Molecular mechanisms of cytochrome P-450 gene regulation

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The role of the mixed function oxidases in cellular metabolism has been studied for more than 30 years. Recently, the use of cDNA cloning and the techniques of molecular biology have resulted in major advances in our understanding of this multi-gene superfamily of isoenzymes. The molecular biology of the cytochromes P-450 has been extensively reviewed [1-4] and aspects of their regulation discussed. As might be expected in a gene superfamily of this size, a variety of regulatory strategies has been observed. This is not surprising when one considers both the tissue specificity of expression shown by most of the cytochrome P450 genes, and the fact that, in many instances, expression is inducible by endogenous and/or exogenous molecules. However, for the non-expert, this variety of levels of regulation can be confusing, especially if the topic is reviewed from the substrate, inducer or gene classification point of view.

This short review, therefore, describes the various levels at which regulation of gene expression can take place in eukaryotes and indicates how the control of cytochrome P450 genes fits in with this general scheme.

The basics of gene expression

DNA in the nucleus of a eukaryotic cell is found in the form of chromatin, in which both histone and non-histone proteins are bound to the genetic material. For a gene to be available for expression, it must be in the activated form available to make mRNA when transcription can take place. The transcribing molecule, RNA polymerase, is then able to synthesize a pre-mRNA copy of the gene, including both the coding (exon) and non-coding (intron) regions. (The exon-intron structure of the gene is the result of gene building during evolution, the exons often coding for different functional domains in the apoprotein.) The pre-mRNA is then processed by a nuclear-splicing mechanism so that the introns are removed and the exons joined together to produce the mature mRNA molecule. This is then transported to the cytoplasm where it is translated by ribosomes into the polypeptide apoprotein. This is usually modified to produce active enzyme which is then targeted to its functional site within the cell. Over the last 10 years, the study of gene regulation in many systems has demonstrated that virtually any of the stages of gene expression can be a site of regulatory control.

Abbreviations used: TCDD, 2,3,7,8-tetrachlorobenz[alpha]-dioxin; Ah, aromatic hydrocarbon.

Gene structure and polymorphism

Quite clearly, an inherited deletion or mutation in a gene can lead to the production of an aberrant mRNA or a defective protein. Such inherited differences between individuals are termed polymorphisms and are of great interest as they may reflect the basis of inter-individual differences in drug or xenobiotic metabolism. Polymorphism may also occur in the control DNA region flanking the gene and result in alteration in the basal or induced rate of mRNA synthesis. A number of rodent polymorphisms have been described [4] and human polymorphisms have been reported in the P450IC, IID, IIIA and XXI subfamilies [5-9]. Polymorphism has also been demonstrated at the aromatic hydrocarbon (Ah) receptor locus in rodents and correlated with differences in IA1 inducibility [2].

Gene activation

The processes of determination and differentiation during the development of an organism involve the activation of genes by the unwinding of condensed, inactive chromatin. This is brought about by a combination of gene-specific demethylation events and the selective binding of activating proteins to the DNA. Thus, while all the cells in an organism will contain the information for the synthesis of the cytochromes P-450, only in some cells will the various genes be in an activated form available to make mRNA when required. To date, developmental stage-specific gene activation has only been clearly demonstrated for rat IIE1 [10], demethylation occurring shortly after birth in the neonate. The possibility that sex, tissue or developmental stage-specific expression of other constitutive or inducible forms of cytochrome P-450 is also controlled at this level, has yet to be demonstrated.

Transcriptional control

Regulation at the level of mRNA synthesis is dependent on two factors: the presence of cis-acting regulatory DNA sequences adjacent to or within the gene, and the synthesis or activation of gene-specific DNA-binding proteins. The DNA-binding proteins dock specifically with their target regulatory DNA sequences and facilitate the entry of RNA polymerase molecules into the mRNA promoter site of the gene. These DNA-binding proteins, or trans-acting factors as they are sometimes known, are the ultimate effectors in a wide variety of regulatory cascades controlling cellular mRNA synthesis. They are present in both the nucleus and cytoplasm and can be activated by ligand binding (drugs or hormones), by phosphorylation (second messenger signalling systems) or, in some instances, by metabolite-induced dimerization/dis-
association [11]. A clear example in the cytochrome P-450 system is the induction of IIA1 by binding of the inducer -Ah receptor complex to an enhancer sequence in the DNA 5' to the gene [12]. However, conclusive proof in the case of other P450 genes, e.g. induction of IIA1 by steroid receptor binding or XIAl induction by binding of cyclic AMP-activated 'steroid hydroxylase inducing protein' [13], has yet to be provided. The presence of a drug-binding receptor mechanism for induction of IVA1 by hypolipidaemic drugs such as clofibrate [14] is also, as yet, unproven. Indeed, it is more likely that cytochromes P-450, such as IVA1, which play a role in intermediary metabolism, are controlled via the same second messenger cascade system as other enzymes in the same metabolic pathway.

Post-transcriptional regulation

Included in this level of control are mechanisms which affect the splicing, transport, stability and translatability of mRNA. Alternative splicing, in particular, has been shown in other systems to be a major strategy by which different mRNAs, with different coding information, stability or translatability, can be derived from the same pre-mRNA primary transcript. In the cytochromes P-450, post-transcriptional mechanisms have been reported to operate in the induction of IAA2 (and possibly IAI) by 3-methylcholanthrene or other systems to be a major strategy by which different mechanisms have been reported to operate in the induction of IIA2 (and possibly IIA1) by troleandomycin or IIB1/2 by hypolipidaemic drugs such as clofibrate. Alternative splicing, in particular, has been shown to provide. The presence of a drug-binding receptor mechanism for induction of IVA1 by hypolipidaemic drugs such as clofibrate [14] is also, as yet, unproven. Indeed, it is more likely that cytochromes P-450, such as IVA1, which play a role in intermediary metabolism, are controlled via the same second messenger cascade system as other enzymes in the same metabolic pathway.

Post-translational control

Regulation of cytochrome P-450 activity at the level of enzyme activity or stability has been shown to operate in various systems. The availability of haem is clearly an important factor and haem synthesis is readily induced following administration of TCD2 [20] or phenobarbital [21]. However, the haem effect is complex, as haem itself may play a role in the transcriptional regulation of some P450 genes [22]. The role of small molecules such as ethanol, acetone or pyrazole in the induction of IIE1 is more clear. Here, there appears to be a specific stabilization of active enzyme protein without any changes in IIE1 mRNA levels [18]. Evidence from experiments in cultured hepatocytes indicates that binding of inducer results in protection of the enzyme and a major increase in half-life [23]. To date, this is the most clear-cut example of post-translational control in the cytochromes P-450. The role of phosphorylation by protein kinase A in the regulation of cytochrome P-450 activity is less clear [24]. Phosphorylation of serine at a conserved target site, ArgArgXaaSerLeu, leads to the inactivation of certain forms including IIB1, IIC2, IIE1 and CI. This cyclic AMP-dependent phosphorylation is competitively inhibited by cytochrome b5 [25]. It is possible that this negative post-translational regulation is involved in balancing the components of the P-450 complex to maximize the number of active substrate-binding complexes.

Conclusions

Application of the techniques of molecular biology and genetic engineering to the study of the mixed-function oxidases has in recent years led to new insights into the mechanism of their regulation. Not surprisingly, in view of the large number of isoenzymes in the gene superfamily, their evolutionary history and their wide range of functions, different genes employ different regulatory strategies. Indeed, the gene for an individual isoenzyme may display more than one type of regulation if the gene is expressed at both basal and induced levels, or if both endogenous and exogenous substrates can be metabolized. Further studies at the molecular level will help to unravel the complexities of regulation in this superfamily of genes and enable us to understand the interplay of the various regulatory mechanisms in the whole organism. Studies on the differences between individuals and between species will help in the prediction of abnormal drug/ xenobiotic reactions, and assist in a rational approach to regulatory decisions. However, there is still much to learn about the importance of superfamily of genes, and probably many surprises remain in store.

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Induction of cytochrome P 450 I and its influences in chemical carcinogenesis

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Man is exposed daily to a plethora of structurally diverse chemicals, many of which induce tumours in animals and, at least some of these, are likely to be carcinogenic to man. The vast majority of these are chemically inert and indeed in systems in vitro they fail to elicit any toxic response. How can these chemically unreactive compounds manifest a carcinogenic response?

Role of metabolism in chemical carcinogenicity

Although inactive per se, chemical carcinogens may be rendered highly reactive through metabolism, largely oxygenation. This activation process generates electrophilic intermediates which readily interact with DNA, initiating the series of events that leads to the formation of tumours. Alternatively, these reactive intermediates may act as radical generators through interaction with oxygen, producing reactive oxygen species which are also capable of causing DNA damage. Metabolites, rather than the parent compound itself are, therefore, responsible for the carcinogenicity of most chemicals.

The activation of a chemical may involve just a single metabolic step, but, more commonly, it is a multistep procedure. The inactive parent compound, termed a precursor, is metabolized to form the proximate carcinogen which, although more reactive than the precursor, is not the entity that readily interacts with DNA. It is the ultimate carcinogen, resulting from further metabolism of the proximate carcinogen, that is responsible for the genotoxicity of the chemical. The precarcinogen is simultaneously converted to more polar, inactive metabolites which are readily excreted following conjugation with endogenous substrates such as glucuronic acid. For example, the carcinogenic aromatic amine 4-aminobiphenyl is activated by N-hydroxylation to the mutagenic hydroxyamine, the proximate carcinogen. However, it also undergoes ring-hydroxylation to yield a number of phenols, which not only are inactive per se, but also cannot be activated through further metabolism, and are thus detoxified following conjugation. Similarly, the proximate and ultimate carcinogens may be also subject to further metabolism through enzymic and non-enzymic conjugation with cellular nucleophiles, such as glutathione, resulting in their deactivation and subsequent excretion, thus limiting the amount available for DNA interaction. For example, the primary epoxides, and to a lesser extent the diol-epoxides, of polycyclic aromatic hydrocarbons, their proximate and ultimate carcinogens, respectively, are deactivated by glutathione conjugation. Clearly the amount of reactive intermediates formed is totally dependent on the net effect of these competing pathways of activation and deactivation. The rates of these processes vary among animal species, so that if an animal favours the activation of a particular chemical it will be vulnerable to its carcinogenicity and vice versa. The guinea-pig is refractive to the carcinogenicity of 2-acetylaminofluorene because it is unable to catalyse its N-hydroxylation, the initial and rate-limiting step in its activation process. Under normal circumstances the activation pathways are relatively minor, most of the chemical being deactivated, and the cell copes easily with the small amounts of reactive intermediates that are produced. However, under certain conditions, e.g. saturation of the deactivation pathways, activation may become a more predominant pathway.

Cytochromes P-450 and metabolic activation of chemical carcinogens

The metabolism of a chemical carcinogen may involve a number of enzyme systems acting in concert, some directing metabolism towards activation and others towards deactivation. At least six enzyme systems participate in the metabolism of benzo[a]pyrene [1]. Undoubtedly, the major enzyme system is the cytochrome P-450-dependent mixed-function oxidases, a ubiquitous system involved in the metabolism of almost every carcinogen. It is a unique system in that it can oxidize a chemical at a number of sites, producing both reactive and inactive metabolites. This ability to catalyse both activation and deactivation was an enigma until in the 1970s, when, following successful isolation and purification, it was realised that cytochrome P-450 is not a single protein, but a superfamily of proteins, each family comprising one or more structurally related proteins [2]. The various families differ from each other in their structure, physiological and immunological properties and especially in their substrate specificities, and this multiplicity of cytochromes P-450 is responsible for its broad substrate specificity. One family may be more closely associated with the activation of a chemical, while another solely catalyses deactivation pathways. Other families of cytochrome P-450 appear to be involved exclusively in the metabolism of endogenous substrates, being, for example, very efficient in the hydroxylation of steroids, and these play no role in xenobiotic metabolism, so that interactions between physiological substrates and synthetic chemicals are avoided.

Of the various cytochrome P-450 families, only three appear to be involved in the metabolism of exogenous chemicals (Table 1). The physiological role of these families remains elusive, but they are capable of metabolizing endogenous substrates such as steroids and prostaglandins, albeit at very low rates. Frequently, a chemical serves as a substrate

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