PCEUT 527
Enzyme Induction: Biochemical Mechanisms
02/11,14/11

1. General Principles
2. Transcriptional Activation
3. Protein degradation
4. Protein stabilization

References

Pavek, P. and Dvorak, Z..

Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the **cytochrome P450 superfamily in human extrahepatic tissues**.

Current Drug Metabolism 2008, 9:129-143.

Rushmore TH, Kong AN.

Pharmacogenomics, **regulation and signaling pathways of phase I and II drug metabolizing enzymes.**

Current Drug Metab. 2002 Oct;3(5):481-90

Why Does Induction Occur?

- An adaptive response of CYPs to xenobiotic exposure or increased levels of endogenous compounds (e.g. hormones)
- Slow regulatory process (compared to CYP inhibition which is rapid)

Oral Contraceptives + St. John's Wort = Miracle babies!

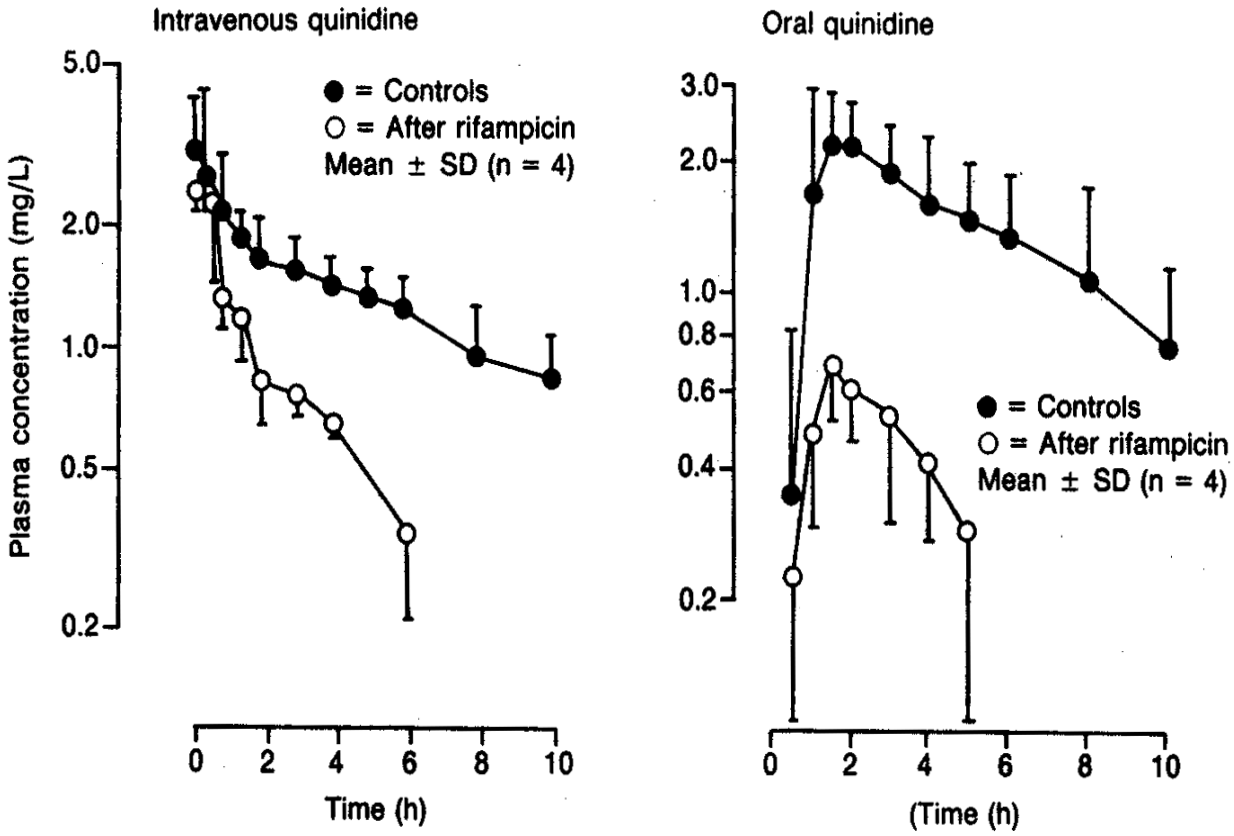
Consequences of Induction

- Change in pharmacological effect because of increased drug metabolism
  - Decreased pharmacological/toxicological effect when activity associated with parent (unchanged drug)
  - Increased pharmacological effect when activity associated with metabolite (increased conversion of prodrug to active metabolite)
- Balance between “toxication” and “detoxification”
  - Decrease in toxicity due to accelerated detoxification
  - Increase in toxicity due to formation of reactive metabolites

Consequences of Induction

- Clinical significance depends on:
  - Magnitude of change in the concentration of the active species (parent, active or toxic metabolites)
  - at the site of pharmacological action, and
  - the therapeutic index of the drug

Rifampin - CYP3A4



NEJM 304:1466-9, 1981

Induction – General Principles

Definition:

- An increase in steady-state concentration of enzyme following exposure to an appropriate stimulus.

Kinetic Considerations:

- For a first-order metabolic process that follows simple Michaelis-Menten kinetics, intrinsic clearance defined as

$$Cl_{\text{int}} = \frac{V_{\max}}{K_m} = \frac{E_t \bullet k_{cat}}{K_m}$$

- Induction accelerates metabolism through an increase in V_{max}

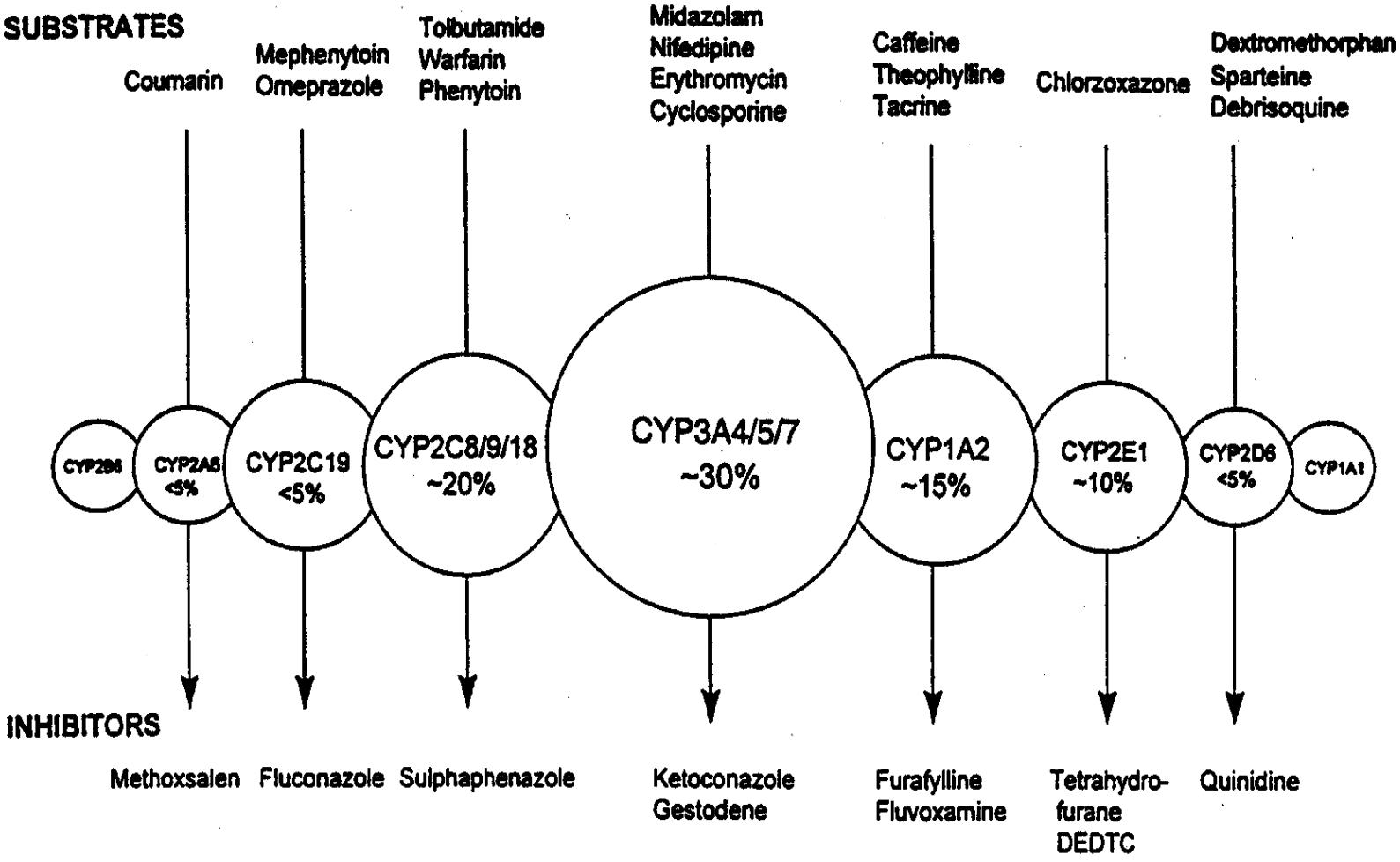
Induction – General Principles

- Enzyme induction can occur by a change in rate of enzyme synthesis or rate of enzyme degradation

$$E_{ss} = \frac{R_o}{k_{\text{degr}}}$$

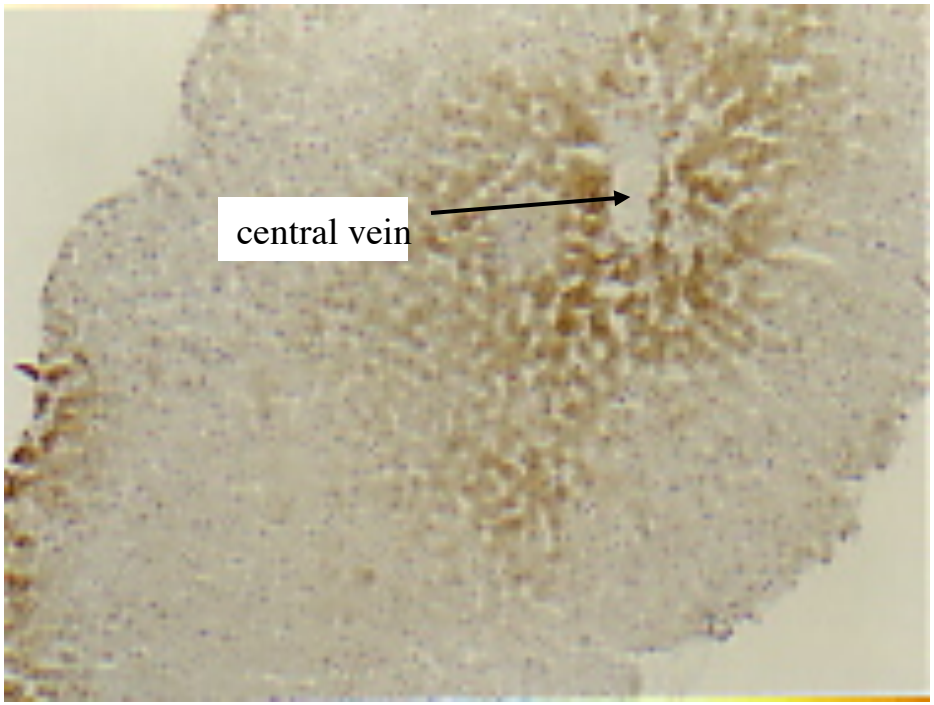
- Synthesis – usually considered zero-order process
- Degradation – first-order process

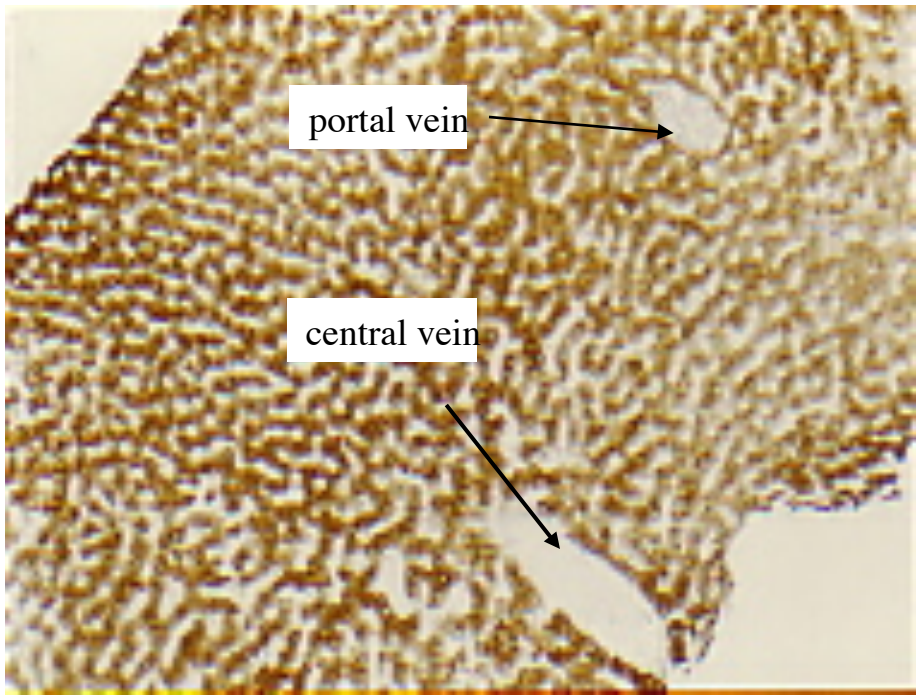
Inducible Human Cytochrome P450s



INDUCERS						
Phenobarb.	Phenobarb. Rifampicin	Phenobarb. Rifampicin	Phenobarb. Rifampicin Dexamethasone Carbamazepine	Omeprazole Tobacco smoke	Ethanol Isoniazid	No known

Induction of Hepatic CYP3A by Phenytoin





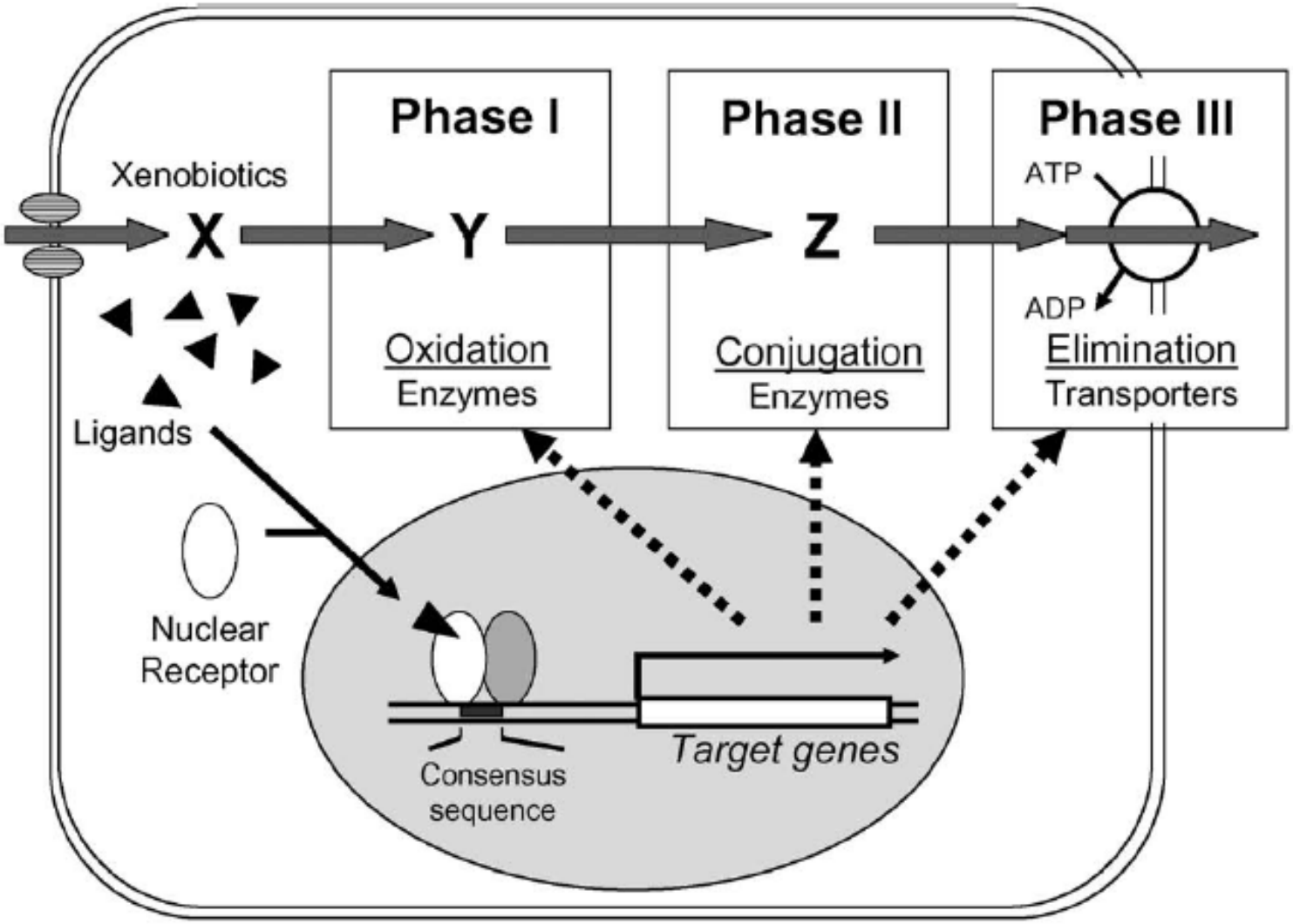
- Biopsies collected from a liver transplant patient placed on phenytoin for seizure control (presumed CsA-induced). Long-term treatment with phenytoin induces enzyme expression in every hepatocyte.

Important Considerations

- Inducers can often induce more than one enzyme
  - Interactions with multiple cell signaling receptors and/or receptor binding to multiple gene targets (e.g., phenobarbital and CAR/PXR and *CYP3A4/CYP2C9/ CYP2B6)*

- A drug can induce Phase I, Phase II and Phase III (transporters) simultaneously (e.g., rifampin and CYPs/UGT/ P-gp)
  - Both parent and metabolite clearance and excretory routes can be affected

Considerations



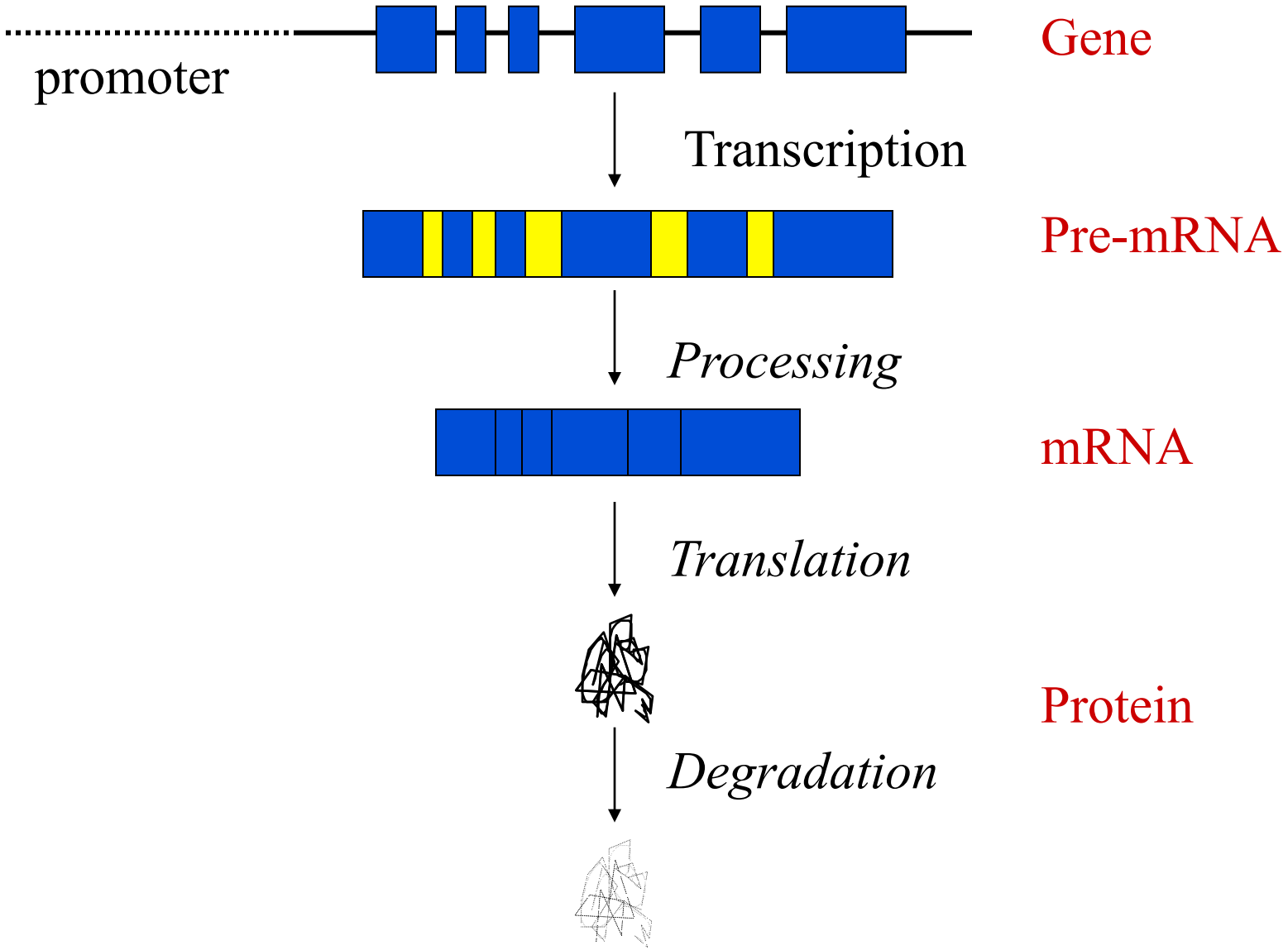
Ref: Nakata, 2006

Considerations

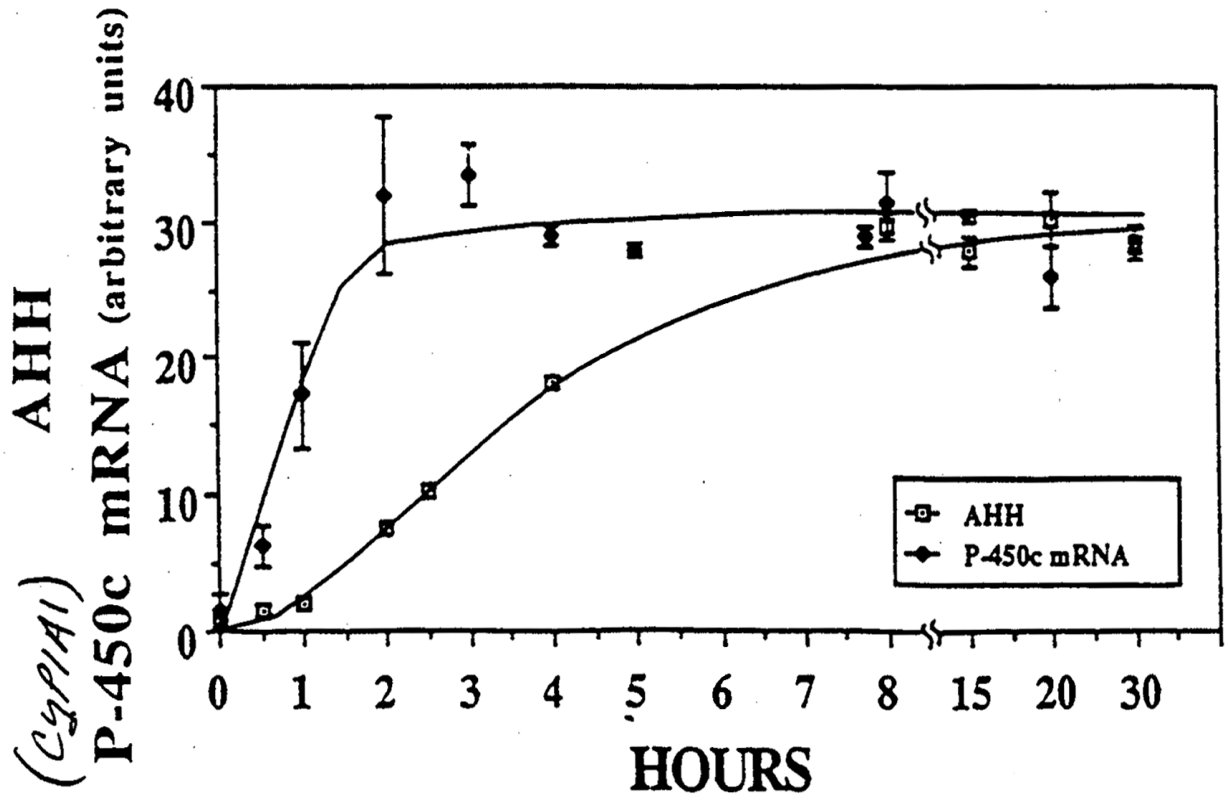
- Some drugs induce their own metabolism ("autoinduction" e.g., carbamazepine), but others act on non-self clearance enzymes

- Induction can occur in multiple tissues, but often associated with tissue-specific receptor or coactivator/repressor expression (ex. PXR-*CYP3A4*)
  - contrast clearance *vs.* toxicological importance

From gene to protein



Time-course of *CYP1A1* Induction in Rat Liver

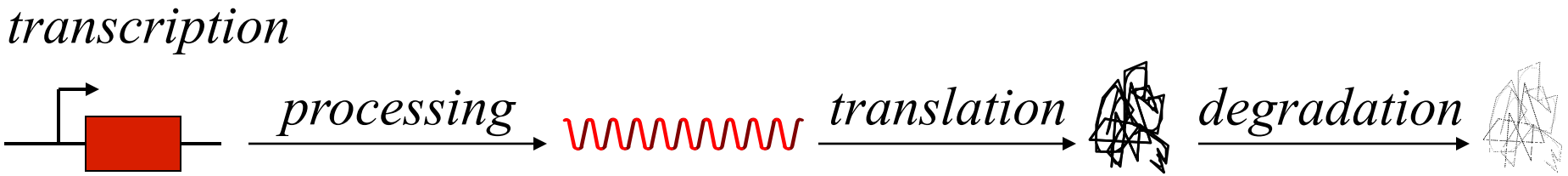


- Transcriptional activation occurs rapidly, followed by increased protein synthesis. mRNA reaches a new steady-state very rapidly (short $t_{1/2}$), protein/activity much later.

Possible steps in Induction

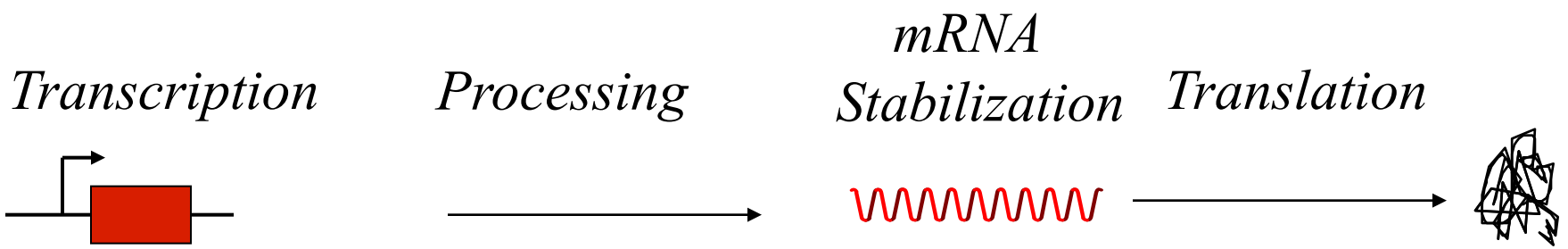
- Multiple steps which can be altered in the presence of an inducer

$$\text{Amt Enzyme}_{ss}\,(\text{mol}) = \frac{\text{Synthesis Rate}(\text{mol}/\text{hr})}{k_{deg}\,(\text{hr}^{-1})}$$



Increased Protein Synthesis

- Receptor-mediated transcriptional activation ***
- Increased efficiency of mRNA processing
- Increased mRNA stabilization
- Reduced miRNA synthesis
- Enhanced mRNA translation efficiency



CYP1A1, 1A2, CYP2A6, 2B6, CYP2C9, 2C19, CYP3A4

CYP1A2
CYP2E1

CYP1A1, CYP2B, 2E1, CYP3A

CYP2E1

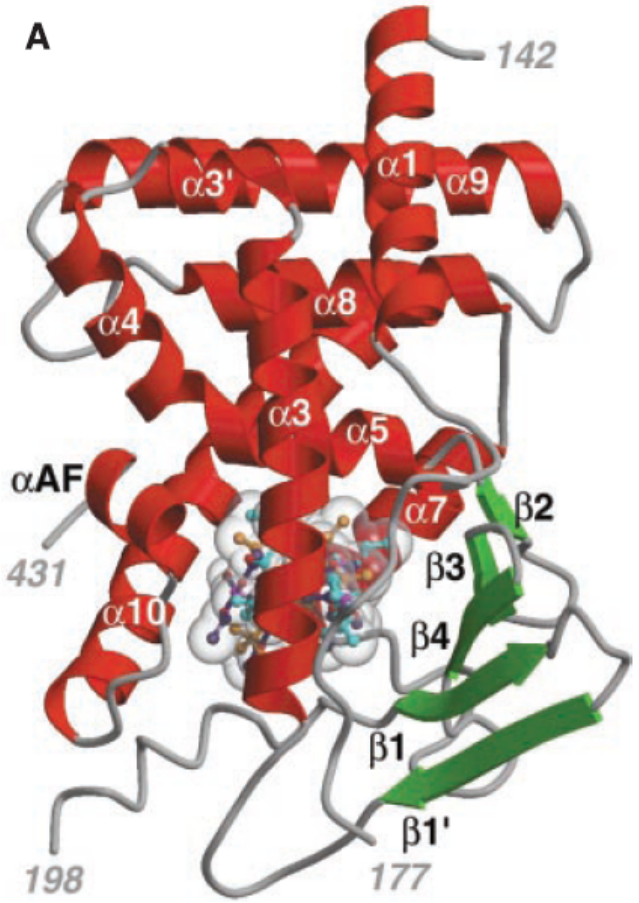
Receptor-Mediated Regulation

- Constitutive, induced and repressed expression of drug metabolizing enzymes and transporters is largely under transcriptional control
- Most common and important mechanism of induction involves nuclear receptor activation
  - P450s
  - UDP glycuronosyltransferases (UGT)
  - Sulfotransferases (SULT)
  - Glutathione S-transferases (GST)
  - Multidrug resistance protein 1 (MDR1)
  - Multidrug resistance-associated proteins (MRP)
  - Organic anion-transporting polypeptides (OATP)

General: Nuclear Receptor Family 1 (NR1)

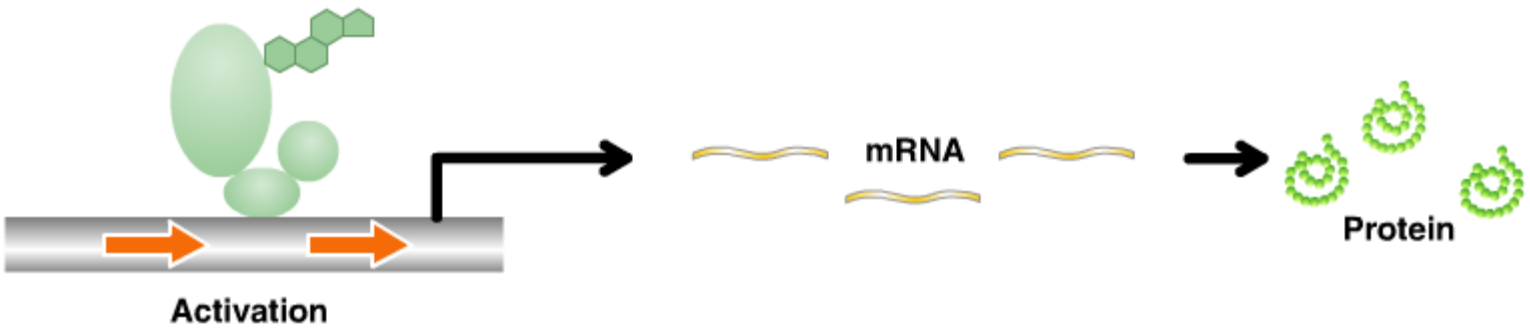
- N-terminal activation function (AF-1)
- Zinc finger DNA binding domain
- Hinge region
- Ligand binding domain
- C-terminal activation function (AF-2)

- Heterodimerizes with 9-*cis* retinoic acid receptor (RXR)



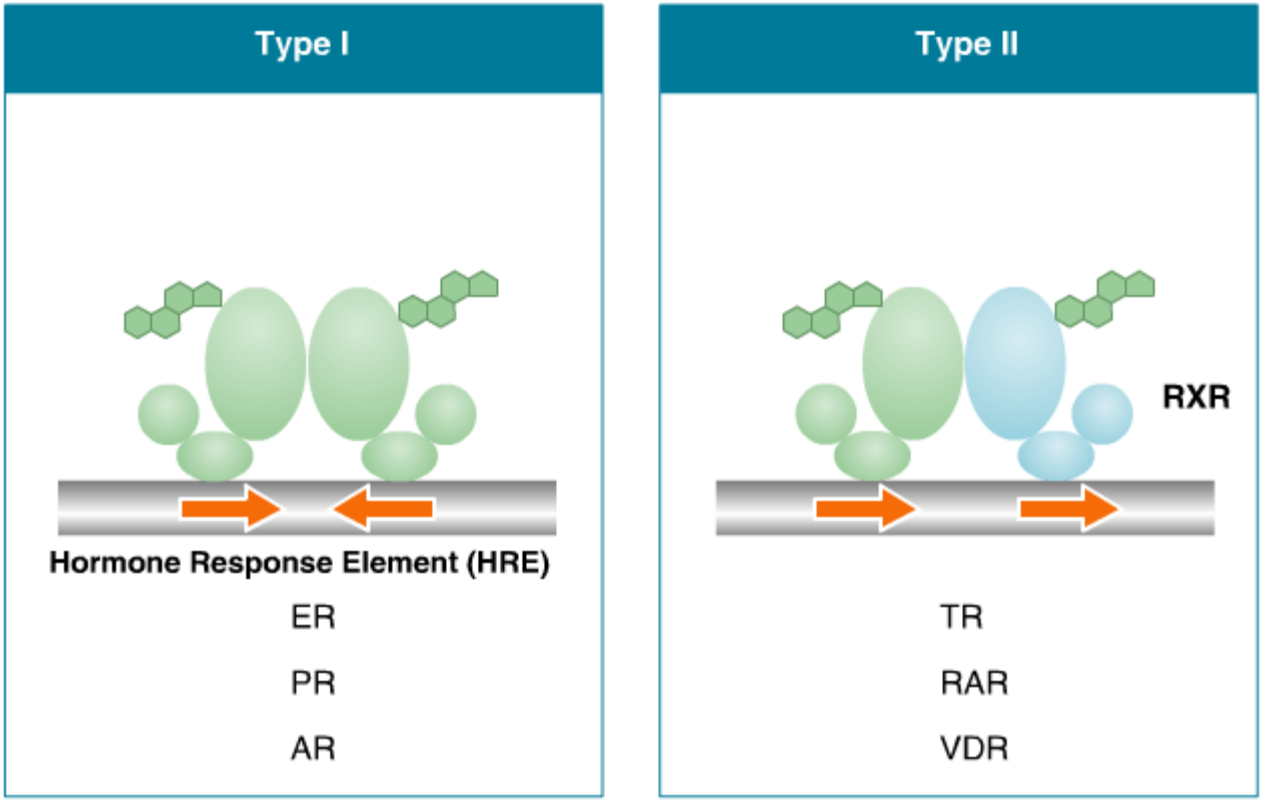
Ref: Whitlock, FASEB, 1996
Redimbo, Science, 2001

Transcriptional Activation – Simplified



- Transcription factors bind to their response elements (5' region of the gene), increase binding/function of polymerase II complex, mRNA is transcribed and translated to protein

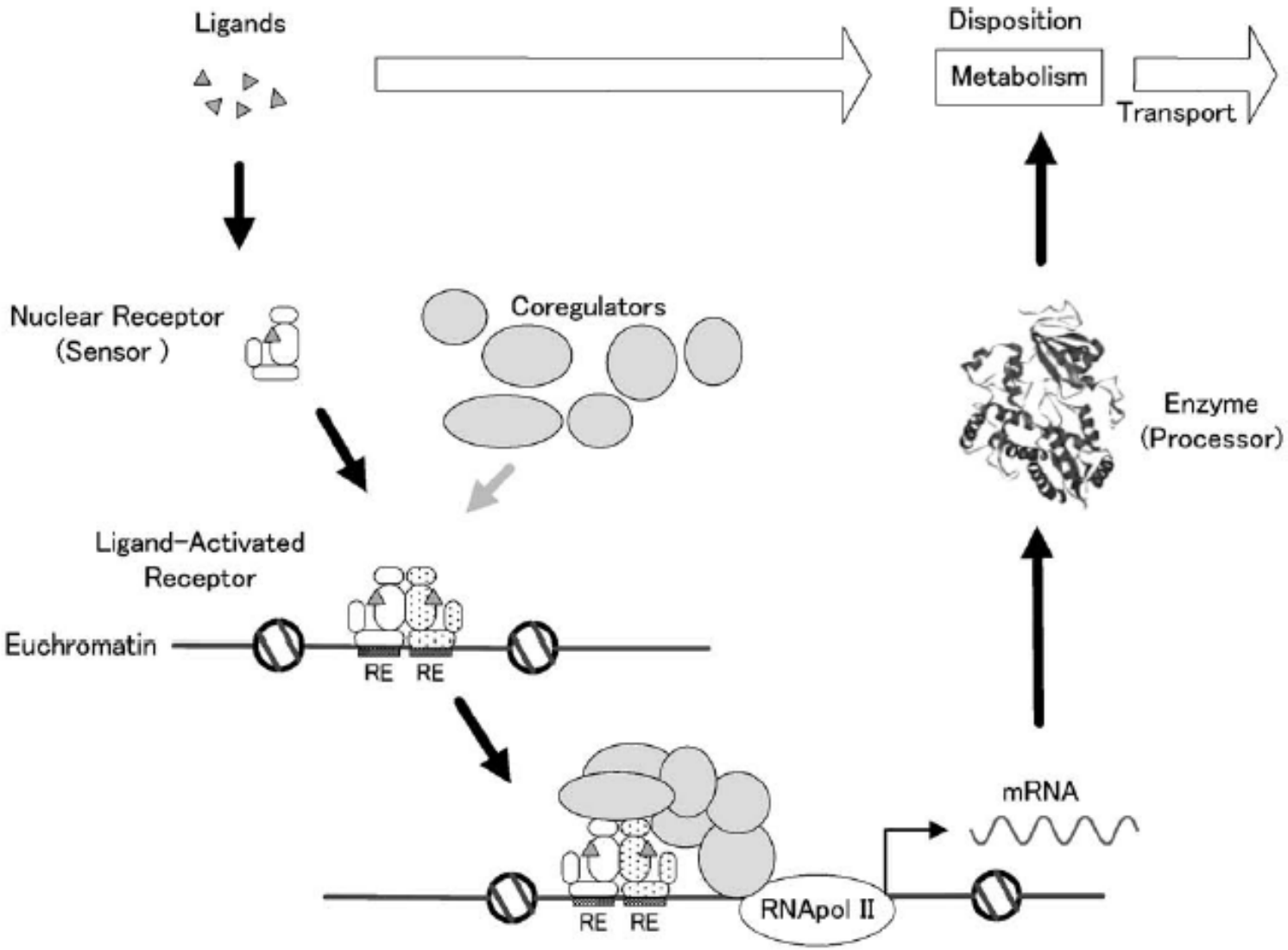
Transcriptional Activation – Response Elements



Transcriptional Activation - Details

- Nuclear receptor associated with corepressors
- Inducer binds and NR dissociates
- Translocation to nucleus (not always)
- Association of with dimerization partner
- Binding of heterodimer to response elements of the target genes
- Release of corepressor proteins
- Recruitment of coactivators and general transcription machinery

Transcriptional Activation – Details

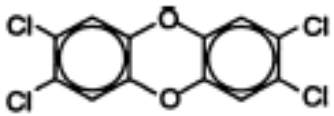


Summary of DME/DTP Nuclear Receptors

CYP Gene Target	Receptor	Inducer
CYP1A1/1A2/1B1	AhR-ARNT	Antiestrogens, PAH
CYP2B6, CYP2C9	CAR-RXRα	Androstanes, bile acids, phenobarbital
CYP3A4	PXR-RXRα	Pregnanes, bile acids, phenytoin, rifampin
CYP4A	PPARα-RXRα	Fibrates, glitazones

AhR

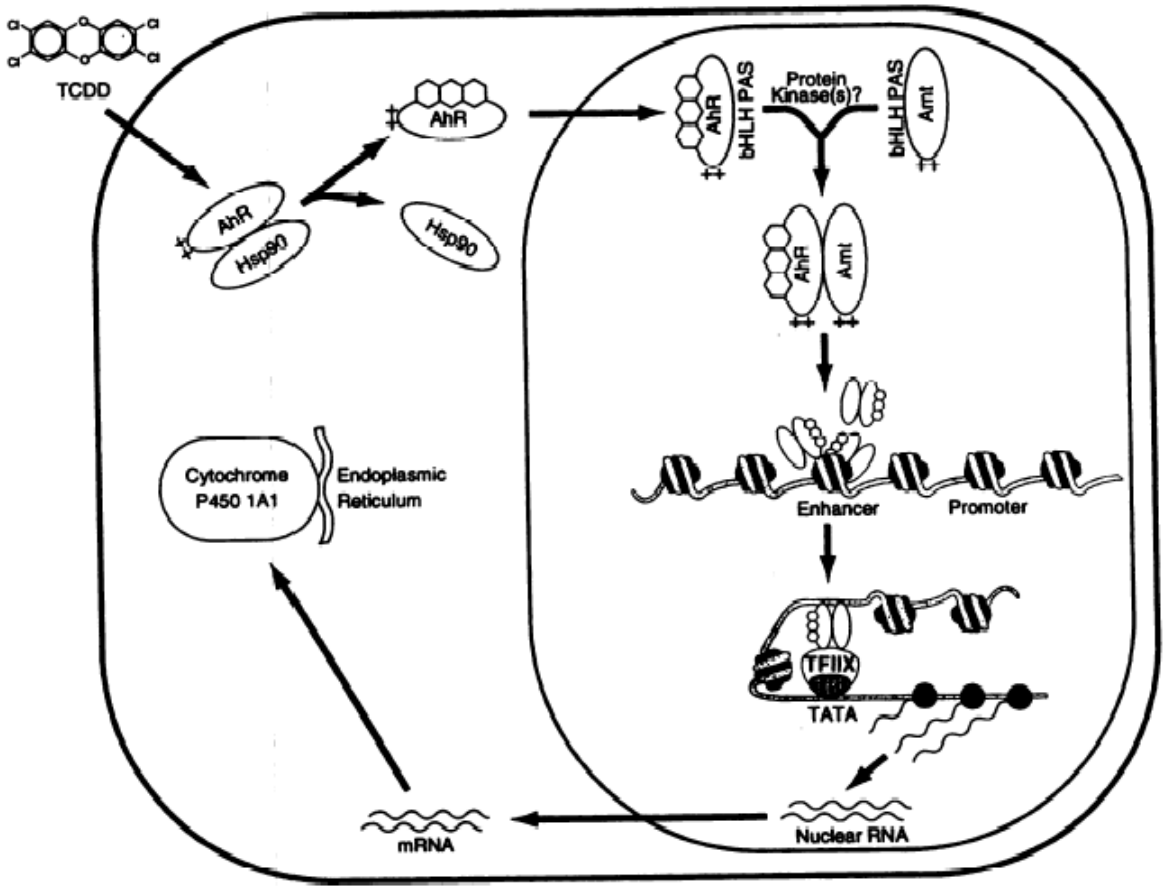
3MC



TCDD

- AhR: Aryl hydrocarbon receptor
  - Response element: XRE
  - CYP1A1, 1A2, 1B1
  - UGT1A1, 1A6
- Activators: planar lipophilic molecules, polycyclic aromatic or halogenated hydrocarbons, β-naphthoflavone, antiestrogens
- Deactivators: 3,4-dimethoxyflavone

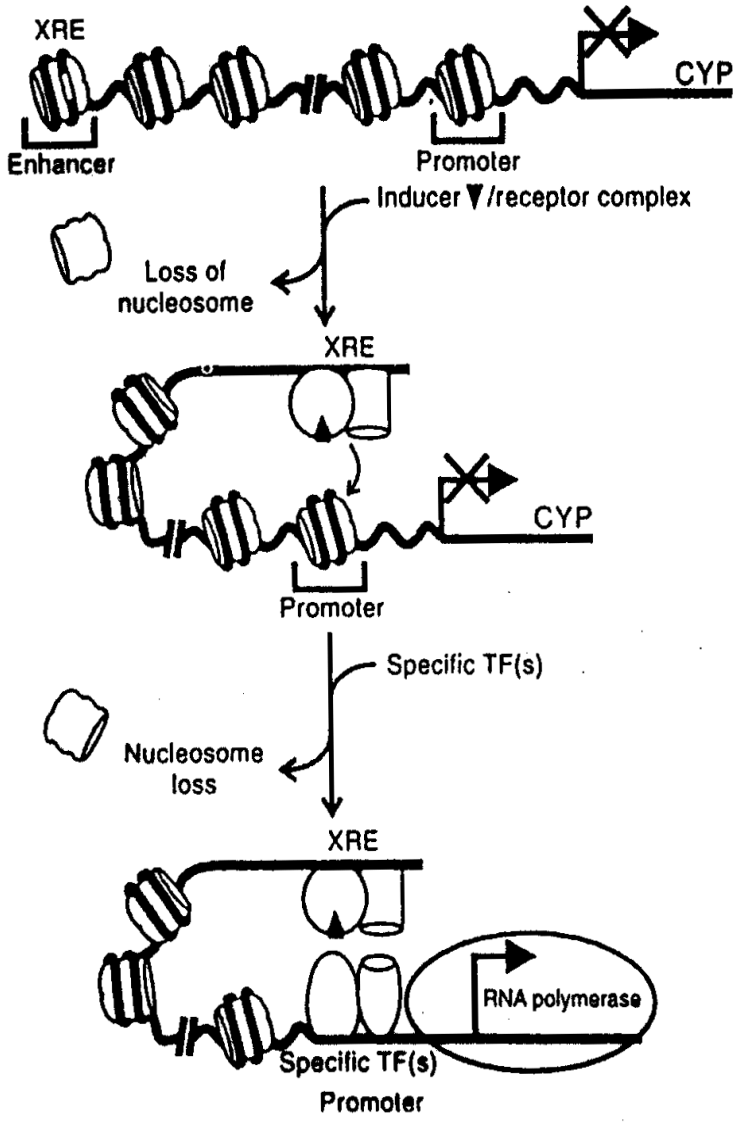
AhR



- Inducer binds
- AhR dissociates with Hsp90
- Translocation to nucleus
- Heterodimerization with Arnt
- Binding to 5'-flanking region of target gene

Ref: Whitlock, FASEB, 1996

Transcriptional Activation: Promoter/Enhancer Effects



- Binding of receptor heterodimer disrupts chromatin structure, permitting binding interactions between promoter and enhancer regions (also requires binding of additional transcription factors, *e.g.,* Sp1)
- The new 3-D structure facilitates the binding of the polymerase II complex and initiation of transcription

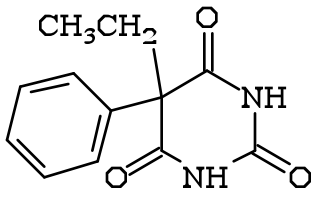
Ref: Clin Exper Pharmacol Physiol, 1998

CAR

- CAR: Constitutive androstane receptor
  - Response elements: DR-3, DR-4, ER-6
  - CYP2A6, 2B1, 2B6, 2C9, 2C19, 3A4
  - UGT1A1
- Constitutively active *in vitro*, quiescent in cytoplasm of hepatocytes *in vivo*
- Treatment with ligand, CAR translocates to nucleus

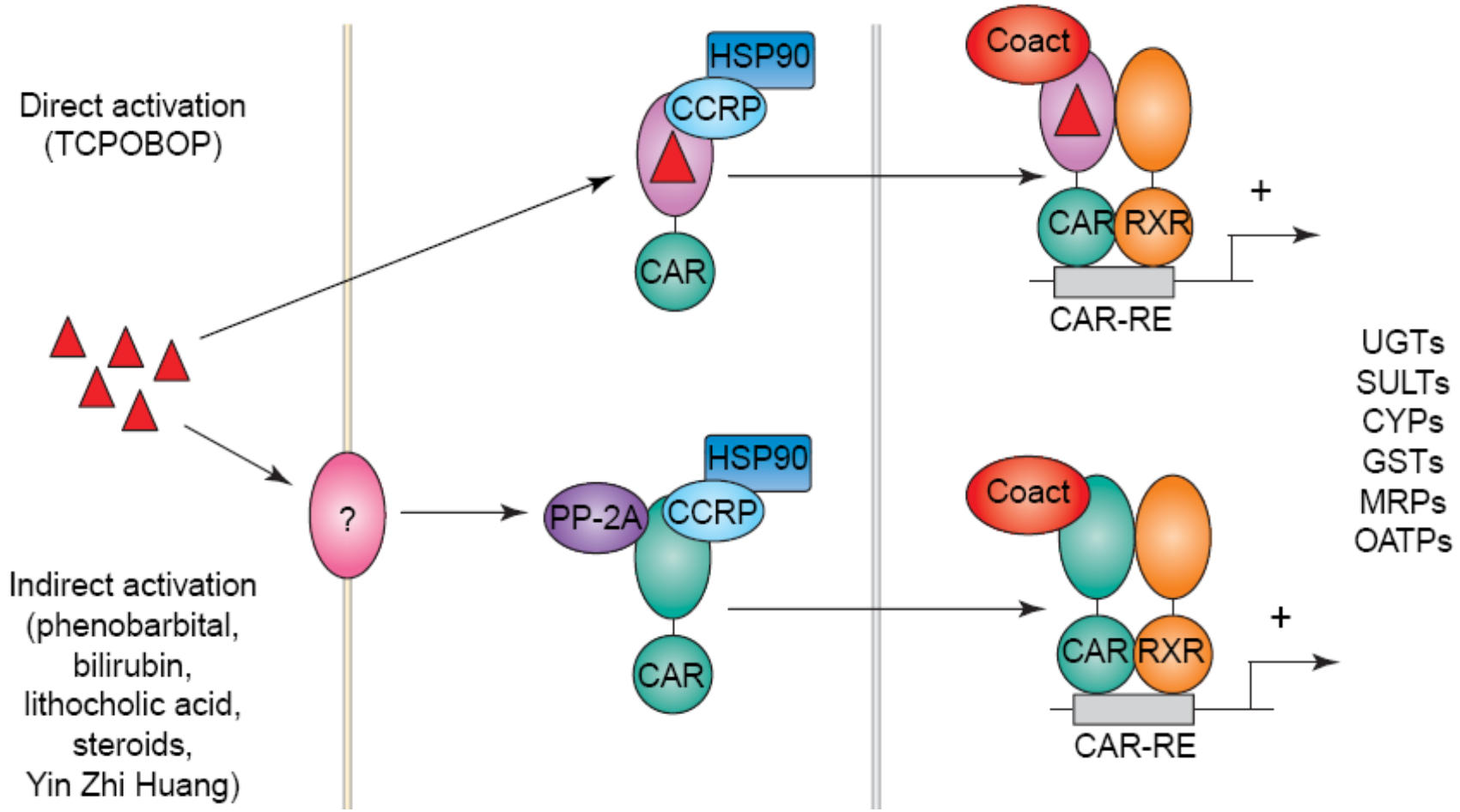
CAR

- Note: phenobarbital, prototypical inducer is not a direct ligand – gene regulation may involve protein phosphorylation, coactivators, cytoplasmic CAR retention protein
- Activators: phenobarbital, TCPOBOP (mice), CITCO (human), clotrimazole, phenytoin, carbamazepine
- Deactivators: Androstanes
- Physiology: bilirubin clearance, bile acid detoxification



phenobarbital

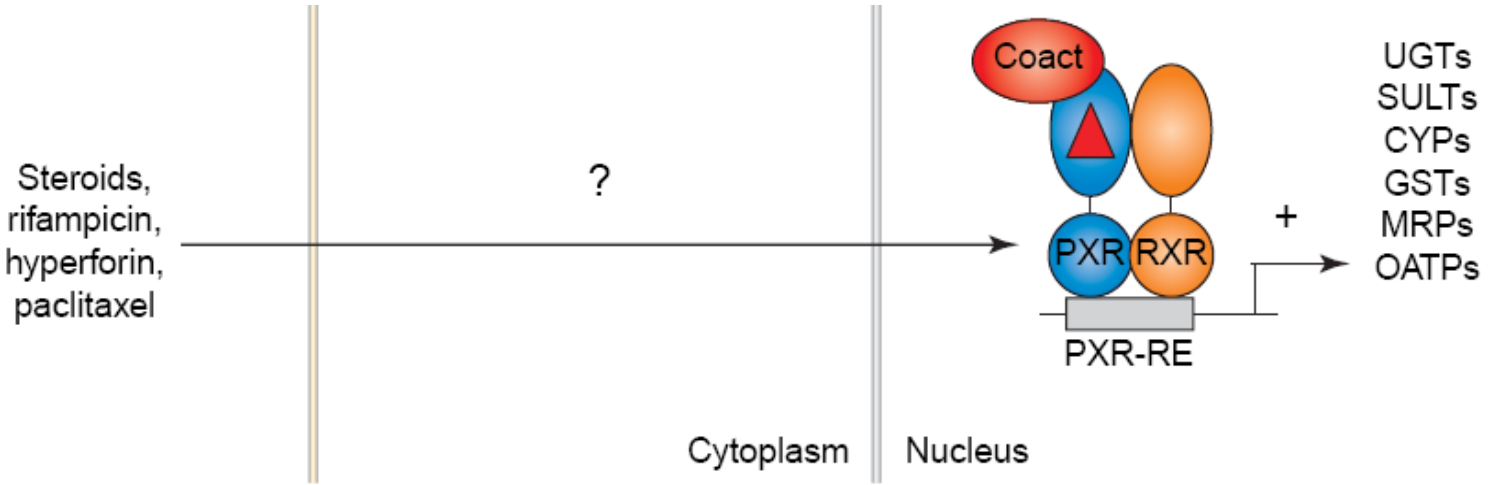
CAR



Ref: Goodwin, Trends Pharmacol Sci, 2004

PXR

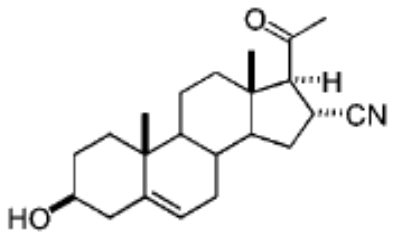
- PXR: Pregnane X receptor
  - Response element: DR-3, DR-4, ER-6, ER-8
  - CYP1A2, 2B6, 2C9, 2C19, 3A4, 3A7
  - SULT2A1, UGT1A1, 1A3, 1A4, MDR1, AHR
  - Represses CYP7A1
- Treatment with ligand, PXR translocates to nucleus (or resides in nucleus)



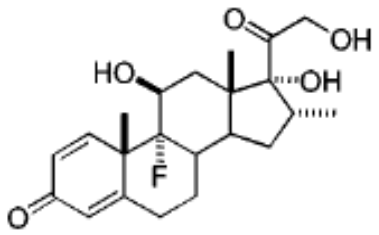
Ref: Goodwin, Trends Pharmacol Sci, 2004

PXR

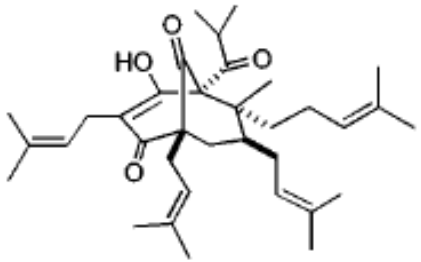
- Crystal structure solved – large binding pocket, promiscuity of PXR towards xenobiotics
- Structurally diverse molecules can induce CYP3A *via* the same biochemical pathway



Pregnenolone 16α-carbonitrile



Dexamethasone



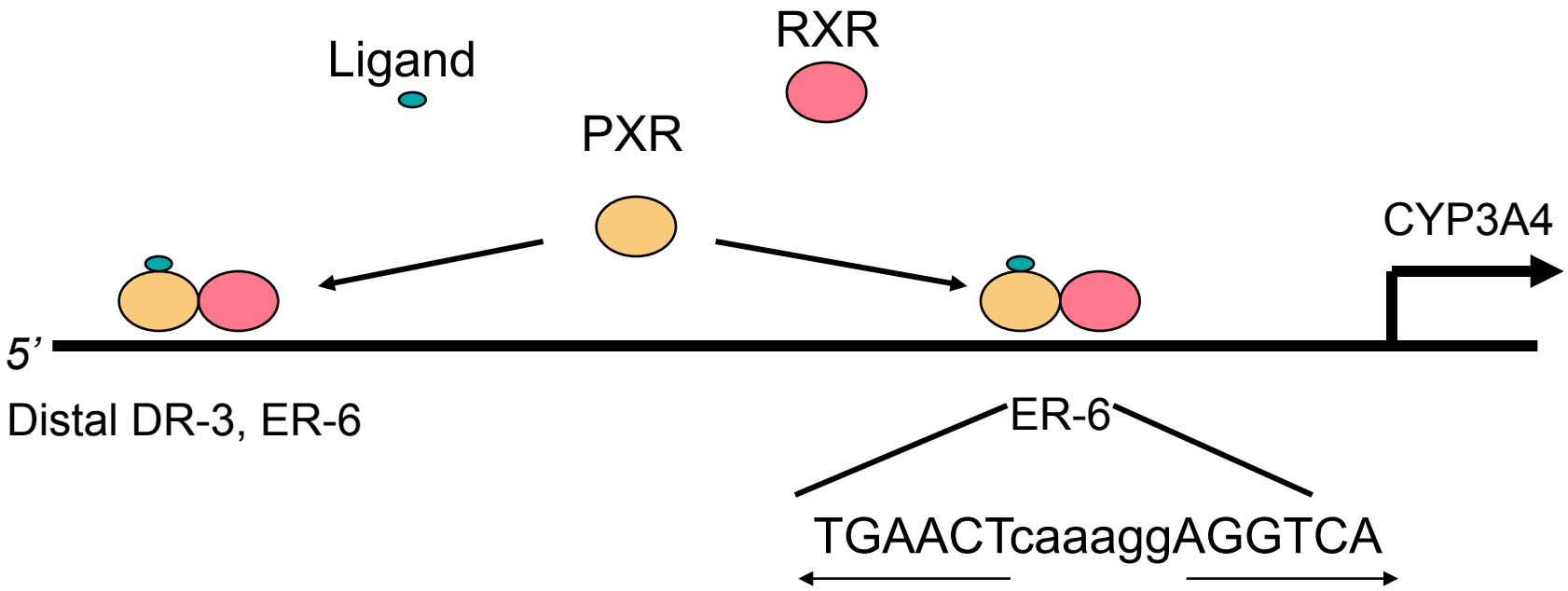
Hyperforin

- Activators: bile acids, rifampin, paclitaxel, nifedapine, clotrimazole*, ritonavir*, glucocorticoids, efavirenz, statins
- Deactivators: ET-743, sulfurafane

* act as inhibitors

Induction of *CYP3A4 via* PXR

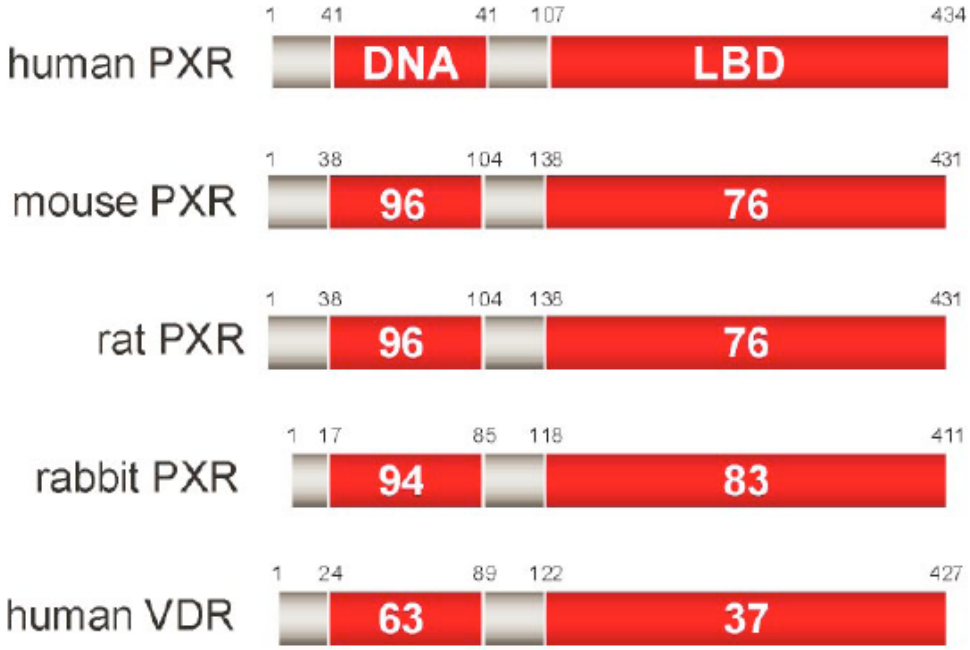
- Maximum induction of CYP3A4: binding of PXR/RXR to distal (DR-3, ER-6) and proximal (ER-6) response elements
- This feature distinguishes *CYP3A4* from the non-inducible *CYP3A5* (lacks distal elements)



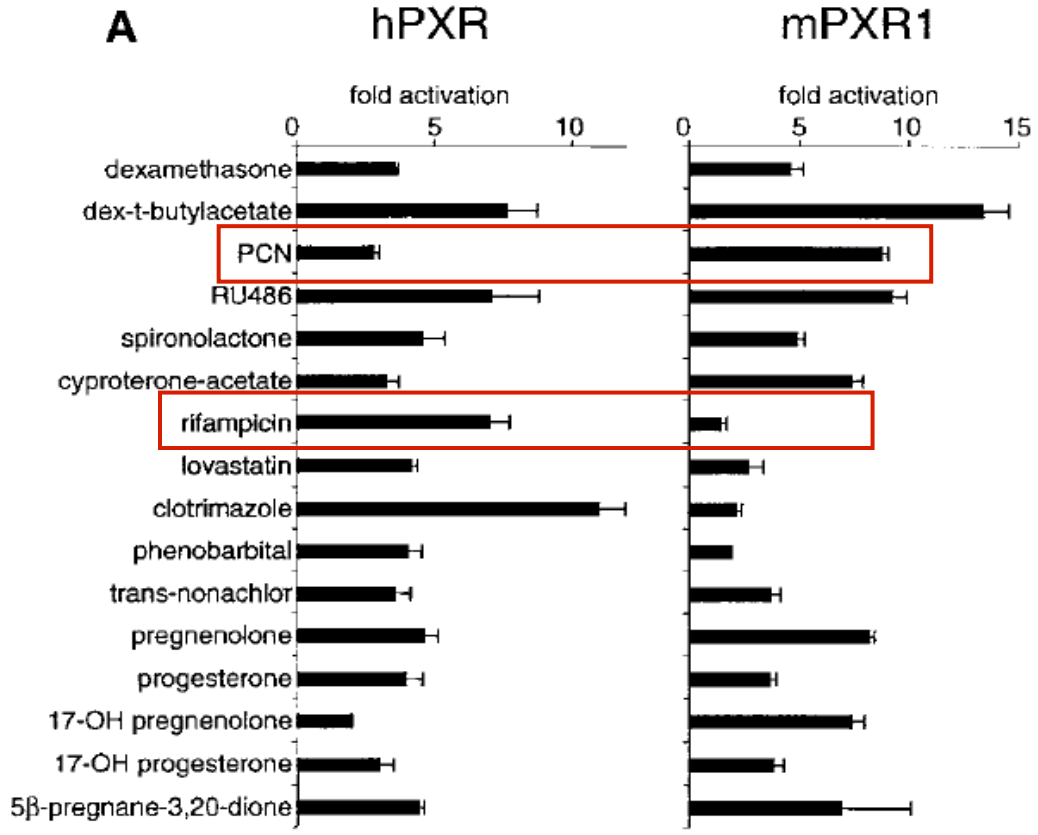
Ref: Goodwin, Mol Pharmacol, 1999

Species Differences in PXR

- Species dependency in CYP3A induction by different inducers (rifampin and PCN) – amino acid sequence difference in ligand binding domains of PXR
- Humanized mice (PXR knockout + human SXR) respond to “human” inducers



Species Differences in CYP3A Induction



- Interspecies differences in the inducibility of CYP3A by xenobiotics can be explained by the difference in binding affinity of the ligand to PXR (ligand binding domain variation).

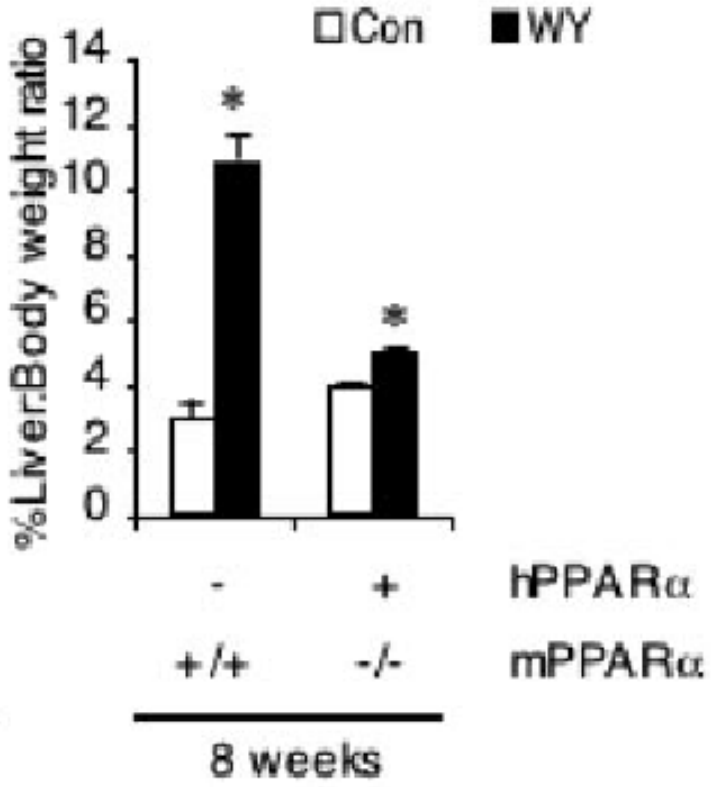
Ref: Goodwin, Ann Rev Pharmacol Toxicol, 2002
Lehmann, J Clin Invest, 1998

PPARα

- PPARα: Peroxisome proliferator-activated receptor
  - Response element: DR-1
  - CYP4A, UGT1A9, 2B4
- PPARα involved in lipid and glucose metabolism
- CYP4A induced – catalyzes ω-oxidation of fatty acids (e.g., lauric acid, arachidonic acid)
- Activators: phthlate ester plasticisers, fibrates, glitazones, certain herbicides, WY-14643

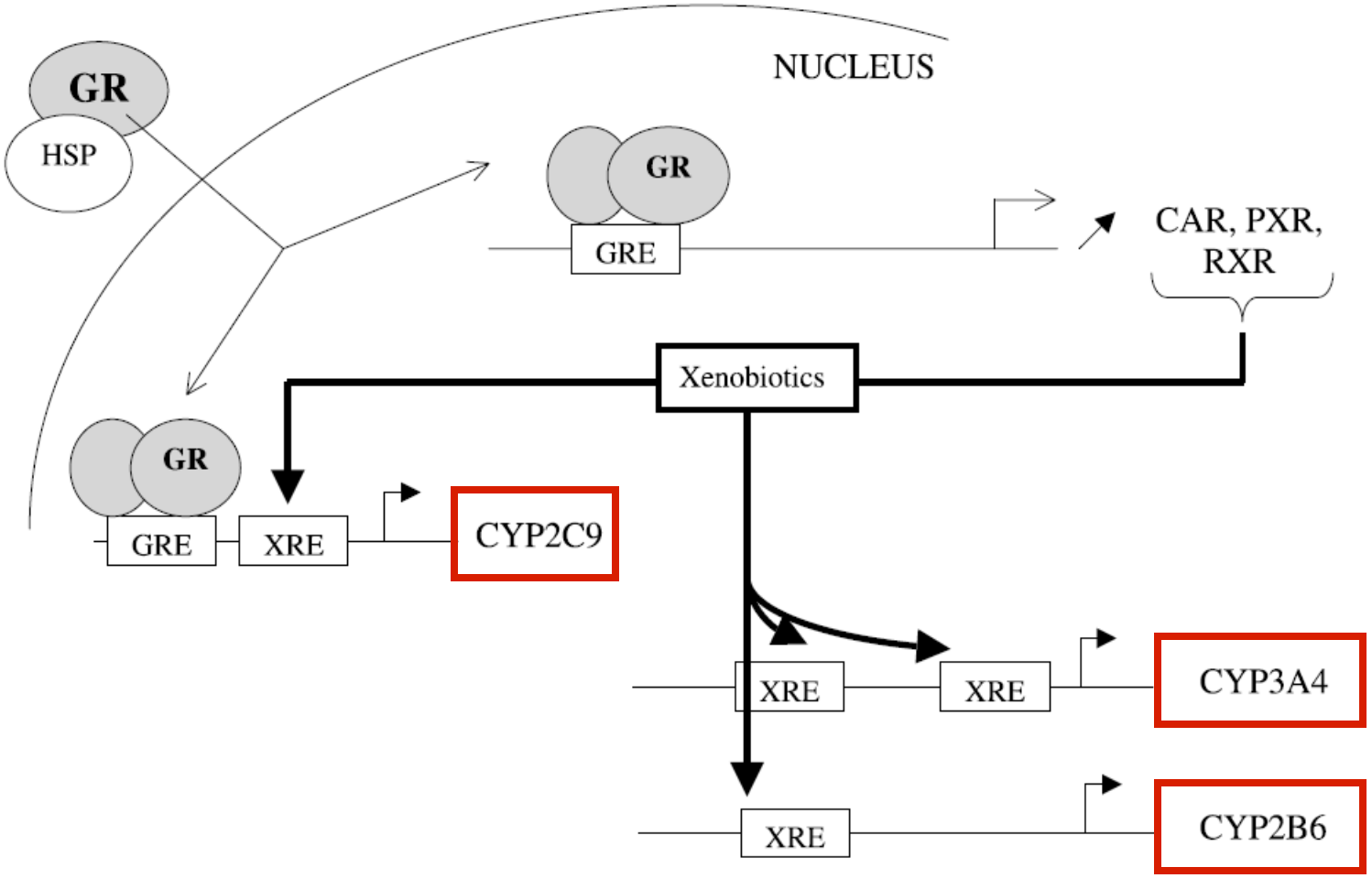
PPARα

- Exposure of rodents to peroxisome proliferators leads to increased size and number of hepatic peroxisomes, hepatomegaly and carcinogenesis
- This does not seem to occur in humans



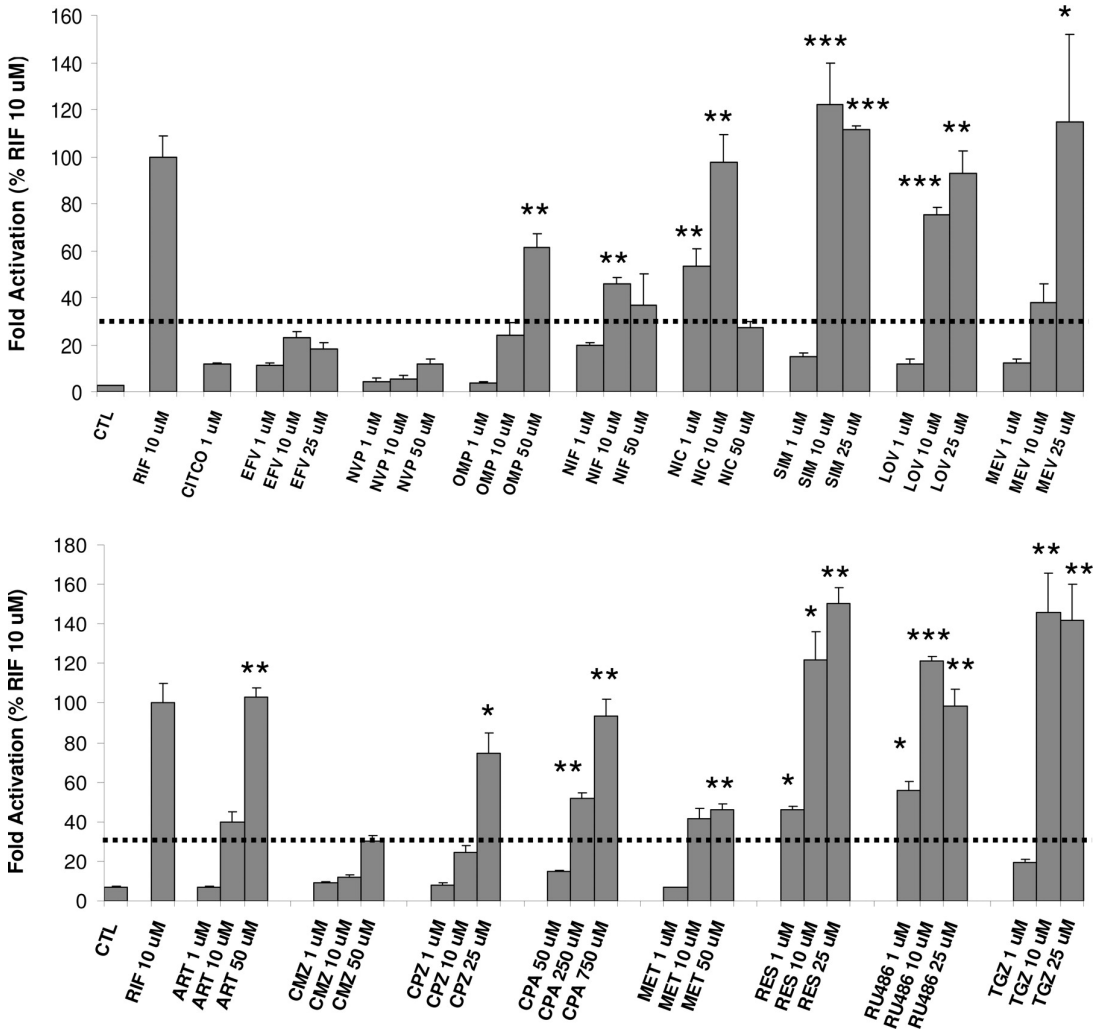
Ref: Cheung, Cancer Res, 2004

Cross-Talk Between Nuclear Receptors



Ref: Pascussi, Biochim Biophys Acta , 2003

Ligand-Selective hPXR Activation



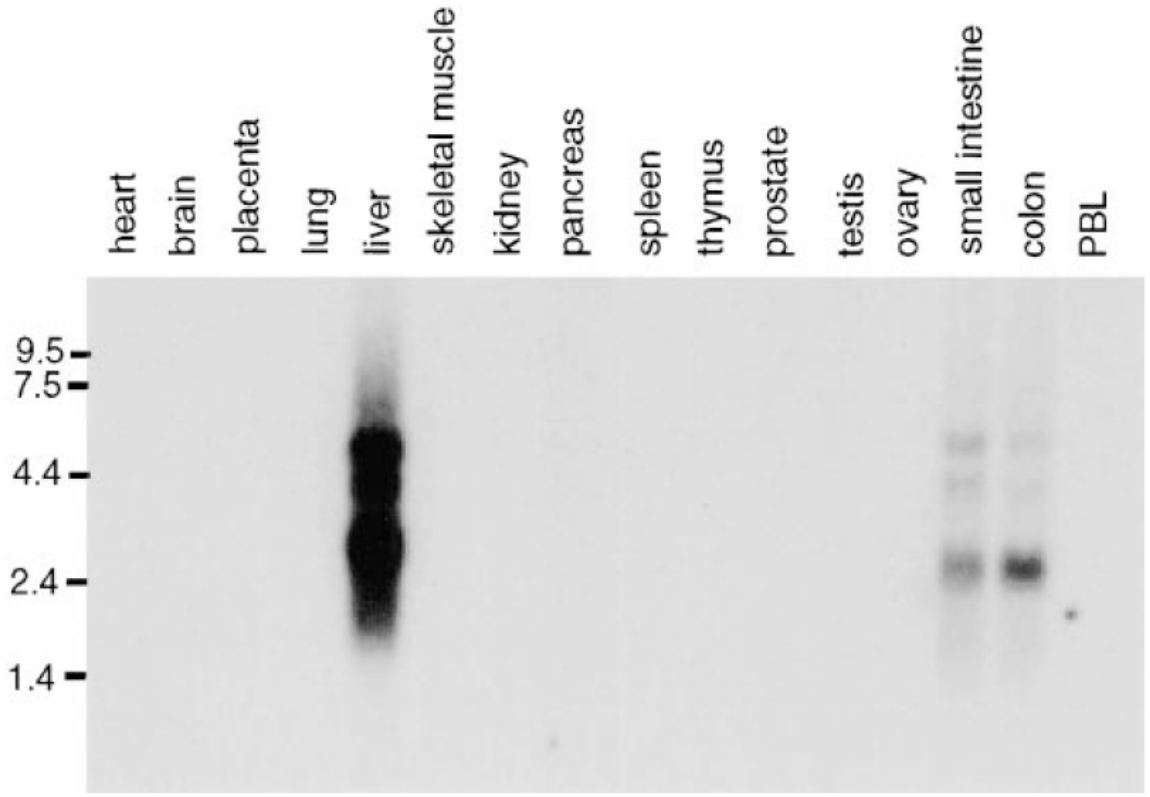
- HepG2 cells transfected with hRXR and a CYP3A4 reporter construct.
- Efavirenz, nivirapine, carbamazepine and phenytoin are poor hPXR activators, but induce CYP3A4 – mediated by CAR activation.

Faucette et al, JPET, 2006

Complications: Where do you see induction?

- Tissue expression of nuclear receptor (PXR)
- Nuclear receptor splice variants
- Response element of target gene inducer (PXR activation of CYP3A4 and P-gp)
- Inducer (PXR activation of CYP3A4 and P-gp)
- Tissue specific corepressors, coactivators, transcription factors

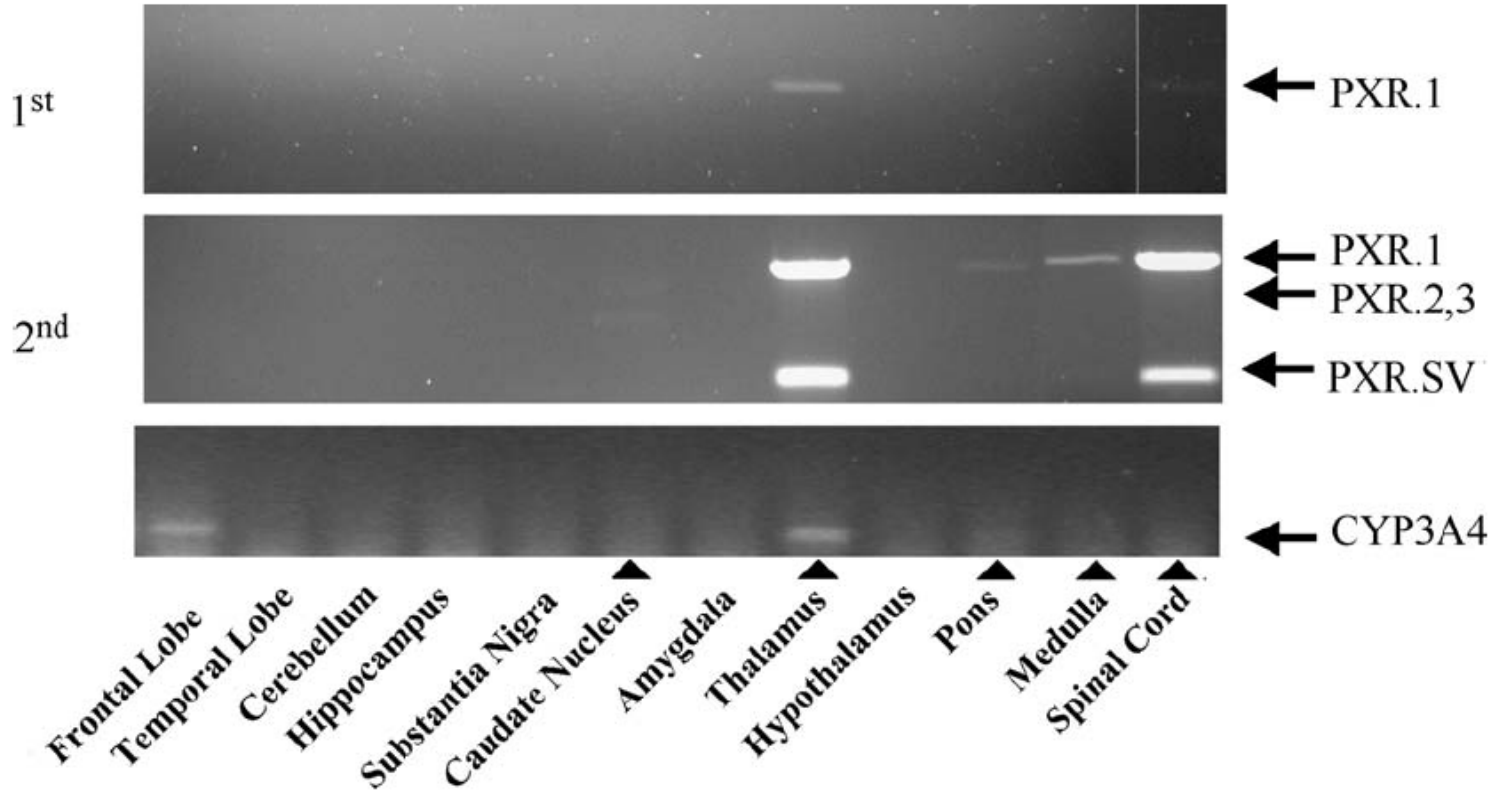
Tissue Expression of hPXR



- Northern blot of PXR hRNA in human tissues
- Major inducible organs express hPXR

Ref: Lehmann, J Clin Invest, 1998

Tissue Expression of hPXR



- Other tissues (such as brain) may express low levels of PXR (or alternatively spliced forms) – detectable by PCR
- Maybe important for P-glycoprotein induction

Ref: Lamba, Toxicol Appl Pharmacol , 2004

Genetic Contribution to Variable CYP3A4 Inducibility

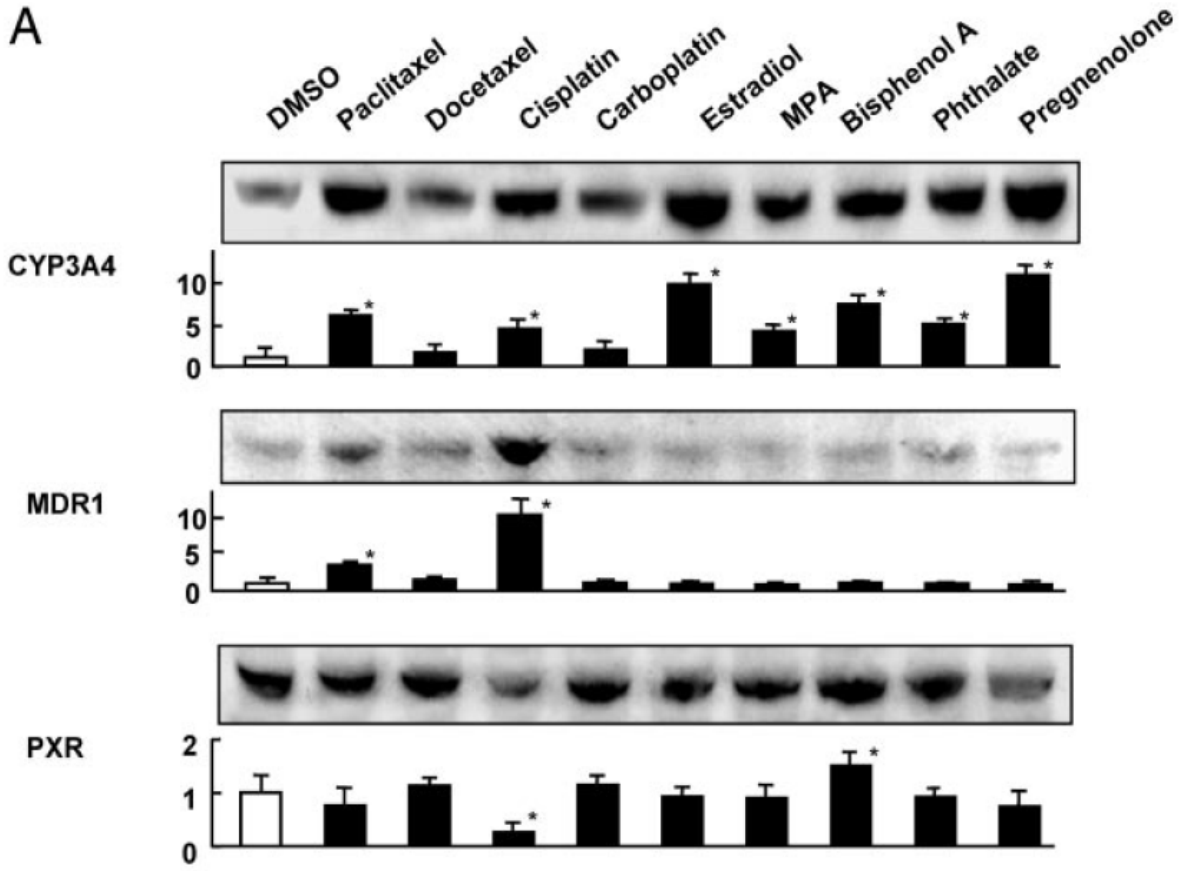
- A number of mutations in the PXR gene have been uncovered recently. Some seem to alter CYP3A4 and CYP2B6 induction response.

Possible mechanisms:

- altered PXR transcription and protein levels
- altered ligand binding to PXR
- altered interaction of heterodimer with response elements

Pharmacogenetics 11:555-72, 2001
Drug Metab Disp 29:1454-9, 2001
Drug Metab Disp 39:92-97, 2011

hPXR Activation: Ligand and Target Gene Effects



- HEC-1 cells (abundant PXR), treated with various ligands, CYP3A4 and P-gp detected by Western blot
- Paclitaxel and cisplatin strongly induced MDR1, whereas CYP3A4 is only weakly induced

Ref: Masuyama, Mol Endo, 2005

Ligand-Specific and Promoter-Specific Induction

- Although multiple genes can be activated by PXR, the magnitude of response for each gene depends on the ligand; this is the result of co-activator specific interactions.

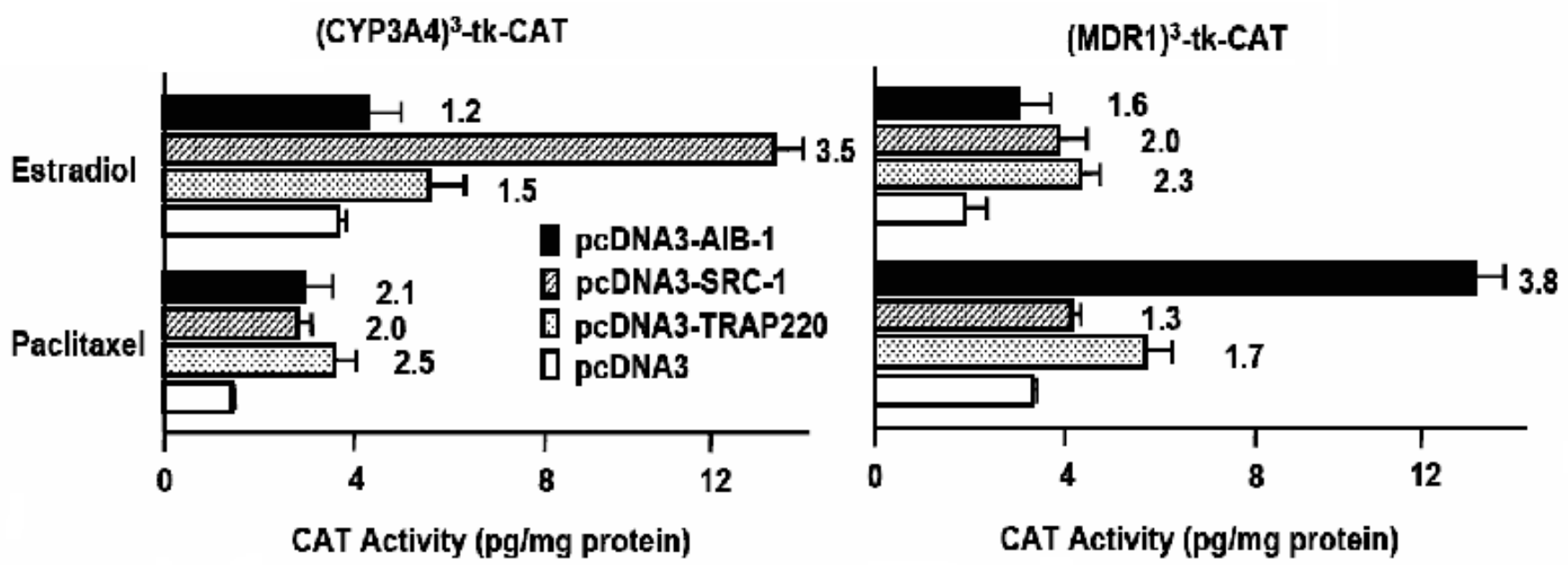
- Note differential effects of PXR ligands on the DR3 and DR4 elements of MDR1 (ABCB1) and CYP3A4 when certain co-activators (SRC-1 and AIB-1) are present

5’-gggtca gca agttca-3’ (DR-3 motif – CYP3A4)

5’-aggtca agtt agttca-3’ (DR-4 motif – MDR1)

Ref: Masutyama, Mol Endocrinol, 2005.

Coactivator-selective Effects



- Transient transfection of coactivator with PXRE–CAT reporter construct
- Note coactivator-selective effects of estradiol on DR3 activation *vs* paclitaxel on DR4 activation

Ref: Masutyama, Mol Endocrinol, 2005.

Ligand-Specific and Promoter-Specific Induction

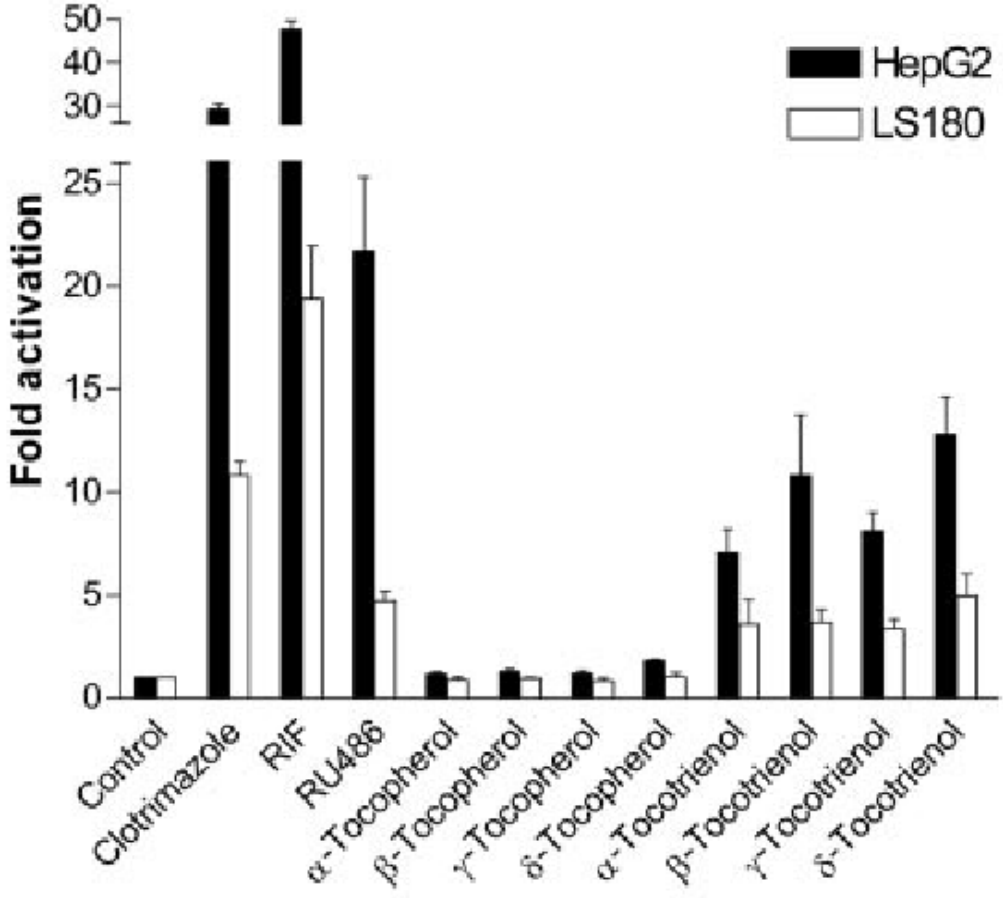
- Ligands for a NR can exhibit both tissue- and gene-selective effects as a result of:
  - Tissue-specific receptor expression
  - Different conformations of the ligand-receptor complex
  - Structural differences in the promoter (RE)
  - Tissues specific expression of nuclear coactivators and corepressors

Tissue-Specific Induction

- Potent CYP3A4 inducers (rifampin) can activate PXR and transcription in liver and intestine
- Weaker PXR ligands have liver selective effects (despite high intestinal concentrations during absorption - phenytoin, efavirenz, troglitazone)
- Effect is gene specific (see MDR1 in LS-180 cells)

- Displacement of corepressor

Tissue-Selective Expression: Corepressors

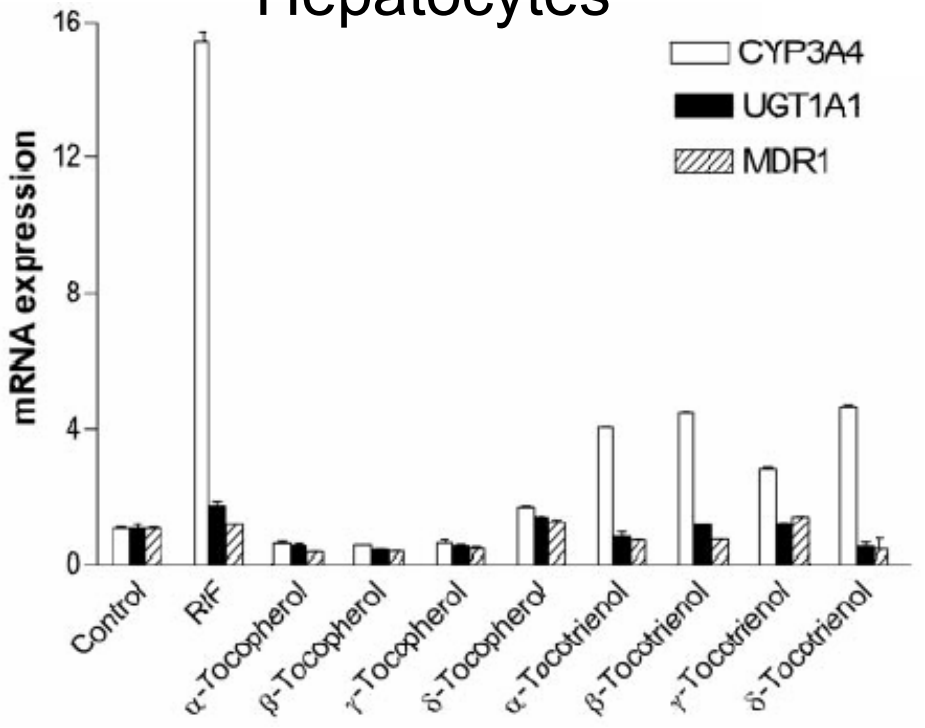


- Cells transfected with PXR and PXRE-reporter
- NCoR, nuclear receptor corepressor highly expressed in LS180 cells (intestine), low in hepatocytes

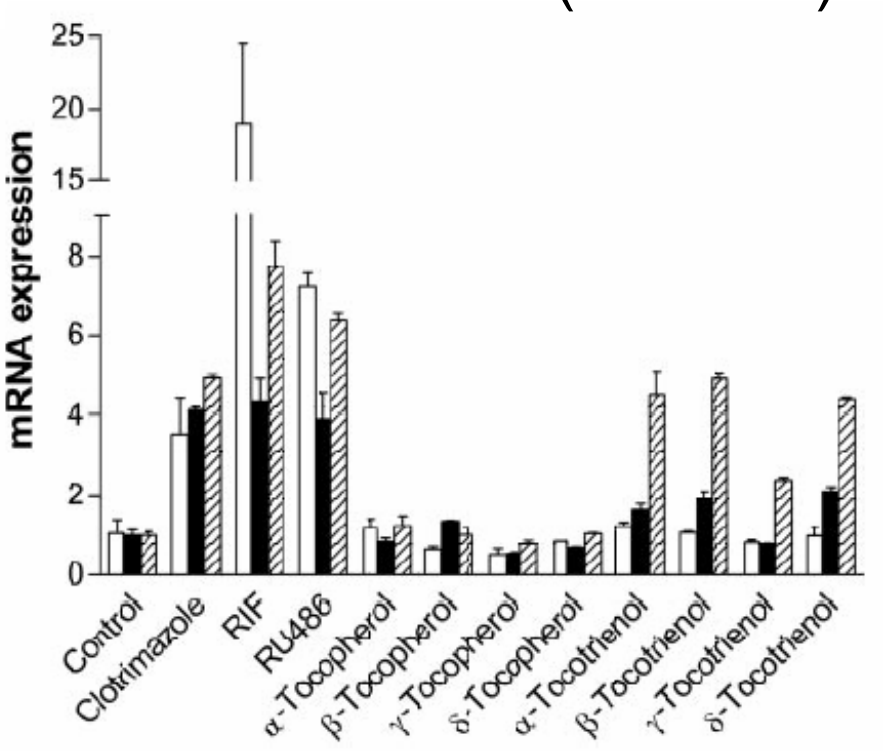
Ref: Zhou, DMD, 2004.

Tissue-, Ligand- and Gene-Specific Induction

Hepatocytes



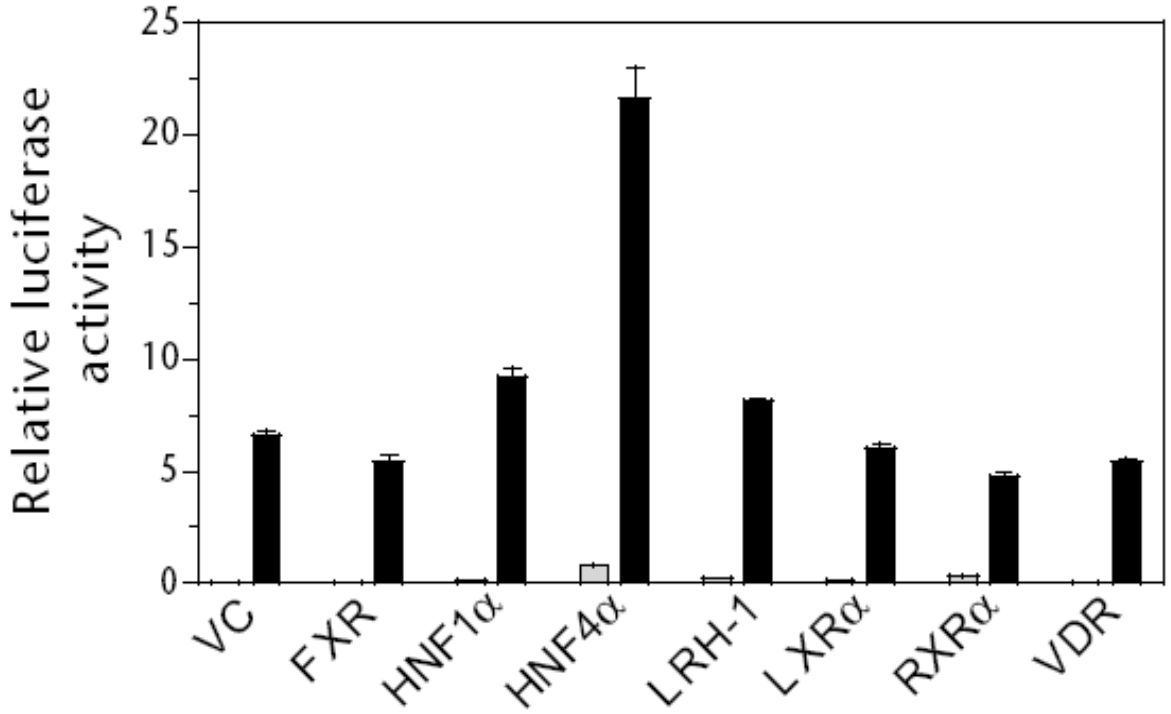
LS-180 cells (intestine)



- Tocotrienols selectively regulate gene expression depending upon tissue (and corepressor expression)

Ref: Zhou, DMD, 2004

Maximum PXR Activation also Requires HNF4α



- HNF4α stimulates transcription 4- to 10-fold above that achieved with PXR alone; (shown basal expression in the absence of exogenous inducer)
- Effect appears to be mediated presumably binding of HNF4α to a DR1 motif in the distal (-7783 and -7771) region of the CYP3A4 gene that contains PXREs (DR3 and ER6).

Ref: Tirona, Nature Medicine, 2003

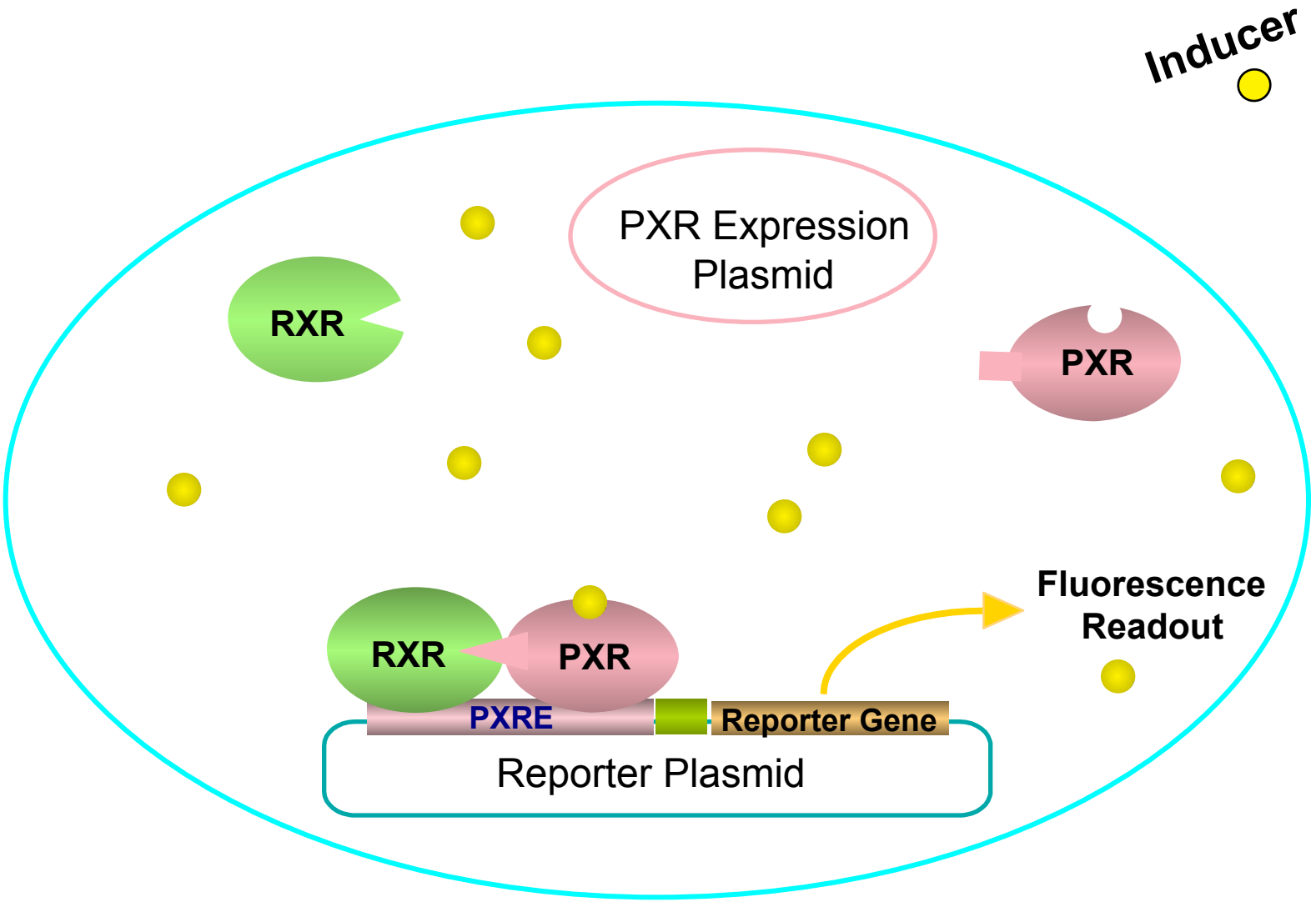
Experimental Techniques: Transcription

Method	Advantages	Limitations
Ligand-PXR Displacement	reproducible, high throughput low cost	false positives, access to technology
Co-transfection (NR & reporter gene)	reproducible, adaptable to enzyme - receptor of interest	single enzyme screen, lower throughput higher costs
Human Hepatocyte	functional kinetic data quantitative RT-PCR multi-enzyme	high variability livers, access to cells, slower turnaround
In vivo animals	accessibility, experience,	species differences* low throughput
In vivo humans	clinical applicability	staging in development, high cost

* May be circumvented with the availability of hPXR animals

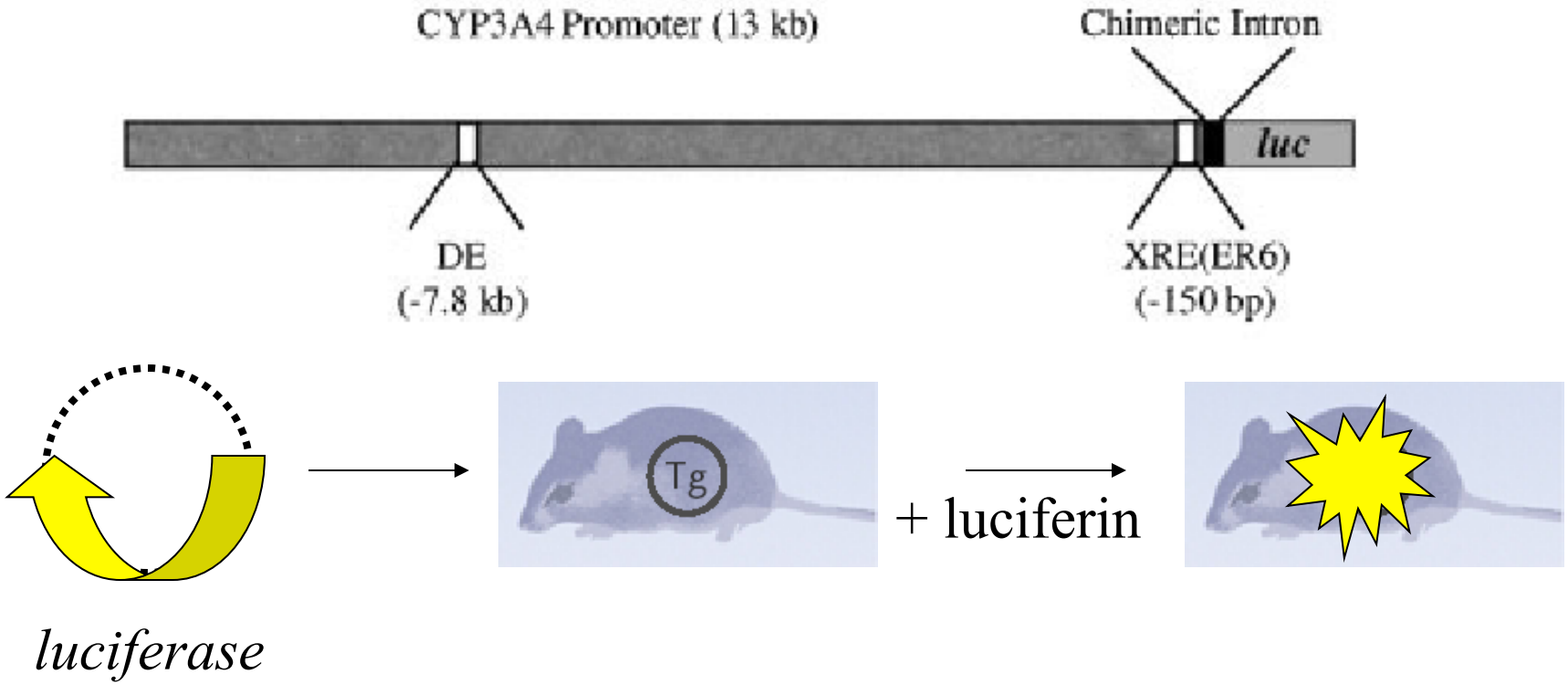
hPXR Transient Transfection in Cells

- Assay of gene activation



CYP3A4 Induction in Genetically Modified Mice

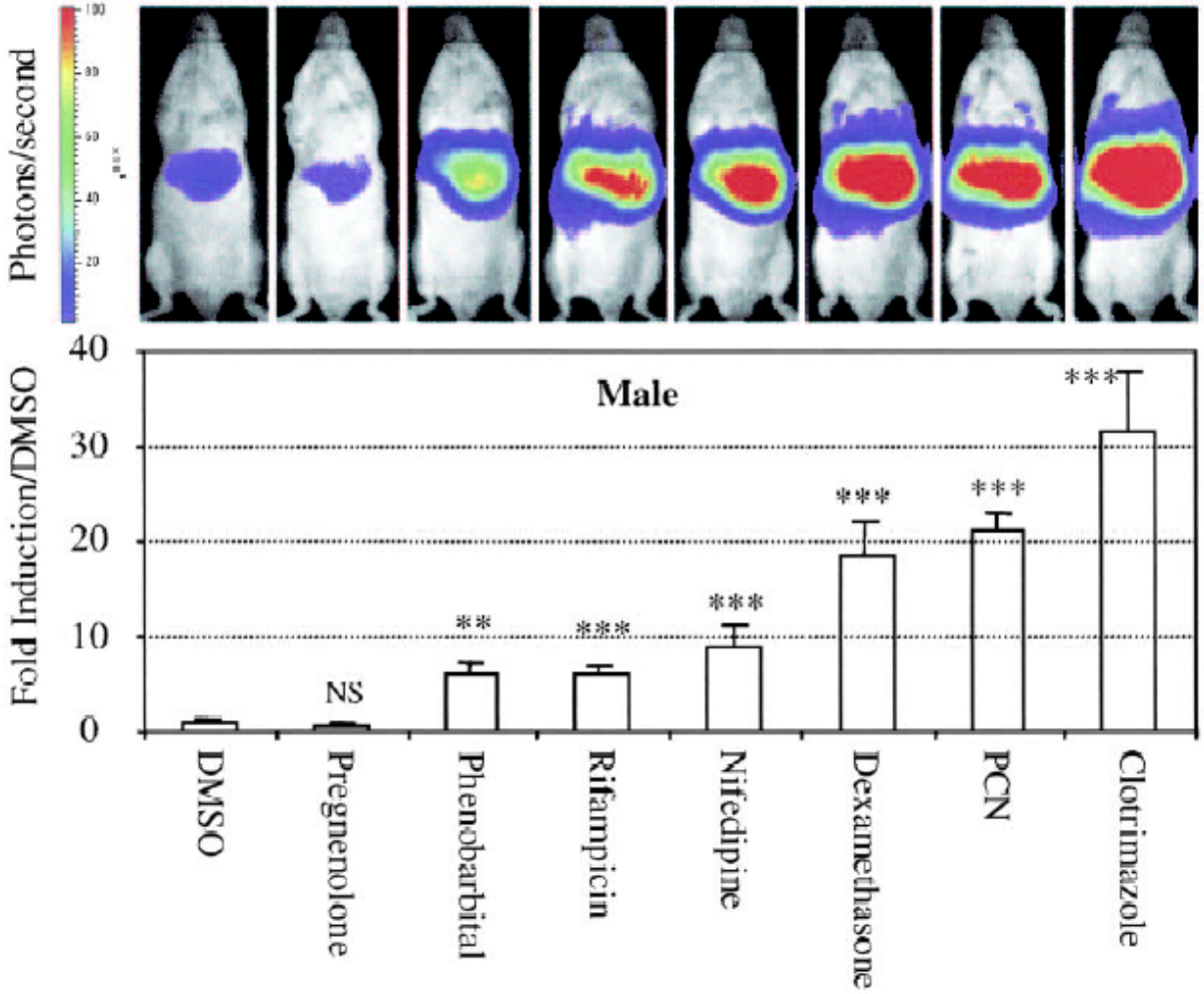
- Generation of transgenic animal expressing human CYP3A4 promoter + luciferase reporter



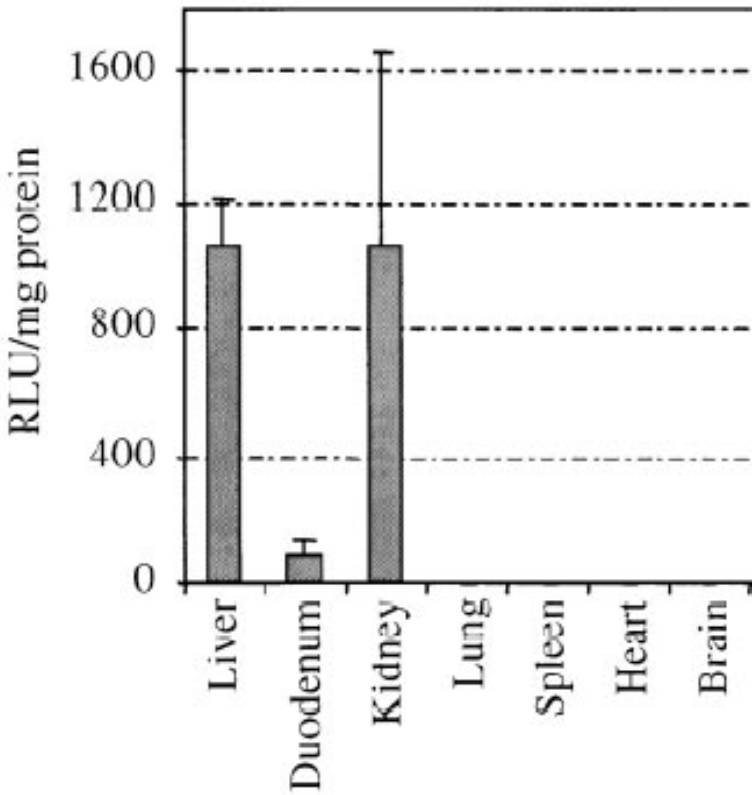
Ref: Zhang, DMD, 2003

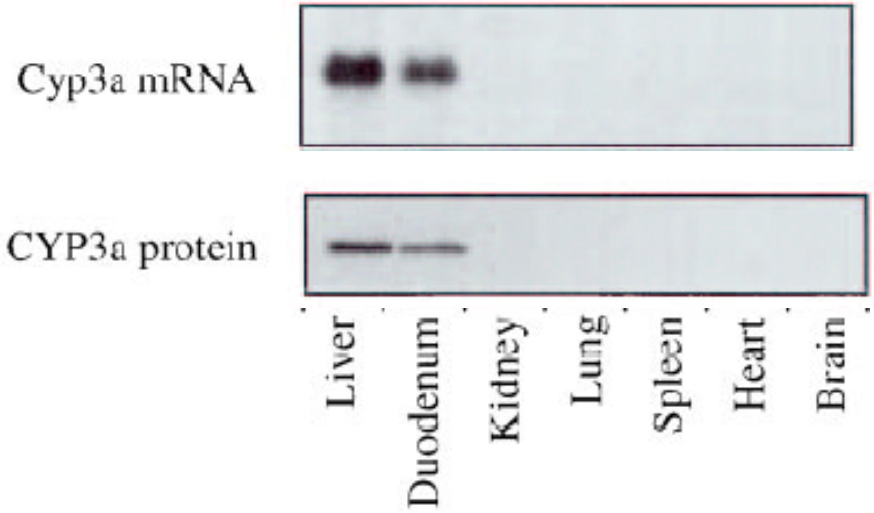
CYP3A4 Induction in Genetically Modified Mice

- Permits in vivo inductive response (mouse PXR with human CYP3A4)



CYP3A4 Induction in Genetically Modified Mice

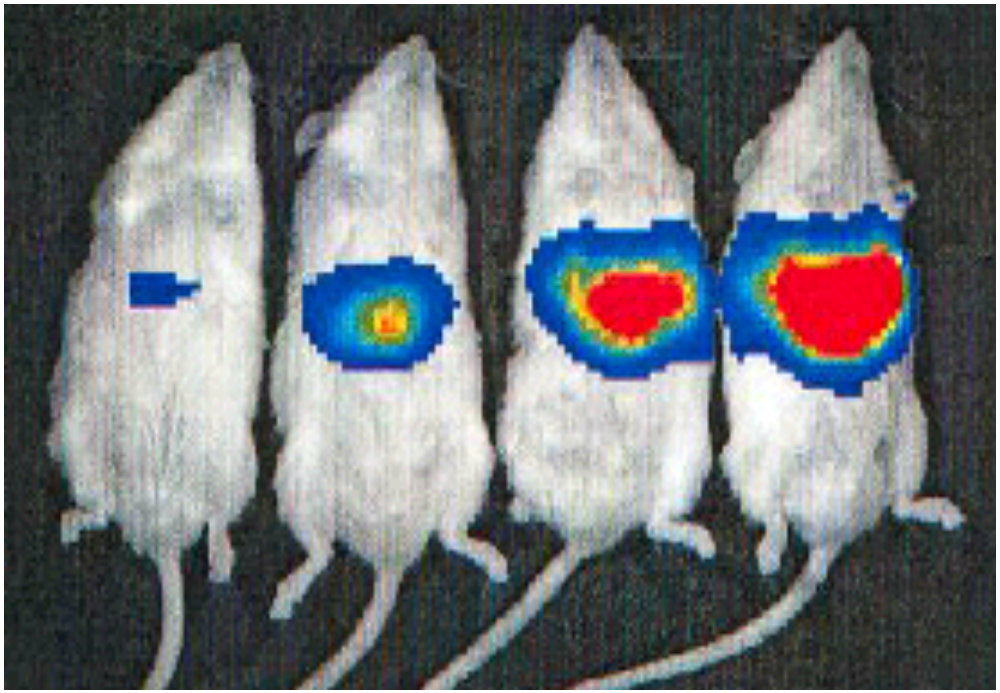




Ref: Zhang, DMD, 2003

Hydrodynamic DNA Infusion: CYP3A/P-gp Induction

rifampin (mg/kg): 0 5 10 50

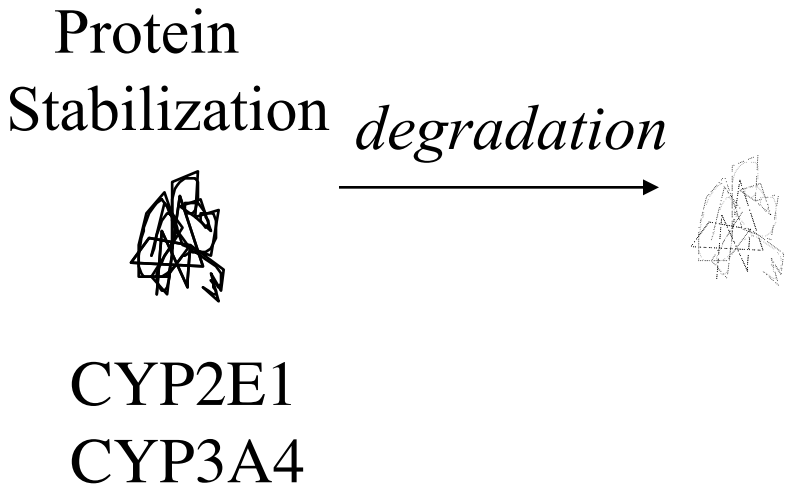


- Transient transduction of hCYP3A4-LUC in mice
- Permit rapid quantitation of inductive response in context of in vivo PK (hPXR or other nuclear receptor, hCYP3A4-LUC or other reporter)

Ref: Schuetz, Mol Pharmacol, 2002

Protein Stabilization: Changes in k_{degr}

- Protein stabilization – decrease in degradation
- Degradation pathways:
  - ubiquitination
  - lysosomal degradation



Protein Degradation

- Quality control system to degrade proteins
  - Unassembled and/or misfolded proteins
  - Metabolic regulation
  - Oxidative damage
- Cytosolic ubiquitin (Ub)-dependent 26S proteasome system
  - (enzymes with short half-lives)
- Lysosomal pathway
  - Recycles membrane proteins, extracellular proteins and proteins with long half-lives

Degradation Pathways

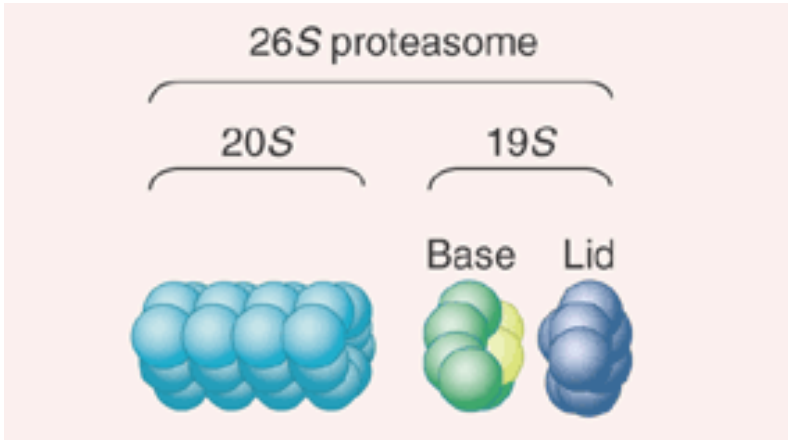
Protein

Ubiquitination

Polyubiquitinylated protein

26S Proteasomal Degradation

Lysosomal Degradation



vacuole

Ref: Murray, Mol Pharmacol, 2002

Ubiquitination & Degradation

- Ubiquitin
  - 76 amino acids (8.5 kD)
  - Highly conserved (present throughout eukaryotic kingdoms)
  - 3 enzymes participate in conjugation of ubiquitin to proteins (ATP-driven)
  - Results in a polyubiquitinylated protein
- Digestion by 26S protease complex
  - ATP-driven multisubunit protease
  - Multiple rounds of ATP hydrolysis enable protease to unfold and processively digest the protein
  - Ubiquitin recycled

Ubiquitination & Degradation

- Proteolysis of ubiquitinylated proteins is a feature of many cellular processes including:
  - Chromosomal stabilization
  - Cell division
  - Apoptosis
  - Cell differentiation
  - Stress response
- Ubiquitin-tagged proteins (that do not undergo proteolysis)
  - Endocytosis
  - Localization of certain proteins in the nucleus

Degradation

- Exhibit asynchronous turnover
- "short $t_{1/2}$" (e.g. CYP3A4) ubiquitin-dependent 26S proteasome pathway
- "long $t_{1/2}$" (e.g. CYP2B1 and OR: $t_{1/2}$ ~ 30 hours) lysosomal degradation
  - Electron micrographs of livers cells of rats treated with leupeptin (serine protease inhibitor) show "lysosomal constipation" and consequent accumulation of CYP2B1and OR

- CYP2E1, biphasic turnover
- t1/2 ~ 7 hours: degradation by proteasomal pathway
- t1/2 ~ 37 hours: lysosomal degradation

Hepatic CYP Half-life

- Although there is no direct data for human CYP half-life in vivo, animal and hepatocyte data suggest values between 6-25 hrs; proteasomal mechanisms associated with a short $t_{1/2}$.

Approximate CYP Half-lives – Cell Culture

Enzyme	t1/2 (hours)	Degradation by Ubiquitination
CYP1A1	15-16	No
CYP1A2	10*	
CYP2B1	19-25	No
CYP2B2	19-25	No
CYP2E1	6-7* 37	Yes No
CYP3A	9-14*	Yes
CYP4A		Yes
NADPH reductase	29-35	No

Adapted from Roberts, JBC 272: 9771-8, 1997

Time-Course of Induction In Vivo

$$\text{Amt Enzyme}_{ss} = \frac{\text{Synthesis Rate}}{k_{deg}}$$

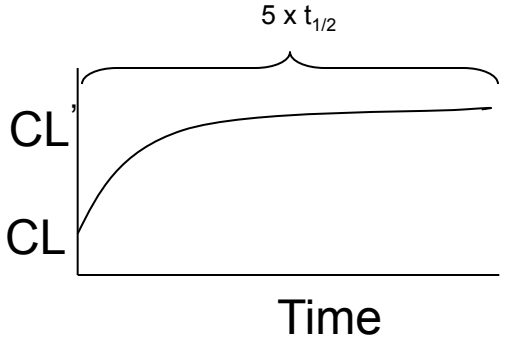
Max effect determined by change in synthesis, so long as k_{deg} is constant.

$$Cl_{int(t)} = Cl'_{int} - \left(Cl'_{int} - Cl_{int}\right) \bullet e^{-k_{deg}' \bullet t}$$

Δ effect

Cl_{int}' is the new (induced) steady-state intrinsic clearance

$$t_{1/2}(enzyme) = \frac{0.693}{k_{deg}}$$



- Assuming constant inducer concentrations (*i.e.,* new, constant synthesis rate), the time to steady-state is controlled by the degradation half-life of the affected enzyme (~ 24-36 hrs).
- Anecdotal observations suggest maximum CYP3A4 induction occurs in 7-14 days; this will depends on the kinetics (steady-state) for the inducing agent(s).

Time Course of CYP3A Induction by Rifampin

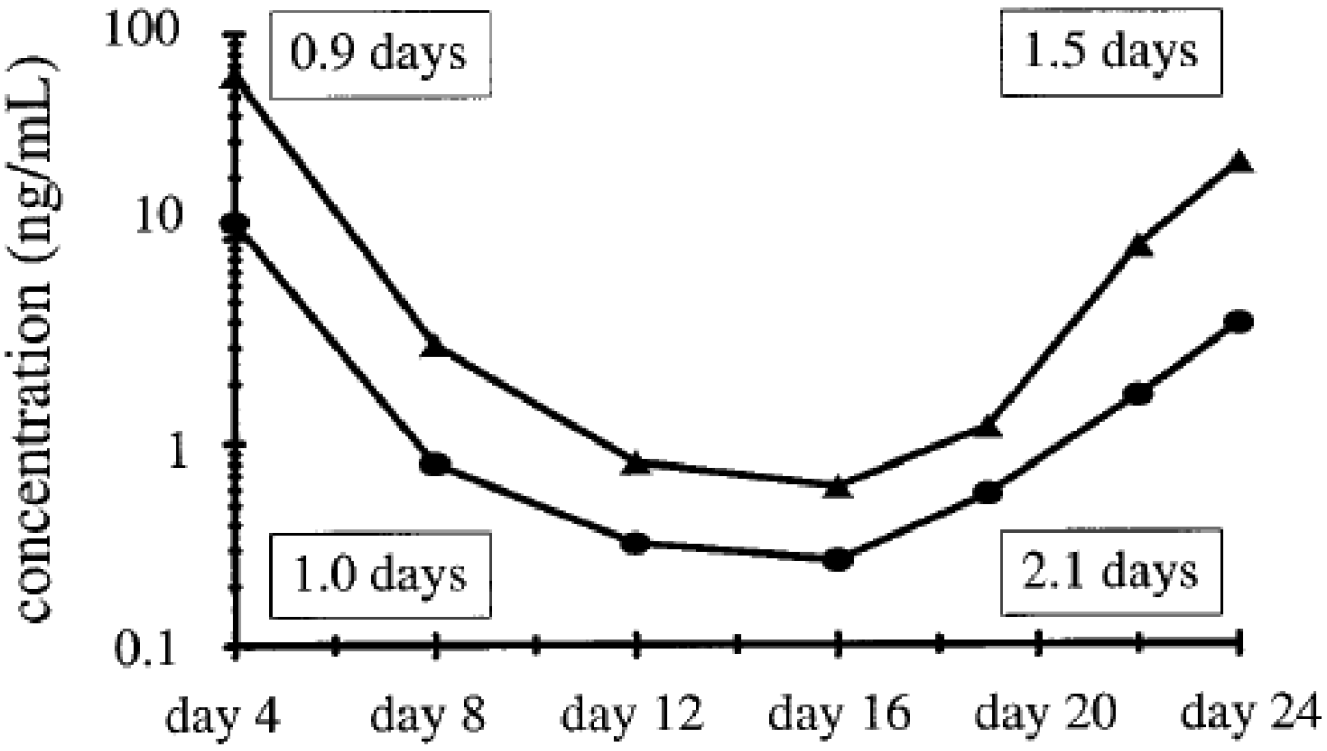
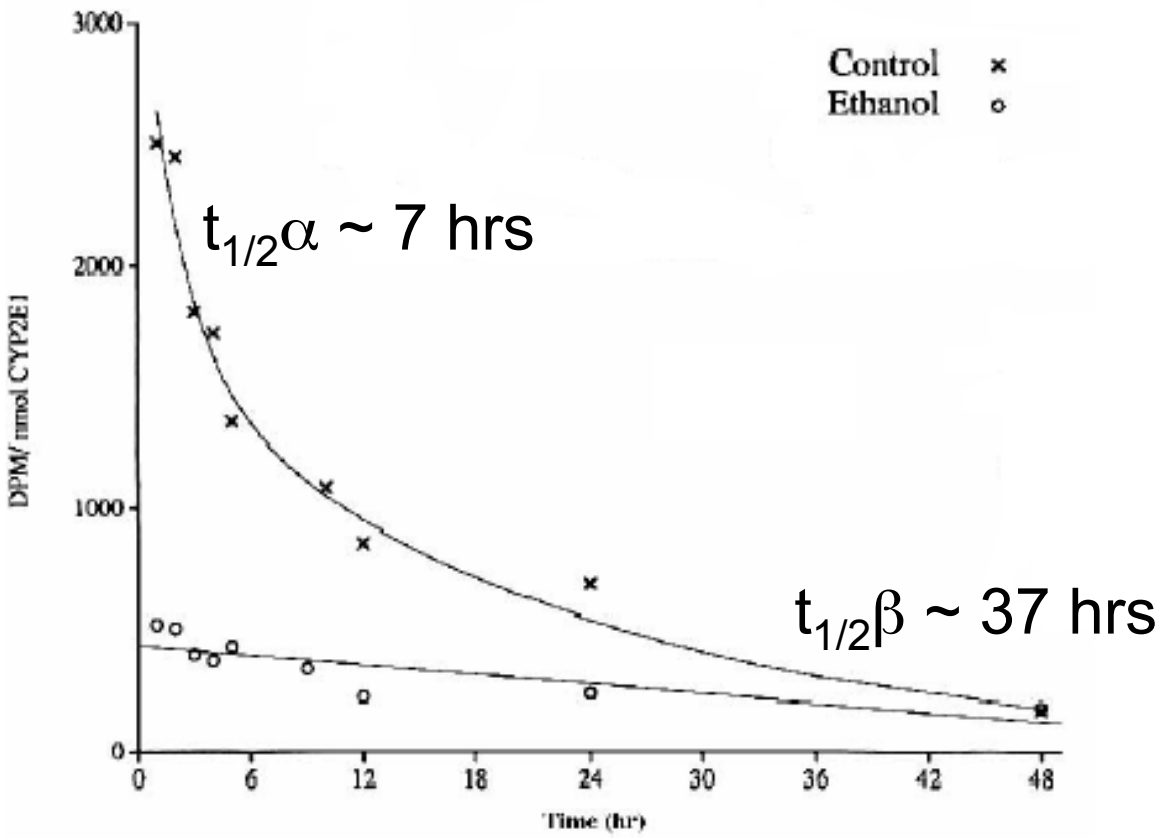


FIG. 2. Mean S-(●) and R-verapamil (▲) trough levels before (day 4), during (day 8 to day 16), and after induction (day 19 to day 24) with rifampin. Half-life of induction (day 4 to day 8) and half-life of decrease in enzyme activity (day 16 to day 24) are given for both enantiomers.

Fromm et al., Hepatology, 1996

Time-course of change in daily trough concentration is inversely proportional to the change in Cl_{int}; a new steady state under "induced" conditions is achieved after several enzyme $t_{1/2}$; note rifampin has a short $t_{1/2}$.

Biphasic Kinetics for CYP2E1 Elimination



- Rats injected with $NaH^{14}CO_3$
- ^{14}C-labeled CYP2E1 (Western blot – scintillation counting of band)

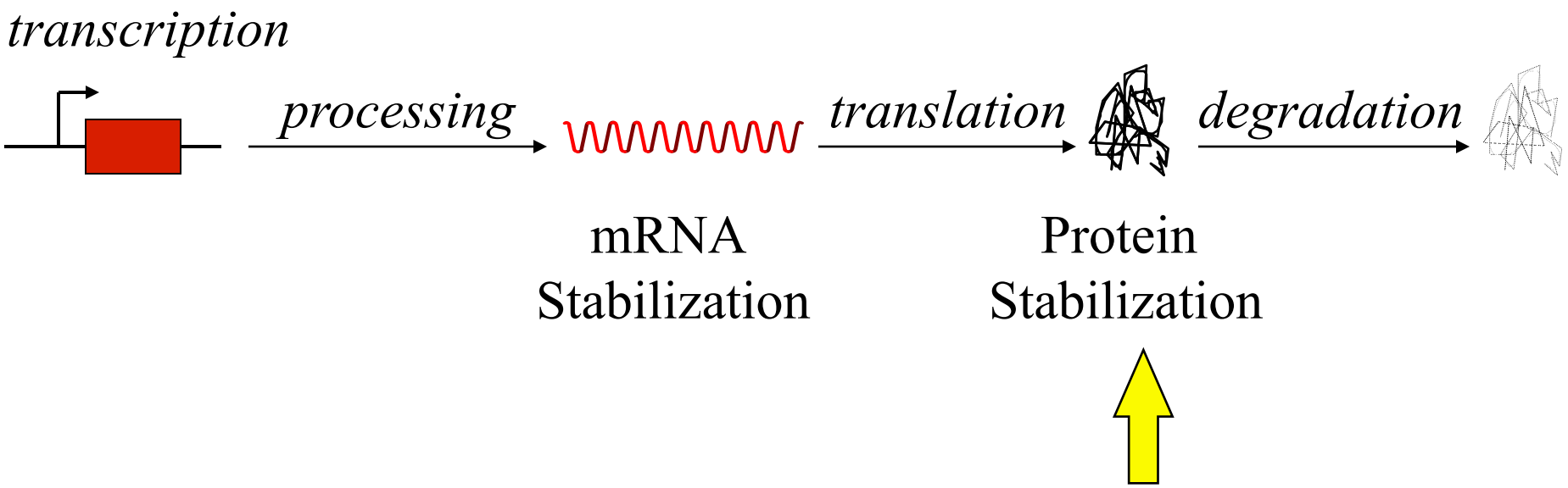
Ref: Roberts, JBC, 1995

Increased Degradation

- Structural changes to CYP (CYP2E1 and CYP3A4) can involve heme oxidation or adduct formation, or protein modification:
  - Oxidation of labile amino acids: Met, Pro, Arg, Lys, His
  - Uncoupled oxidation - generating reactive oxygen species
  - Phosphorylation of Ser129 (CYP2E1)
  - Ubiquitination

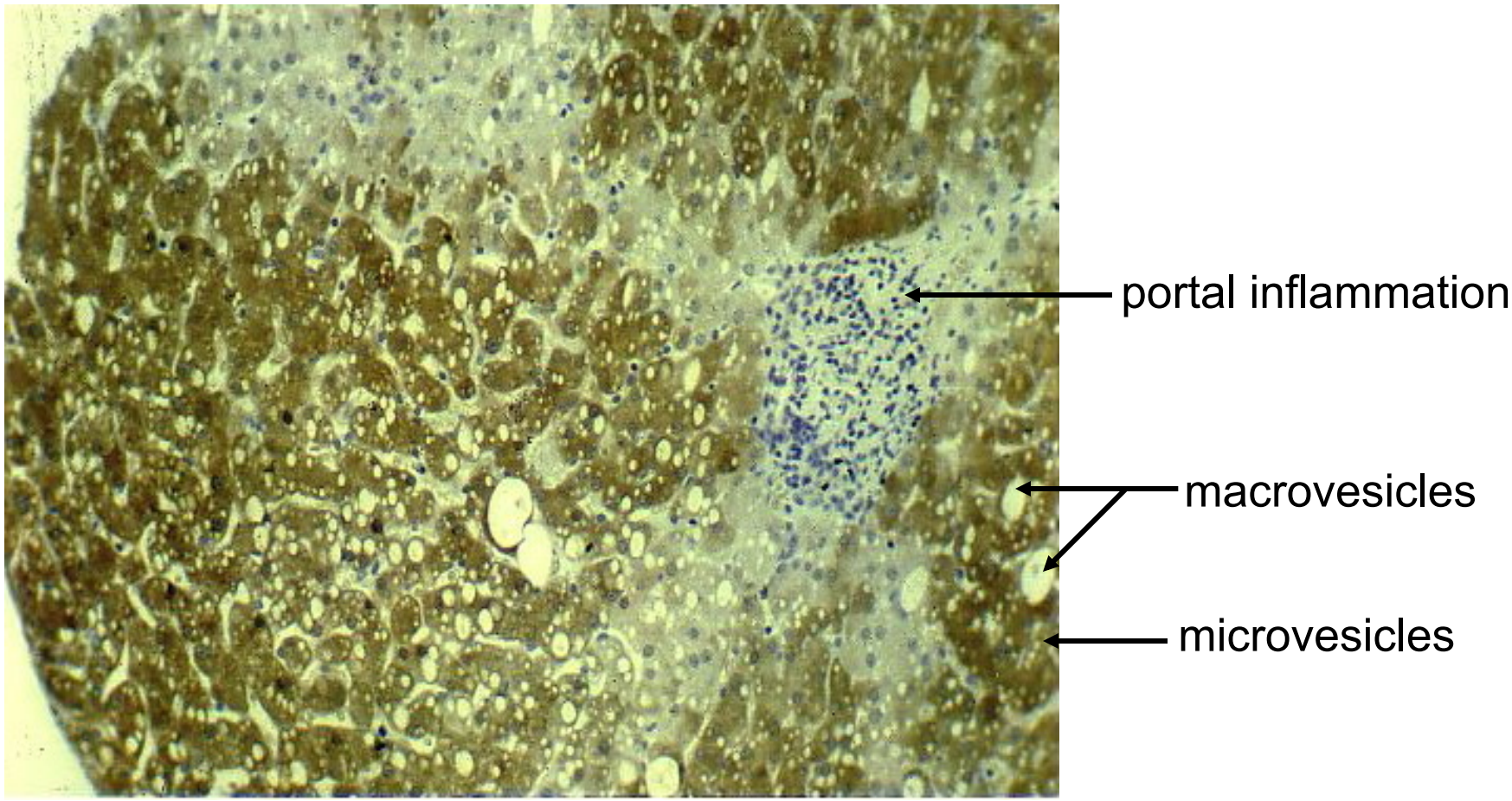
- Once modified, protein destruction occurs rapidly

Induction by Protein Stabilization



$$E_{ss} = \frac{R_o}{k_{\text{degr}}}$$

Induction of CYP2E1 in Steatotic Liver



- Immunohistochemistry of CYP2E1 (brown stain)
- Hepatic steatosis occurs in ~ 5-10% of the population; most commonly seen with obesity (90% with morbid obesity)

Conditions Inducing CYP2E1

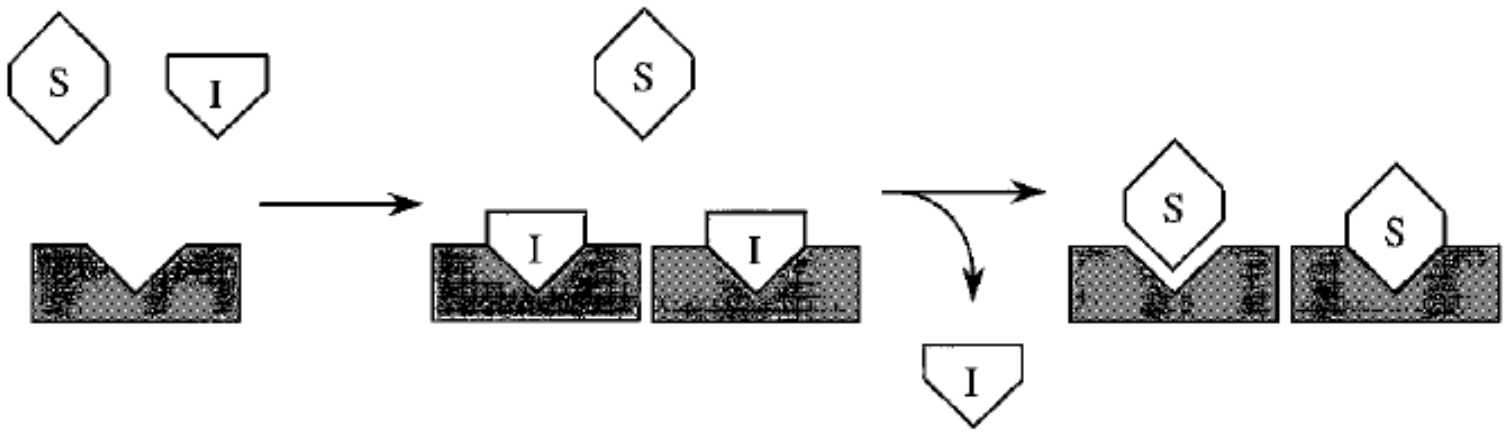
- Xenobiotics
  - Ethanol, acetone
  - Pyrazoles, pyridines, primary alcohols
- Pathophysiological Conditions
  - Chronic fasting
  - Steatosis
  - Diabetes
  - Birth

Transcriptional Activation of CYP2E1

- Most studies conducted in adults have failed to find evidence of increased mRNA synthesis following treatment with CYP2E1 inducers (ethanol, pyridine, acetone, pyrazole)
- Only birth triggers gene activation
- CYP2E1 mRNA in hamsters may be increased by ethanol and pyrazole. 2-stage induction process:
  - high BAC - increased mRNA (stabilization?; miRNA suppression)
  - low BAC - protein stabilization
- There is also evidence that mRNA translation efficiency may be enhanced by inducers (blocked by translation inhibitors - NaF)

Ref: BBRC 150:304-10, 1988
Eur J Pharmacol 248:7-14, 1993

Stabilization of CYP2E1 by Active Site Occupation



Enzyme: Baseline → "Stabilization" → Accumulation

Substrate: Baseline → Reduced clearance → Increased clearance

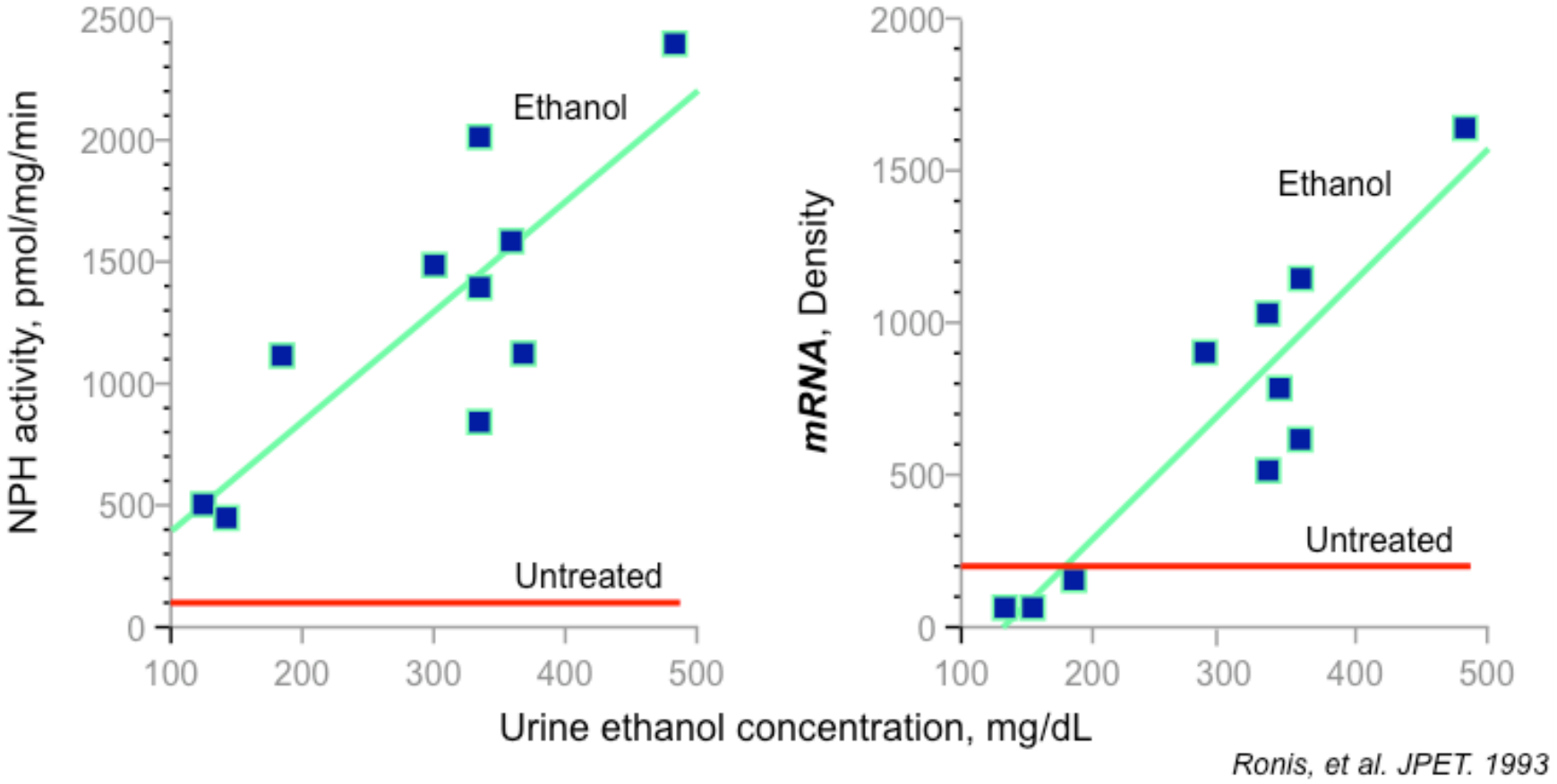
Ref: Chien, DMD, 1997

Induction by Stabilization of Other CYPs - 3A?

- Both ubiquitin-dependent and lysosomal degradation of CYP3A enzymes has been described
- Earlier studies described biphasic inhibition/induction of CYP3A by clotrimazole and miconazole
  - mechanism of induction may have involved, in part, protein stabilization
  - However, in vivo in humans, only inhibition has been described

Ref: Ritter, DMD, 1987

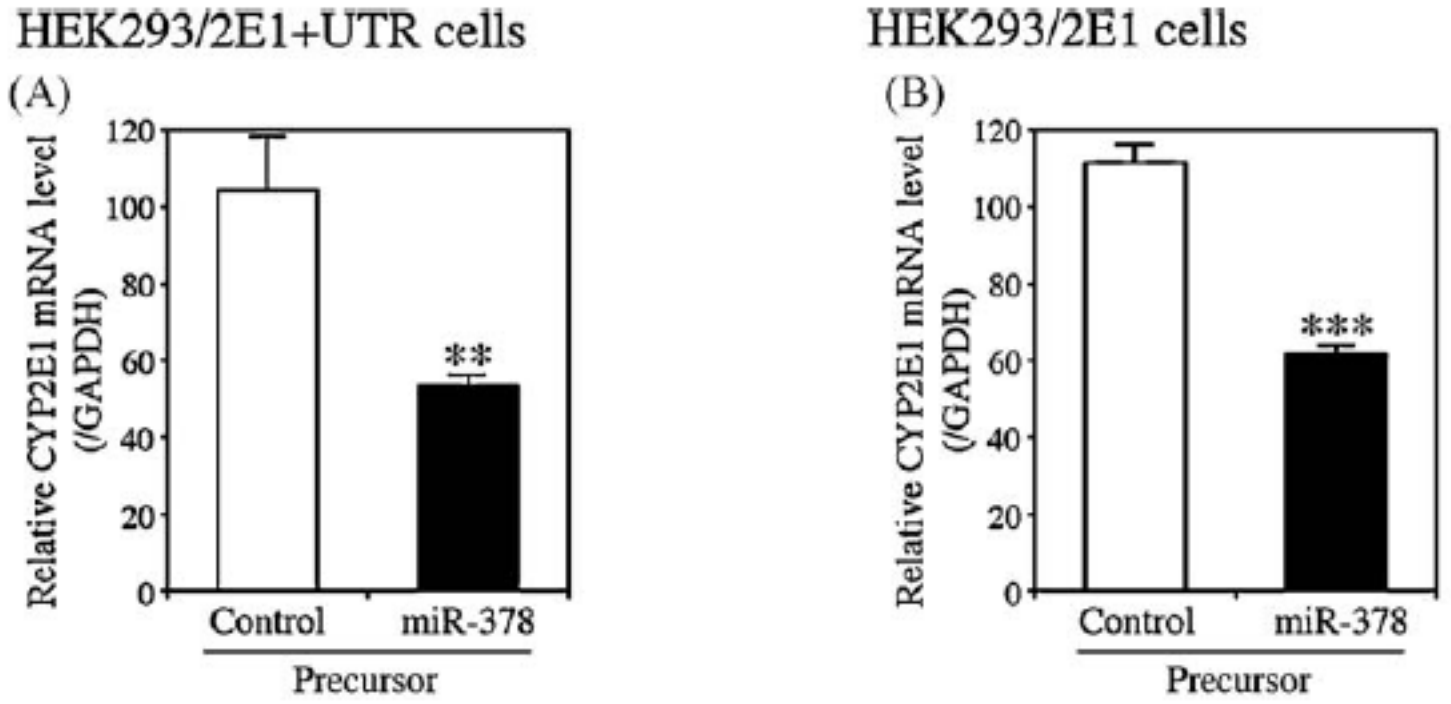
Effect of Ethanol on *CYP*2E1 Synthesis and Ex Vivo Activity



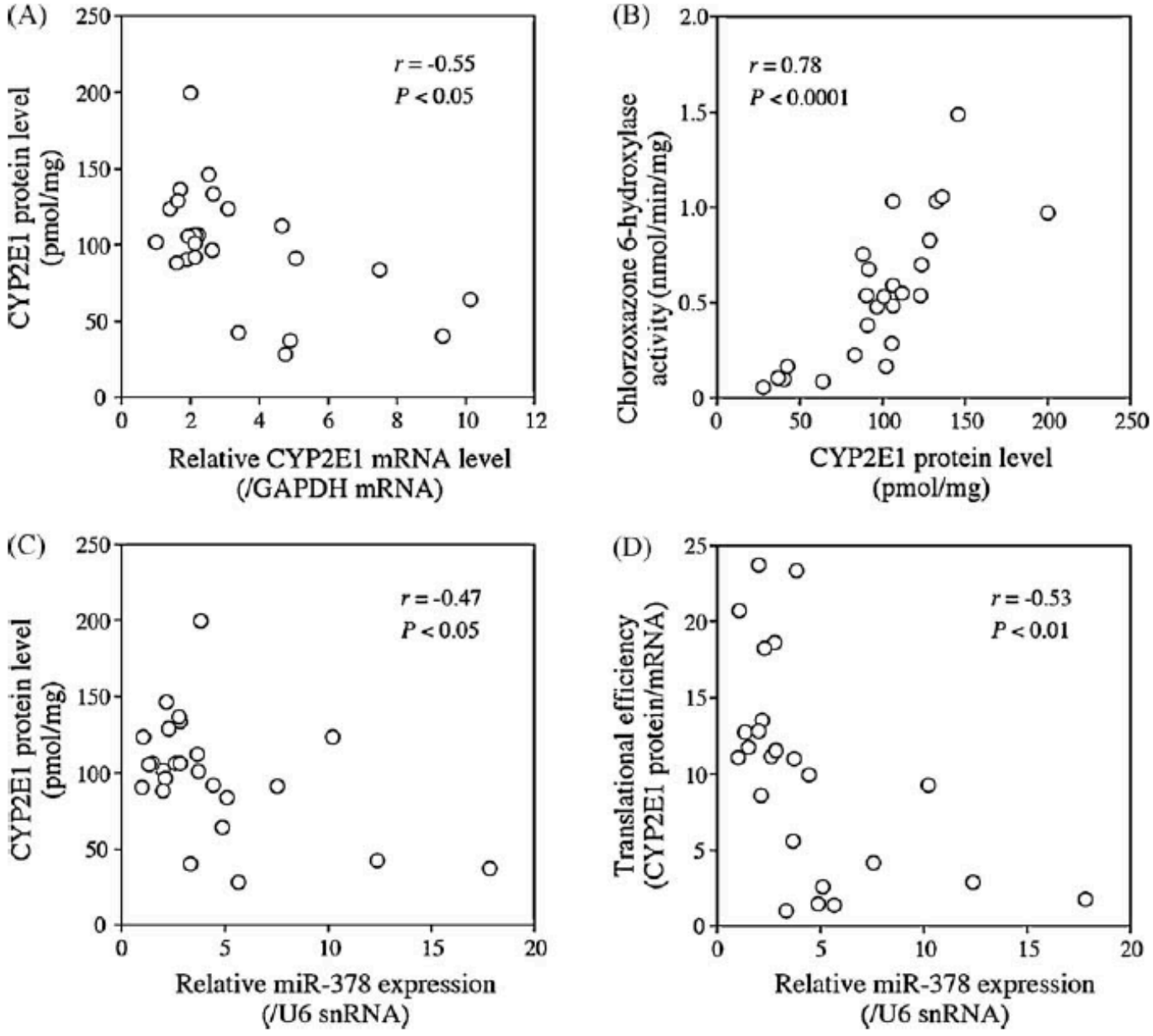
Ronis, et al. JPET. 1993

- induction of ex vivo hepatic CYP2E1 activity correlates with ethanol exposure; interestingly, there is with discontinuity in mRNA (300 mg/dL)

Regulation of CYP2E1 by miRNA



- Mohri et al (Biochem Pharmacol, 2010) provided evidence that CYP2E1 is regulated by miRNA-378
- Speculated that the effects of xenobiotics and disease (diabetes, steatosis) on CYP2E1 levels may be mediated by repression of miRNA-378



- mRNA, miRNA, protein and activity analysis of human livers