Introduction

K. RUCKPAUL and H. REIN

Scientific knowledge — as a simplified rule — passes from the discovery of a new phenomenon (fact, subject, reaction) through its disintegrative analytical phase, to the integration of resolved detailed data and on into a more or less reconstituted system of mutual relationships. These general characteristics may similarly refer to cytochrome P-450 research which started about thirty years ago. Now it is one of the most intensively studied enzymes. In the last decade alone, more than 10,000 papers relating to cytochrome P-450 have been published. This abundance of information requires a comprehensive review to condense the data to the very essentials.

In the beginning, cytochrome P-450 investigations mainly dealt with physiological, pharmacological and toxicological aspects. These comprised studies on its occurrence in different species and tissues together with analyses of its localization within the cell. Finally, examinations of its implications in detoxifying and toxifying processes, including the response to inducers and the quantitation of the degree of induction.

The analytical phase is characterized by the isolation and purification of the essential components of the different cytochrome P-450 systems, and the determination of their molecular weights, their spectral and other physicochemical properties. It is further characterized by studies on the broad substrate specificity of the catalytic activity and the analysis of the different pathways of oxygen activation. This period led to the recognition of the reaction cycle and the detection of the functional versatility and the structural multiplicity including sequencing of the primary structure of cytochrome P-450. The full-length (or nearly full-length) cDNA and/or amino acid sequences for about 80 gene products have currently been analysed. One of the high-lights in this period was the analysis of the 3-dimensional structure of cytochrome P-450cam in 1985.

The phase of a comprehensive treatise is characterized by an evaluation of accumulated analytical data, the integration of which permits deeper insights into the complex relationships which constitute the structural and more so the functional integrity of cytochrome P-450 systems. Molecular biology and its novel techniques have become of special importance by accelerating the

process, thus enabling the alignment of different complete primary structures and the quantitation of overall homologies among these structures resulting in the statement that cytochromes P-450 exhibit a gene superfamily which — dependent on the inducer — can be divided into families and subfamilies. In addition, new experimental approaches have become available to investigate comparative and integrative considerations at different levels of integration.

Problems inherent to this stage include:

- which structural features of the multitude of compounds that interact with cytochromes P-450 determine their capability to control oxygenatic and oxidative pathways of the enzyme?
- how is the catalytic activity of the complex enzyme system regulated?
- at what level of cytochrome P-450 biosynthesis is the induction process regulated in dependence on different inducers — and what consequences result with regard to active interference?
- to what extent is hormonal control of importance for the induction of sex specific cytochromes P-450, what is triggering that process and by what route is it accomplished?

The first volume of 'Frontiers in Biotransformation', following the considerations outlined above, is intended to review selected topics which all contribute to the concept of 'Regulation'.

Regulation is conceivable at different levels of integration. At the molecular level interactions can be amended by low molecular effectors (substrate, co-substrate) and by high molecular effectors (reductase, cytochrome b_5). The sum of the interactions results in changes of either conformation, redox behaviour and/or turnover rates of cytochrome P-450. Due to the enzyme's membrane bound character the surrounding phospholipids provide a matrix which itself has been proposed as regulating enzymatic activity by affecting the e^- -transfer through influencing the stability of the cytochrome P-450/reductase-complex and the organization of the enzyme system within the matrix. Finally, the physiologically most important regulation proceeds at the cellular level. Induction, inducibility as well as sex, species and tissue specificity determine via the isozymic distribution and the enzyme concentration the extent of toxification and detoxification.

A reliable comprehensive review naturally requires much accumulated data. Looking at the current state-of-the-art for various cytochromes P-450 it can be seen that the level of knowledge achieved to date differs greatly. As compared to the remarkable progress in the elucidation of structural and functional details in cytochrome P-450cam and the mammalian cytochromes P-450, the analytical period of microbial and plant cytochromes has just begun. This implies that a sufficient amount of data necessary for a comprehensive review of those cytochrome P-450 systems is still missing.

Due to its pharmacological and toxicological origin it is not surprising that the liver system and especially that of the endoplasmic reticulum has been most

intensively studied. Consequently most papers published so far are concerned with this system. That emphasis is likewise reflected in the articles of volume 1. The concentration on the endoplasmic system, however, implies correspondingly less research on other systems. An extrapolation of mechanisms found valid for one distinct system has therefore to be carefully carried out taking into account the biochemical diversity of cytochrome P-450 dependent monooxygenatic systems so far characterized (soluble — membrane bound; endoplasmic — mitochondrial).

The present volume is introduced by an article which is mainly concerned with regulation of the liver exoplasmic cytochrome P-450 LM2 (cytochrome P-450 II B1 according to recently published recommendations for a unified nomenclature). Several considerations favour this chapter as an introductory one. (1) Studies on an isolated cytochrome P-450 isozyme which represents a simplified system are suited to the elucidation of principle mechanisms of action and regulation. (2) A remarkable amount of data on cytochrome P-450 II B1 has been accumulated which provides a reliable basis for such a review. (3) Cytochrome P-450 II B1, due to its involvement in biotransformation reactions of a huge number of exogenous substrates, drugs and environmental contaminants, is of high relevance with regard to pharmacological and toxicological implications.

In the following articles the scope outlined above is detailed, starting with a comprehensive contribution on model systems in chapter 2. In general, utilization of models has proved an appropriate means to convert complex biological systems into relevant subjects which can be experimentally examined. Therefore, metalloporphyrins are surveyed, the aim being to study the physicochemical, geometrical and electronic requirements for a catalytically-active metalloporphyrin and to derive conclusions on the chemical reactivity and functional diversity of the heme group in the enzyme. Moreover, the studies on models of cytochrome P-450 also represent a possible line which may initiate future developments of engineered synthetic enzymes having optimized functions.

A comprehensive review which is aimed at elucidating regulation phenomena has to consider the structural suppositions which are necessary for the catalytic function. Therefore, structural multiplicity which at least in part originates from the existence of isozymes is an essential for understanding the functional versatility and — based on these structure/function-relationships — to understand further the molecular basis of regulation. The data accumulated so far are summarized in chapter 3.

Cytochrome P-450-catalyzed reactions depend on the enzyme's capability to activate and split molecular oxygen by insertion of electrons. This unique function brings about functional versatility in that the insertion of a different number of electrons leads to differential degrees of oxygen activation, generating either an oxygenatic or an oxidative pathway. Due to their reactivity, uncoupled activated oxygen species are of toxicological importance. Besides direct toxicological effects on cytochrome P-450, the formation of activated

oxygen species represents an important side effect of monooxygenatic reactions, the regulation of which is discussed in chapter 4.

A more complex mechanism of cytochrome P-450 regulation proceeds at the cellular level. It is hormonal in nature and involved in sexually differentiated steroid drug metabolism. Great efforts in the last 5 years have revealed an immense progress in insight, not only into the molecular basis of the sex-specific isozymes but also into the regulation of their biosynthesis. The detailed knowledge about that differentiation may be assumed to have practical pharmacological consequences on sex-differentiated biotransformation recativities in the near future (chapter 5).

Molecular biology and especially genetic engineering will allow not only new experimental approaches to the analysis of structure/function relationships by site directed mutagenesis but also the construction of arteficial (e.g. chimeric) cytochromes P-450. By way of these new techniques the molecular basis of genetic relatedness of structural multiplicity and the mechanisms of its regulation have become available. Further these investigations include studies on the phylogenetic relations of evolutionarily distant cytochromes P-450. Based on the results derived therefrom, their application to clinical problems may become relevant.

The articles of this volume, being mainly concerned with endoplasmic cytochrome P-450, present comprehensive information on the principles of regulation. Therefore they contribute knowledge and strategies for integrational treatment of cytochrome P-450 systems outside this selection. Following these lines, data will be elaborated which may prove the general validity of the outlined regulation principles as challenging task for future progress.