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THIRD INTERNATIONAL SYMPOSIUM:
CYTOCHROME P450 BIODIVERSITY

Final Report

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Abstract

The Symposium was held on October 8-12, 1995 at the Marine Biological Laboratory in Woods Hole Massachusetts. Other international symposia promote cytochrome P450 research but have a primary focus on mammalian systems. This symposium is exclusively devoted to research in other organisms, and major topics reflect the distribution and dominance of non-mammalian species in the biosphere.

The five sessions focused on basic mechanism, regulation, biodiversity, host-parasite interactions, and practical applications. 170 Scientists contributed 38 oral presentations and 91 posters, with a truly international composition of the symposium. Practical applications were a recurring feature, linking reports on mechanism and regulation to studies on the engineering of substrate specificity, microorganisms to degrade halogenated hydrocarbons and herbicides, and the production of in vitro P450 electrochemical bioreactors.

At the time of the symposium there were 477 cytochrome P450 sequences in the database. Expansion of the known plant P450 genes was reported, with 20 new plant P450 families added in the last 3 years. Of these only 5 families have a physiological function associated with them. A growing number of identified invertebrate P450s was documented, where in insects, the forms identified are primarily involved in inducible xenobiotic metabolism and detoxification of toxic plant substances.

Report of Third International Symposium: Cytochrome P450 Biodiversity, held at Woods Hole MA October 8-12, 1995 with funding support from U. S. Department of Energy grant DE-FG02-95ER61969.

Background

Cytochromes P450 are a superfamily of heme-containing enzymes with a wide range of monooxygenase activities that make them critically important in virtually all life forms. Their importance in human health has resulted in a pronounced research emphasis upon mammalian forms of these proteins and their structural genes.

However, it is evident that essential P450s in plants, microbes, insects and other non-mammalian forms participate in novel functions and pathways unique to these organisms. P450s in these systems have become the objects of bioengineering as elegant regio- and stereoselective catalysts for fine organic synthesis, as key enzymes of crop improvement, as targets of antifungal agents for both plant and human pathogens, and as enzymes that function in development and differentiation and in the biodegradation of organic pollutants.

Five years ago scientists in Central and Western Europe active in microbial biotechnology organized a symposium to provide a new forum on P450 research (First International Symposium on Cytochromes P450 of Microorganisms), held in July 1991 in Berlin, Germany. Japan, with its historical strengths in research on P450s and on bacterial and fungal fermentation, hosted the second symposium in Tokyo in June 1993, and expanded the program to address plant P450 research. Whereas other international symposia promote cytochrome P450 research with a primary focus on mammalian systems, this symposium series is devoted to research in other organisms, and major topics reflect the distribution and dominance of non-mammalian species in the biosphere.

Third International Symposium

A goal of this symposium was to provide the first convenient opportunity for both established and for more junior level U.S. scientists to engage in international discussion of data on non-mammalian P450 research. The program addressed the continuing expansion of basic research and biotechnology involving P450s of microbes and plants, and included new findings on P450s of insects and other non-mammalian life forms, under its title Cytochrome P450 Biodiversity. It was held at the Swope Conference Center at the Marine Biological Laboratory, Woods Hole, MA October 8-12, 1995, with support from U.S. Department of Energy, U.S.

National Institute of Environmental Health Sciences, U.S. Department of Agriculture, University of Cincinnati, Purdue University, The DuPont Company, Roussel UCLAF, Pioneer Hi-Bred International, American Cyanamid Company, Ciba Geigy Corporation, DowElanco, Sandoz Agro Inc.

National and international leaders in their respective aspects of P450 research and biotechnology assembled with a cross-section of young scientists from the United States, Canada and Mexico, and overseas. The group included 15 U.S. students who received support from the Young Scientist Awards committee, based upon the committee's evaluation of submitted abstracts and supporting letters. The 170 scientists in attendance contributed 38 oral presentations and 91 posters in the symposium.

The five sessions focused on basic mechanism, regulation, biodiversity, host-parasite interactions, and practical applications. In fact practical applications were a recurring feature, linking reports on mechanism and regulation to studies on the engineering of substrate specificity, microorganisms to degrade halogenated hydrocarbons and herbicides, and the production of in vitro P450 electrochemical bioreactors.

At the time of the symposium there were 477 cytochrome P450 sequences in the database. Expansion of the known plant P450 genes was reported, with 20 new plant P450 families added in the last 3 years. Of these only 5 families have a physiological function associated with them (F. Durst, CNRS, Strasburg). A growing number of identified invertebrate P450s was documented, where in insects, the forms identified are primarily involved in inducible xenobiotic metabolism and detoxification of toxic plant substances (J. Fogelman, University of Denver; R. Feyereisen, University of Arizona; M. Schuler and M. Berenbaum, University of Illinois).

Another feature is the number of new and unusual forms of cytochrome P450 that have been found. Indeed most generalizations about a highly conserved feature of this superfamily would lead to a discussion of the exceptions at this conference. In some cases, such as with bacterial P450BM3, the unusual aspects of a P450 come under such intense scrutiny that the structural features and regulatory aspects become an archetype for more "normal" members of the superfamily (J. Peterson, Dallas; A. Fulco, UCLA). In the case of P450_{tyr} from sorghum, a new protein has been found which catalyzes multiple steps in the biosynthetic pathway of the cyanogenic glycoside, dhurrin (B. Halkier, Copenhagen, Denmark). Here, a seemingly complex process is simplified by finding a single catalyst for several steps. Similar cases of proteins which seem to make complex reactions simple are found in fungi: the CYP56 involved in the biosynthesis of dityrosine in *Saccharomyces cerevisiae* (M. Breitenbach, Salzburg, Austria), and the alkane oxidizing CYP52A3 can oxidize a hydrocarbon to a dicarboxylic acid (W.-H. Schunck, Berlin). Regulation of CYP52 gene expression in *Candida maltosa* is controlled by a recognizable 90 bp alkane responsive region of the promoter (M. Takagi, University of Tokyo, Japan). Other cases of novel P450s from the plant kingdom came from R. Backhaus (Arizona State University) with an allene oxide synthase P450 being an unexplained but integral component of *Guayule*

rubber particles, and the description by M. Zenk and T. Kutchan (University of Munich) of the plant P450s involved in formation of methylenedioxy bridges, normally associated with classical P450 inhibitors like piperonyl butoxide.

In contrast to some of the extremely novel P450s found in the biosphere, at least one, the sterol 14- α -demethylase seems to be found in nearly all eukaryotic species (Y. Yoshida, Mukogawa Women's University, Japan). This enzyme is most commonly studied in fungi as the target site for a wide range of inhibitors used both as drugs and agricultural chemicals (S.L. Kelly, Sheffield University, UK). Fungi constitute a major group of organisms that are the topic of discussion at this conference. Eukaryotic microorganisms such as these yeast and fungi provide a distinct advantage for P450 studies; they contain a diverse number of P450-mediated reactions as found in other eukaryotes, and they retain the relative simplicity of study found in microorganisms. Thus it is possible to study the host-pathogen interactions with members of the CYP57 family which are responsible for pisatin demethylation and consequent virulence of *Nectria haematococca* (H. vanEtten, Arizona). Filamentous fungi have novel functioning P450s as well, including the prokaryote-like P450nor from *Fusarium oxysporum* (H. Shoun, Tsukuba, Japan), which functions as a nitric oxide reductase with no accessory electron transfer proteins.

S.G. Sligar (University of Illinois), R. L. Ornstein (Pacific Northwest Laboratory), and W.L. Alworth (Tulane University) spoke about efforts to engineer substrate specificity into bacterial P450s, and T. Shimizu (Tohoku University, Japan) and I. Nagy (Catholic University Leuven, Belgium) described microorganisms with P450 capacities to degrade halogenated hydrocarbons and atrazine, respectively. P450s also have a major impact in pesticide resistance and papers in this session revealed a growing understanding of the genetics of herbicide resistance in maize (Michael Barrett, University of Kentucky), and biotechnological approaches to identifying the P450 genes involved (Danielle Werck-Reichhart, Strasbourg, France), and producing herbicide-resistant crops (H. Ohkawa, Kobe University, Japan). Similar advances have been made in insect studies, and Jeffrey Scott (Cornell University) described the CYP6D1 gene from housefly which is responsible for pyrethroid resistance.

Electron transfer from putidaredoxin to P450CAM is strongly influenced by alteration of the heme redox potential, as discussed by Y. Ishimura (Keio University, Japan). The coupling of oxygen reduction and substrate hydroxylation was the topic of a talk by John Dawson (University of S. Carolina). Denis Pompon (CNRS, Gif-sur-Yvette, France) discussed recognition rules which govern the interaction between eukaryotic P450s, reductase, and cytochrome b5 in his studies of heterologous expression in yeast. Ron Estabrook (Dallas) described the progress towards a P450 electrochemical bioreactor in his laboratory using a fused reductase - P450BM3 and a novel redox mediator. In principle, this could be used either as a bioreactor or as a detector element on a biosensor. Introducing a petunia flavonoid 3',5'-hydroxylase gene into carnations is an approach to redirect flower pigment biosynthesis to produce genetically engineered novel flower colors (T. Holton, Florigene Pty Ltd, Australia). The problems in the P450 mediated reactions facing the industrial production of steroids were introduced by M. Spagnoli (Roussel UCLAF, France).

The above material was provided to scientific organizations and societies for use in their news letters as a meeting report. Coverage as it ran in the Current Topics section of the ASM News is appended to this report.

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