 14, 2013

New Trends in Cytochrome P450 Research at the Half-Century Mark*

Published, JBC Papers in Press, April 30, 2013, DOI 10.1074/jbc.R113.466821

F. Peter Guengerich[1]

From the Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

Cytochrome P450 enzymes have major roles in the metabolism of steroids, drugs, carcinogens, eicosanoids, and numerous other chemicals. The P450s are collectively considered the most diverse catalysts known in biochemistry, although they operate from a basic structural fold and catalytic mechanism. The four minireviews in this thematic series deal with the unusual aspects of catalytic reactions and electron transfer pathway organization, the structural diversity of P450s, and the expanding roles of P450s in disease and medicine.

In a sense, the field of cytochrome P450 research first began in the 1940s with observations on oxygenation reactions in the metabolism of drugs, steroids, and carcinogens (1–3). Although P450 spectra had been reported in 1958 (4, 5), P450 was not really first characterized until the publication of a communication by Tsuneo Omura and Ryo Sato in *The Journal of Biological Chemistry* in 1962 (6), followed by two extensive papers in 1964 (7, 8). These pioneering studies in mammalian systems were soon complemented by work on a bacterial P450 system by Irwin C. Gunsalus and associates (9).

Having passed the 50-year anniversary of P450 last year, the field is a mature one, in a sense. Through the human genome project, we now know that humans have 57 P450 (*CYP*) genes. The genes have been organized into families (denoted by the first identification number) and subfamilies (denoted by letters). Thus, "2D6" denotes family 2, subfamily D, and individual P450 6 (genetic variants are denoted *1 ("wild type"), *2, etc.) (10). (In some cases, the same designation across species is used for a P450, but not always.) The Protein Data Bank contains nearly 500 P450 structures, including 30 different mammalian P450s (20 of the human P450s).

Most of the reactions catalyzed by P450s are mixed-function oxidation with the following general stoichiometry: $NADP(P)H + H^+ + O_2 + R \rightarrow NADP^+ + H_2O + RO$, where R is the substrate. The catalytic mechanism is understood to involve a so-called "Compound I" high-valent iron species (formally FeO^{3+}, with the iron in the Fe^{IV} state and the remaining positive charge distributed in the porphyrin ring), endogenous to that characterized in peroxidase chemistry. The elusive Compound I intermediate was elegantly described in a P450 by Rittle and Green in 2010 (11).

Today, the P450 field has made tremendous contributions in numerous areas. The understanding of P450s has revolutionized aspects of drug development and agriculture. We now understand genetic diseases in endocrinology, and P450 played a leading role in fields as diverse as pharmacogenetics, chemical carcinogenesis, molecular epidemiology, bioremediation, plant breeding, and insect control.

Nevertheless, important questions remain, and we have an opportunity to further apply our understanding of P450s to important problems. With >18,000 known P450 sequences available and the number increasingly rapidly, it is humbling to realize that we understand the functions of only a fraction of these P450s (12). The flexibility of the P450 proteins is now recognized, and prediction of catalytic activities for individual P450s is still difficult.

This thematic series consists of four minireviews (13–16), selected to present the current state of knowledge in the field of P450 and to stimulate interest in the challenges of the future.

In the first minireview (13), Andrew W. Munro and I discuss the unusual features of P450s to show the extent of variation that can be accommodated among a set of enzymes that use a rather common chemical mechanism, that of the Compound I oxidation already mentioned. Part of this minireview deals with seemingly unusual reactions, and part deals with unusual P450 systems, showing diversity in pathways of electron transfer, etc.

The second minireview in the series, written by Michael T. Green and co-workers (14), describes the long quest for the reactive intermediate in the P450 that catalyzes the oxygenation of the substrate. This field has a long history, with early hypotheses about mobile forms of reactive oxygen being involved and moving on to the concept of a high-valent iron-oxygen complex solving the problem of spin-forbidden reactions of oxygen. Recent work on the intermediate Compound I (11, 16) is discussed, including alternative proposals.

The third minireview, written by Eric F. Johnson and C. David Stout (15), addresses a different aspect, that of three-dimensional structures. As with the catalytic mechanism and the electron transfer pathways (13), there is an inherent commonality in the P450 structures but also considerable divergence in many of the details, especially in the active site. The active site volumes of human P450s vary at least 7-fold, and several have been found to contain two substrates. This diversity in the active sites is one reason for the hundreds of thousands of P450 substrates.

The fourth minireview in the series, written by Irina A. Pikuleva and Michael R. Waterman (16), deals with the roles of P450s in diseases (aside from the prominent roles for P450s in drug metabolism) (17). Numerous examples of genetic defects in the metabolism of sex steroids, glucocorticoids, cholesterol, bile acids, vitamin D, and eicosanoids are now understood in

* This work was supported, in whole or in part, by National Institutes of Health Grant R37 CA090426.

[1] To whom correspondence should be addressed. E-mail: f.guengerich@vanderbilt.edu.

the context of our knowledge of P450s. Most of these are acute or otherwise obvious maladies, and a challenge still continues to understand roles of P450 contributions to chronic diseases such as cancer and hypertension.

When Professor Ryo Sato retired in 1987, the P450 field was at the quarter-century mark, and a retirement ceremony was held in Nara, Japan, with the title "Cytochrome P450: New Trends" (18). The 25 years since then have seen an explosion of new knowledge that even those in the field really did not anticipate. What the future holds for P450 discovery in the next 25 or 50 years, with "New Trends," will undoubtedly interest and surprise us.

REFERENCES

1. Mueller, G. C., and Miller, J. A. (1948) The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. *J. Biol. Chem.* **176,** 535–544
2. Williams, R. T. (1947) *Detoxication Mechanisms*, 1st Ed., John Wiley & Sons, Inc., London
3. Ryan, K. J. (1958) Conversion of androstenedione to estrone by placental microsomes. *Biochim. Biophys. Acta* **27,** 658–669
4. Klingenberg, M. (1958) Pigments of rat liver microsomes. *Arch. Biochem. Biophys.* **75,** 376–386
5. Garfinkel, D. (1958) Studies on pig liver microsomes. I. Enzymic and pigment composition of different microsomal fractions. *Arch. Biochem. Biophys.* **77,** 493–509
6. Omura, T., and Sato, R. (1962) A new cytochrome in liver microsomes. *J. Biol. Chem.* **237,** 1375–1376
7. Omura, T., and Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239,** 2370–2378
8. Omura, T., and Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J. Biol. Chem.* **239,** 2379–2385
9. Katagiri, M., Ganguli, B. N., and Gunsalus, I. C. (1968) A soluble cytochrome P450 functional in methylene hydroxylation. *J. Biol. Chem.* **243,** 3543–3546
10. Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C., and Nebert, D. W. (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers, and nomenclature. *Pharmacogenetics* **6,** 1–42
11. Rittle, J., and Green, M. T. (2010) Cytochrome P450 compound I: capture, characterization, and C–H bond activation kinetics. *Science* **330,** 933–937
12. Guengerich, F. P., and Cheng, Q. (2011) Orphans in the human cytochrome P450 family: approaches to discovering function and relevance to pharmacology. *Pharmacol. Rev.* **63,** 684–699
13. Guengerich, F. P., and Munro, A. W. (2013) Unusual cytochrome P450 enzymes and reactions. *J. Biol. Chem.* **288,** 17065–17073
14. Krest, C. M., Onderko, E. L., Yosca, T. H., Calixto, J. C., Karp, R. F., Livada, J., Rittle, J., and Green, M. T. (2013) Reactive intermediates in cytochrome P450 catalysis. *J. Biol. Chem.* **288,** 17074–17081
15. Johnson, E. F., and Stout, C. D. (2013) Structural diversity of eukaryotic membrane cytochrome P450s. *J. Biol. Chem.* **288,** 17082–17090
16. Pikuleva, I. A., and Waterman, M. R. (2013) Cytochromes P450: roles in diseases. *J. Biol. Chem.* **288,** 17091–17098
17. Guengerich, F. P. (2005) Human cytochrome P450 enzymes. in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (Ortiz de Montellano, P. R., ed) pp. 377–530, 3rd Ed., Kluwer Academic/Plenum Press, New York
18. Sato, R., Omura, T., Imai, Y., and Fujii-Kuriyama, Y. (eds) (1987) *Cytochrome P-450: New Trends: Yamada Conference XVII, Nara Hotel, Nara, Japan, May 11–13, 1987*, Yamada Science Foundation, Nara, Japan