The Cytochrome P450's and Their Genes

Cloning of the genes for the P450 enzymes may help to explain genetic differences in susceptibilities to cancer and toxic chemicals

The heme-containing enzymes known as the cytochrome P450's are a major component of the defenses that protect living organisms against the toxic chemicals in their environment. These include not just man-made chemicals, which arrived only recently on the evolutionary scene, but also those in natural foods, in waste and putrefaction products, and in smoke from grass and forest fires.

The P450 enzymes add oxygen to a wide range of compounds. They are needed for the synthesis of endogenous biologically active agents, including steroid hormones and the prostaglandins, but also work on foreign chemicals, helping to convert them to a form that can be more readily excreted from the body. The P450 system is as important in defending the body against foreign chemicals as the immune system is in dealing with foreign pathogens. Paradoxically, however, some of the foreign chemicals, which are initially not capable of causing cancer, are converted to active carcinogens by P450-catalzyed reactions.

Genetically determined differences in the production or activity of P450 enzymes can thus influence individual susceptibilities to cancer and also to adverse drug reactions. In addition, a defect in one of the genes has recently been linked to a severe genetic disease, congenital adrenal hyperplasia, in which production of the steroid hormone cortisol is deficient. Defects in other P450 genes might also cause steroid hormone deficiencies. Consequently, a better understanding of the molecular biology of the P450 system may be of practical value for identifying individuals at increased risk of developing cancer or experiencing dangerous drug reactions. It may also aid in the diagnosis and treatment of genetic disorders.

Not surprisingly then, the cloning of P450 genes has become a growth industry, especially during the past year or two. This research was the topic of the first "International Workshop on the P450 Genes and Their Regulation," which was held last month at Airlie House.*

One overwhelming impression conveyed by the workshop is that there are many P450 genes. In a fashion reminiscent of the genes of the immune system, the P450 genes constitute a "superfamily," so far including five different families. Additional families are likely. "How many other families we are going to find is not clear, but we are speaking of 30 to 200 genes in the superfamily at this time," explains Daniel Nebert of the National Institute of Child Health and Human Development (NICHD), who organized the workshop with Milton Adesnik of New York University School of Medicine and Yoshiaki Fujii-Kuriyami of the Japanese Foundation for Cancer Research in Tokyo. This diversity of enzymes having different, but sometimes overlapping specificities, helps the organism to deal with so many different foreign chemicals, just as the immune system may handle a virtually unlimited number of foreign substances.

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Much of the discussion at Airlie centered on how the multiplicity of P450 enzymes might have arisen. A few years ago, Nebert suggested that the enzyme diversity might be generated by some of the same strategies used to generate diversity in the immune system. This includes, among other things, the assembly of a single complete antibody gene by joining three or four separate DNA segments.

Superficially at least, the P450's show a structural resemblance to antibody proteins. For example, investigators from several laboratories have determined the nucleotide sequences of a number of genes from the phenobarbital-inducible P450 family of the rat. This is one of the larger P450 families; it includes some 20 genes divided among two subfamilies. The original members of the family, as the name suggests, were identified because they are turned on by the drug phenobarbital.

Adesnik points out that the phenobarbital-inducible P450's, like antibody proteins, appear to consist of variable and constant regions. However, there is no evidence that the P450 enzymes are encoded, as antibodies are, by separate variable and constant gene segments that must eventually be joined to form a complete coding sequence. Each P450 enzyme appears to have its own complete gene. "It was a provocative, testable hypothesis," Nebert says of his proposal, "but now we know that is unequivocably wrong."

Nevertheless, the formation of the P450 variants may resemble that of antibody and other immune molecules in one way. Adesnik presented evidence suggesting that gene conversion may have contributed to the evolution of the multiple phenobarbital-inducible genes. Gene conversion involves the replacement of a DNA segment from one gene with a sequence from another. It can lead to the maintenance of multiple copies of similar gene sequences, and Adesnik suggests that this type of gene conversion may have produced the constant region of the phenobarbital-inducible P450's.

However, gene conversion, by introducing new DNA segments into genes, can also generate diversity, which apparently occurs in antibody and other immune system genes. Adesnik also has evidence that this type of gene conversion contributed to the diversity in one of the phenobarbital-inducible subfamilies. "I propose that gene conversion has markedly speeded up the evolution of the P450 genes," he says. "A P450 subfamily may be more able to withstand change, because other members can take up the slack."

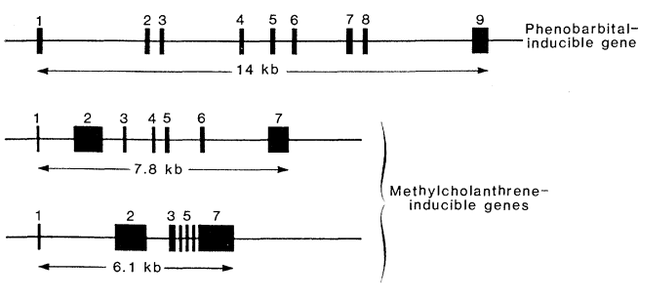
There was also a great deal of discussion at the Airlie workshop about whether the various P450 gene families evolved from the same ancestral gene or from separate, unrelated ancestral genes. The data available so far are apparently contradictory on this issue. Comparison of the amino acid sequences of phenobarbital- and methylcholanthrene-inducible enzymes shows sufficient similarity to suggest that the same ancestral gene

*The workshop was sponsored by the National Institute of Child Health and Human Development and held in Airlie, Virginia, from 31 March to 3 April.

gave rise to both families, Fujii-Kurijami points out, but the intron-exon patterns of the two types of genes are different enough to indicate that they evolved from separate ancestors. Sequences of genes from more species and P450 families may help to resolve the issue.

Control of P450 gene expression is another current topic of interest. In particular, the regulation of the methylcholanthrene-inducible enzymes is attracting attention because a high production of these enzymes may contribute to cancer development.

Among the compounds that induce members of this family is dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), which cooperates with other chemicals to produce cancer in experimental animals. The dioxin-inducible P450 gene appears to encode the enzyme known as aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH), Nebert reported. Epidemiological studies suggest that people who produce large amounts of AHH in response to inducing chemicals are at increased risk of developing lung cancer. The risk is especially great for people who smoke cigarettes. The hypothesis is that AHH converts aryl hydrocarbons, which are inhaled with the cigarette smoke, to active carcinogens. The degree of inducibility of the enzyme is genetically determined.



Exon-intron patterns for P450 genes from two different families. The heavy bars represent the protein-coding exons.

Work from Nebert's laboratory and from that of James Whitlock, Jr., of Stanford University School of Medicine shows that the control of the dioxin-inducible enzyme is complex, involving negative as well as positive regulatory sites. Chemical inducers, such as dioxin, diffuse through the outer cell membrane and combine with specific receptors in the cytoplasm. The dioxin-receptor complex then moves into the nucleus and binds to the gene's positive control site. In addition, there appears to be a short-lived protein that binds to a negative regulatory sequence and represses the activity of the gene.

The genetic explanation for the individual variations in the responses of the dioxin-inducible enzyme to activating chemicals is currently unknown, but is being avidly sought because it would help to explain why some individuals are more prone to developing cancer than others. It might also lead to tests for identifying high-risk individuals, who could then take steps to minimize their exposure to carcinogens—by not smoking, for example.

In addition, genetically determined deficiencies in one or another of the P450 enzymes have been linked to adverse, sometimes life-threatening, reactions to therapeutic drugs. A way of identifying the individuals who are likely to have such a reaction because they are unable to make a P450 enzyme or make one that is defective would also be desirable.

The availability of the cloned P450 genes is helping investigators to approach these problems. Differences in the activity or inducibility of a P450 enzyme may reflect variations in the nucleotide sequences of the gene or its control regions. If that is the case, then the variations might be detectable with restriction enzymes that split DNA at specific sites. The cloned genes are needed to identify the fragments.

The Nebert group has done preliminary experiments of this type with the human dioxin-inducible gene. Although they were unable to detect any consistent differences in the fragment patterns produced by digesting high-inducible genes and those produced by digesting low-inducible genes, the total number of samples was small and more work will be needed to confirm this result.

However, the variations in gene expression need not be the result of alterations in the P450 gene itself. They might instead result from changes in regulatory genes, such as those coding for the AHH receptor or the repressor protein of the dioxin-inducible gene. In fact, Eric Johnson of the Research Institute of Scripps Clinic presented results at the workshop that suggest that altered control might be the cause of the genetically determined variations in the production by rabbits of 21-hydroxylase, a P450 enzyme needed for the synthesis of the steroid hormone cortisol.

A 21-hydroxylase gene deficiency in humans has recently been linked by Perrin White and his colleagues at Memorial Sloan-Kettering Cancer Center and Cornell University Medical Center to congenital adrenal hyperplasia, a genetic disease that afflicts approximately one infant in every 5000 births. Identification of the genetic defect of this condition should lead to improved prenatal diagnosis and therapy. Severely affected infants can die of salt loss if not treated promptly with the hormone and masculinization of females is common. However, treatment of the mother with the synthetic steroid dexamethasone can prevent these abnormalities.

White and his colleagues find that there are two 21-hydroxylase genes in the human genome, each of which is located just to the right of a complement gene in the major histocompatibility complex (MHC), which encodes many of the genes needed for immune cell function. Their evidence indicates that only one of the two 21-hydroxylase genes is active in cortisol synthesis. Its deletion gives rise to a severe form of congenital adrenal hyperplasia. The adjacent complement gene may also be lost in these patients. However, not all of the patients have the 21-hydroxylase gene deletion. They may have a less extensive defect. The second 21-hydroxylase gene appears to be inactive in humans.

A similar arrangement of 21-hydroxylase and complement genes is also seen in the mouse MHC, according to Jonathan Seidman of Harvard Medical School, and in the bovine MHC, according to Walter Miller of the University of California School of Medicine in San Francisco. A gene duplication, occurring before these mammalian species diverged, presumably gave rise to this pattern. Why two such dissimilar genes as those coding for complement proteins and 21-hydroxylase are so closely linked is unclear.

Finally, Yoshiyasu Yabusaki of Sumitoma Chemical Company, Ltd., in Hyogo, Japan, reported that a rat P450 gene could be introduced into yeast cells where it directs the synthesis of a functional enzyme product. And Benjamin Unger of the University of Illinois has obtained expression in the bacterium *Escherichia coli* of a P450 gene from another bacterium. Workshop participants were enthusiastic about these developments because they make it possible to produce P450 enzymes that have been altered by specific gene mutations. In this way the contributions of the various portions of a P450 molecule to its enzymatic activity and other properties can be evaluated. —**JEAN L. MARX**