**P50**

**JAK KINASE, STAT TRANSCRIPTION FACTORS, AND GROWTH HORMONE (GH) REGULATION OF SEX-DEPENDENT LIVER P450 GENE EXPRESSION:**

D.J. Waxman, J.H. Park, P.A. Ram (Boston Univ., Boston MA and GH Unit, RP Towers, BG Snell, RJ Wilkins, HW Davis (AResearch, Hamilton, New Zealand).

GH exerts sexually dimorphic effects on liver P450 gene transcription that are regulated by the temporal pattern of pituitary GH release, which is intermittent in male rats and nearly continuous in females. We previously identified STAT5b, a liver-expressed latent cytoplasmic transcription factor that is tyrosine phosphorylated by JAK3 kinase and undergoes nuclear translocation to a high level in response to GH pulses in male rats. Intermittent GH pulsation also activates STAT5b's DNA-binding activity toward an upstream DNA element of the GH pulse-induced, adult male-expressed rat CYP2C11 gene. By contrast, in female rats, continuous plasma GH desensitizes the STAT5b activation pathway. STAT5b protein is thus proposed to be an important mediator of the effects of intermittent GH pulses on the male-specific pattern of liver P450 gene expression (J 1995) J Biol Chem, 270: 13262.

GH also activates, stimulates STAT5b-binding, and induces nuclear translocation of SHP-1, a phosphotyrosine phosphatase that is proposed to catalyze dephosphorylation leading to deactivation of STAT5b following a GH pulse. The importance of STAT5b for the effects of GH pulses was established using a mouse gene knockout model. Major loss of multiple sexually differentiated responses associated with the sexually dimorphic pattern of pituitary GH secretion was observed in STAT5b gene disrupted mice. Male-characteristic body growth rates and male-specific liver gene expression were decreased to wild-type female levels in STAT5b/ mice thus appear to be GH pulse-insensitive, providing strong support for the hypothesis that STAT5b is the major, if not the sole STAT protein that mediates the sexually dimorphic effects of GH pulses on the liver and perhaps other target tissues. (Supported by NIH grant DK33765 and New Zealand Foundation for Research Science and Technology).

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**P51**

**INFLUENCE OF PROTEIN KINASE C AND TYROSINE KINASE ON HUMAN CYP1A1 GENE EXPRESSION:**

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Analysis of CYP1A1 gene control by Ah receptor ligands has been facilitated by the use of recombinant cell lines that stably express luciferase reporter genes under the control of the human CYP1A1 promoter. Human HepG2 and MCF7 cells that stably express CYP1A1-luciferase have been constructed and shown to be responsive to a number of Ah receptor ligands. Interestingly, the ability of the Ah receptor to modulate activation of the CYP1A1 gene in these different cell lines is in part dependent upon intracellular events that are controlled by protein kinase C (PKC) and tyrosine kinase. In HepG2 cells, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a more efficient overall inducer of CYP1A1 than is 3-methylcholanthrene (3MC), while 3MC appears to be more efficient as a transcriptional activator of CYP1A1 in MCF7 cells. However, inhibition of PKC activity in both cell lines completely blocks the ability of the receptor to initiate ligand dependent CYP1A1 activation. Thus inhibition of PKC does not influence Ah receptor function, since nuclear uptake and DNA binding are not altered by PKC inhibition. Other inducers of CYP1A1 such as PCBs and omeprazole are also blocked when PKC is inhibited in MCF7 cells, the inhibition of tyrosine kinase activity dramatically inhibits transcriptional activation of the CYP1A1 gene, suggesting that tyrosine kinase-dependent pathways play an important role in Ah receptor mediated transcriptional response. However, inhibition of tyrosine kinase activity in HepG2 cells has little effect on TCDD directed transcription, but dramatically enhances transcriptional activity by 3MC. Taken together, it would appear that cell specific signaling events underlie the ability of the Ah receptor to modulate transcriptional control of the CYP1A1 gene. (Supported USPHS grant GM36590).

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**P52**

**CONTROL OF STEROIDOGENIC P450 GENE EXPRESSION BY A ORPHAN NUCLEAR RECEPTORS**

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The adrenal cortex, testis, and ovary are major steroid hormone synthetic tissues in which steriodogenic P450s catalyze reactions from cholesterol to various steroid hormones. In recent studies, it has been investigated how Ad4BP/SF-1 and DAX-1 are implicated in the regulation of the steriodogenic tissue functions. In particular, extensive studies have been performed to elucidate the transcriptional activities of the nuclear factors and the transcriptional regulation of the genes encoding these factors. These nuclear factors show similar distributions in the steriodogenic tissues with a few exceptions, and show functional correlation. With respect to the regulation of these transcription factor genes, Ad4BP/SF-1 has been found to be implicated in the regulation of both genes. Ad4BP/SF-1 seems to function as a key factor for differentiation and maintenance of the steriodogenic tissue through regulating a variety of steriodogenic tissue specific genes.

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**P53**

**SUPRAMOLECULAR MODEL OF CYTOCHROME P-450**

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A synthetic model of membrane-bound Cytochrome P-450 is described which incorporates the important features of the natural system, namely molecular oxygen as the oxidant, a metalloporphin as catalyst, an electron donor, and a membrane system enclosing these components. The model was created by anchoring to a synthetic vesicle membrane both a CYP101(Co(I)) and a hemin complex, which catalyzes the formation of a rhodium(I) hydride species which reacts with manganese(II) to form manganese(III). The latter complex rapidly binds molecular oxygen, and transfers it to an alkene substrate which incorporates the important features of the natural system, namely the ability of the receptor to initiate ligand dependent CYP1A1 activation. Thus inhibition of PKC and tyrosine kinase activity in HepG2 cells has little effect on TCDD directed transcription, but dramatically enhances transcriptional activity by 3MC. Taken together, it would appear that cell specific signaling events underlie the ability of the Ah receptor to modulate transcriptional control of the CYP1A1 gene. (Supported USPHS grant GM36590).

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**CHEMICAL MODELS (P53-P54)**

**P54**

**HYDROGEN BONDS AT THE THIOLATE SITE**

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The structure of cytochrome P-450cm has suggested the presence of weak double NH...S hydrogen bonds between S of Cys 357 and two amide NHs. A remarkable effect on the electrochemical properties by NH...S hydrogen bond on the CYP1A1 promoter. The inability of the receptor to modulate activation of the CYP1A1 gene (Support USPHS grant GM36590)