

## Minireview

# Pregnane X Receptor and Constitutive Androstane Receptor at the Crossroads of Drug Metabolism and Energy Metabolism

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Received July 18, 2010; accepted August 23, 2010

### ABSTRACT:

The pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) are two closely related and liver-enriched nuclear hormone receptors originally defined as xenobiotic receptors. PXR and CAR regulate the transcription of drug-metabolizing enzymes and transporters, which are essential in protecting our bodies from the accumulation of harmful chemicals. An increasing body of evidence suggests that PXR and CAR also have an endobiotic

function that impacts energy homeostasis through the regulation of glucose and lipids metabolism. Of note and in contrast, disruptions of energy homeostasis, such as those observed in obesity and diabetes, also have a major impact on drug metabolism. This review will focus on recent progress in our understanding of the integral role of PXR and CAR in drug metabolism and energy homeostasis.

### Pregnane X Receptor and Constitutive Androstane Receptor As Master Regulators of Drug Metabolism and Drug Transporter

Over the long period of evolution, every organism has developed a complex defense system to prevent the accumulation of toxic xenobiotics and endogenous metabolites. Although many of the water-soluble chemicals are readily eliminated by transporter proteins, lipophilic compounds often require biotransformation to become more water-soluble before being excreted. The enzymes responsible for biotransformation include Phase I and Phase II enzymes. The cytochrome P450 (P450) enzymes belong to a superfamily of heme-containing Phase I enzymes that catalyze monooxygenase reactions of lipophilic compounds facilitated by the reducing power of the NADPH P450 oxidoreductase. Phase II enzymes catalyze the conjugation of water-soluble groups to xeno- and endobiotics. Conjugation reactions include glucuronidation, sulfation, methylation, and *N*-acetylation. In many cases, biotransformation leads to metabolic inactivation of chemicals. However, biotransformation may also activate

the so-called prodrugs to pharmacologically active products or even to toxic metabolites (Handschin and Meyer, 2003; Pascucci et al., 2008).

Most drug-metabolizing enzymes are inducible in response to xenobiotics, which represent an adaptive response of our bodies to chemical insults. The molecular basis for this inducible defense system remained largely unknown until 1998, when pregnane X receptor [(PXR) alternatively termed steroid and xenobiotic receptor, or SXR, in humans] was discovered (Blumberg et al., 1998; Kliewer et al., 1998). PXR is expressed predominantly in the liver and intestine, and it is activated by a wide variety of natural and synthetic compounds. Upon activation, PXR forms a heterodimer with retinoid X receptor and activates the transcription of drug-metabolizing enzyme and transporter genes. Examples of PXR target genes include Phase I *CYP3A*s and *CYP2C*s, Phase II UDP-glucuronosyltransferase 1A1 (*UGT1A1*), and sulfotransferases (*SULT*s), and drug transporters *MDR1* and *MRP2*. PXR has since been established as a xenosensor and master regulator of xenobiotic responses. The essential role of PXR in xenobiotic regulation and in dictating the species specificity of xenobiotic responses was confirmed through the creation and characterization of PXR null mice (Xie et al., 2000; Staudinger et al., 2001) as well as humanized PXR transgenic mice (Xie et al., 2000; Ma et al., 2007).

The constitutive androstane receptor (CAR) is a sister xenobiotic receptor of PXR. Purified first from hepatocytes as a protein bound to the phenobarbital responsive element in the *CYP2B* gene promoter,

This work was supported in part by the National Institutes of Health National Institute of Environmental Health Sciences [Grant ES014626]; the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant DK076962]; and a Molecular Pharmacology Fellowship funded by the University of Pittsburgh Department of Pharmacology and Chemical Biology.

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.110.035568.

**ABBREVIATIONS:** P450, cytochrome P450; PXR, pregnane X receptor; SULTs, sulfotransferases; CAR, constitutive androstane receptor; PB, phenobarbital; TCPOBOP, 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene; LXR, liver X receptor; NASH, nonalcoholic steatohepatitis; SREBP-1, sterol regulatory element-binding protein 1; AMPK, AMP-activated protein kinase; PAR bZip, PAR-domain basic leucine zipper; PGC-1 $\alpha$ , peroxisome-proliferator-activated-receptor- $\gamma$ -coactivator-1 $\alpha$ ; FOXO1, Forkhead box O1 protein; CPR, cytochrome P450 reductase; PPAR $\gamma$ 2, peroxisome proliferator-activated receptor  $\gamma$ 2; TH, thyroid hormone; PCN, pregnenolone-16 $\alpha$ -carbonitrile; CPT1 $\alpha$ , carnitine palmitoyltransferase 1 $\alpha$ ; HMGS2, 3-hydroxy-3-methylglutarate-CoA synthase 2; FoxA2, Forkhead box A2; CREB, cAMP response element-binding protein; PEPCK, phosphoenolpyruvate carboxykinase; AICAR, 5-amino-1- $\beta$ -D-ribofuranosyl-1*H*-imidazole-4-carboxamide.

CAR was subsequently shown to bind to the *CYP2B* gene promoter as a heterodimer with retinoid X receptor. In general, it is believed that endogenous CAR resides in the cytoplasm of hepatocytes. Upon exposure to its agonist phenobarbital (PB) or 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), CAR translocates from the cytoplasm to the nucleus and triggers the transcription of its target genes. Transfected CAR exhibited a high basal activity and was once termed a "constitutively active receptor." The name of constitutive androstane receptor was conceived due to the binding and inhibition of CAR activity by androstanes (Forman et al., 1998). CAR null mice showed a lack of induction of *Cyp2b10* and many other Phase I and Phase II enzymes and drug transporters by TCPOBOP and PB in the liver and small intestine (Wei et al., 2000).

### Drug Metabolism Can Be Affected by Energy Metabolism

Drug metabolism can be affected by various pathophysiological factors, including diabetes and liver diseases that may affect the expression or activity of drug-metabolizing enzymes and transporters. Nuclear receptors, including the xenobiotic receptors PXR and CAR and sterol sensor liver X receptor (LXR), may function as the links between drug metabolism and energy metabolism.

P450 enzyme down-regulation has been well documented in animal models of obesity, steatosis, and nonalcoholic steatohepatitis (NASH) (Leclercq et al., 1998; Su et al., 1999). Down-regulation of constitutive P450 expression in rat liver was proportional to the extent of hepatic lipid accumulation. Moreover, the capacity of drug metabolism was reported to be impaired in human patients with obesity, hepatic steatosis, and NASH (Fiatarone et al., 1991; Blouin and Warren, 1999; Cheymol, 2000). Liver lipid accumulation, especially in the early stage of steatosis, can significantly down-regulate several important P450s (Zhang et al., 2007), which is consistent with the decreased P450 expression in liver microsomes derived from patients with steatosis and NASH (Donato et al., 2006, 2007; Fisher et al., 2009). A recent report showed that the polyunsaturated fatty acids can down-regulate PB-induced *CYP2B* expression in a CAR-dependent manner in rat primary hepatocytes (Finn et al., 2009), which was consistent with an earlier report that polyunsaturated fatty acids can attenuate PB-induced nuclear accumulation of CAR (Li et al., 2007). Because hepatic steatosis often leads to increased free fatty acid levels, the inhibitory effect of free fatty acids on CAR provides a plausible explanation for the negative effect of steatosis on drug metabolism. Hepatic steatosis is often associated with increased lipogenesis, in which the sterol regulatory element-binding protein 1 (SREBP-1) is a key lipogenic transcription factor. It has been reported that SREBP-1 inhibited the transcriptional activities of PXR and CAR by functioning as a non-DNA binding inhibitor and blocking the interaction of PXR and CAR with nuclear receptor cofactors (Roth et al., 2008a).

The effect of hepatic steatosis on drug metabolism can also be mediated by the lipogenic nuclear receptor LXR through the cross-talk between LXR and CAR. LXRs, both the  $\alpha$  and  $\beta$  isoforms, were defined as sterol sensors. In rodents, LXR activation increases hepatic cholesterol catabolism and formation of bile acids by inducing cholesterol 7 $\alpha$ -hydroxylase (*Cyp7a1*) (Peet et al., 1998). LXRs were later found to be an important regulator of SREBP-1c and lipogenesis (Repa et al., 2000). It has been reported that LXR-deficient mice fed with a high-cholesterol diet showed significantly higher induction of *Cyp2b10* mRNA level upon PB treatment compared with wild-type mice, suggesting that LXR may repress PB-mediated CAR activation and *Cyp2b10* induction in vivo (Gnerre et al., 2005). Our laboratory recently showed that LXR $\alpha$  and CAR are mutually suppressive and functionally related in vivo. In particular, loss of CAR increased the

expression of lipogenic LXR target genes, leading to increased hepatic triglyceride accumulation; whereas activation of CAR inhibited the expression of LXR target genes and LXR ligand-induced lipogenesis. In contrast, a combined loss of LXR  $\alpha$  and  $\beta$  increased the basal expression of xenobiotic CAR target genes; whereas activation of LXR inhibited the expression of CAR target genes and sensitized mice to xenobiotic toxicants. The mutual suppression between LXR $\alpha$  and CAR was also observed in cell culture and reporter gene assays (Zhai et al., 2010).

The AMP-activated protein kinase (AMPK) can also mediate the effect of energy metabolism on drug metabolism. As an important energy sensor, AMPK is activated in response to stresses, such as starvation, exercise, and hypoxia, which deplete cellular ATP supplies. Upon activation, AMPK increases energy production such as lipid oxidation, whereas it decreases energy-consuming processes such as gluconeogenesis and lipogenesis, to restore energy balance (Long and Zierath, 2006). Recent reports suggest that activation of AMPK is essential for PB induction of drug-metabolizing enzymes in mouse and human livers (Rencurel et al., 2005, 2006; Shindo et al., 2007). The induction of *Cyp2b10* by PB in primary mouse hepatocytes was associated with an increased AMPK activity, and the treatment of AMPK agonist 5-amino-1- $\beta$ -D-ribofuranosyl-1H-imidazole-4-carboxamide (AICAR) triggered the nuclear accumulation of CAR in mouse livers (Shindo et al., 2007). In primary human hepatocytes, treatment with AICAR or metformin, another AMPK agonist, alone can induce *CYP2B6* and/or *CYP3A4* expression as efficient as PB, whereas overexpression of the dominant-negative mutant AMPK $\alpha$ 1 or use of AMPK inhibitor completely blocked the PB induction of *CYP2B6* and *CYP3A4* (Rencurel et al., 2006). The essential role of AMPK in the induction of *Cyp2b10* was further demonstrated in AMPK $\alpha$ 1/ $\alpha$ 2 liver-specific knockout mice, in which the PB and AICAR induction of *Cyp2b10* was blunted (Rencurel et al., 2006). Note that liver tissue from AMPK $\alpha$ 1/ $\alpha$ 2 liver-specific knockout mice had markedly increased basal mRNA expression of *Cyp2103a11*, which were not observed in isolated primary mouse hepatocytes. In an independent report, AICAR was shown to prevent the nuclear translocation of CAR in isolated primary rat hepatocytes (Kanno et al., 2010). It is unclear whether the discrepancies between the Shindo et al. (2007) and Kanno et al. (2010) studies were due to the differences in animal species or experimental conditions. The link between PB treatment and AMPK activation remains to be firmly established. It was proposed that PB may alter mitochondrial function and trigger the generation of reactive oxygen species, which subsequently activate LKB1 kinase and AMPK phosphorylation (Blättler et al., 2007). The expression of CAR and its target genes has been reported to be regulated by the circadian clock-controlled PAR-domain basic leucine zipper (PAR bZip) family of transcription factors. Mice deficient of three PAR bZip proteins were hypersensitive to xenobiotic compounds, and the deficiency in detoxification may contribute to their early aging (Gachon et al., 2006). AMPK can also regulate the circadian clock by phosphorylating and destabilizing the clock component cryptochrome 1 (Lamia et al., 2009). Together, these reports suggest the connections between molecular clocks, energy metabolism, and nuclear receptor signaling pathways.

Diabetes, a major manifestation of the metabolic syndrome, also has an impact on drug metabolism. Drug clearance was significantly increased in untreated patients of type 1 diabetes, which can be reversed by insulin treatment (Zysset and Wietholtz, 1988; Goldstein et al., 1990). This result was consistent with the observation in a streptozotocin-induced type 1 diabetic mouse model, in which the mice exhibited an increased *Cyp2b10* basal expression that can be corrected by insulin treatment (Sakuma et al., 2001).

The increased *Cyp2b* expression in type 1 diabetic mice seemed to be CAR-dependent and was associated with increased activities of peroxisome-proliferator-activated-receptor- $\gamma$ -coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) and AMPK (Dong et al., 2009a). The increased expression of *Cyp2b* in type 1 diabetes is reminiscent of starvation conditions during which the insulin level is decreased, and the expression of both *Cyp2b10* and *CAR* is induced (Maglich et al., 2004). Consistent with the observations in type 1 diabetes, insulin deprivation enhanced both dexamethasone- and  $\beta$ -naphthoflavone-induced expression of *Cyp3a* and *Cyp1a* in rat primary hepatocytes (Sidhu and Omiecinski, 1999). In contrast, the expression of *Cyp2b* and *2e* was suppressed by insulin in rat hepatoma cells (De Waziers et al., 1995). The molecular mechanism for the interaction between the insulin pathway and PXR/CAR activity was suggested by a recent study showing that Forkhead box O1 protein (FOXO1), a member of the insulin-sensitive transcription factor family, can interact with and function as a coactivator to CAR- and PXR-mediated transcription (Kodama et al., 2004). It was believed that in conditions of insulin deficiency, such as starvation and type 1 diabetes, FOXO1 translocates into the nucleus, becomes activated, and increases the transcriptional activity of CAR and PXR.

### Energy Metabolism Can Be Affected by Drug Metabolism and Xenobiotic Receptors PXR and CAR

#### Effects of P450s and PXR/CAR on Hepatic Lipid Metabolism.

P450 enzymes are known to be involved in the biotransformation of both xenobiotics, such as drugs, as well as endobiotics, such as cholesterol, steroid hormone, bile acids, and prostanoids. Although the role of P450s in drug metabolism has been well recognized and extensively studied, the endobiotic functions of P450s and to what extent they will affect systemic or tissue-specific homeostasis are less understood. Because all P450s receive electrons from a single donor, cytochrome P450 reductase [(CPR) NADPH:ferrihemoprotein reductase] (Smith et al., 1994), deletion of CPR will in principle inactivate all P450s. Mouse models with liver-specific deletion of CPR were generated by two independent groups (Gu et al., 2003; Henderson et al., 2003). Liver CRP null mice, in which the Cre expression was under the control of the albumin promoter, showed dramatically decreased liver microsomal P450 and heme oxygenase activities as expected (Pass et al., 2005). We were surprised to find that the liver of CPR null mice exhibited hepatomegaly and massive hepatic steatosis by two months of age, accompanied by severely reduced circulating cholesterol and triglyceride levels. These results have clearly implicated P450 activity in hepatic lipid homeostasis. Given the fact that the liver CPR null mice have their CPR gene deletion occur neonatally, as controlled by the albumin promoter, a subsequent study was designed to investigate the effect of P450 inactivation in adult mice by using a conditional knockout strategy. The conditional knockout of CRP was achieved by crossing the CRP floxed mice with the CYP1A1-Cre transgenic mice, in which the expression of Cre recombinase is controlled by the rat *CYP1A1* gene promoter. The CYP1A1-Cre transgene has a low basal expression, but its expression can be markedly induced by a pharmacological administration of 3-methylcholanthrene, an aryl hydrocarbon receptor agonist, leading to a time-dependent and hepatic-specific deletion of CRP (Finn et al., 2007). Associated with the time-dependent reduction of CPR expression in the liver after the treatment of 3-methylcholanthrene in adult mice, these animals developed fatty liver and had reduced nonfasting plasma cholesterol and triglyceride levels. The hepatic steatosis is mainly accounted by the accumulation of triglycerides. The source of accumulated triglycerides was believed to be dietary fatty acids,

because a fat-deficient diet can reverse the steatotic phenotype (Finn et al., 2009).

The xenobiotic receptor CAR also plays an important role in hepatic lipid homeostasis. Activation of CAR by its agonist TCPOBOP has been reported to inhibit the expression of hepatic lipogenic genes and alleviate hepatic steatosis in high-fat diet fed mice and *ob/ob* mice (Dong et al., 2009b; Gao et al., 2009). Several possible but not mutually exclusive molecular mechanisms have been proposed to explain the inhibitory effect of CAR on lipogenesis. In one study, it was suggested that activation of CAR or PXR reduced the level of SREBP-1 by inducing Insig-1, a protein blocking the proteolytic activation of SREBPs (Roth et al., 2008b). Our own study suggested that CAR may inhibit lipogenesis by inhibiting the LXR agonist responsive recruitment of LXR $\alpha$  to the *Srebp-1c* gene promoter (Zhai et al., 2010).

In contrast to the inhibitory effect of CAR on lipogenesis, activation of PXR seemed to promote lipogenesis. Transgenic mice expressing a constitutively activated PXR showed hepatomegaly and marked hepatic steatosis, and treatment of mice with a PXR agonist elicited a similar effect. PXR-induced lipogenesis was independent of the activation of SREBP-1c and was associated with the induction of fatty acid translocase (FAT/CD36), peroxisome proliferator-activated receptor  $\gamma$ 2 (PPAR $\gamma$ 2), and stearoyl-CoA desaturase-1 (Zhou et al., 2006). Further promoter analyses showed that both *CD36* and *PPAR $\gamma$ 2* are direct transcriptional targets of PXR (Zhou et al., 2006, 2008). A recent report showed that the thyroid hormone (TH)-responsive spot 14 protein, the expression of which correlates with lipogenesis, was a PXR target gene (Breuker et al., 2010). Therefore, the induction of spot 14 may have also contributed to the lipogenic effect of PXR. Treatment of mice with the PXR agonist pregnenolone-16 $\alpha$ -carbonitrile (PCN) is known to alleviate the lithocholic acid-induced hepatotoxicity (Staudinger et al., 2001; Xie et al., 2001). It was recently reported that the PCN-mediated stimulation of lipogenesis contributes to the protection from lithocholic acid-induced hepatotoxicity (Miyata et al., 2010).

The steatotic effect of PXR was also associated with suppression of several genes involved in fatty acid  $\beta$ -oxidation. Activation of PXR by its agonist PCN can inhibit lipid oxidation by down-regulating the mRNA expression of carnitine palmitoyltransferase 1 $\alpha$  (CPT1 $\alpha$ ) and mitochondrial 3-hydroxy-3-methylglutarate-CoA synthase 2 (HMGCS2), two key enzymes involved in  $\beta$ -oxidation and ketogenesis, in a PXR-dependent manner. Mechanistically, it has been shown that PXR can directly interact with Forkhead box A2 (FoxA2) and prevent FoxA2 binding to the *Cpt1a* and *Hmgcs2* gene promoters (Nakamura et al., 2007). Finally, unlike CAR, activation of PXR had little effect on the lipogenic effect of LXR (Zhai et al., 2010).

#### Effects of PXR and CAR on Hepatic Glucose Metabolism.

Chronic treatment with PB has been reported to decrease plasma glucose levels and improve insulin sensitivity in diabetic patients (Lahtela et al., 1985). Treatment with PB suppressed the expression of gluconeogenic enzymes PEPCK1 and G6Pase in mouse liver (Ueda et al., 2002) and in primary rat hepatocytes (Argaud et al., 1991). The regulation of the gluconeogenic pathway by PXR was initially suggested by the suppression of PEPCK and G6Pase in VP-hPXR transgenic mice (Zhou et al., 2006). Treatment of wild-type mice with the PXR agonist PCN also suppressed cAMP-dependent induction of G6Pase in a PXR-dependent manner (Kodama et al., 2004). Several possible but not mutually exclusive molecular mechanisms have been proposed to explain the inhibitory effect of PXR and CAR on gluconeogenesis. It was reported that PXR can form a complex with phosphorylated cAMP response element-binding protein (CREB) in a ligand-dependent manner to prevent CREB binding to the cAMP

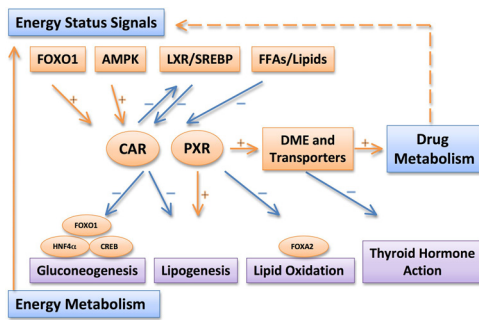


FIG. 1. Summary of the major interactions between drug metabolism and energy metabolism and the central roles of PXR and CAR in these cross-talks. Note that the drug metabolism and energy status can be affected by pathophysiological conditions, such as obesity, diabetes, and fatty liver. DME, drug-metabolizing enzymes; FFA, free fatty acids; HNF4 $\alpha$ , hepatocyte nuclear factor 4 $\alpha$ .

response element and inhibit CREB-mediated transcription of G6Pase (Kodama et al., 2007). PXR and CAR can also physically bind to FoxO1 and suppress its transcriptional activity by preventing its binding to the insulin response sequence in the gluconeogenic enzyme gene promoters (Kodama et al., 2004). PXR and CAR may also inhibit hepatocyte nuclear factor-4 $\alpha$  activity by competing for the DR1 (direct repeat spaced by one nucleotide) binding motif in the gluconeogenic enzyme gene promoters (Miao et al., 2006). The *in vivo* significance of CAR-mediated suppression of gluconeogenesis was supported by two recent reports that activation of CAR ameliorated hyperglycemia and improved insulin sensitivity in *ob/ob* mice and high-fat diet fed wild-type mice (Dong et al., 2009b; Gao et al., 2009).

**CAR May Impact Energy Metabolism and Fasting Response by Affecting Thyroid Hormone Metabolism.** It has been reported that the expression of CAR was induced during long-term fasting, possibly due to the up-regulation of PGC-1 $\alpha$  (Ding et al., 2006). Moreover, CAR-deficient mice were defective in fasting adaptation and lost more weight during calorie restriction (Maglich et al., 2004). One possible mechanism by which CAR affects fasting response is through the effect of CAR on TH metabolism. Treatment of wild-type mice with a CAR agonist decreased serum T<sub>4</sub> level (Qatanani et al., 2005). The decrease in T<sub>4</sub> level was associated with a concomitant increase in the serum thyroid-stimulating hormone level in wild-type but not in CAR(-/-) mice. The effect of CAR on TH homeostasis was believed to be achieved through the regulation of TH-metabolizing Phase II enzymes. Activation of CAR induced the expression of glucuronosyltransferases UGT1A1 and 2B1 and sulfotransferases SULT2A1, 1C1, and 1E1 in wild-type mice but not in CAR(-/-) mice. UGT1A1 and UGT2B1 are responsible for T<sub>4</sub> and T<sub>3</sub> glucuronidation, respectively (Visser et al., 1993a,b). SULTs are important for the inactivation of THs by blocking outer ring deiodination and causing irreversible inactivation of THs (Visser, 1994). However, the net effect of CAR on TH homeostasis remains controversial. It was reported that activation of CAR decreased the serum reverse T<sub>3</sub> level, resulting in the desuppression of thyroid hormone target genes during partial hepatectomy (Tien et al., 2007).

### Conclusions and Perspectives

Figure 1 summarizes the major interactions between drug metabolism and energy metabolism and the central roles of PXR and CAR in these cross-talks. Induction or suppression of P450s and other drug-metabolizing enzymes can alter drug metabolism and clearance. Our understanding of the regulation of drug metabolism and activities of PXR and CAR by liver energy status may offer possible explanations for the variation of drug responses associated with metabolic diseases

and provide rationale for appropriate dose adjustment. On the other hand, activation of PXR and CAR could have a significant impact on energy homeostasis by affecting lipogenesis, gluconeogenesis, and fatty acid oxidation. It is tempting to speculate that pharmacological modulation of PXR and CAR may be beneficial in managing metabolic diseases.

Among the remaining challenges, although PXR has been shown to affect lipid and glucose metabolism, the *in vivo* role of PXR in obesity and type II diabetes remains to be demonstrated. The endogenous ligands for PXR and CAR that elicit the metabolic functions of these two receptors also remain to be identified.

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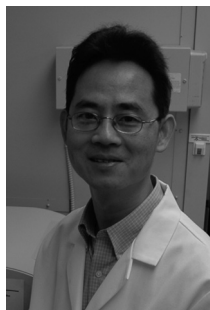
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