The role of xenobiotic-responsive transcription factors in the gene expression of drug-metabolizing enzymes

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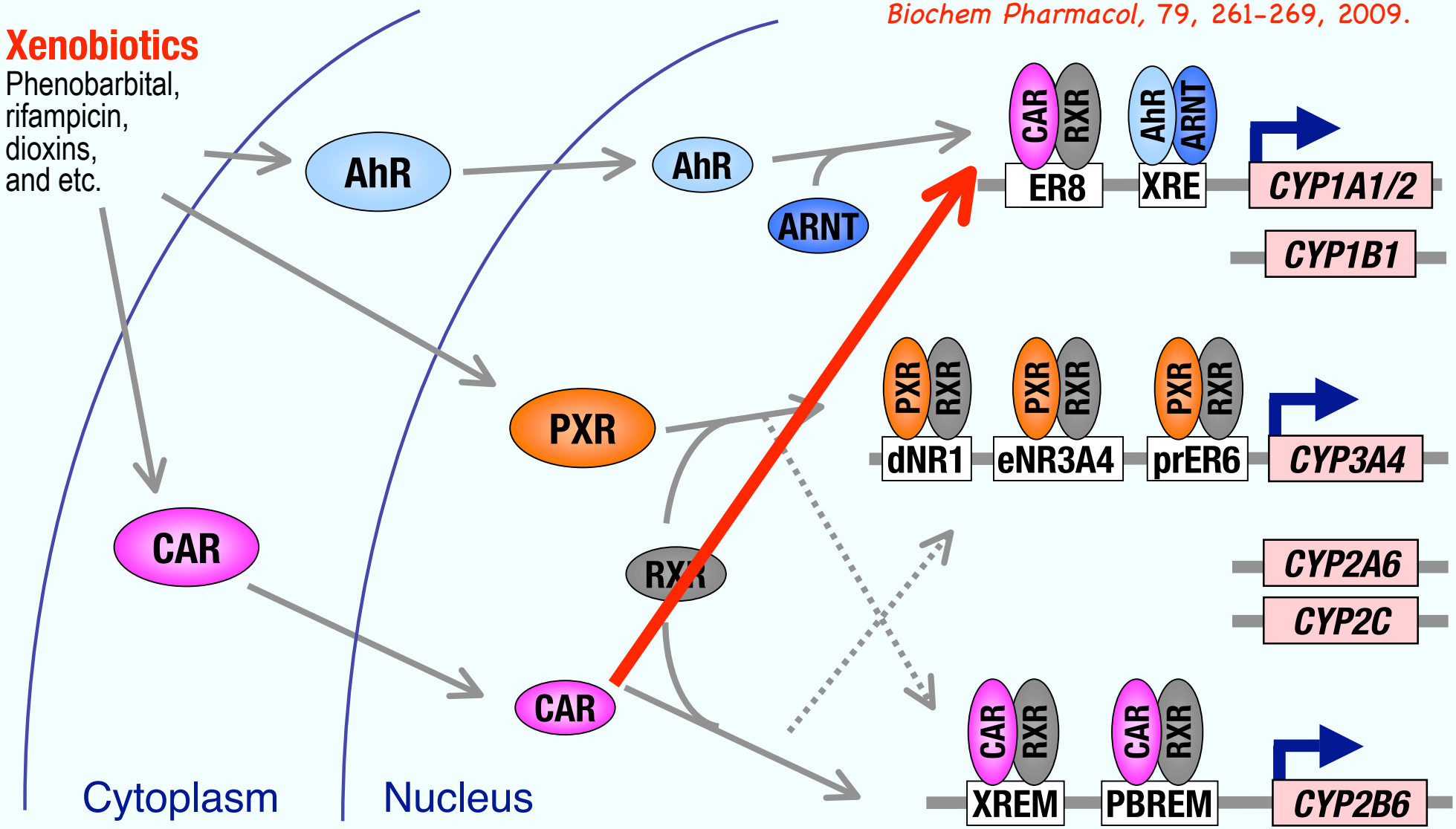
Young Investigator's Award Presentation

Xenobiotic-induced expression of CYP genes through xenobiotic-responsive nuclear transcription factors

Yoshinari et al.,
Biochem Pharmacol, 79, 261-269, 2009.



AhR; aryl hydrocarbon receptor, XRE; xenobiotic-responsive element, XREM; xenobiotic-responsive enhancer module, PBREM; phenobarbital-responsive enhancer module,

Summary

1. HSP90 and protein phosphatase PP2A are associated with nuclear translocation of CAR.

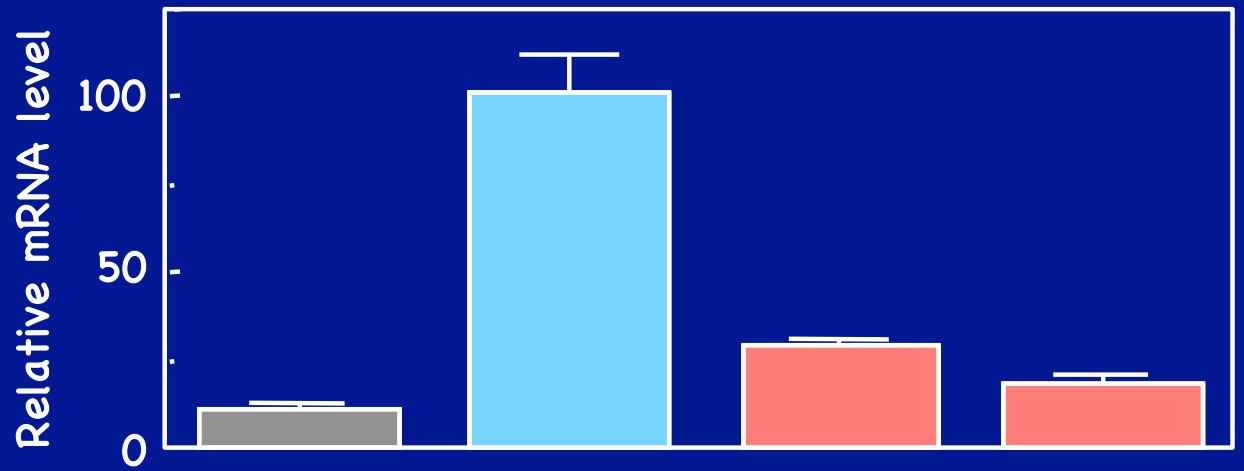
2. CAR is a major determinant for the variations of CYP2B expression in rodent livers.

3. Cyp3a expression is attenuated in the liver and intestine of obese mice fed a high-fat diet.

4. AhR ligands alter the adipose gene expression profile for the lipid/carbohydrate metabolism as well as drug metabolism.

Hsp90 inhibitor geldanamycin (GA) and protein phosphatase inhibitor okadaic acid (OA) inhibit Cyp2b10 induction and CAR nuclear translocation in mouse hepatocytes

Cyp2b10 mRNA



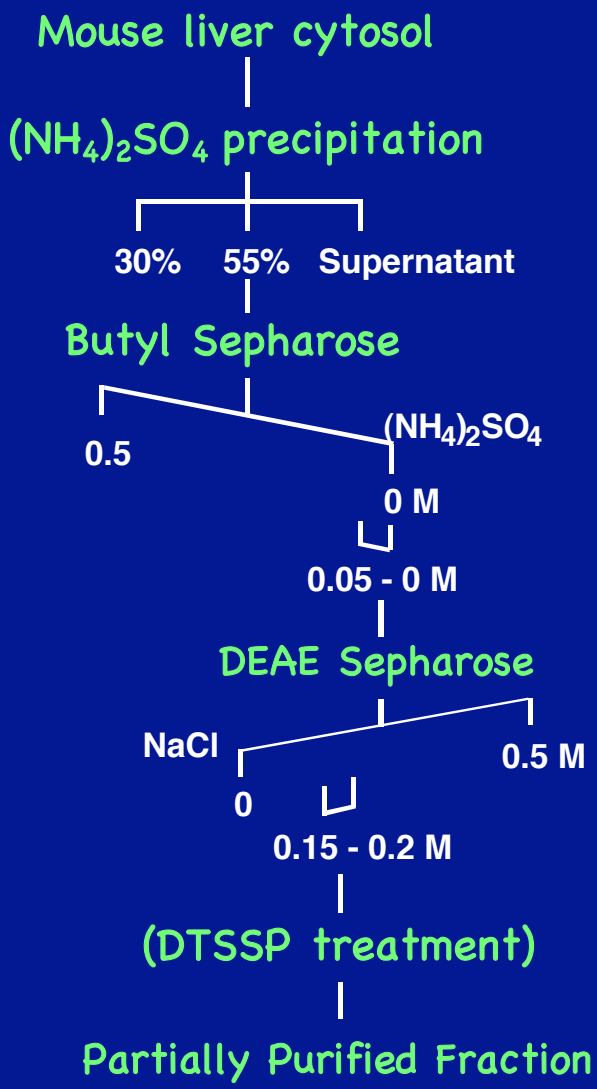
Nuclear CAR

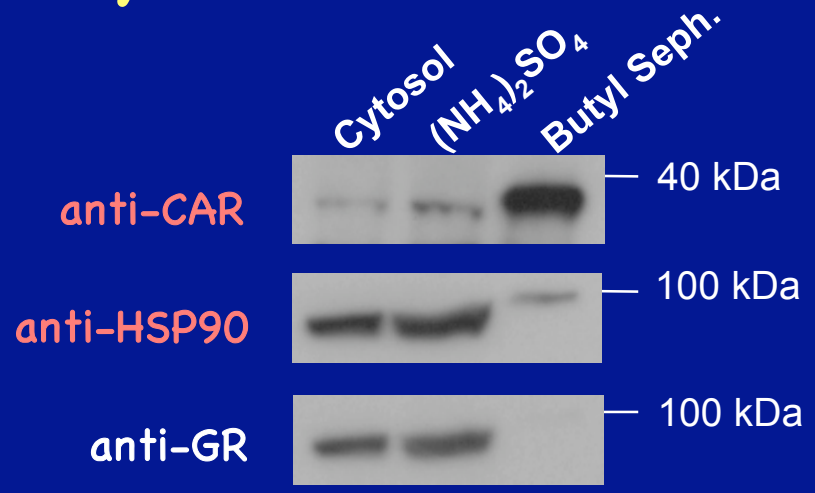
Pretreatment:	–	–	GA (20 μM)	OA (10 nM)
Inducer:	Vehicle	TCPOBOP (0. 25 μM)		

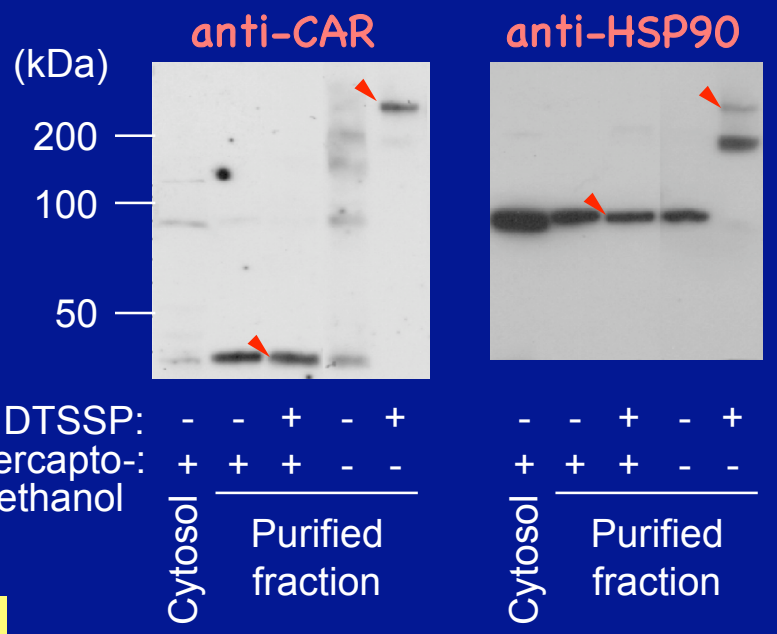
TCPOBOP; 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene.

Yoshinari et al., *FEBS Lett* (2003) 548, 17-20.

Partial purification of CAR complex from mouse liver cytosol





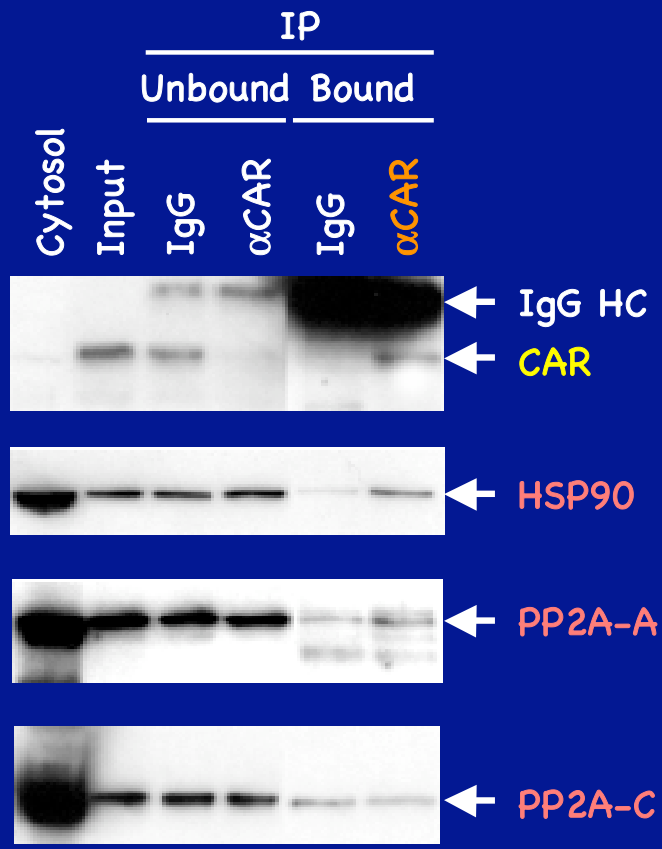


DTSSP; 3,3`-dithiobis(sulphosuccinimidyl propionate):
Water-soluble and thiol cleavable homobifunctional crosslinker

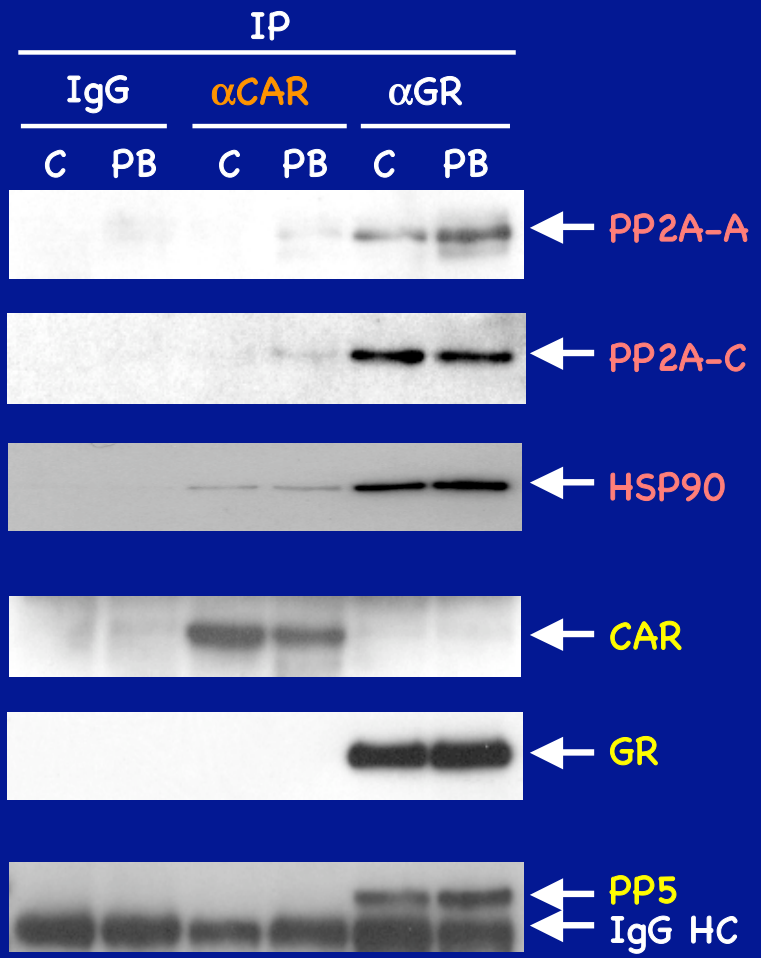
Yoshinari et al., *FEBS Lett* (2003) **548**, 17-20.

Presence of Hsp90 and protein phosphatase PP2A in the cytoplasmic CAR complex

Partially purified fraction



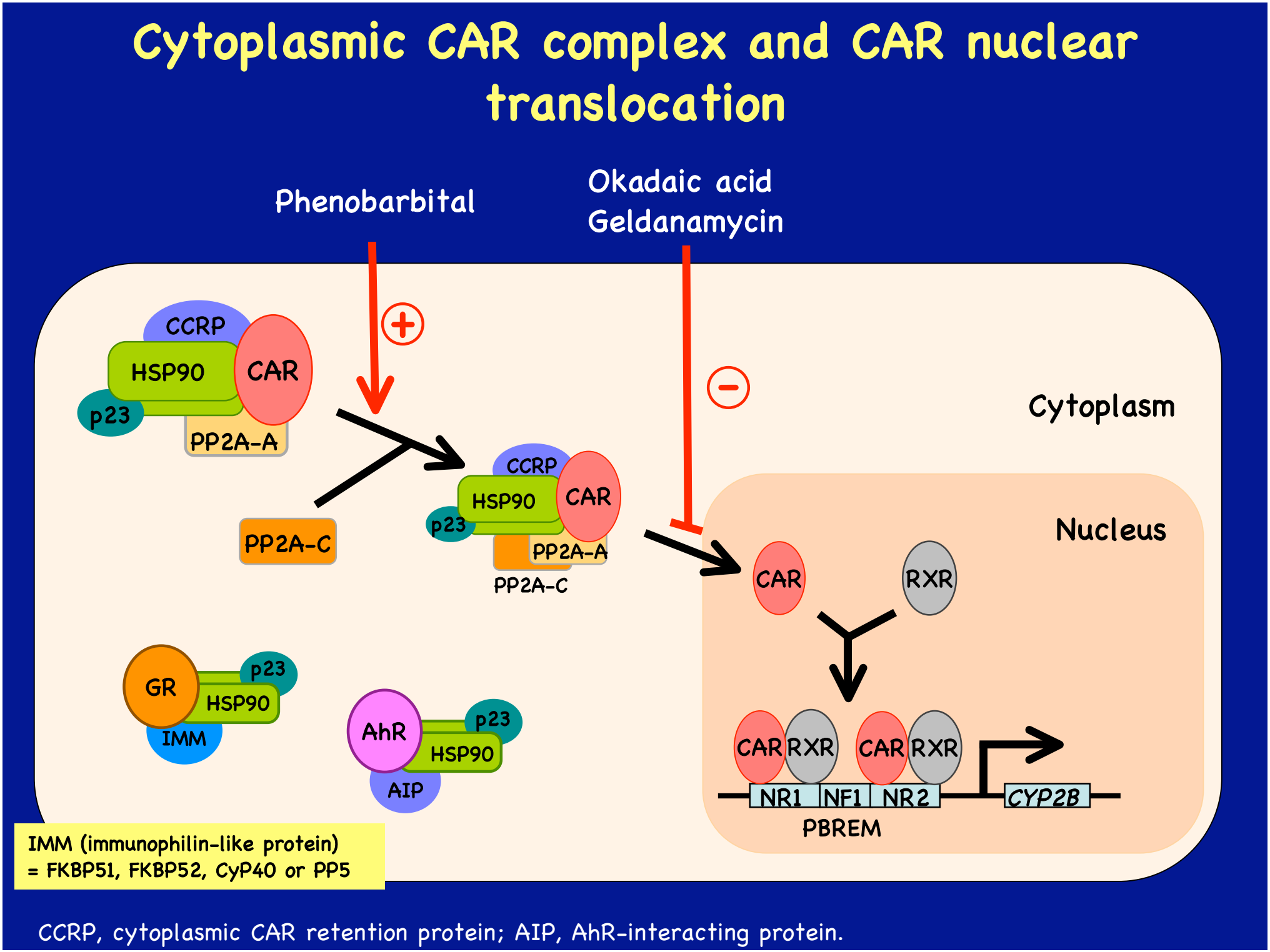
Mouse liver cytosol



IP, immunoprecipitation; HC, heavy chain; PP, protein phosphatase.

Yoshinari et al., *FEBS Lett* (2003) 548, 17-20.

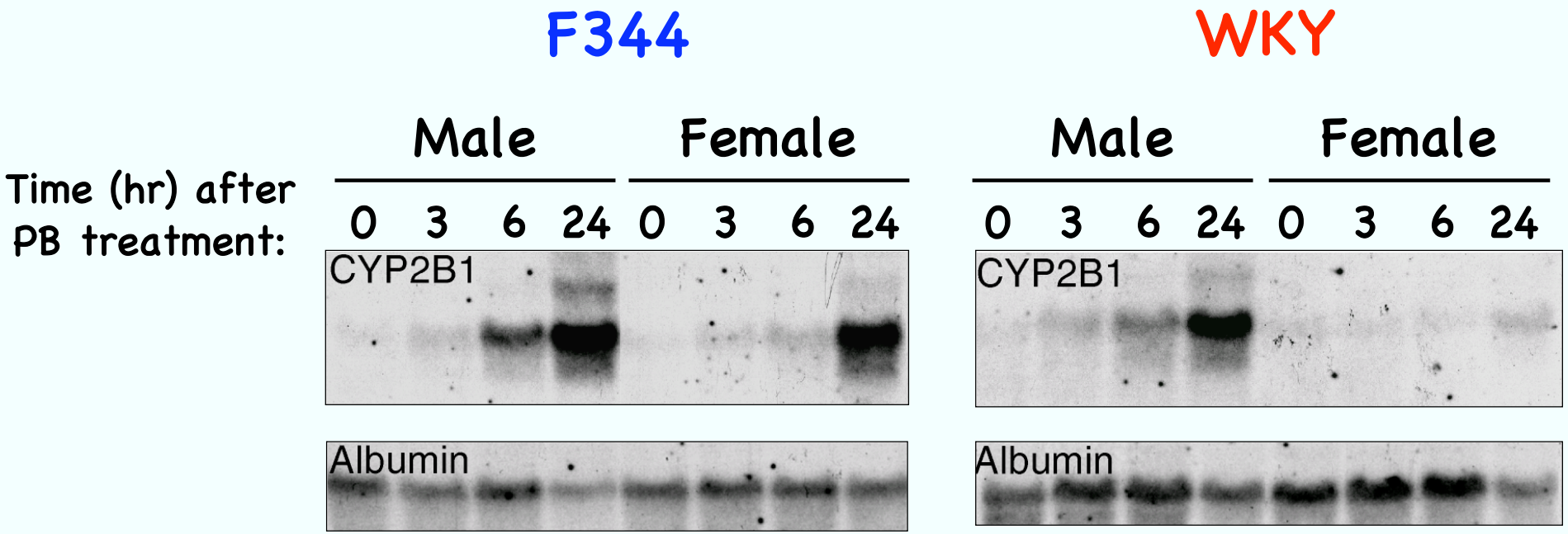
Cytoplasmic CAR complex and CAR nuclear translocation



CCRP, cytoplasmic CAR retention protein; AIP, AhR-interacting protein.

Sex- and strain-dependent differences in phenobarbital induction of CYP2B1 in rat livers

CYP2B1 mRNA levels

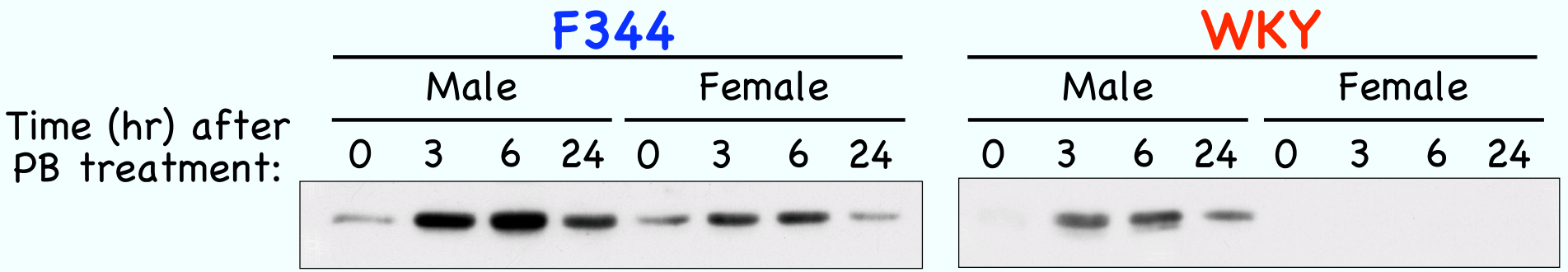


WKY; Wistar-Kyoto
PB; phenobarbital

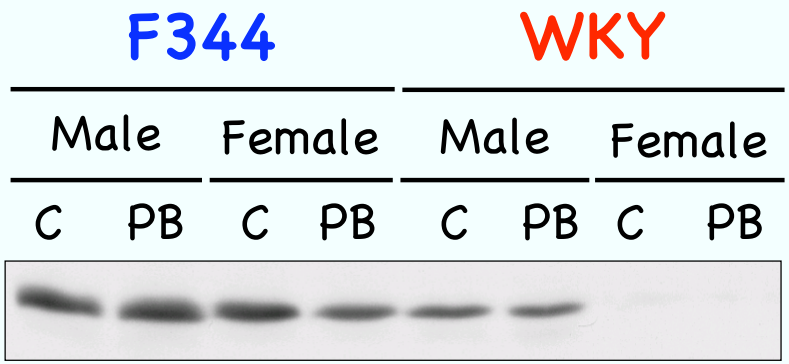
Yoshinari et al., *Mol Pharmacol* (2001) **59**, 278-284.

Sex- and strain-dependent differences in CAR expression in the liver of F344 and WKY rats

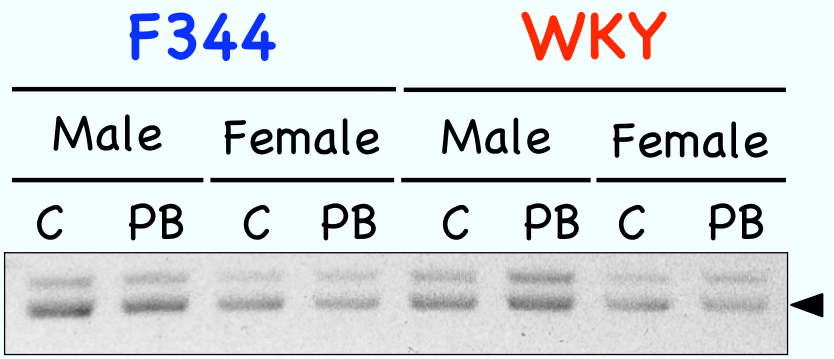
Nuclear CAR (NR1-bound)



CAR total protein



CAR mRNA

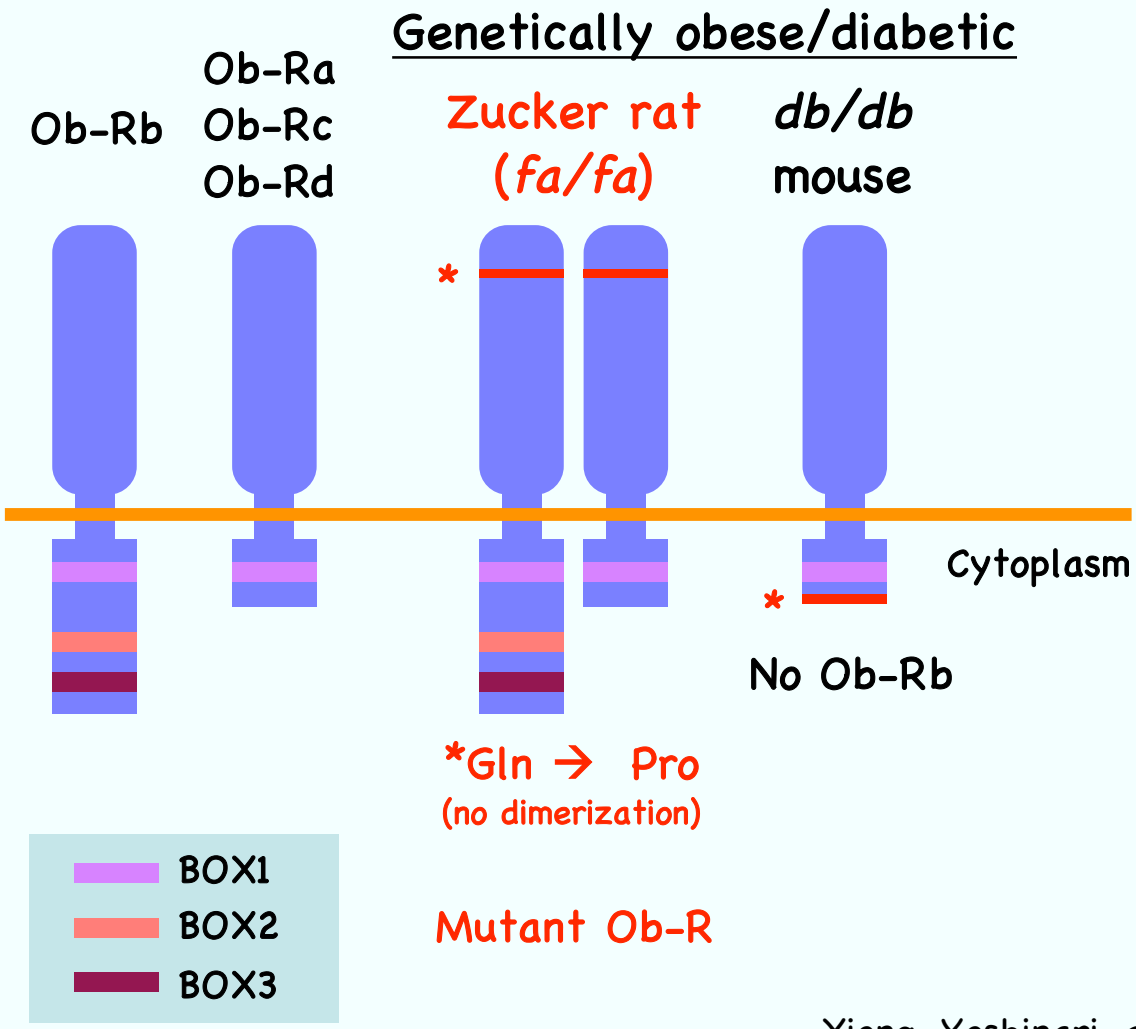


Lack of PB induction of CYP2B1 in the liver of female WKY rats is attributed to the strikingly reduced CAR protein levels.

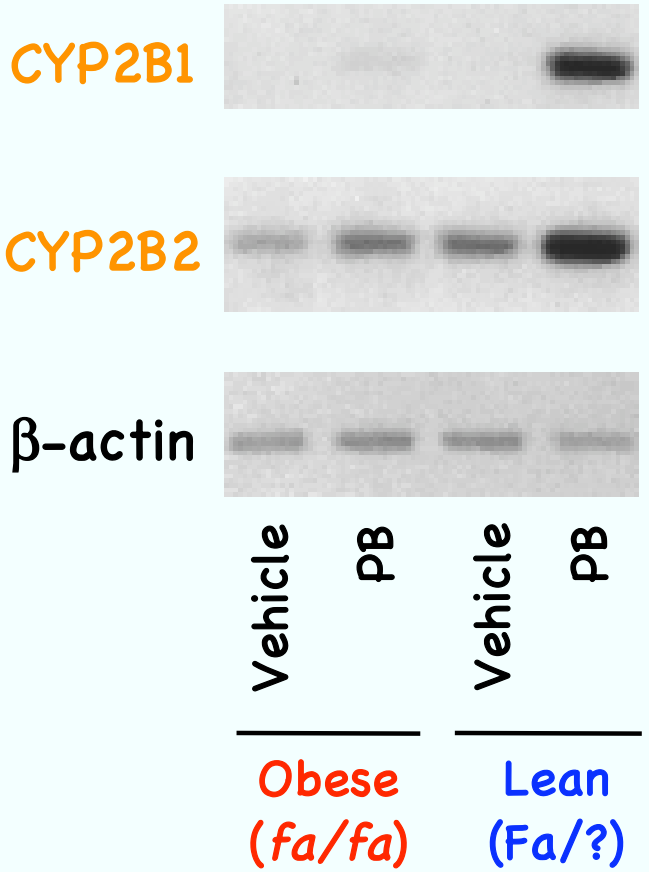
Yoshinari et al., *Mol Pharmacol* (2001) **59**, 278-284.

Expression of CYP2B1/2B2 in the liver of genetically obese/diabetic Zucker rats

Leptin receptor (Ob-R)



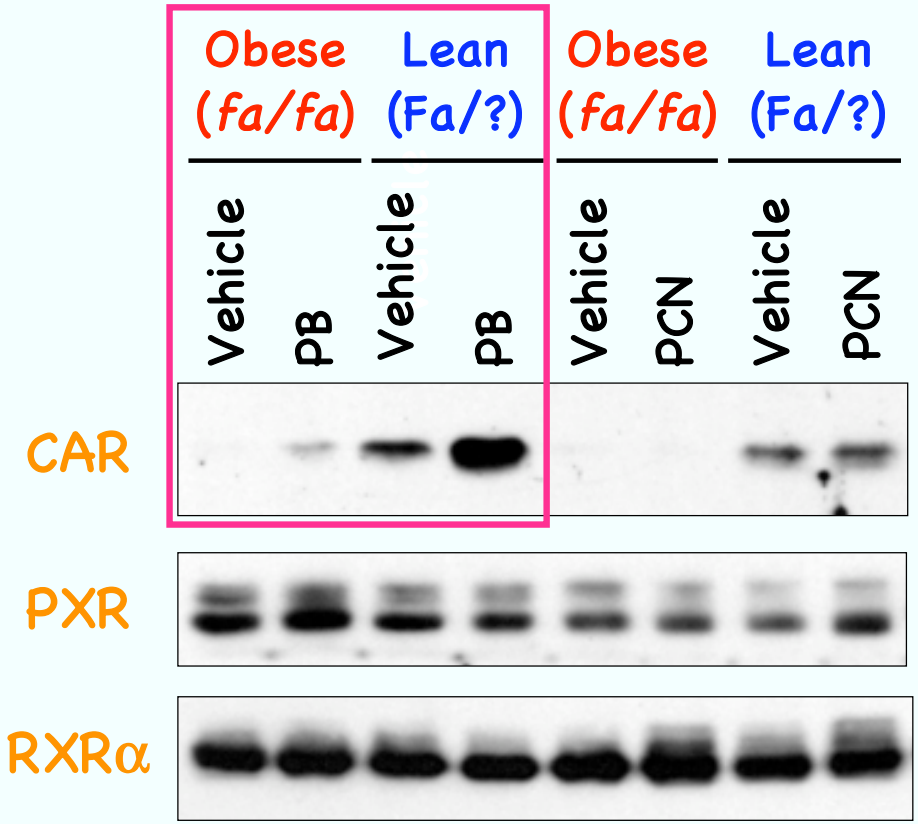
mRNA levels

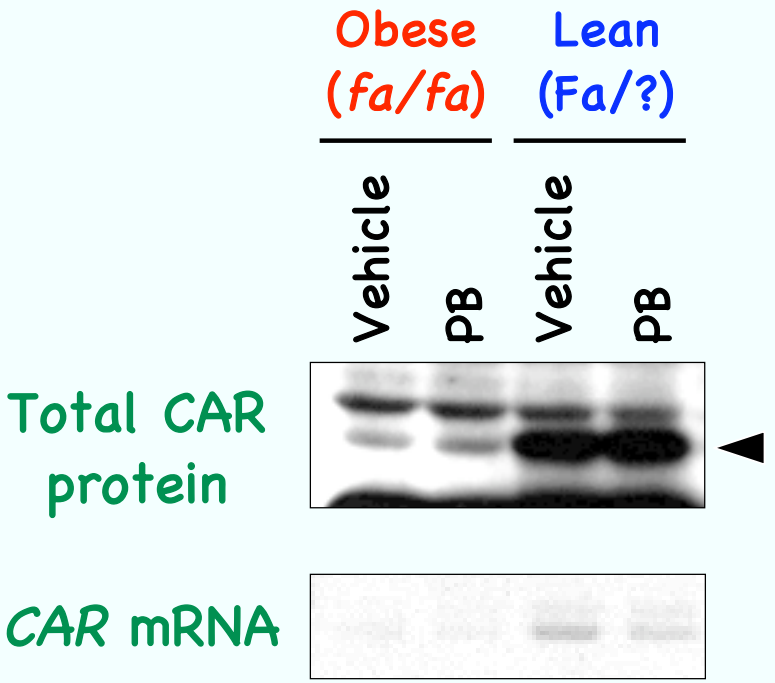


Xiong, Yoshinari, et al., *Drug Metab Dispos* (2002) **30**, 918-923.

CAR expression levels in Zucker fatty rat livers

Nuclear CAR protein



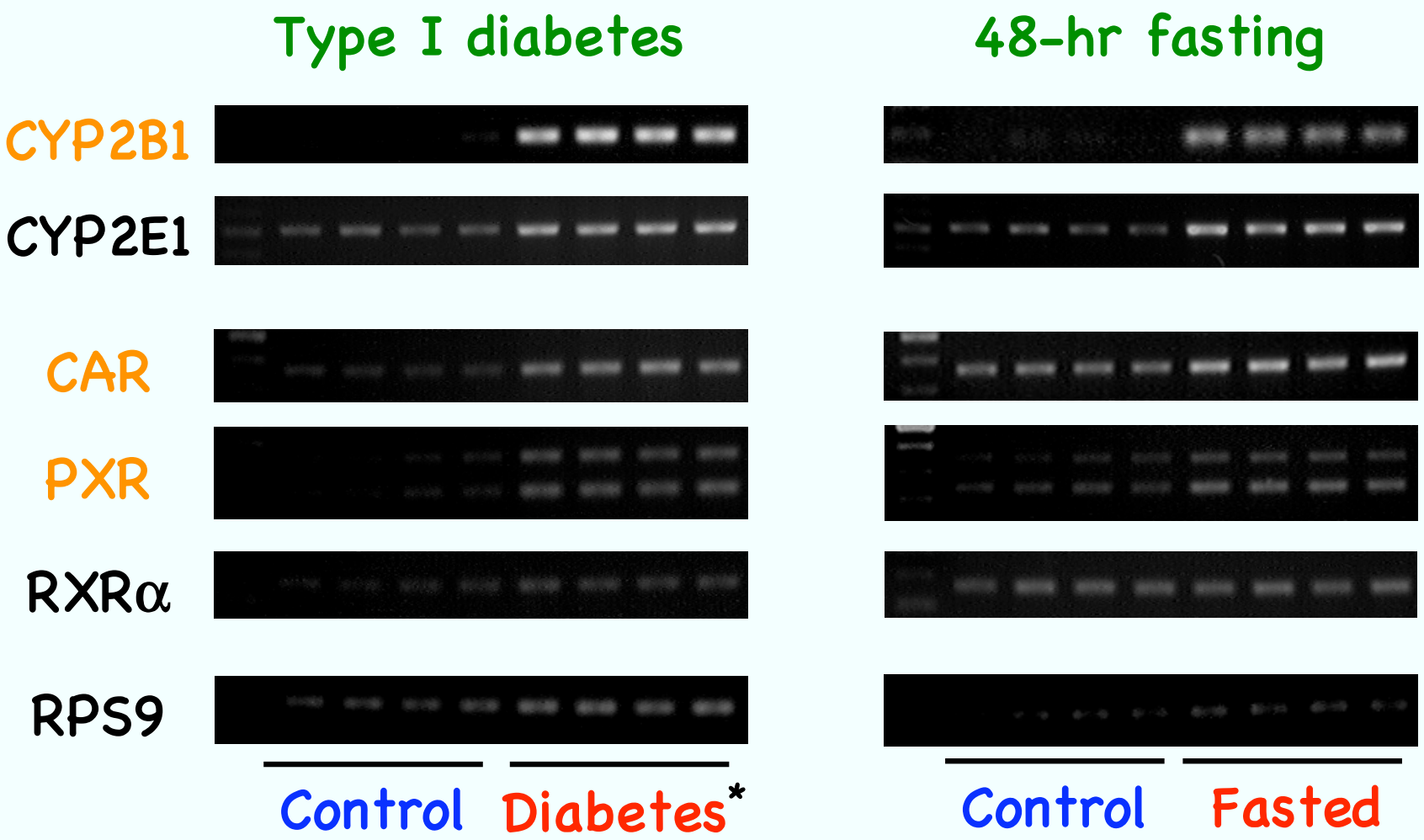


CAR expression is reduced in the liver of Zucker fatty rats, which leads to low basal expression and PB induction of CYP2B1/2B2.

PCN; pregnenolone-16α-carbonitrile.

Xiong, Yoshinari, et al., *Drug Metab Dispos* (2002) **30**, 918-923.

Changes in the mRNA levels of CYPs and nuclear receptors in the liver of diabetic and fasted rats



*Male SD rats were treated with streptozotocin (65 mg/kg, i.p.) and killed 2 weeks later.

Hepatic CYP levels in obese/diabetic animals

Model animal	Hepatic CYP mRNA levels
STZ-induced diabetes (type I)	CYP2B/2E/4A ↑
Zucker fatty rat (leptin receptor mutation)	CYP2E ↑, CYP2B/3A ↓
db/db mouse (leptin receptor mutation)	CYP2B/2E ↑, CYP3A→
ob/ob mouse (leptin mutation)	CYP2B/2E ↑
High-fat diet feeding (high calorie intake)	?

Production of obese mice with high-fat diet

Male ICR mouse
(5-wk-old)

35 - 40 d

HFD or control chow

Assays:
Serum biochemistry
Quantitative RT-PCR
Western blotting

High-fat diet #1 (HFD1)

Ingredient	%
Casein	24.0
L-Methionine	0.3
Lard	36.0
Cornstarch	10.0
Maltose	10.0
Sucrose	15.0
AIN76 vitamin	1.0
AIN76 mineral	3.5
Choline bitartrate	0.2

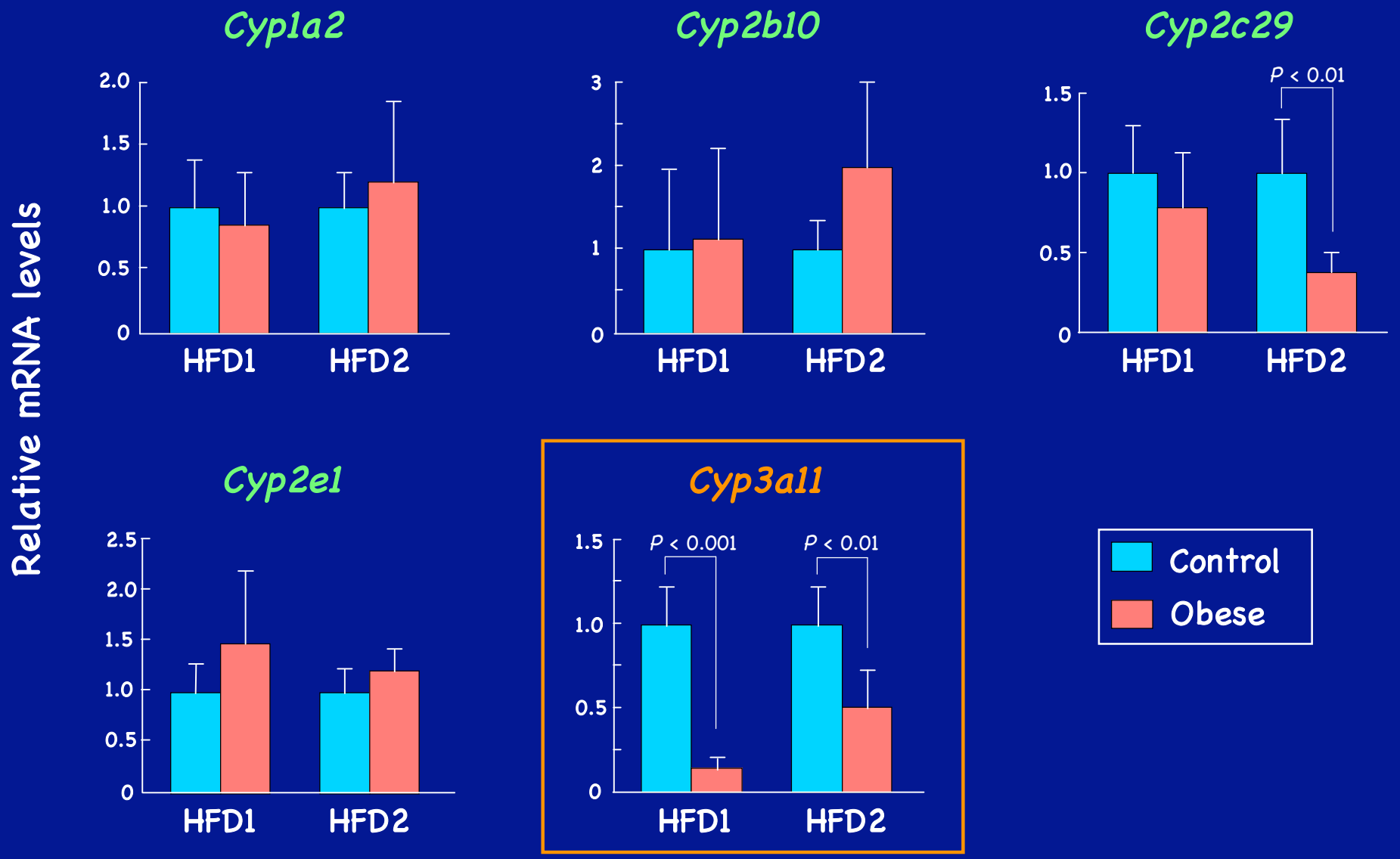
High-fat diet #2 (HFD2)

Ingredient	%
Casein	24.5
Egg albumin	5.0
L-Cystine	0.43
Lard	15.9
Safflower oil	20.0
Cellulose	5.5
Maltodextrin	8.25
Lactose	6.93
Sucrose	6.75
AIN93 vitamin	1.4
AIN93G mineral	5.0
Choline bitartrate	0.36
Butylhydroquinone	0.002

HFD, high fat diet.

Yoshinari, et al., *Pharm Res* (2006) **23**, 1188-1200.

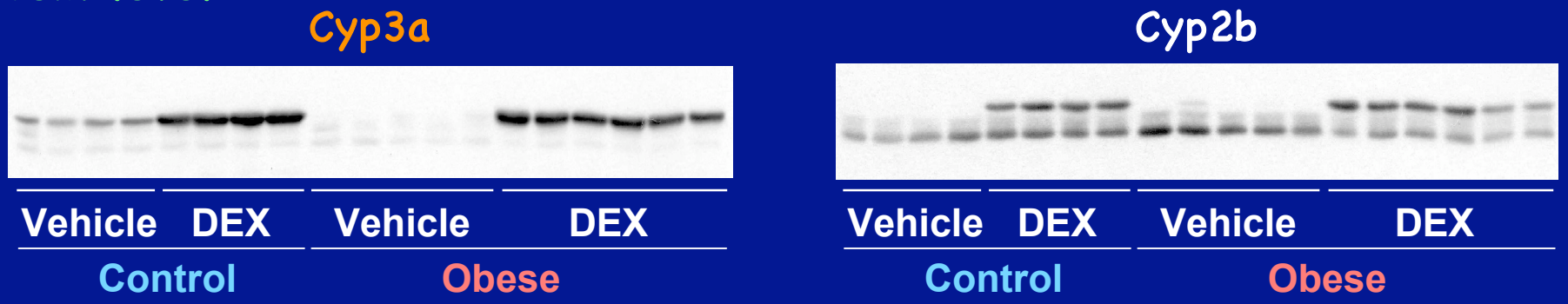
Hepatic CYP mRNA levels in obese mice fed a high-fat diet



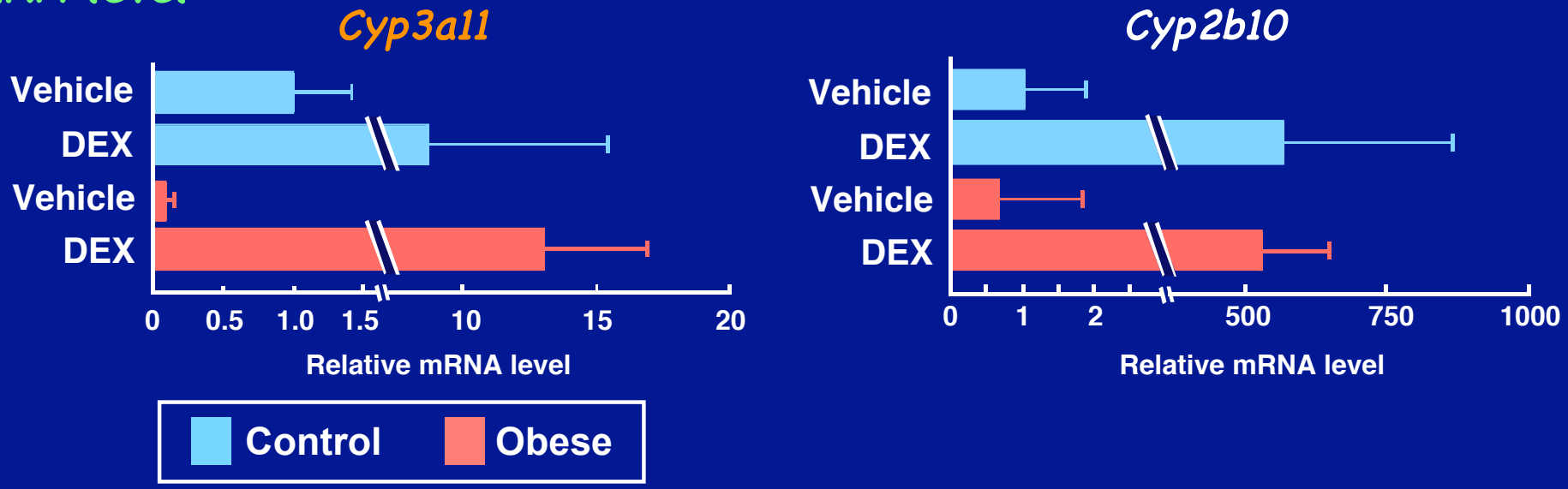
Yoshinari, et al., *Pharm Res* (2006) **23**, 1188-1200.

Unchanged Cyp3a induction by dexamethasone (DEX) in the liver of obese mice fed high fat diet

Protein level



mRNA level

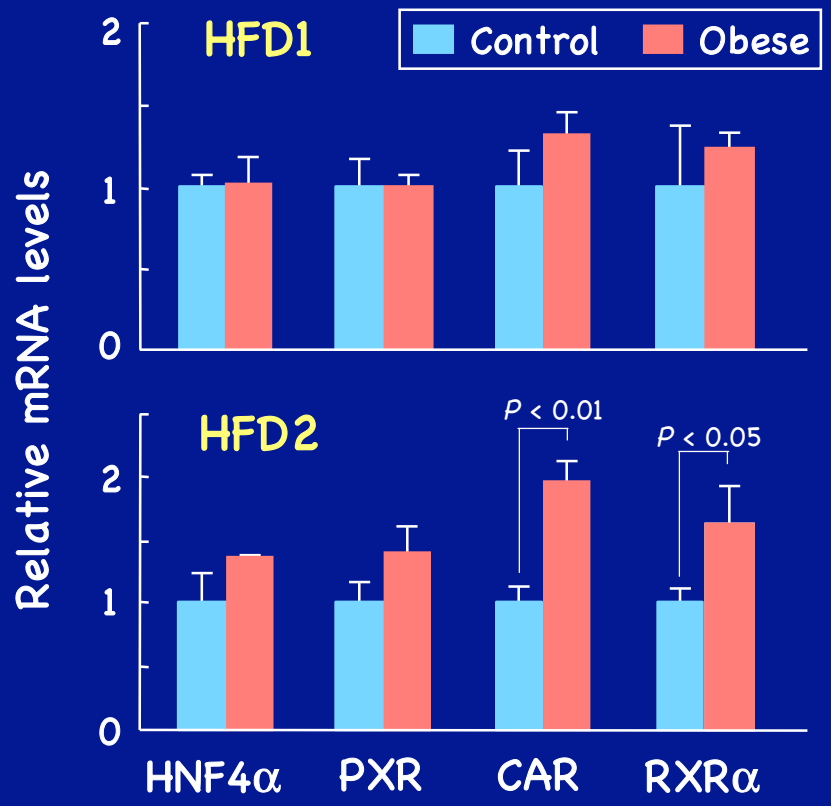


Mice fed a control chow or HFD1 for 5 wk were treated with DEX (100 mg/kg) or vehicle (corn oil) intraperitoneally 24 hr before sacrifice, and microsomes and total RNA were prepared from the liver.

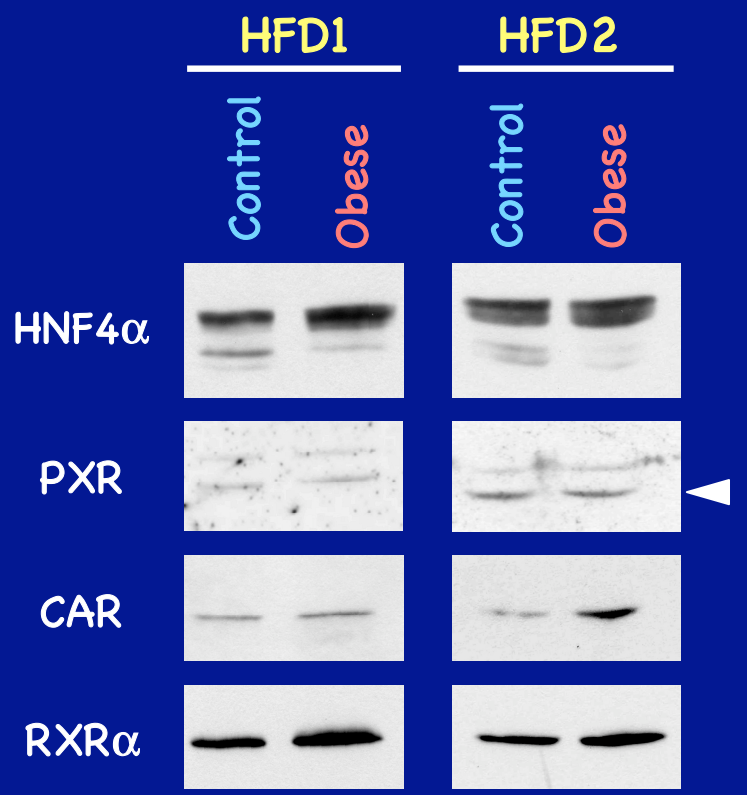
Yoshinari, et al., *Pharm Res* (2006) **23**, 1188-1200.

Nuclear receptor levels in obese mouse livers

mRNA level



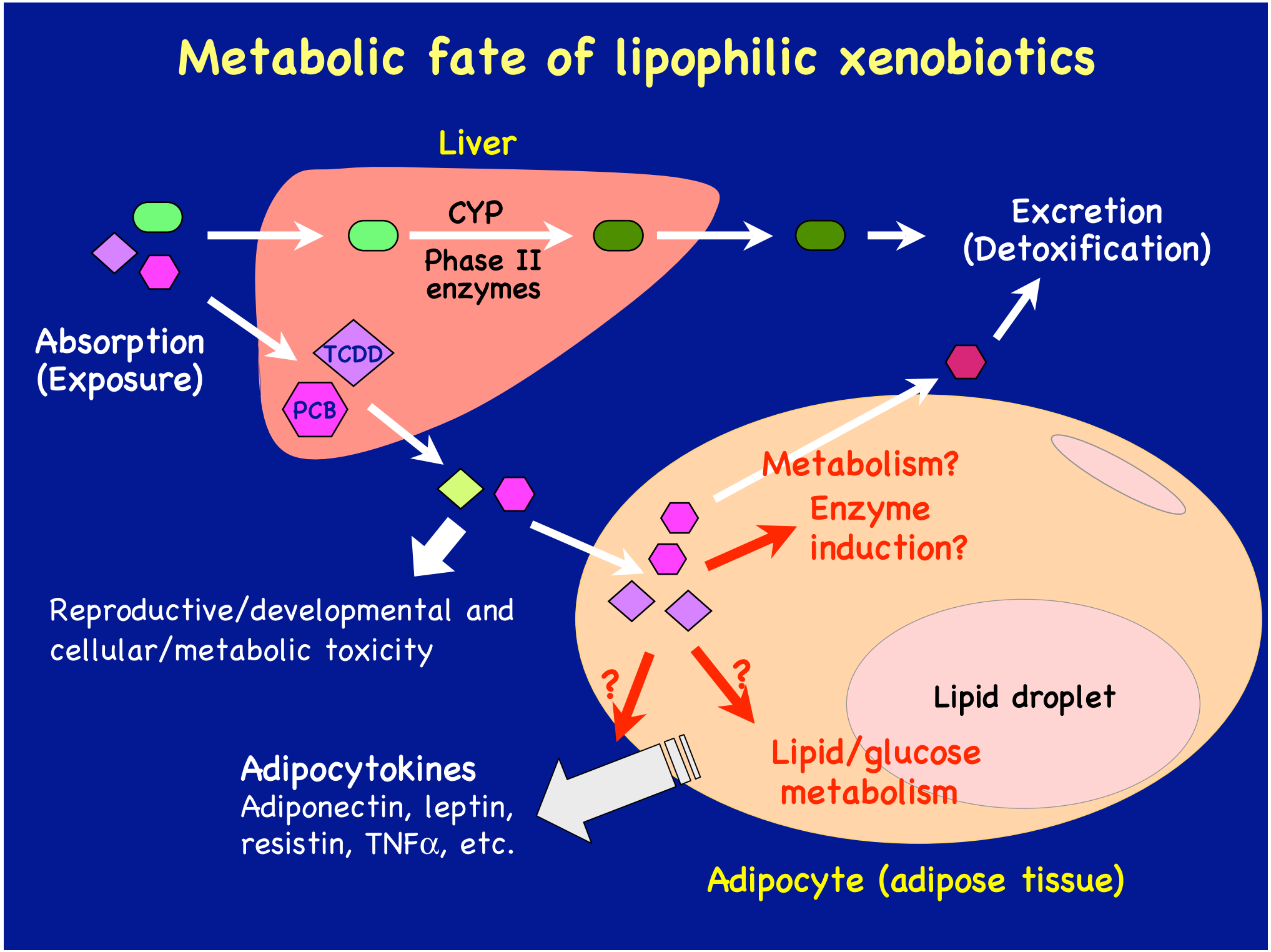
Nuclear protein level



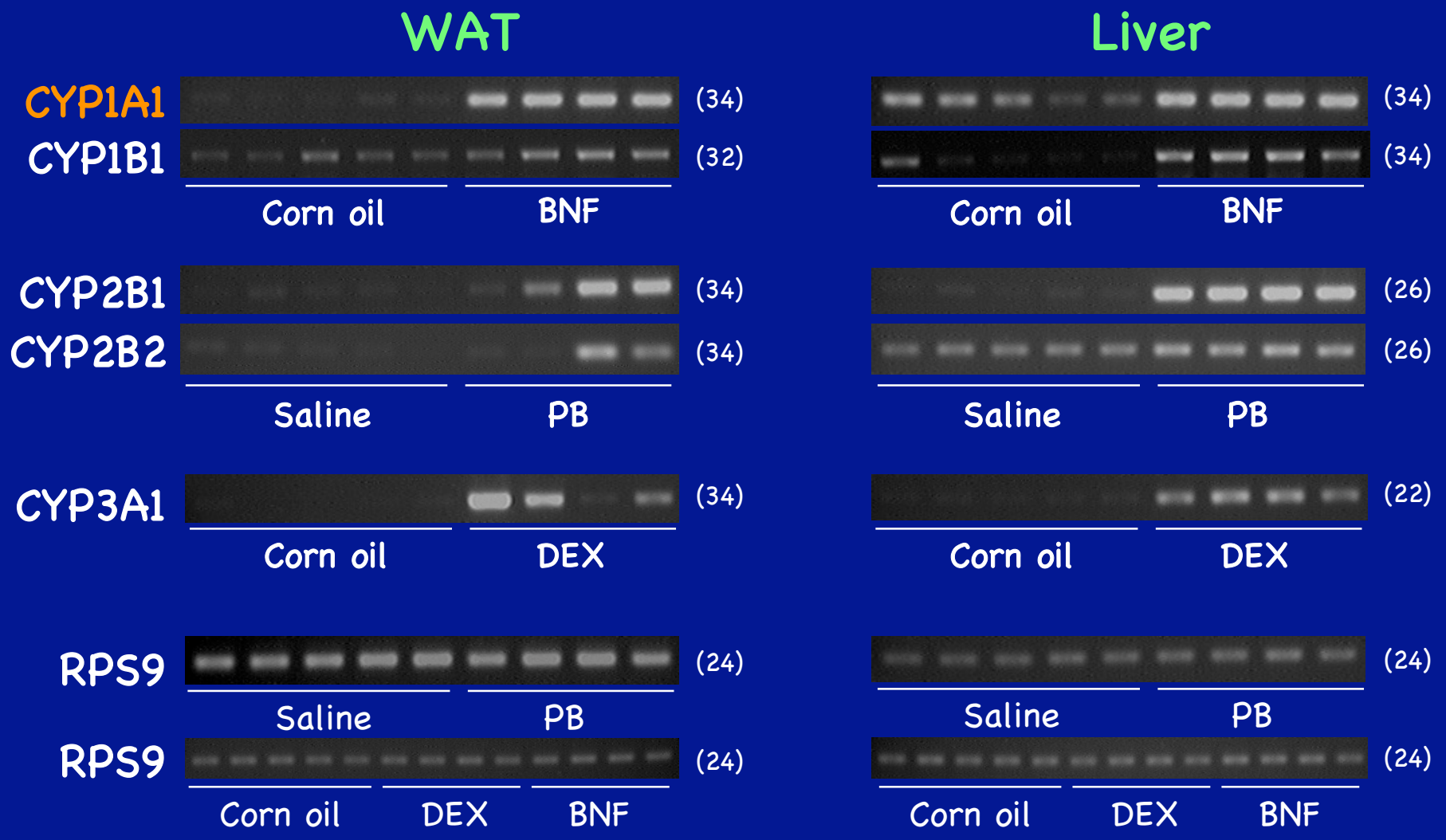
Cyp3a expression is attenuated in the liver of obese mice fed a high-fat diet in a nuclear receptor-independent manner.

HNF4α, hepatocyte nuclear factor-4α.

Yoshinari, et al., *Pharm Res* (2006) **23**, 1188-1200.


Metabolic fate of lipophilic xenobiotics
Liver
CYP
Phase II enzymes
Excretion (Detoxification)
Absorption (Exposure)
TCDD
PCB
Metabolism?
Enzyme induction?
Reproductive/developmental and cellular/metabolic toxicity
?
?
Lipid droplet
Lipid/glucose metabolism
Adipocytokines
Adiponectin, leptin, resistin, TNFα, etc.
Adipocyte (adipose tissue)

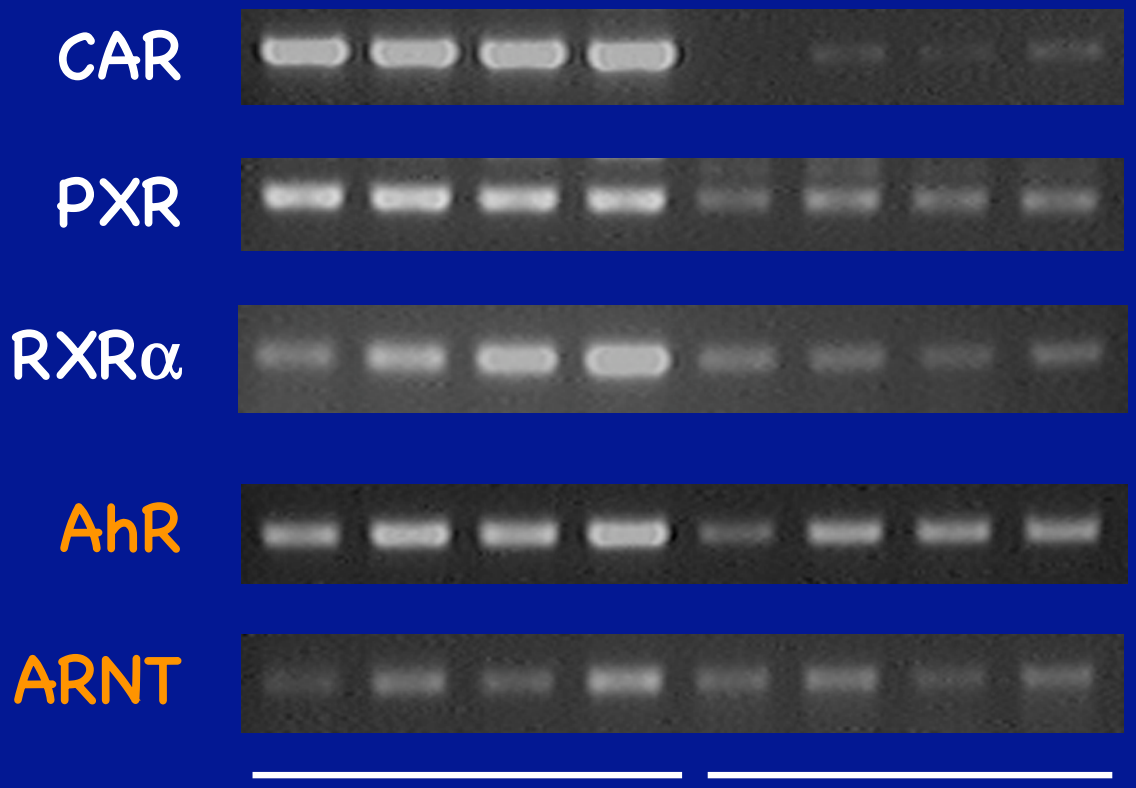
CYP mRNA levels in rat epididymal WAT and livers after treatment with typical CYP inducers



Male SD rats were treated intraperitonealy with β-naphthoflavone (BNF; 40 mg/kg/day), PB (80 mg/kg/day) or DEX (40 mg/kg/day) for 3 days. The numbers in parentheses indicate the number of PCR amplification cycles.

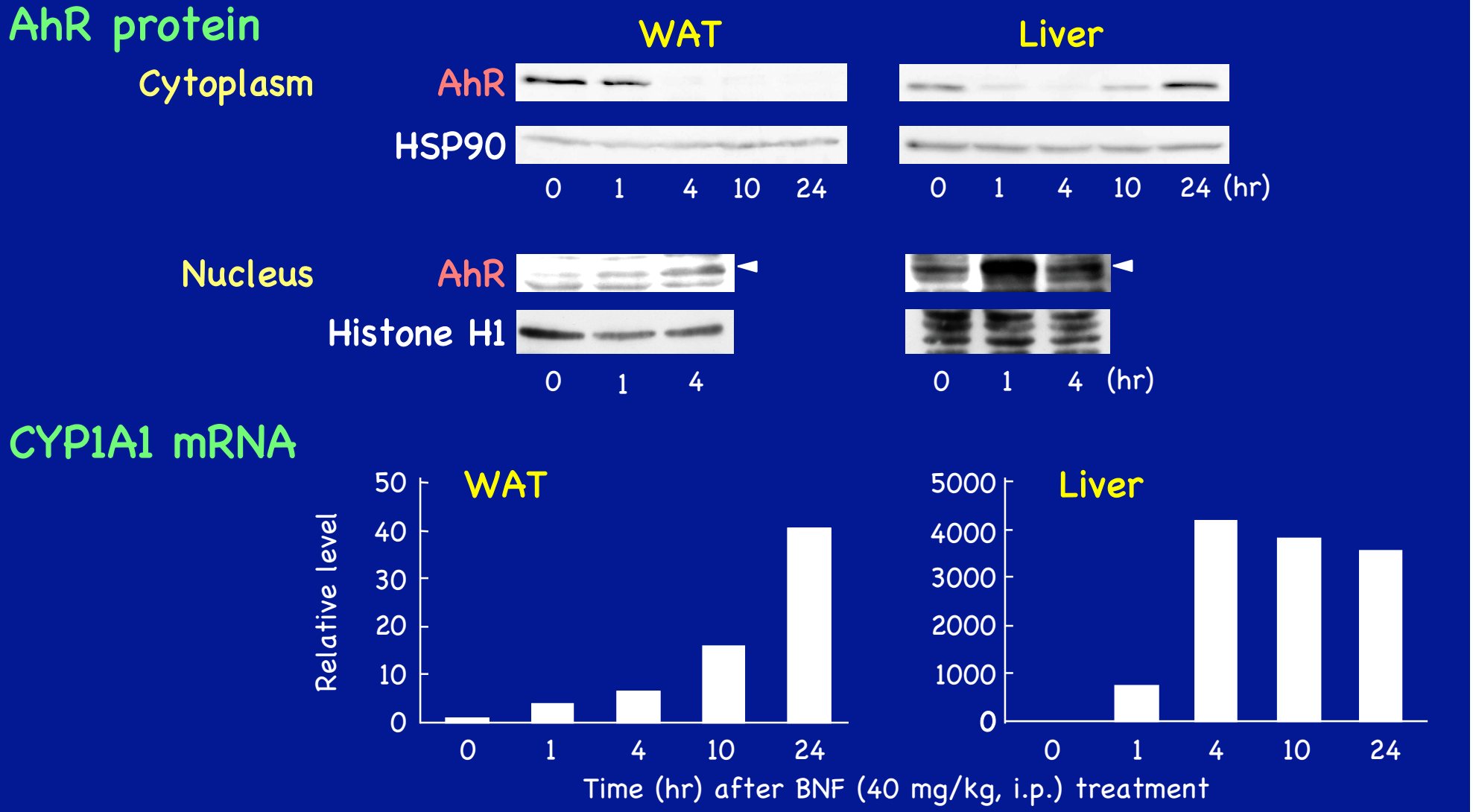
Yoshinari et al., *J Pharmacol Exp Ther*, 2004, **311**, 147.

mRNA levels of transcription factors in rat liver and epididymal WAT



Yoshinari et al., *J Pharmacol Exp Ther*, 2004, **311**, 147.

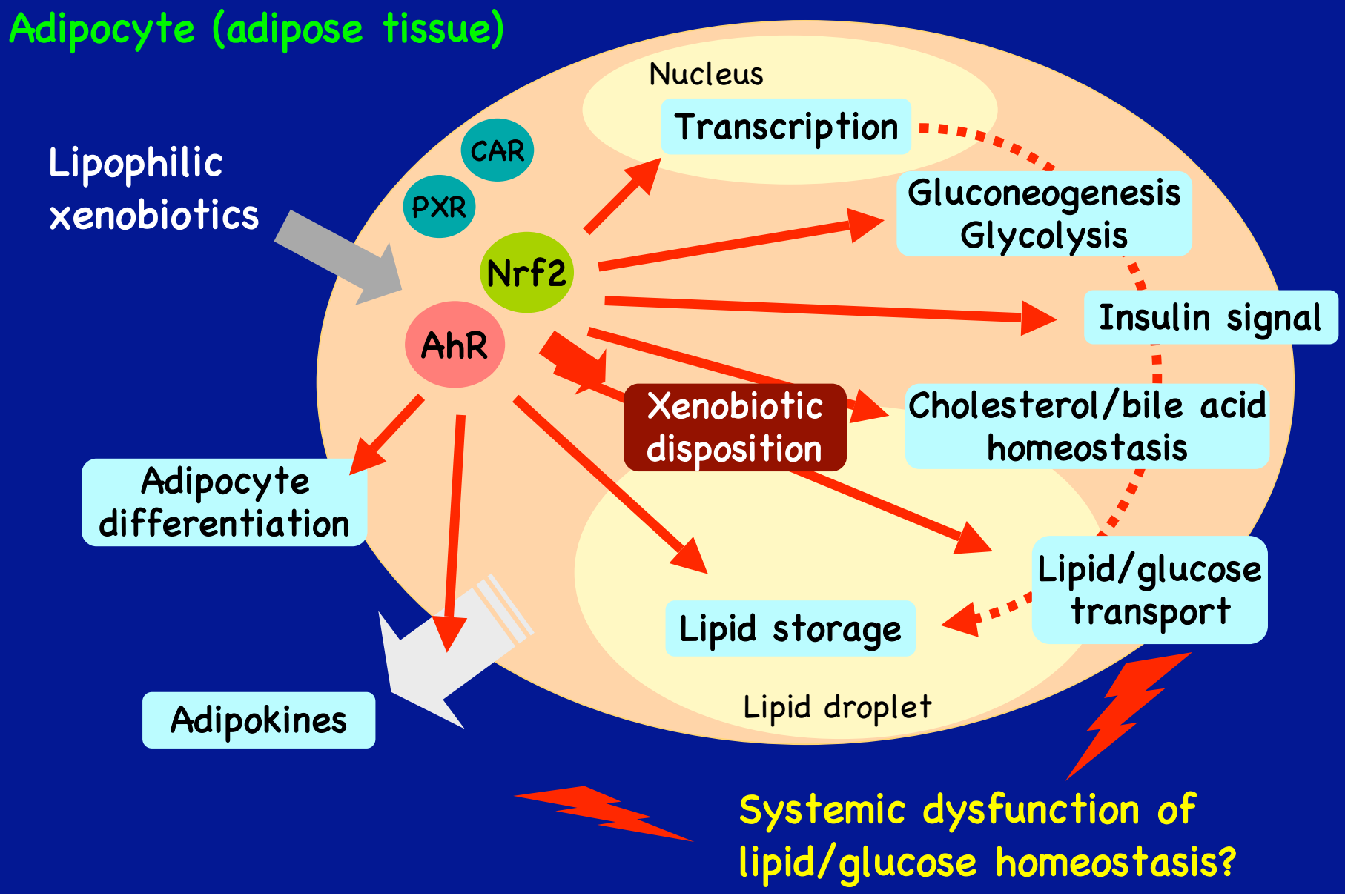
BNF-induced nuclear translocation of AhR in rat WAT



Lipophilic AhR ligands activate AhR in rat WAT to enhance the expression of CYP1A1.

Yoshinari et al., *Drug Metab Dispos*, 2006, **34**, 1081.

Lipophilic xenobiotic may affect adipocyte function through changing its gene expression profile



Summary

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