Humanized Mouse Models in the Context of Drug Development

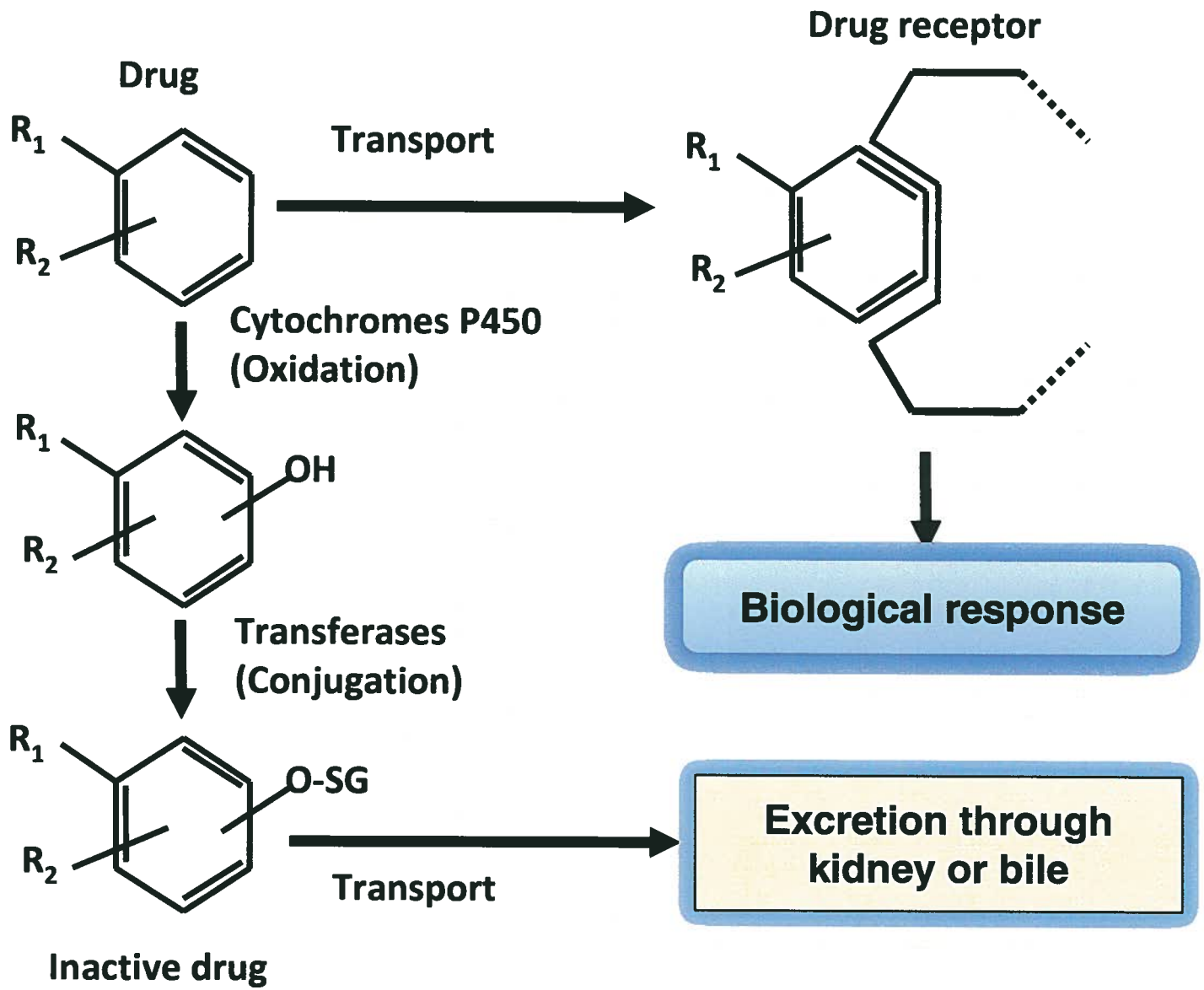
Introduction

CYP3A4

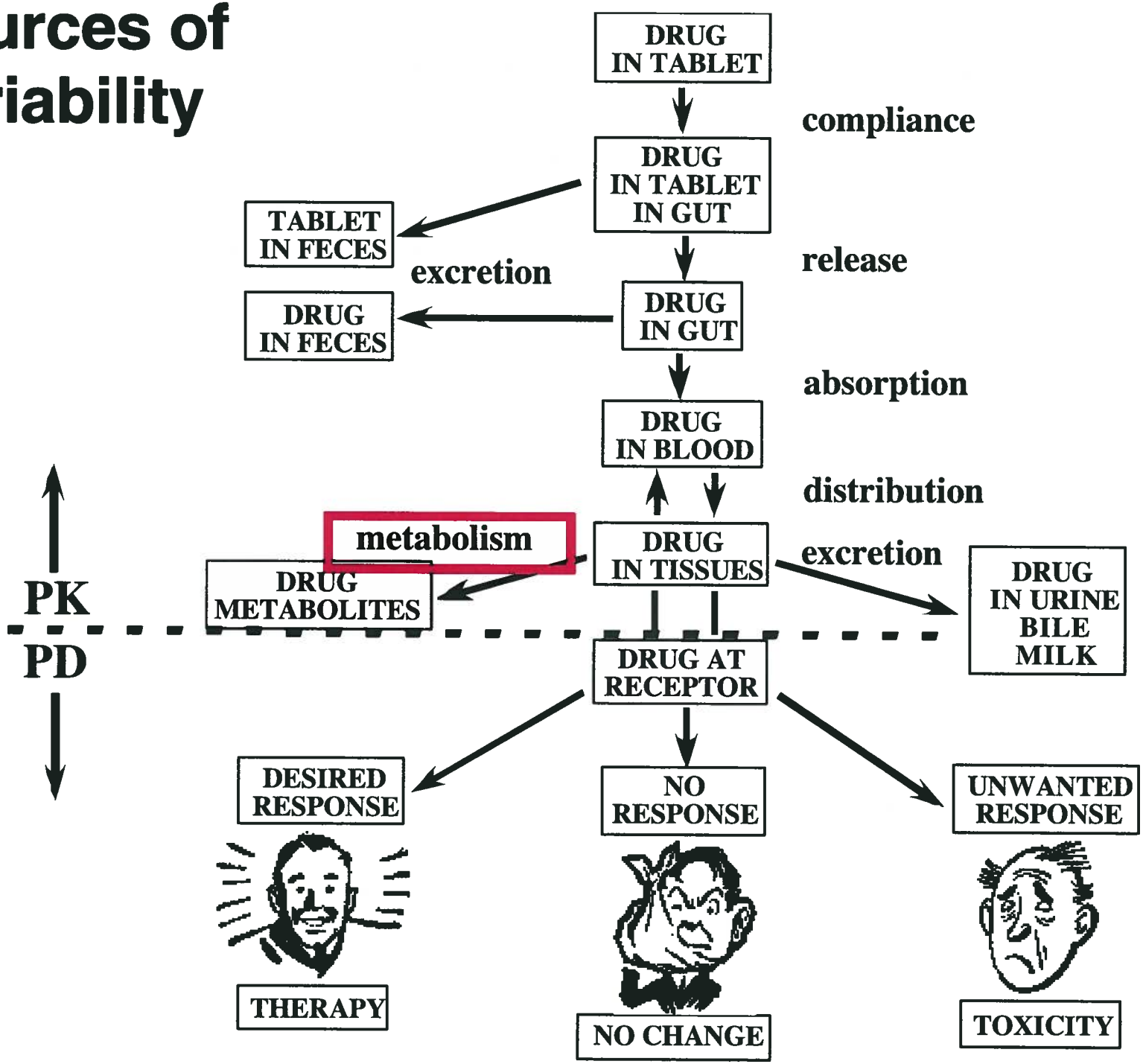
Pregnane X receptor (PXR)

Peroxisome proliferator-activated receptor α (PPARα)

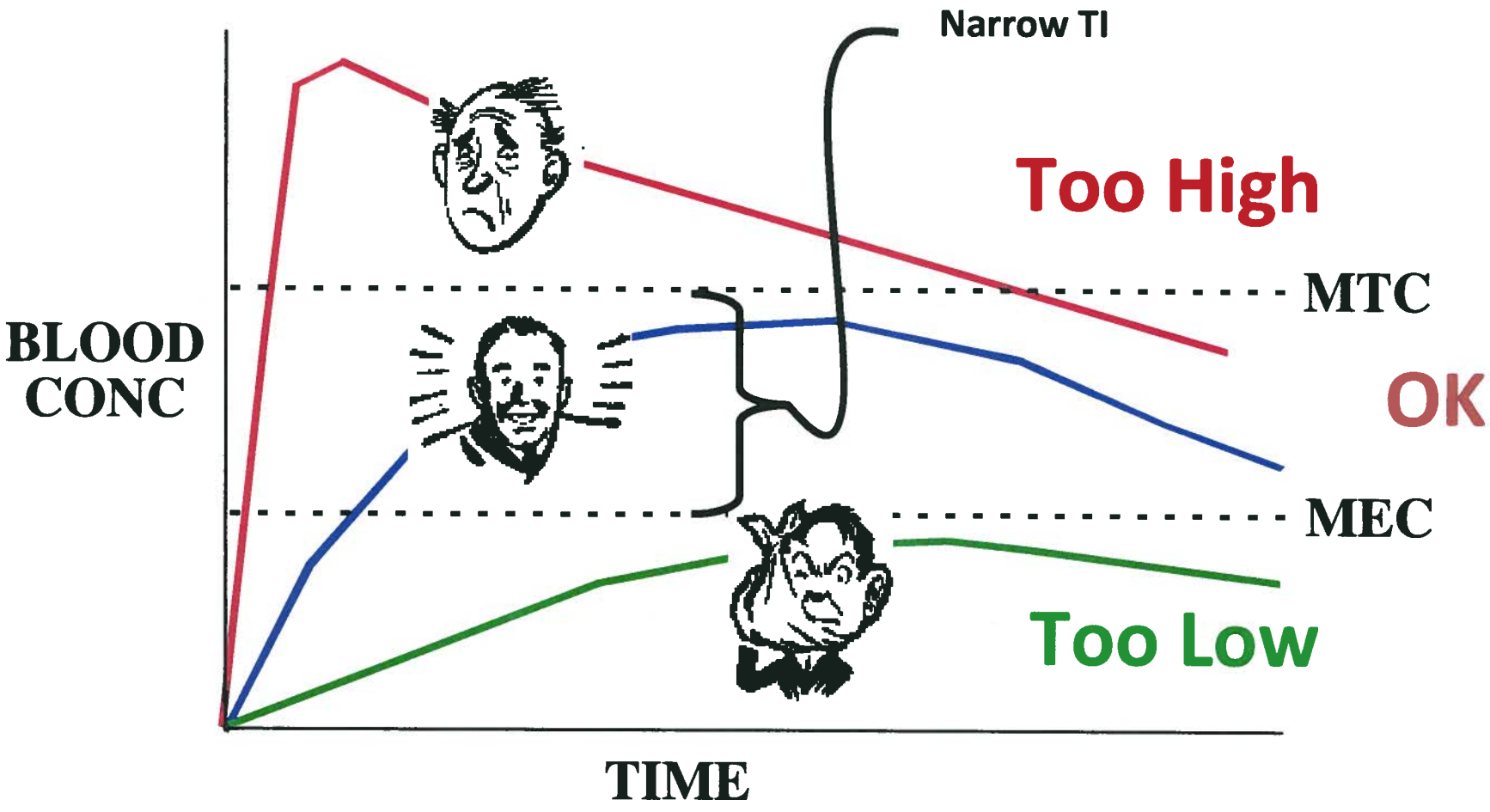
Metabolism vs Drug Action



Sources of Variability



Therapeutic Range/Index (TI) of Drug

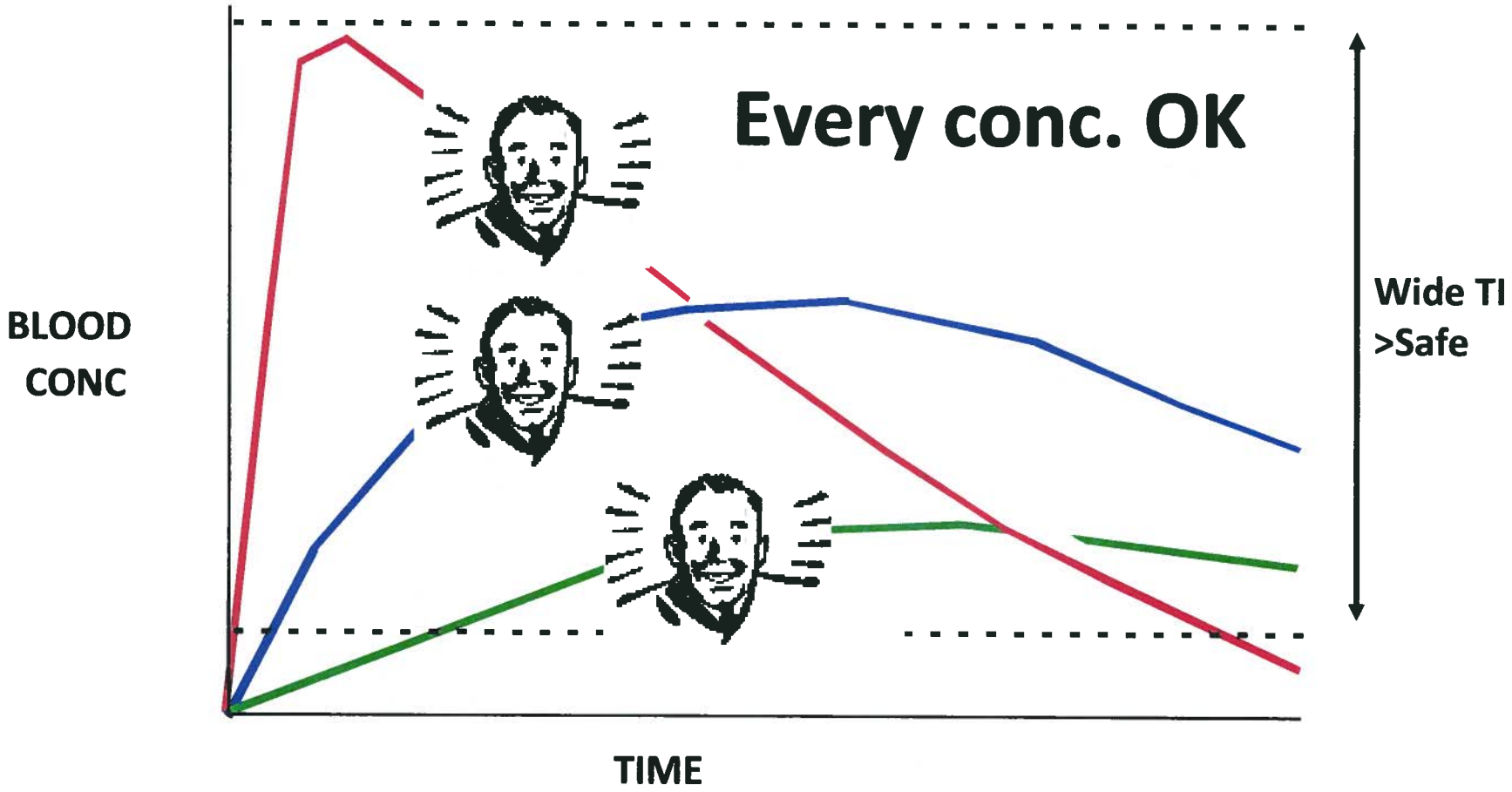


MTC = Maximal Tolerated Concentration

MEC = Minimal Effective Concentration

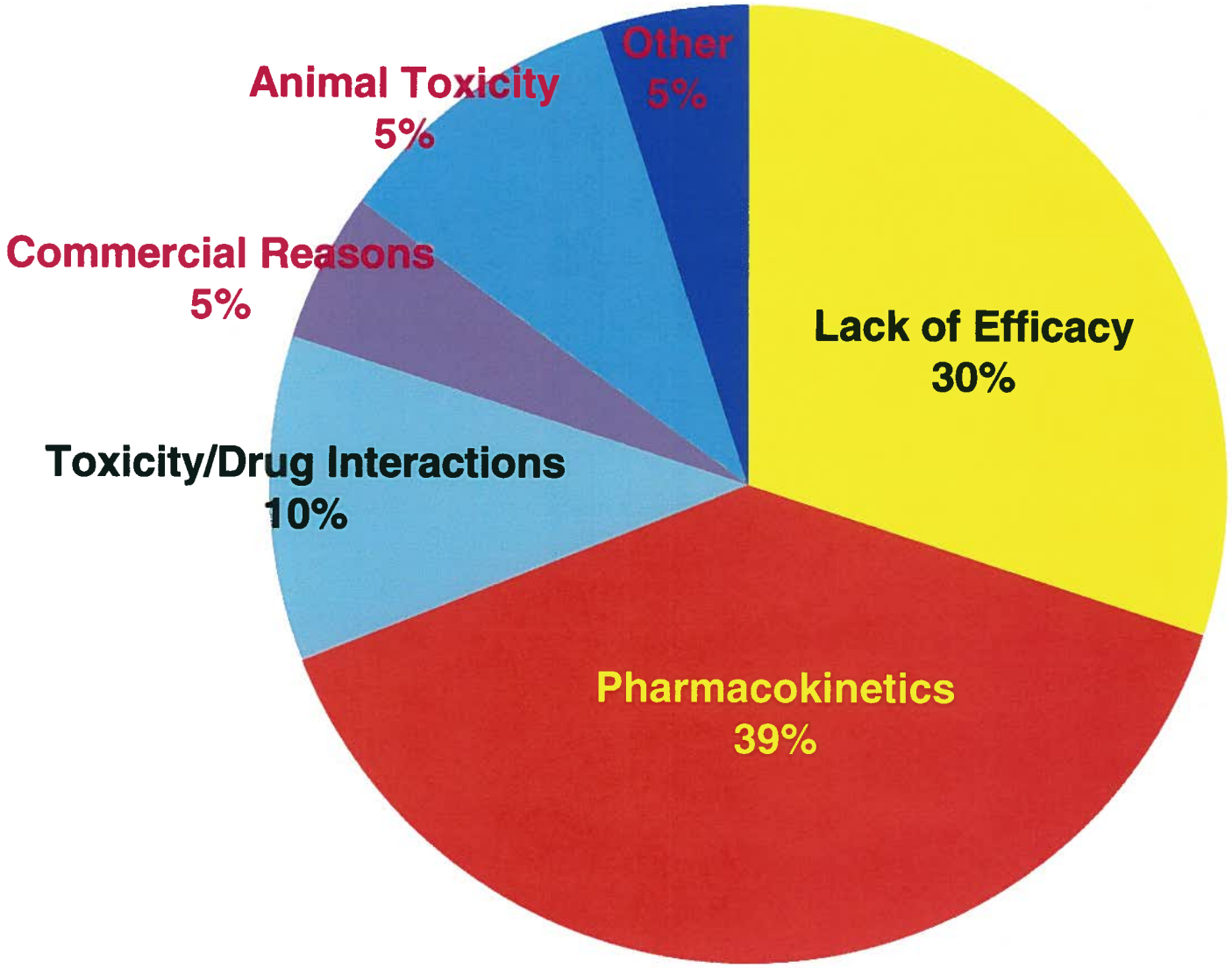
Antiarrythmics: Fluoxitine
Cancer drugs: 6-Thioguanine
Antipsychotics: Imipramine

Wide Therapeutic Range > Metabolism of No Consequence



Anti-inflammatories: NSAIDs (Sulindac)
Cholesterol lowering: Statins (Lipitor)
Type 2 diabetes: Thiazolidindiones (Pioglitazone)

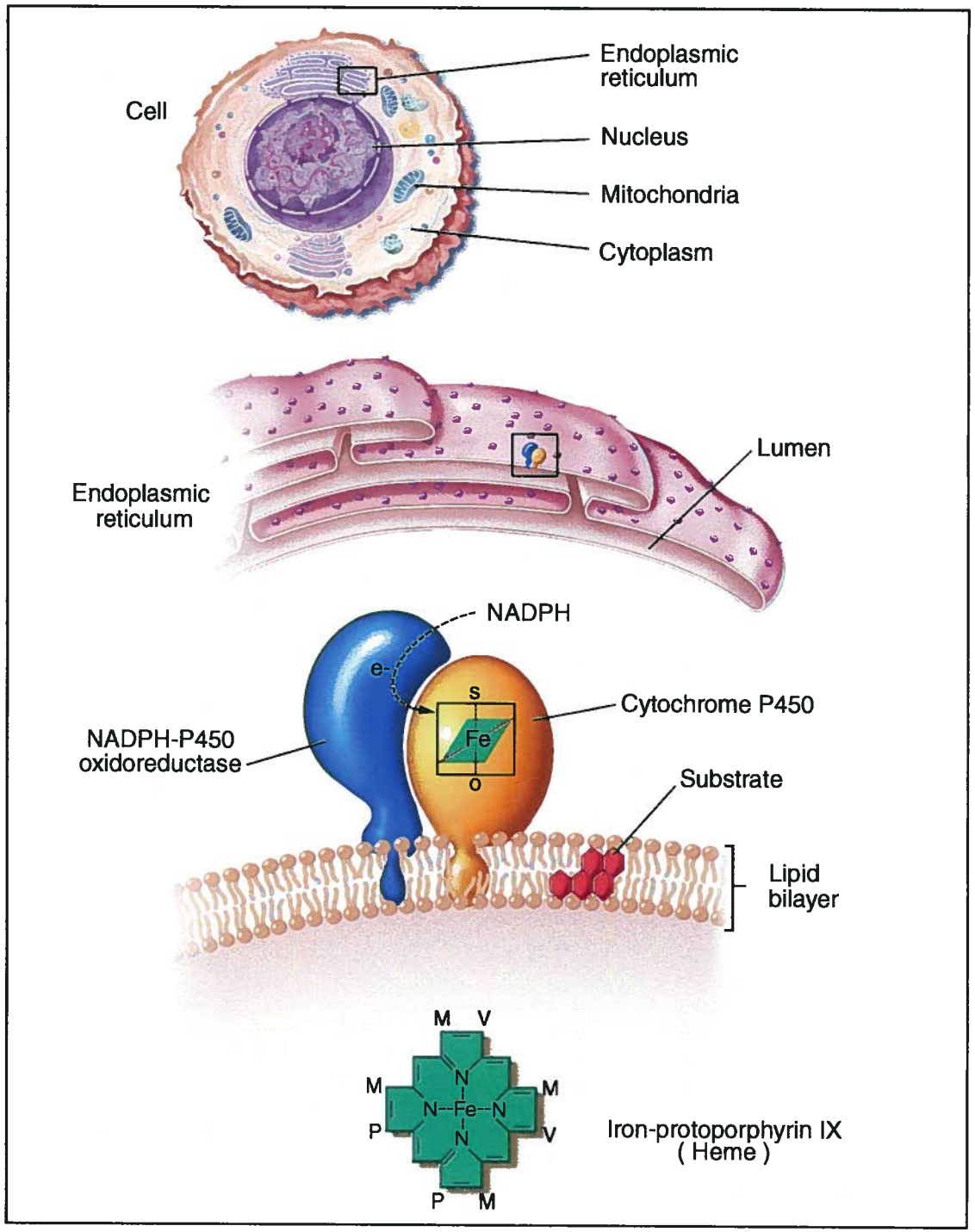
Reasons for Attrition (Failure) in Drug Development



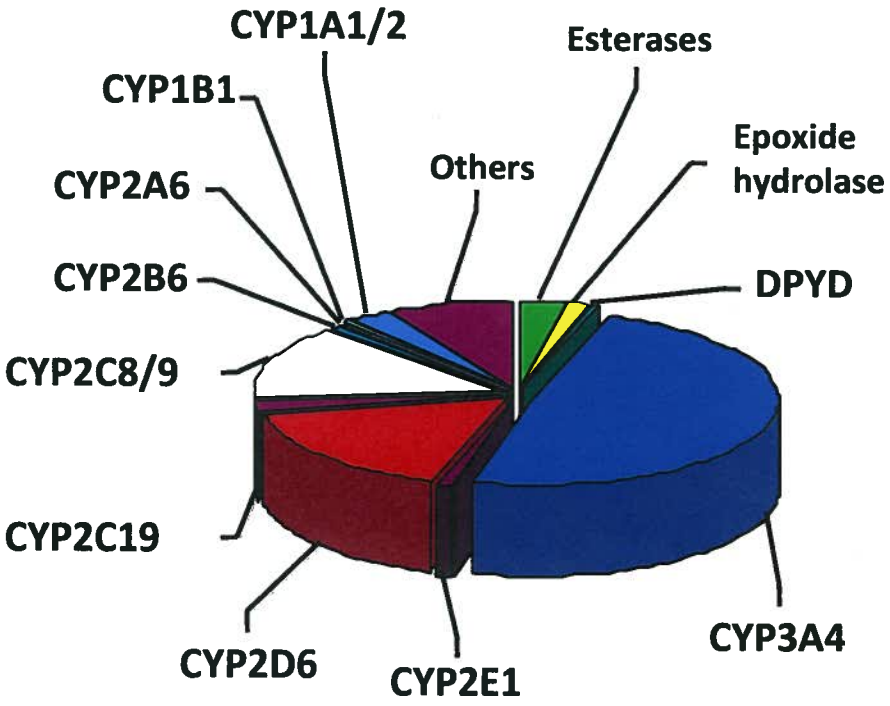
Cytochromes P450 (CYPs)

Liver enriched
Endoplasmic reticulum

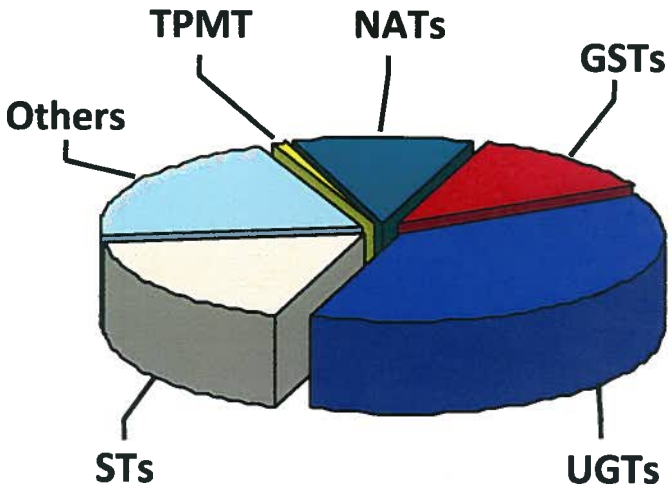
Certain transferases also localized to the ER



Hepatic Human Drug Metabolizing Enzymes (average abundance)

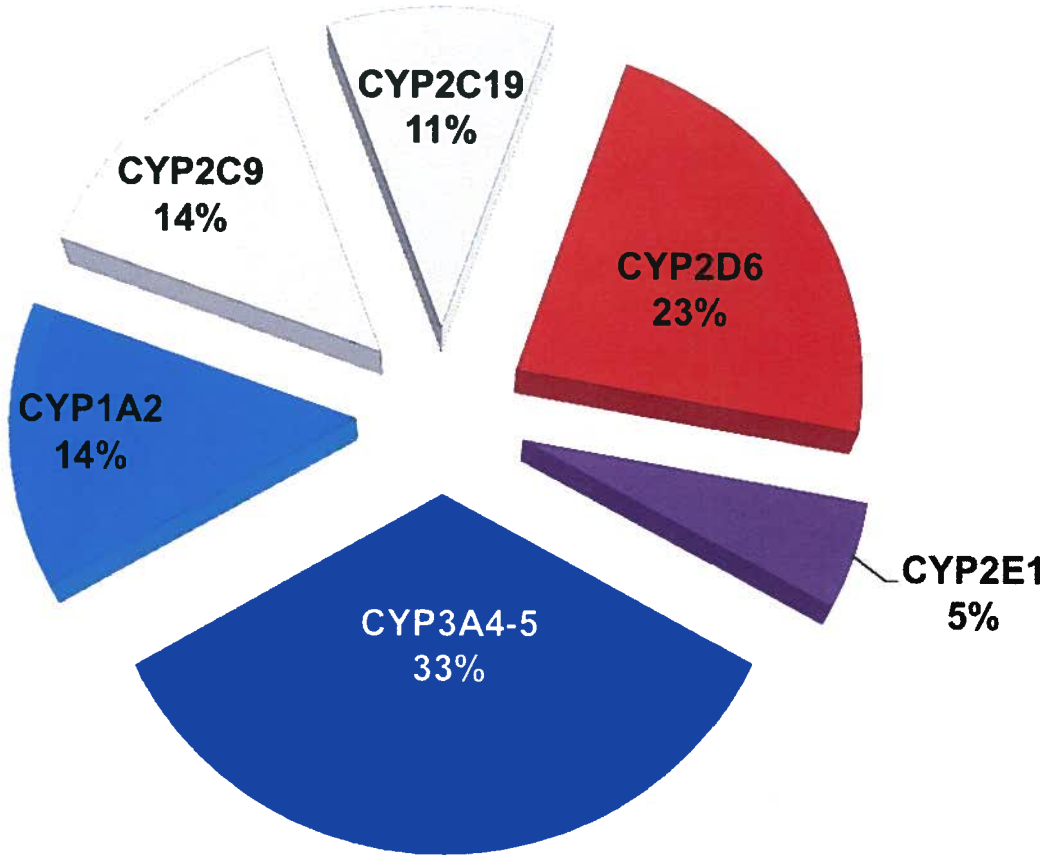


P450s
Marked Variability



Transferases
Little Variablity

% Drugs Metabolism by CYP Enzymes



Preclinical

- Drug discovery – Find a compound with activity
- Lead optimization (alter original compound)
  - Optimize activity (target receptor binding)
    - Binding, on-off rates, Clinical activity (pharmacodynamics)
  - Optimized metabolism (and elimination)
    - Rate of metabolism
    - Enzymology of metabolism
    - Avoid potential drug interactions
      - Toxicity
      - Efficacy

Preclinical Drug Metabolism Studies

- Determine the nature of metabolites

  Stable metabolites > good

  Electrophiles > bad

  Bind to cellular nucleophile - DNA, RNA and protein

  Cause cell death or transformation - cancer

Drug Metabolism Studies

- Which P450s are involved in metabolism of the drug candidate?

  Several P450s > good

  One P450 > bad
  CYP2D6 - polymorphism
  CYP3A4 - drug interactions

Drug Interactions

Major Human P450s

P450 Genes	Substrates
CYP1A1-CYP1A2*	Carcinogens/toxins
CYP1B1*	Carcinogens/toxins
CYP2A6*	Carcinogens/toxins
CYP2B6	Drugs (limited)
CYP2C8, 9, 19	Drugs
CYP2D6*	Drugs
CYP2E1*	Carcinogens/toxins
CYP3A4*,5 ,7*,43	Drugs

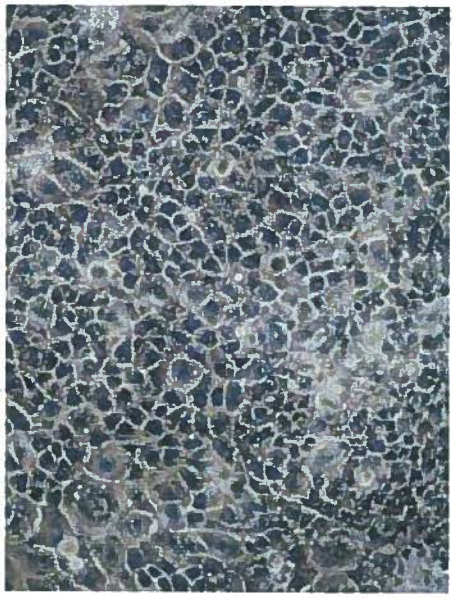
* Humanized mice available

In vitro Studies to Predict Drug Metabolism/Toxicity in Humans

Human liver cell fractions

Human hepatocytes
- Slices
- Primary cultures

Recombinant human CYPs



"ADME"

- Drug absorption (A)
- Drug distribution (D)
- Drug-receptor (transporter) interaction
- Drug metabolism (M)
- Drug excretion (E)

>Mouse models can be of great value for preclinical ADME

Transgenes

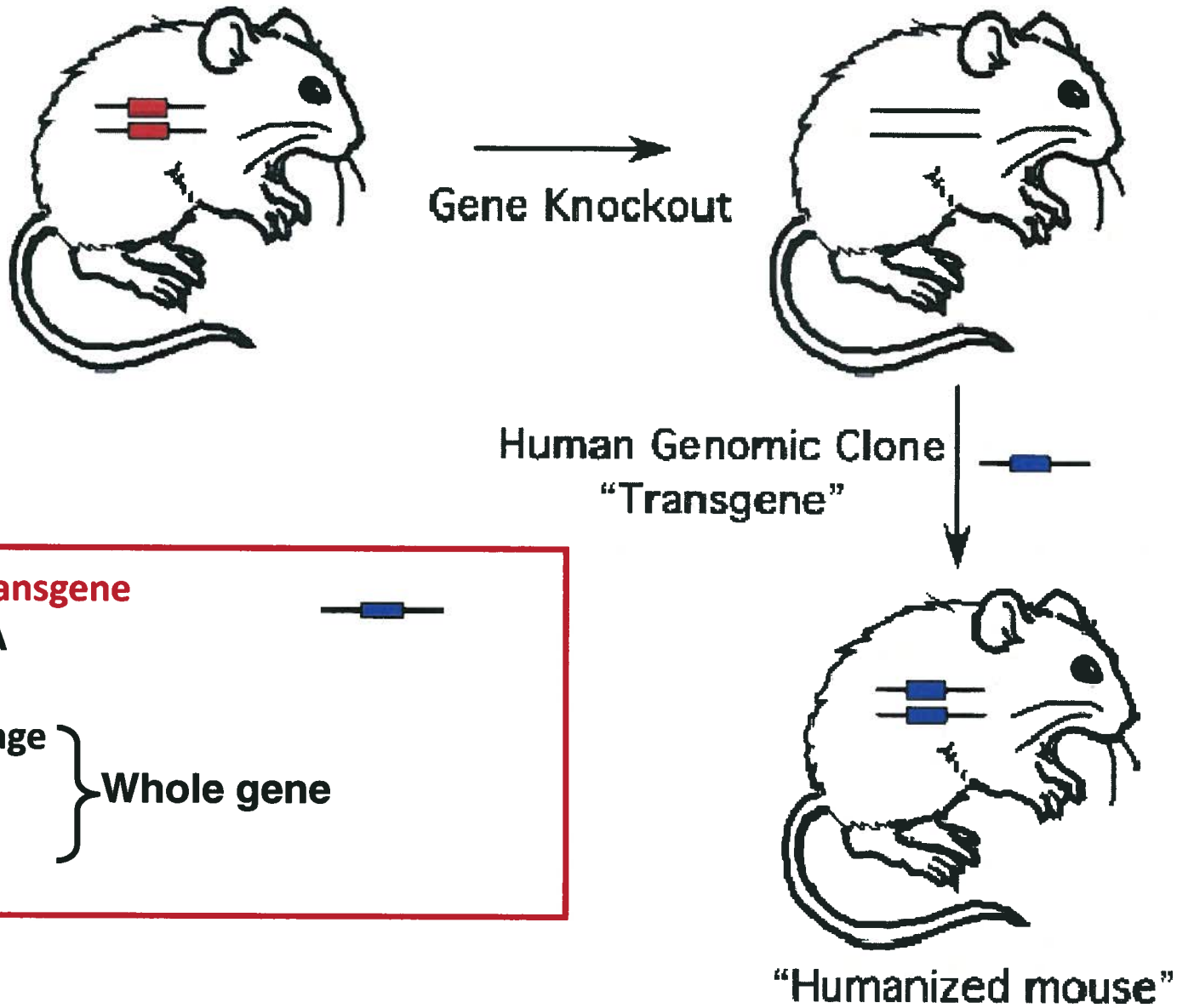
1. cDNAs driven by heterologous promoters
- Tissue-specific expression (albumin promoter – liver)
- Controlled expression (Tet-OFF System)
- Tissue-specific and controlled

2. cDNAs driven by native promoters (knock-in)

3. Complete genes under control their own promoters
- Natural tissue expression patterns
- Native regulatory controls

4. Knock-in of complete genes (size limitation)

Production of Gene Knockout and Humanized Mice

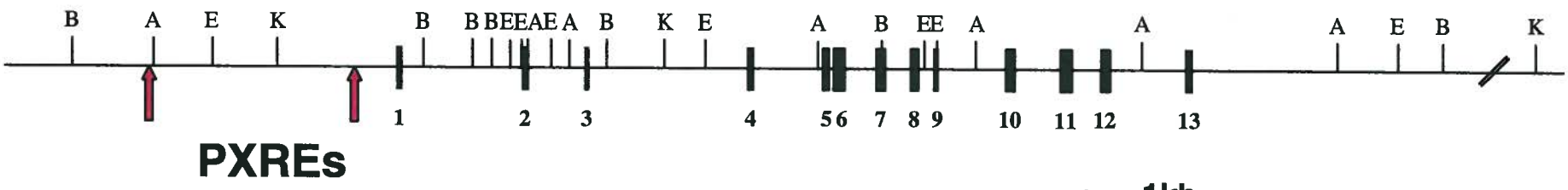


Human transgene

- cDNA
- λ phage, BAC, PAC } Whole gene

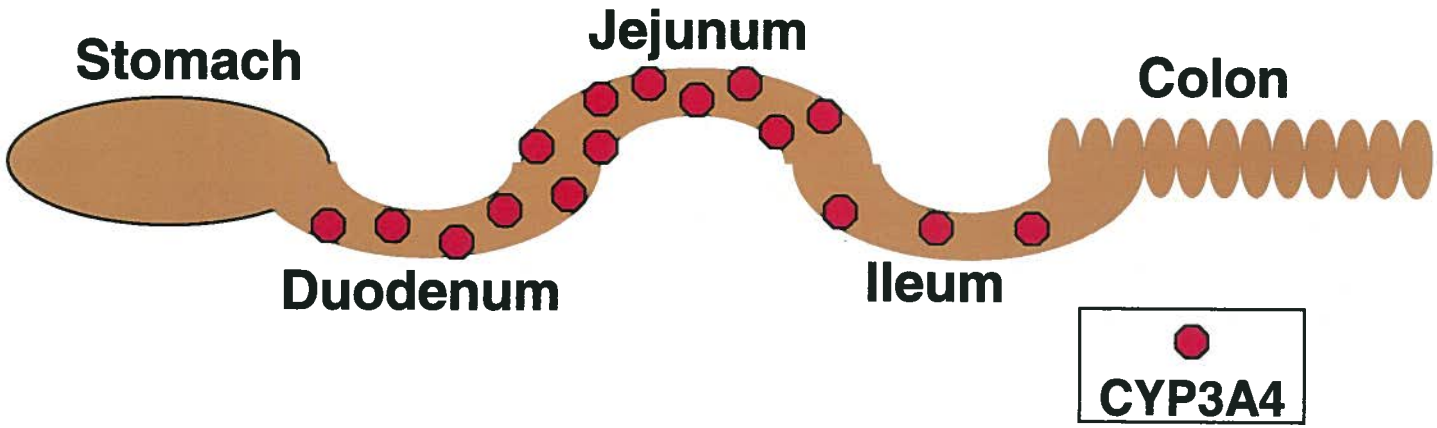
Expression of CYP3A4 in the Gut of hCYP3A4 Mice

CYP3A4 BAC clone

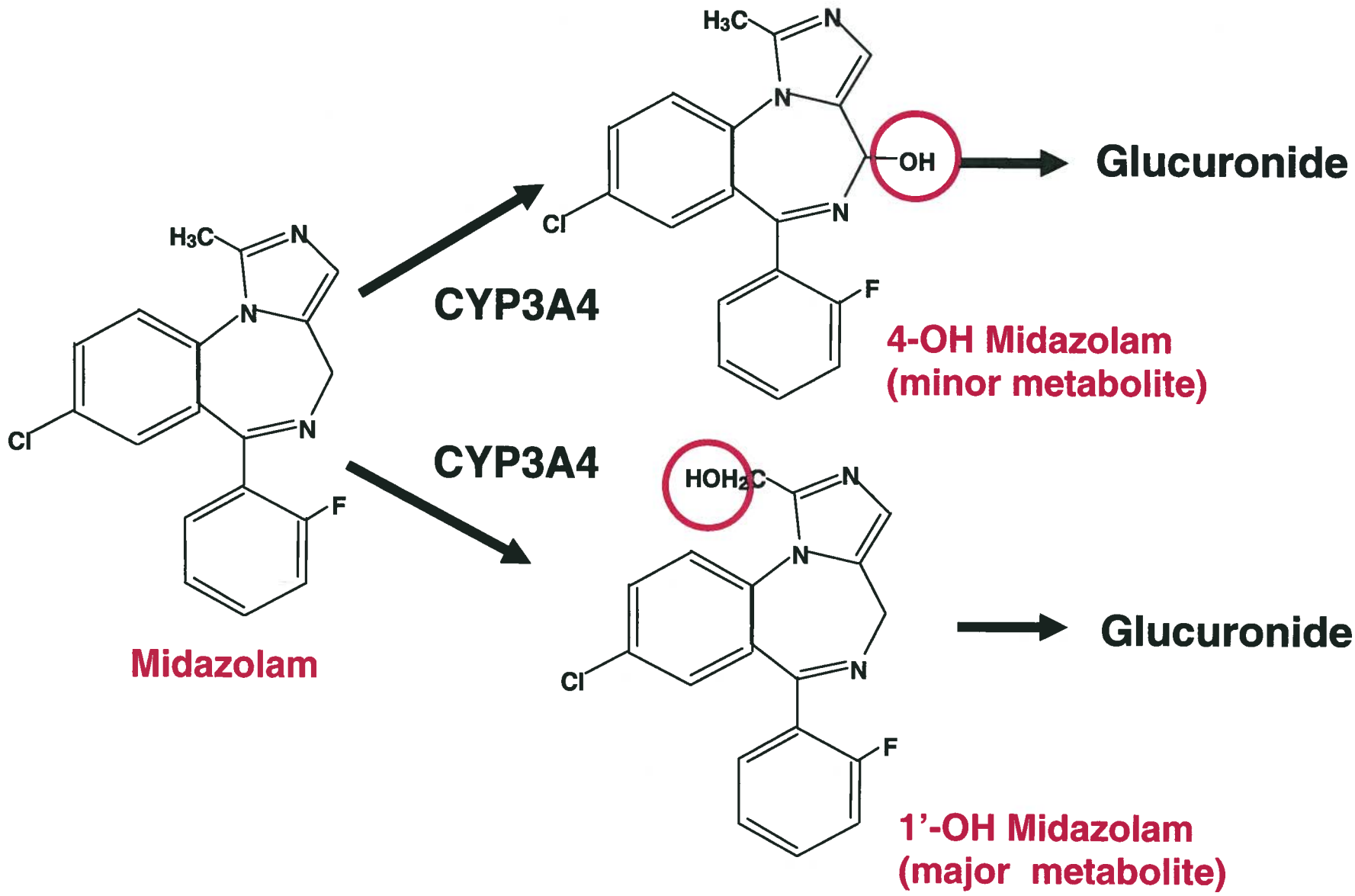


PXREs

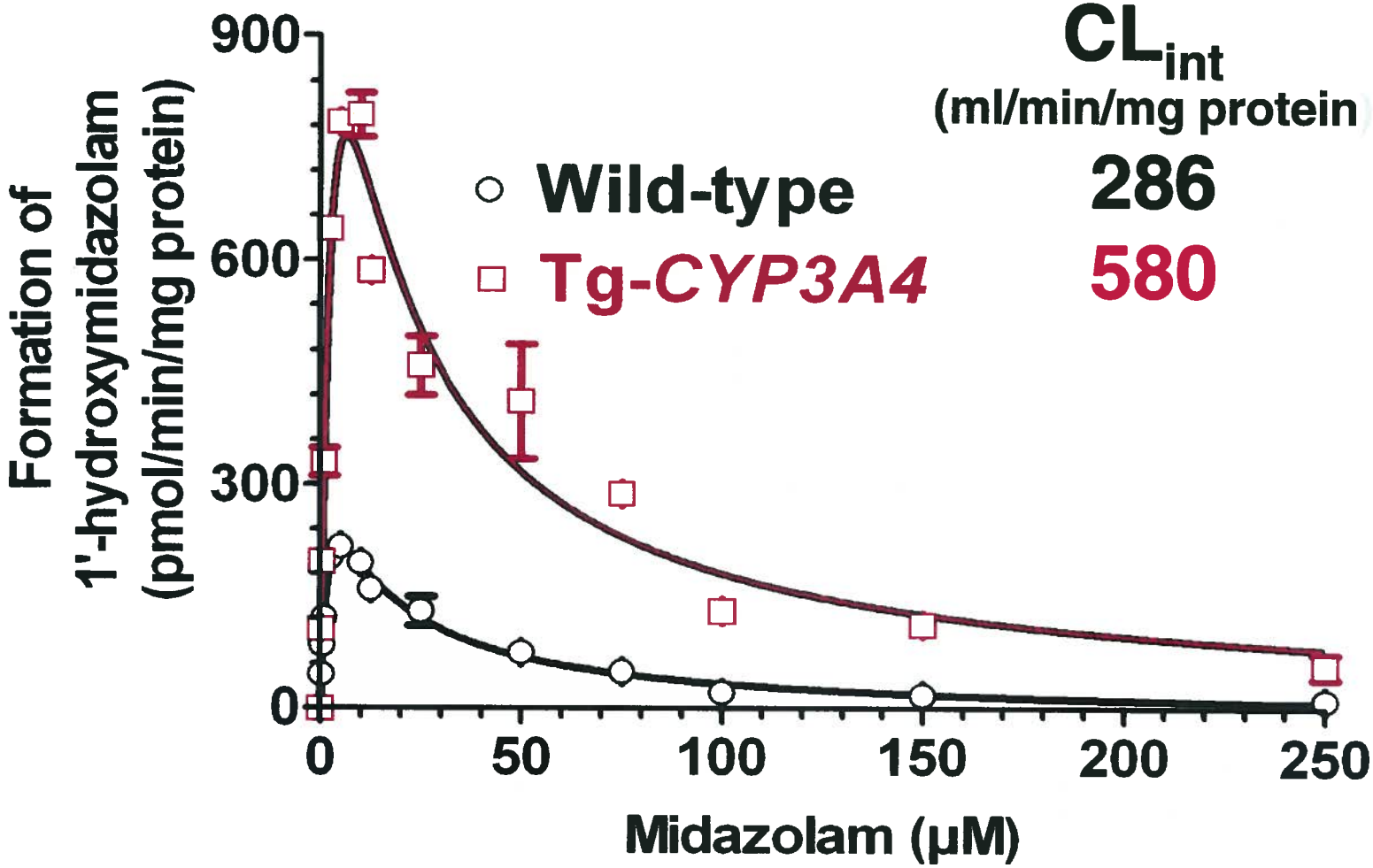
1kb



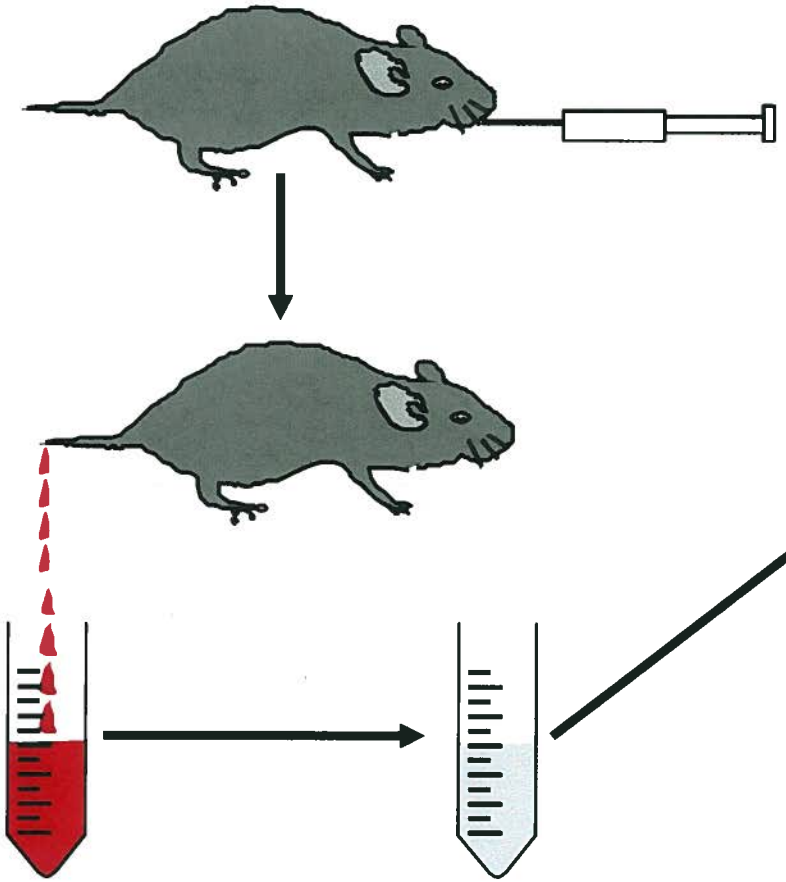
Metabolism of Midazolam by CYP3A4



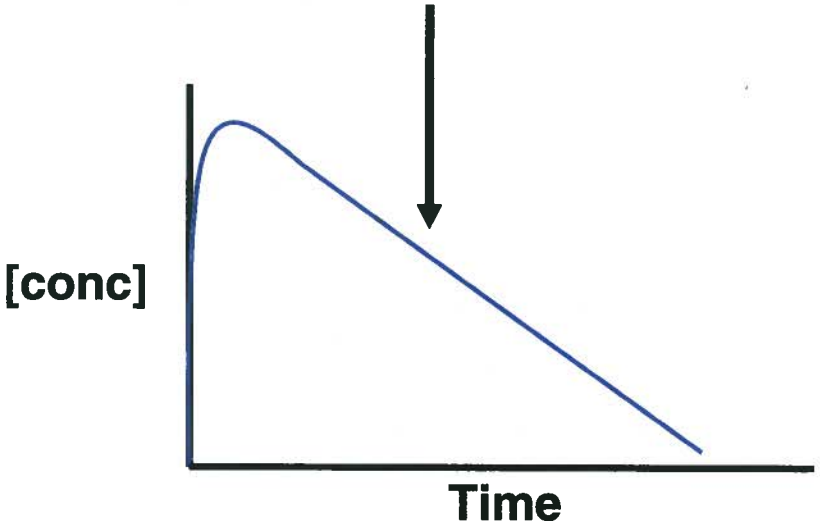
Midazolam 1'-hydroxylation in h*CYP3A4* and Wild-type Mouse Gut Microsomes



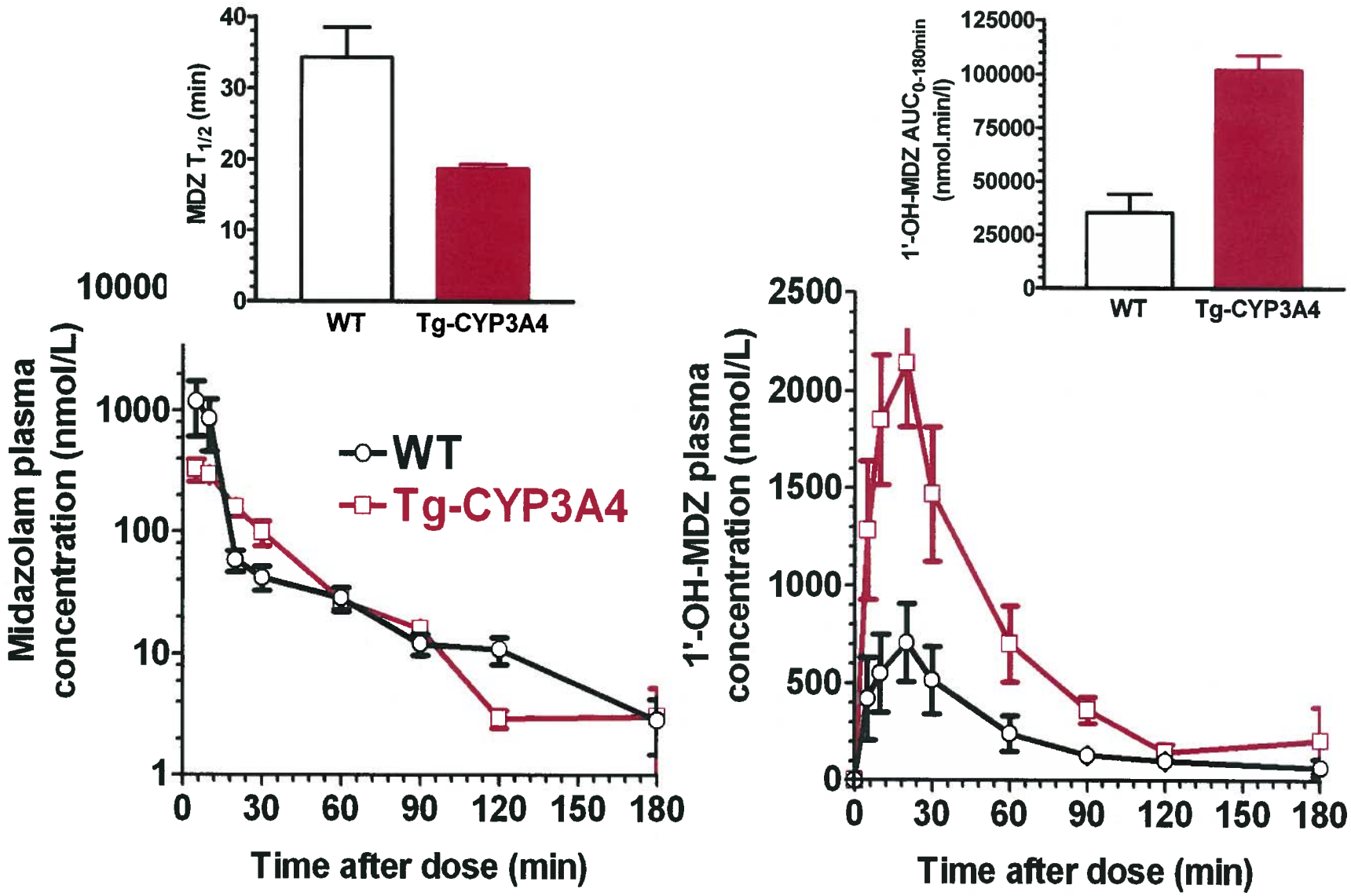
Pharmacokinetics



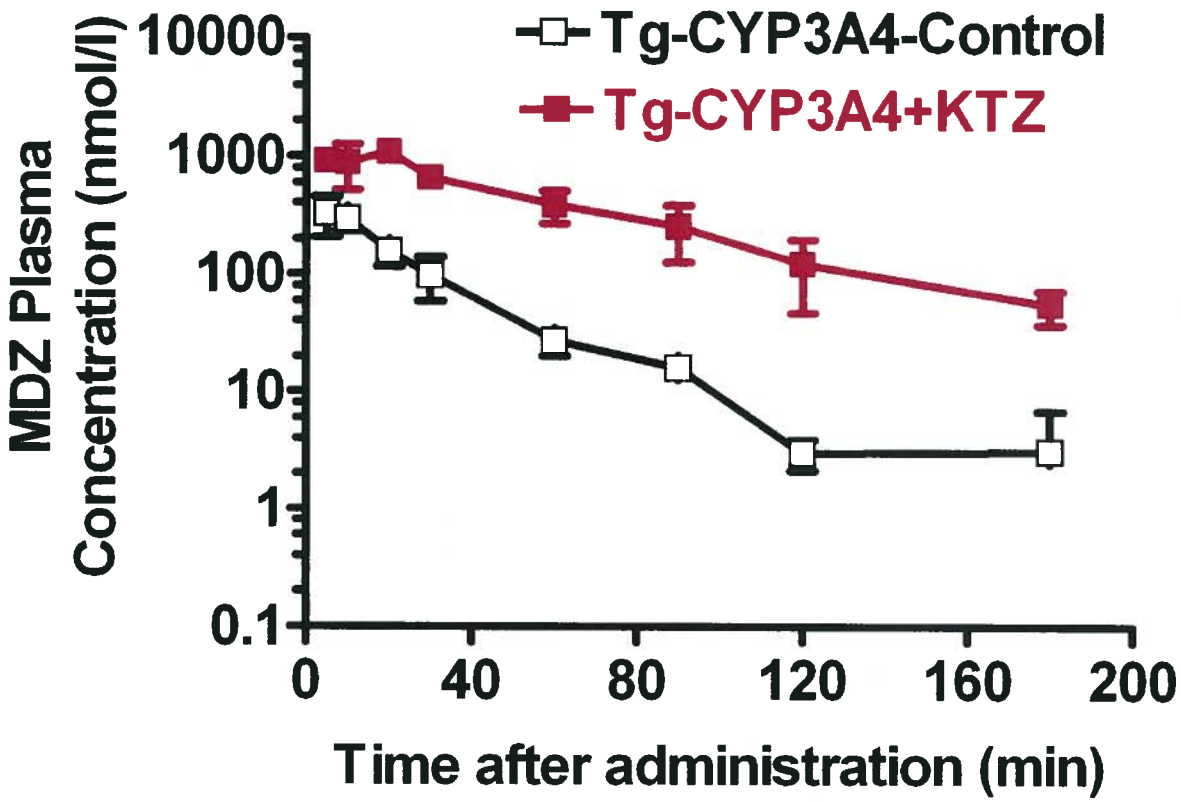
LC-MS/MS



Midazolam Disposition in h*CYP3A4* Mice After Oral Dosing (2.5 mg/kg)



Drug-Drug Interactions: Ketoconazole Inhibits Midazolam Disposition in h*CYP3A4* mice



$AUC_{0-\infty}$: 7-fold ↑

$T_{1/2}$: 2-fold ↑

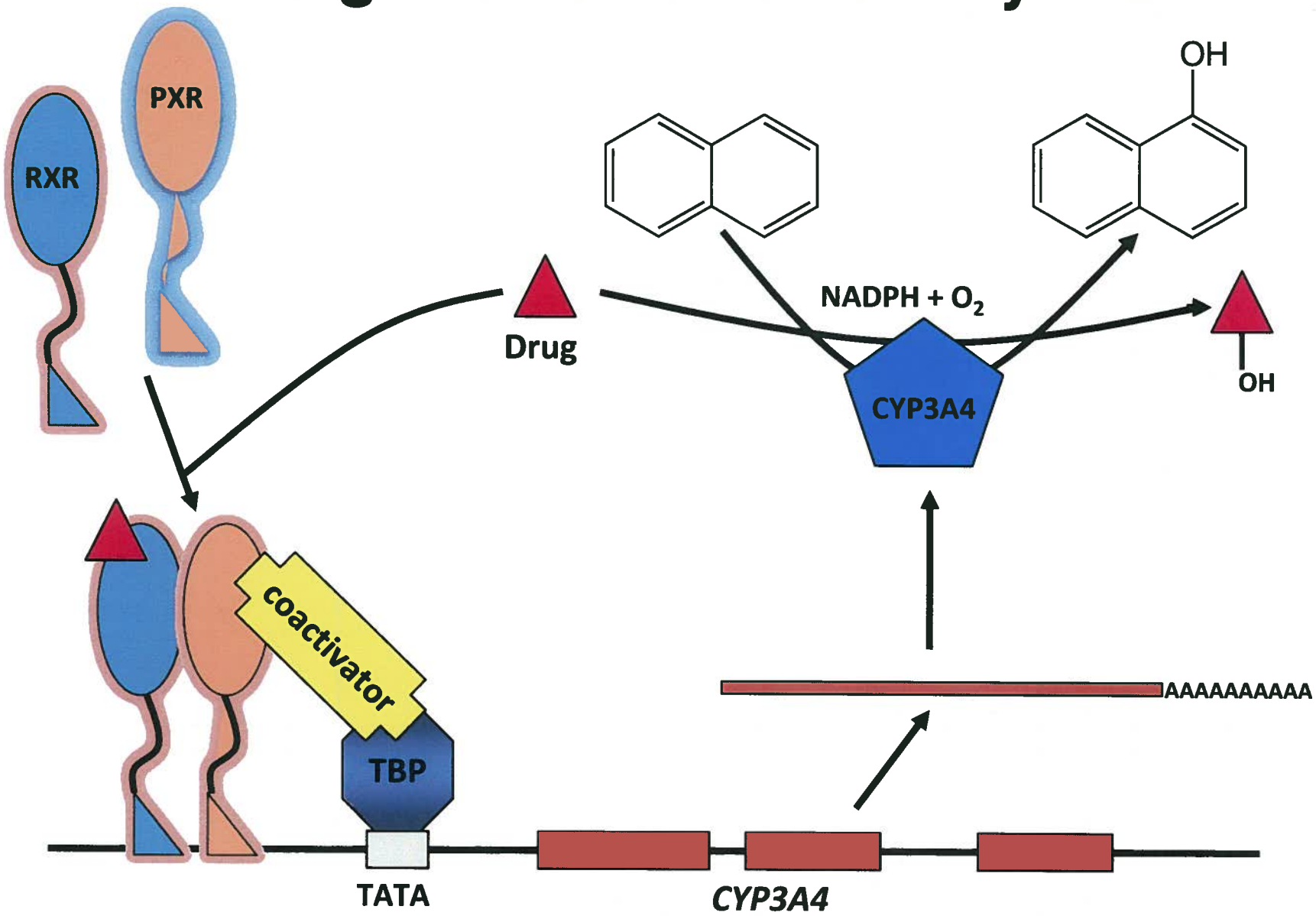
CL_{oral}: 10-fold ↓

Conclusions

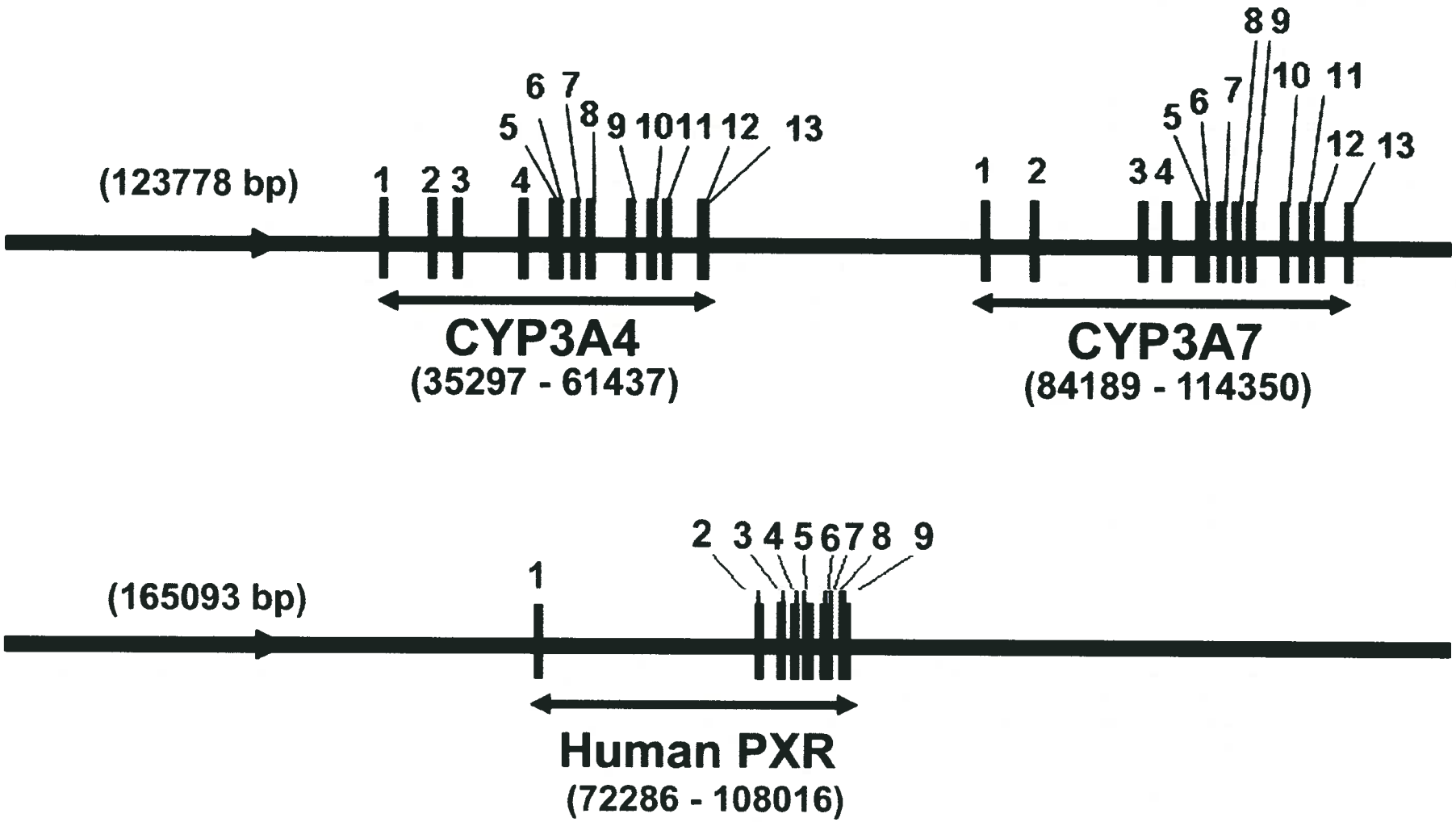
CYP3A4-humanized mouse

- CYP3A4 expressed in small intestine and liver
  - Influences PK of oral drugs
  - Impacts drug interactions
- Induced by PXR and CAR activators (R.Wolf)
- Regulated sex-specifically by GH

Regulation of CYP3A4 by PXR



Transgenes – P1 Phage Artificial Chromosomes

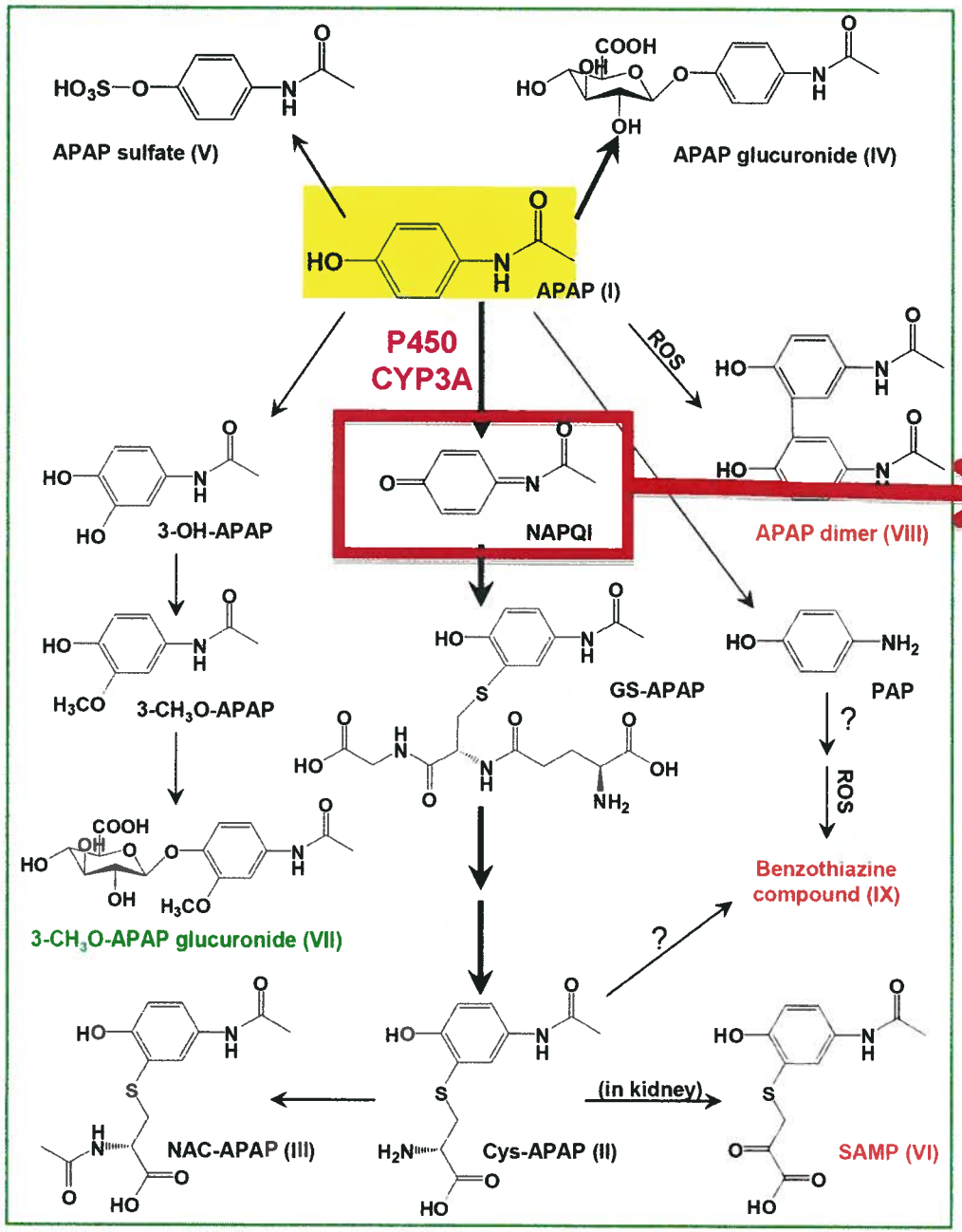


Acetaminphen Toxicity

- Dosage (adults): 325-650 mg/kg every 4-6 hr, maximum dose is 4 g/day.
- Overdose >

  acute liver/kidney injury

Accounting for 39% of cases related to drug-induced acute liver failure in the USA.

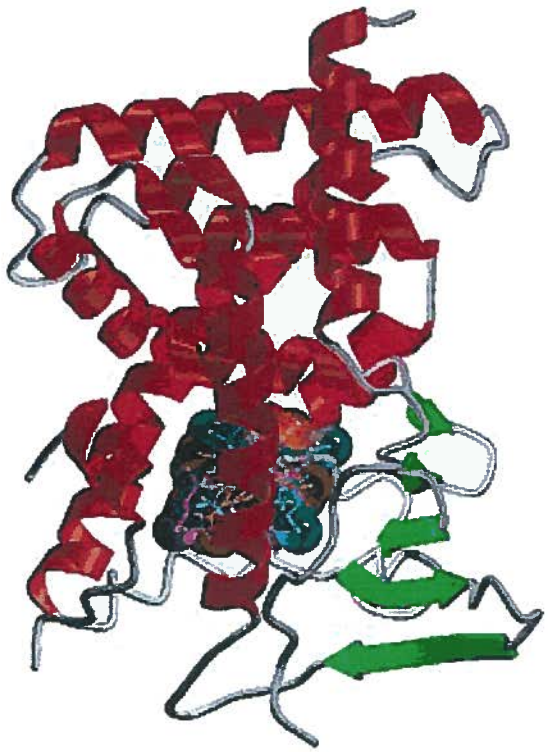


Toxic Electrophile

↓

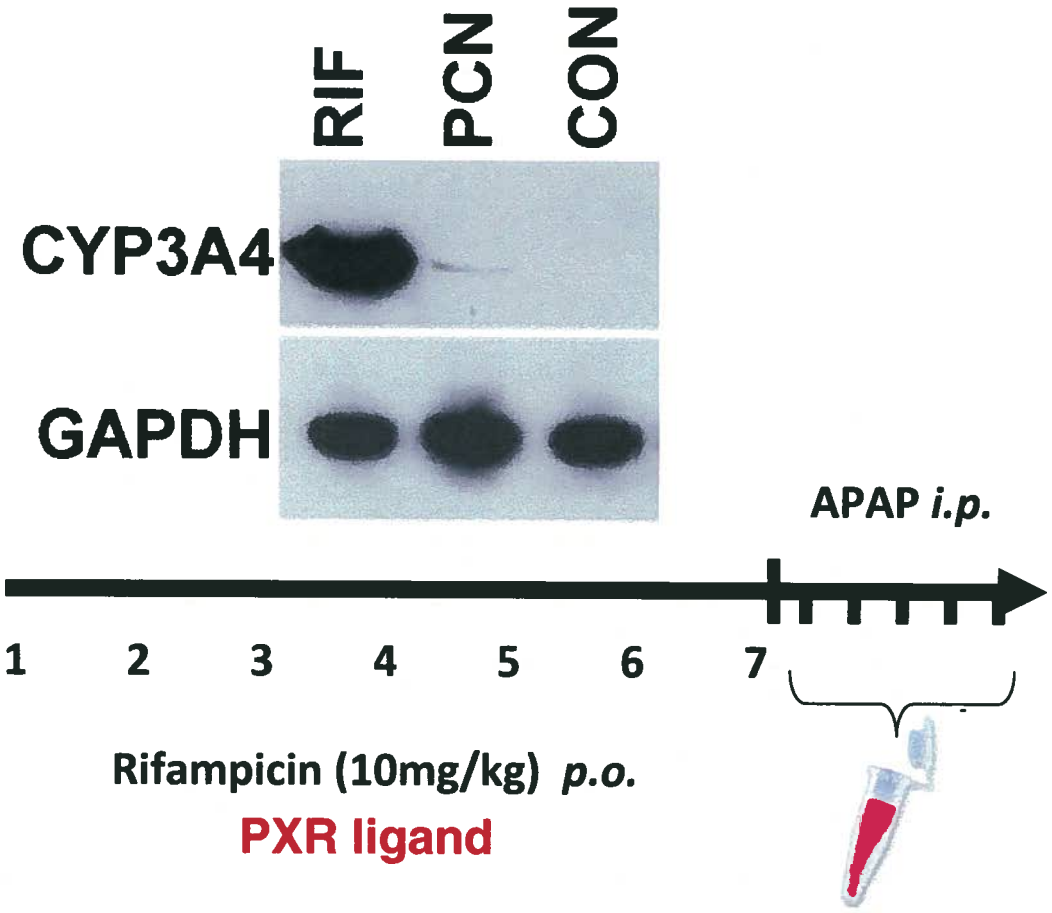
Mitochondrial Damage

Experimental Design

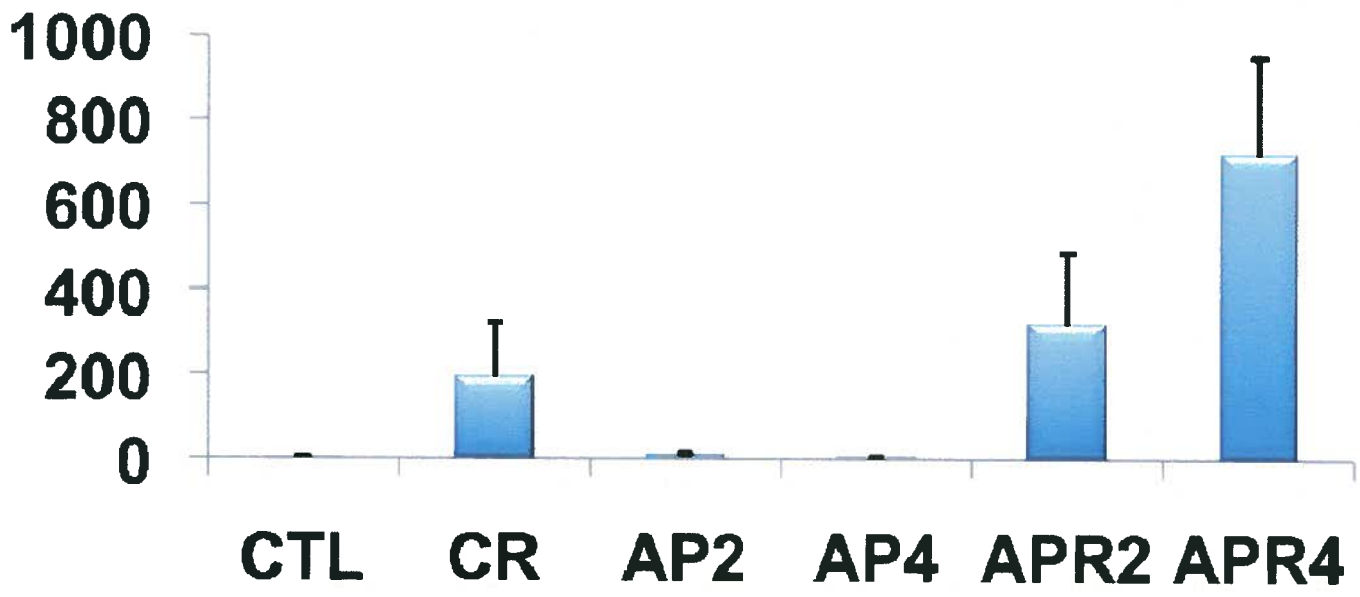


Human PXR

Rifampicin
antibiotic specific ligand for
human PXR

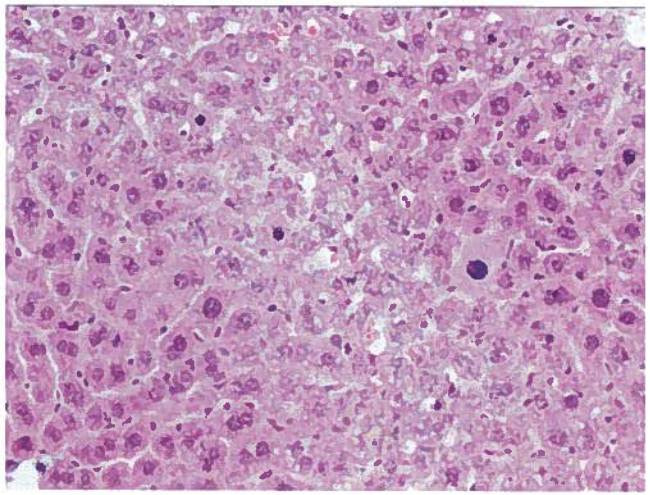


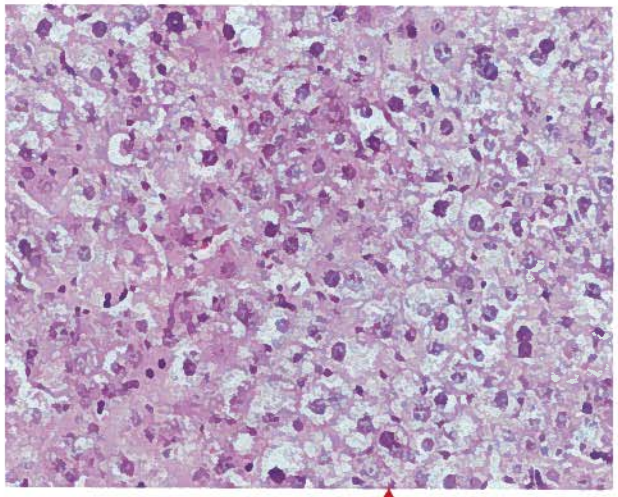
Increased CYP3A4 mRNA Expression (qPCR units)

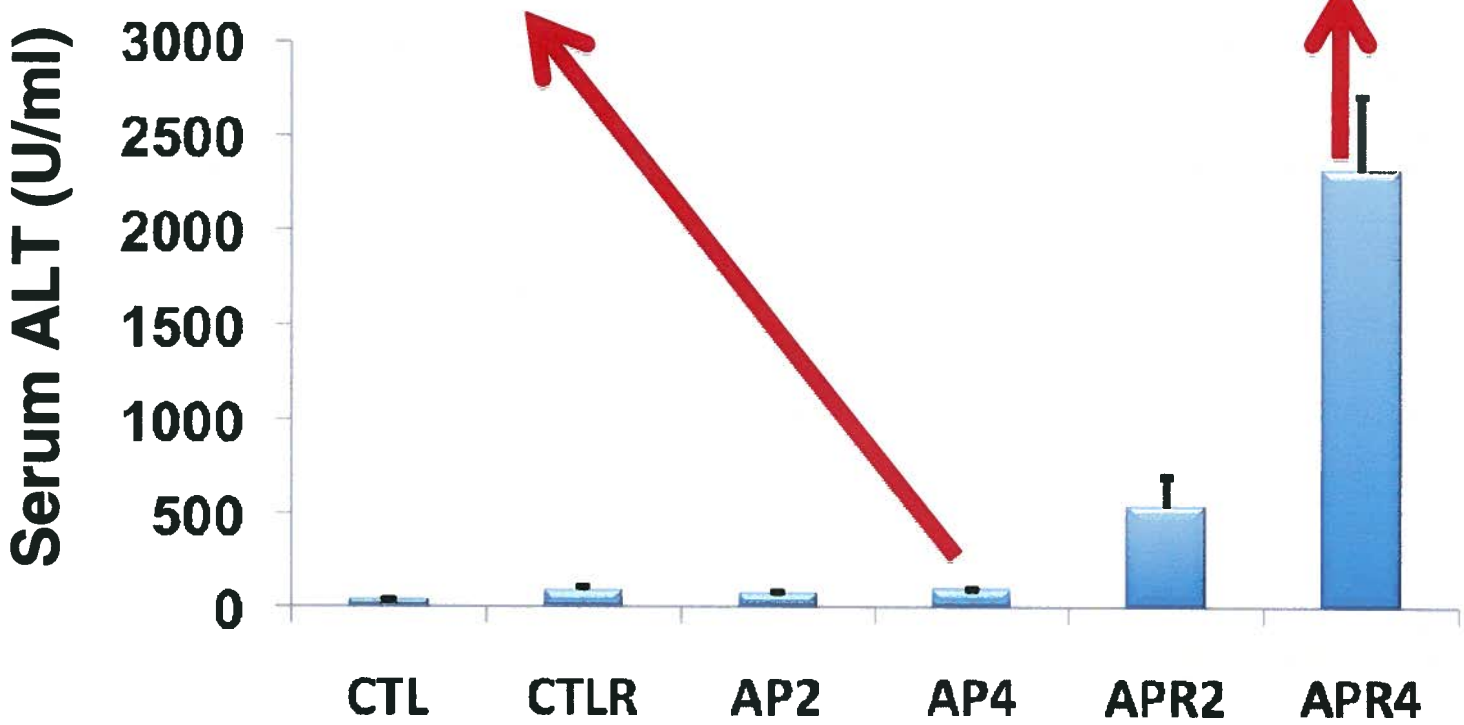


AP – acetaminophen
R – rifampicin
2 – 200 mg/kg AP
4 – 400 mg/kg AP

Liver Damage







Conclusions

1. Stable expression of a two PAC transgene in appropriate target tissues

    Human PXR and it main target gene CYP3A4

2. Prediction of potential fatal drug interaction between an antibiotic, rifampicin, and an analgesic, acetaminophen

CITATIONS:

Drug Metab Dispos. 31: 548-58, 2003
Drug Metab Dispos. 36: 2506-12, 2008
J Pharmacol Exp Ther. 327: 288-99, 2008
Drug Metab Dispos. 37: 1611-21, 2009

Peroxisome Proliferators

- Plasticizers (di(2-ethylhexyl)phthalate (DEHP)
- Solvents (trichloroacetic acid, trichloroethylene)
- Herbicides (lactyofen, haloxyfab)
- Endogenous hormones (dehydroepiandosterone sulfate)
- Fatty acids (leukotriene B4)
- Fibrate drugs (clofibrate, fenofibrate, gemfibrozil)

Ligands/activators of Peroxisome Proliferator-Activated Receptor α (PPARα)

NUCLEAR RECEPTOR SUPERFAMILY

Steroid

GR
MR
AR
PR
ERα, β γ
VDR
TRα, β
RARα, β γ

Sensors

PPARα, β, γ
LXRα, β
FXR
PXR
CAR

RXRα, β, γ

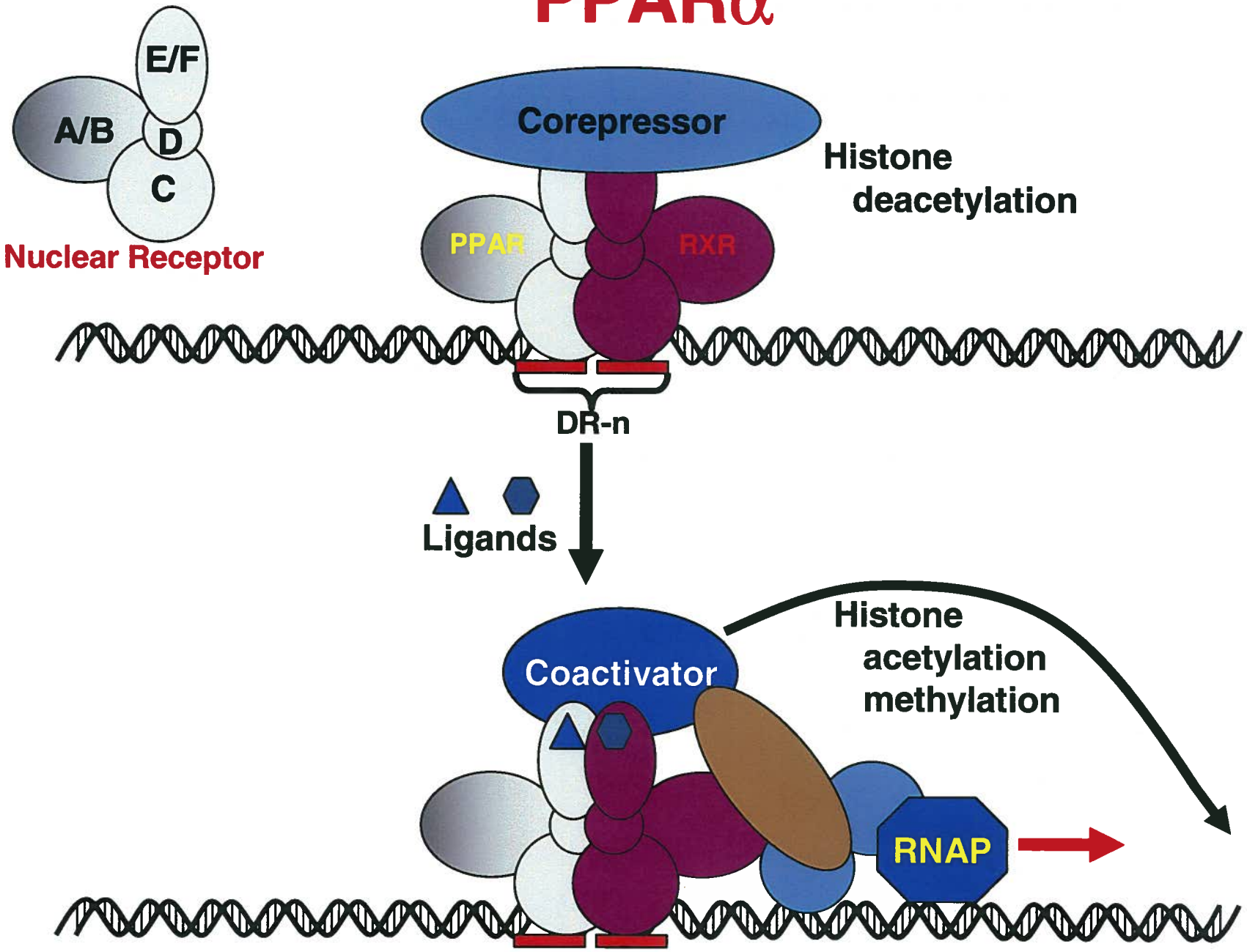
HNF4α, γ

Orphans

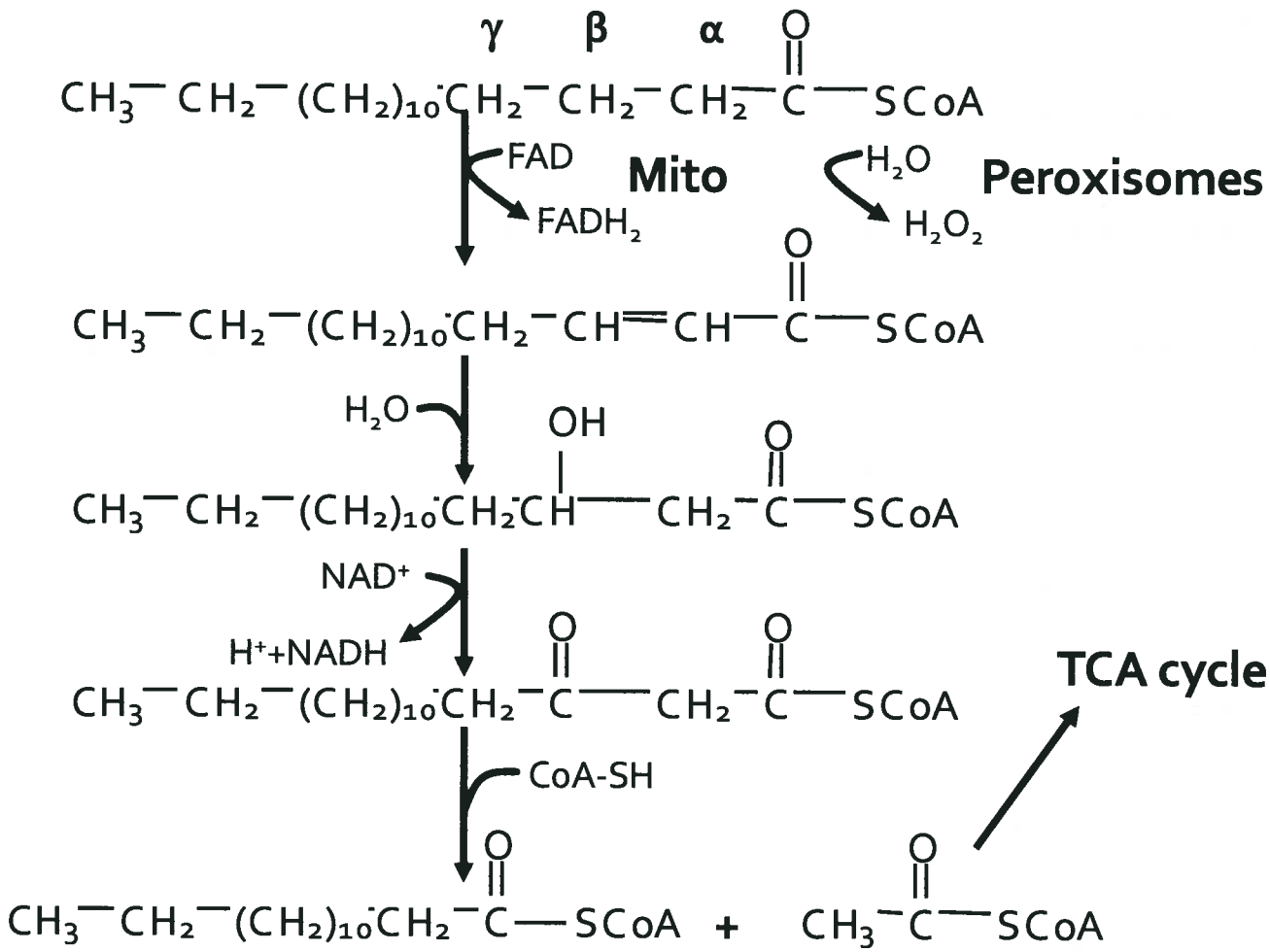
ARP-1
COUP-TF
EAR2
SCP46
REVERBα, β
SF-1
LRH-1
NGFI-B
NURR1
TR2
TR4
RZRα, β
ERR1
Others.....

6 Families
26 Subfamilies
> 50 genes

PPARα

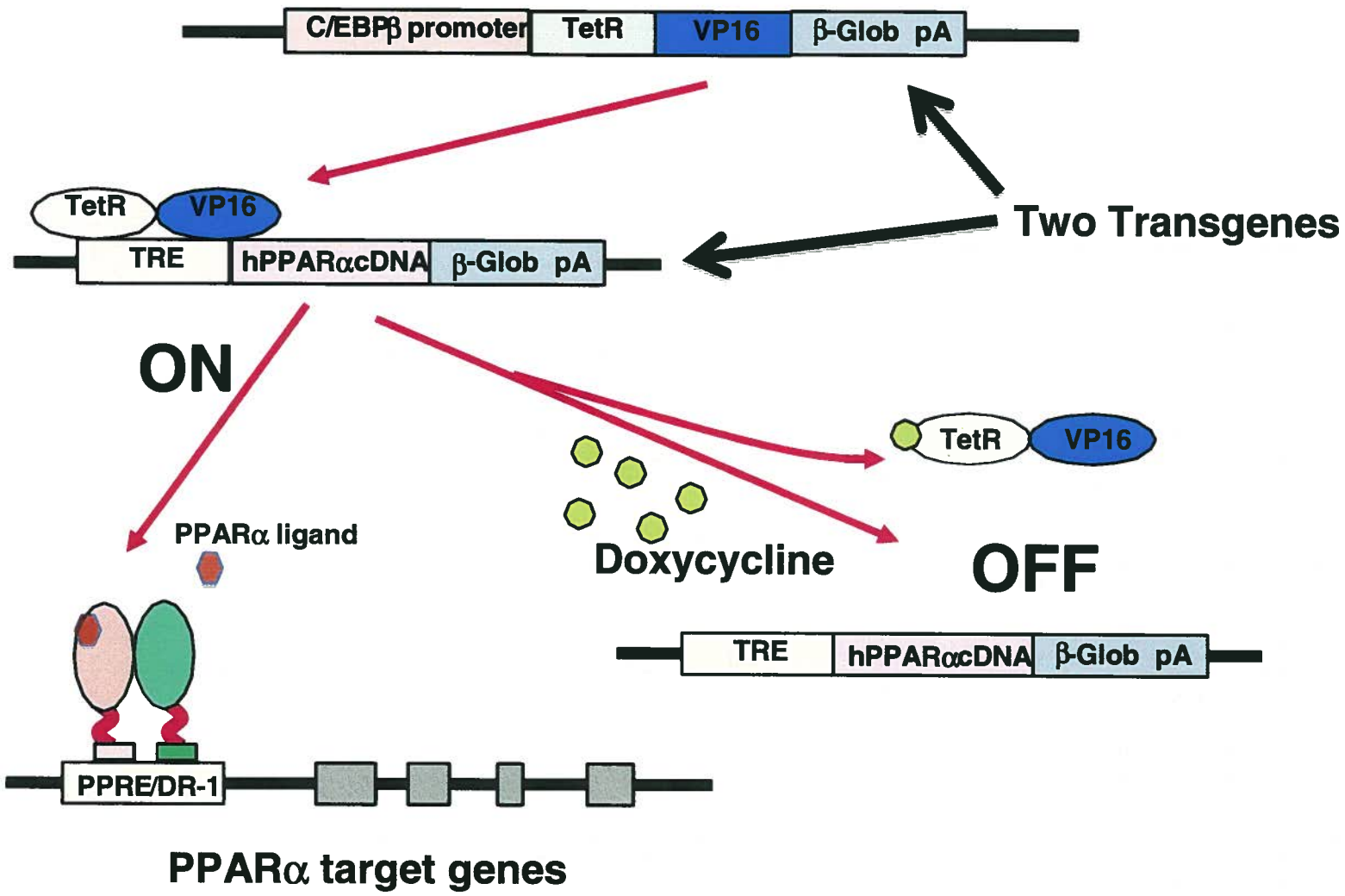

E/F
A/B
D
C
Nuclear Receptor
Corepressor
Histone
deacetylation
PPAR
RXR
DR-n
Ligands
Coactivator
Histone
acetylation
methylation
RNAP

PPARα Activation Induces Genes Involved in Mitochondrial and Peroxisomal Fatty Acid β-Oxidation



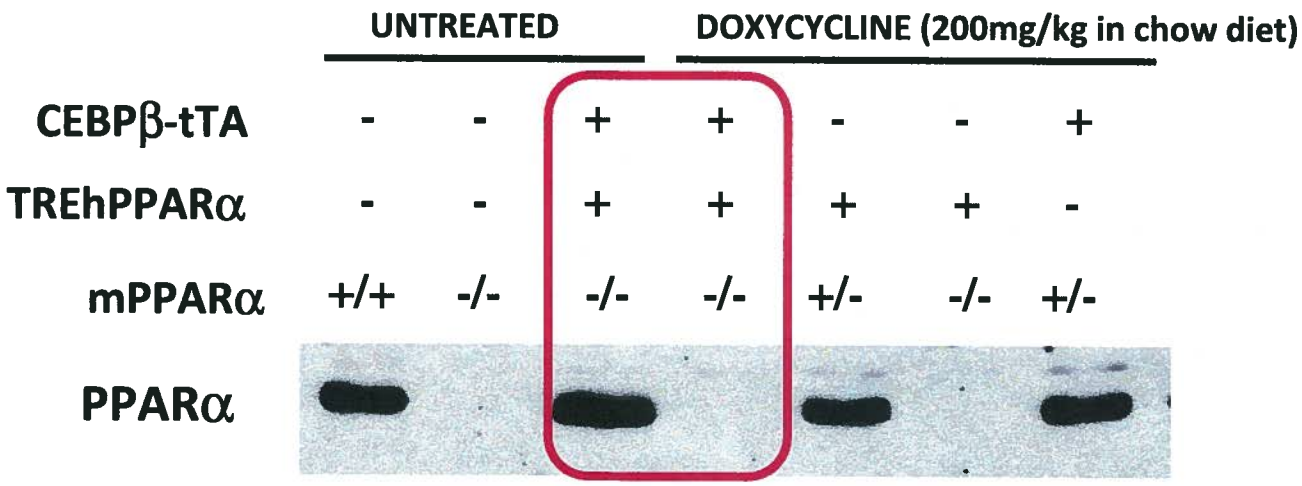
> Reduce serum triglycerides

Expression of hPPARα with the Tet-OFF System

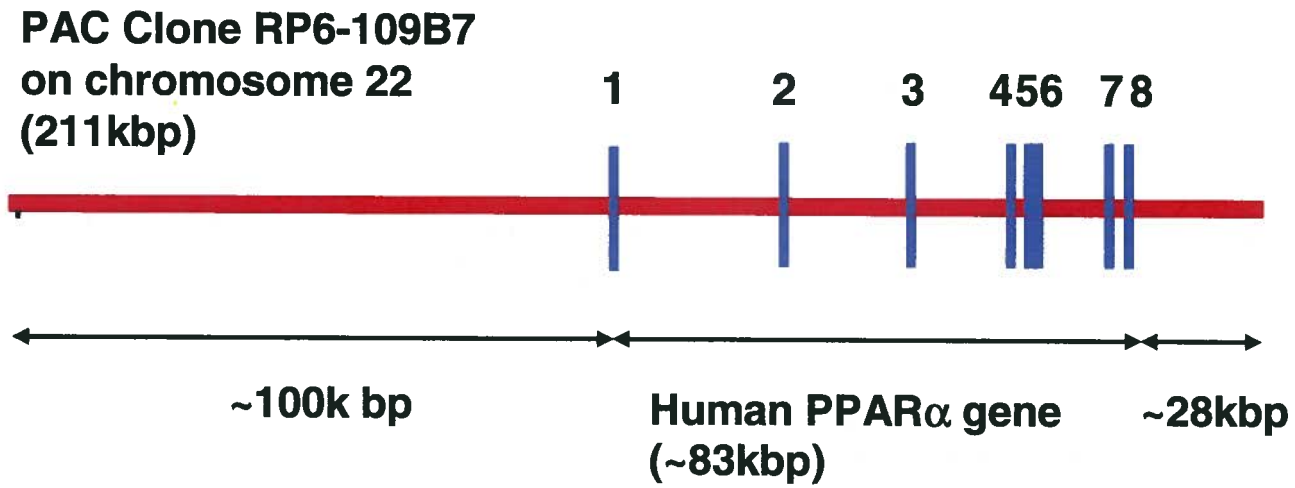


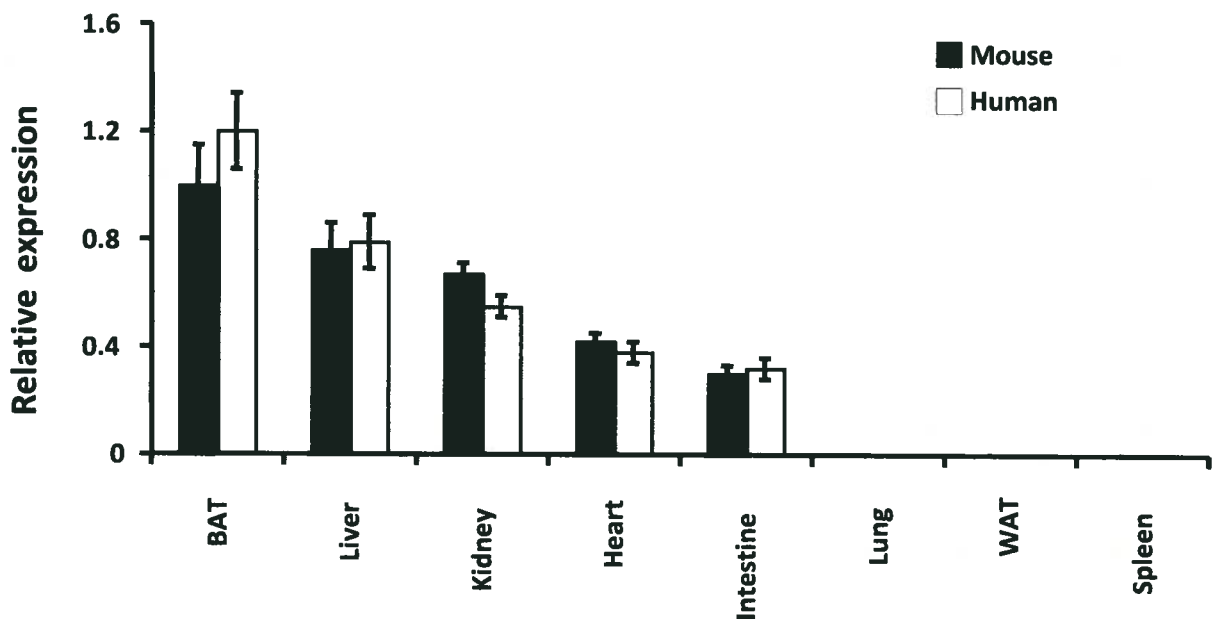
> Liver-specific, regulatable expression of human PPARα

Tet-off mice express hPPARα protein

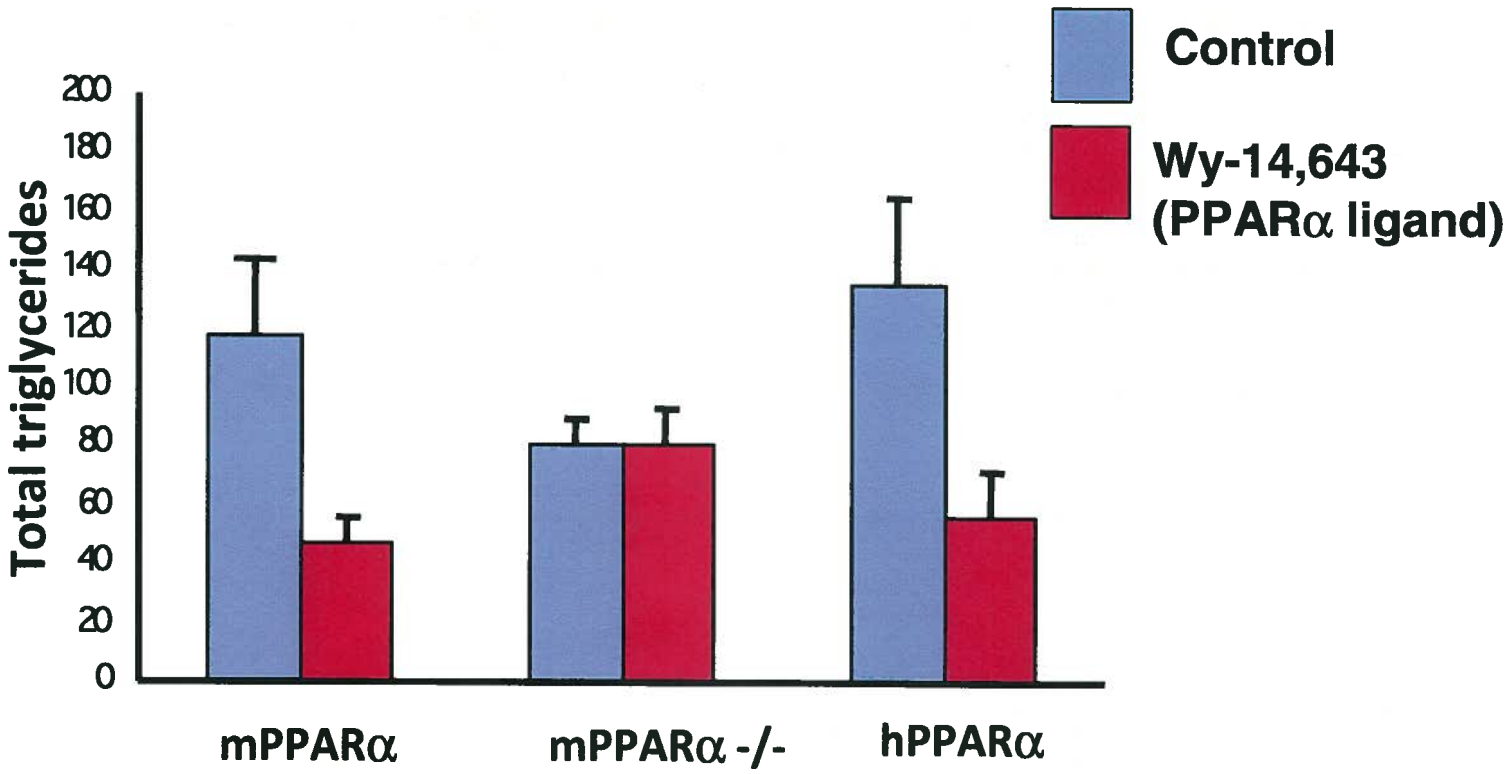


PPARα Humanized Mice

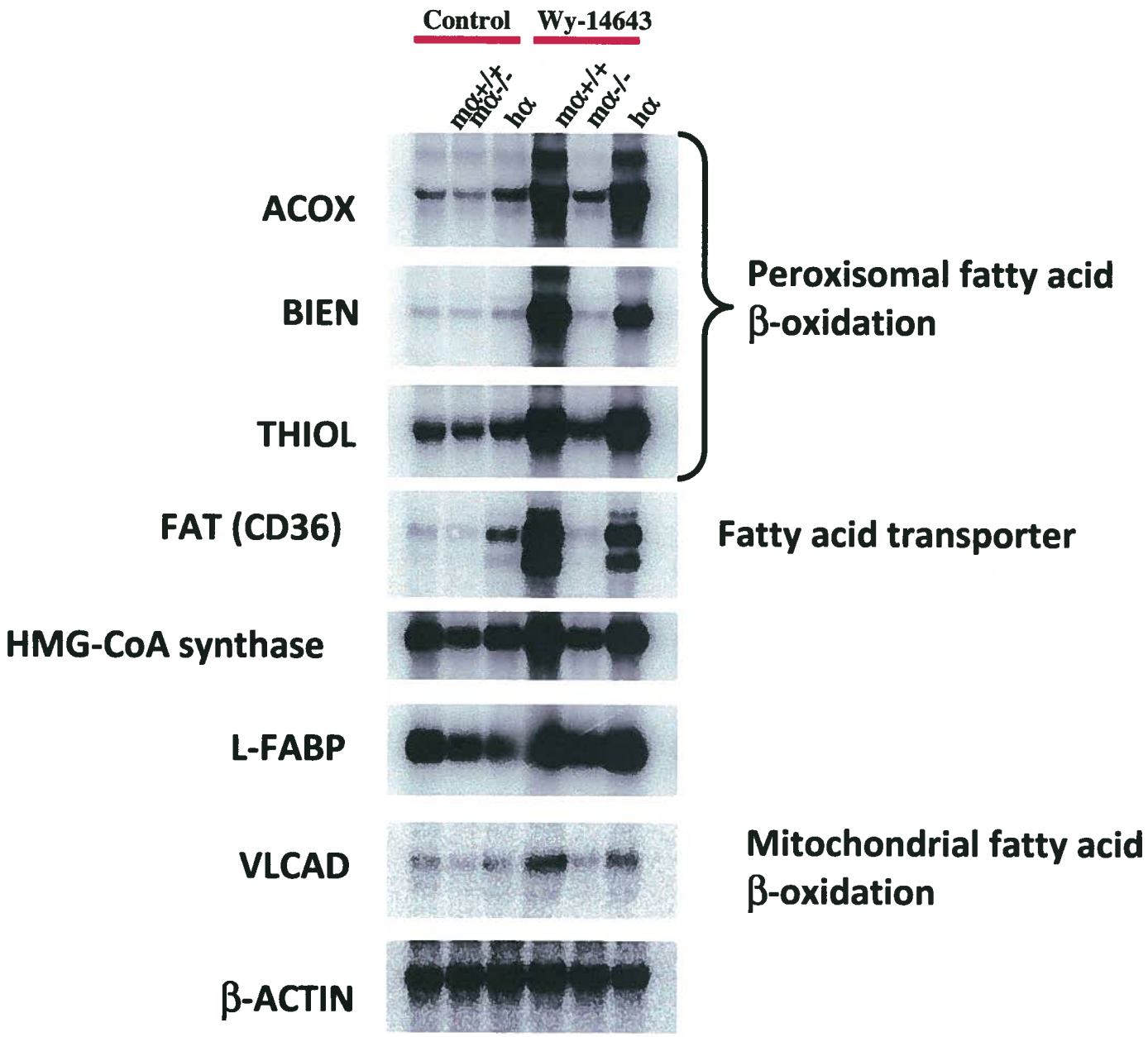


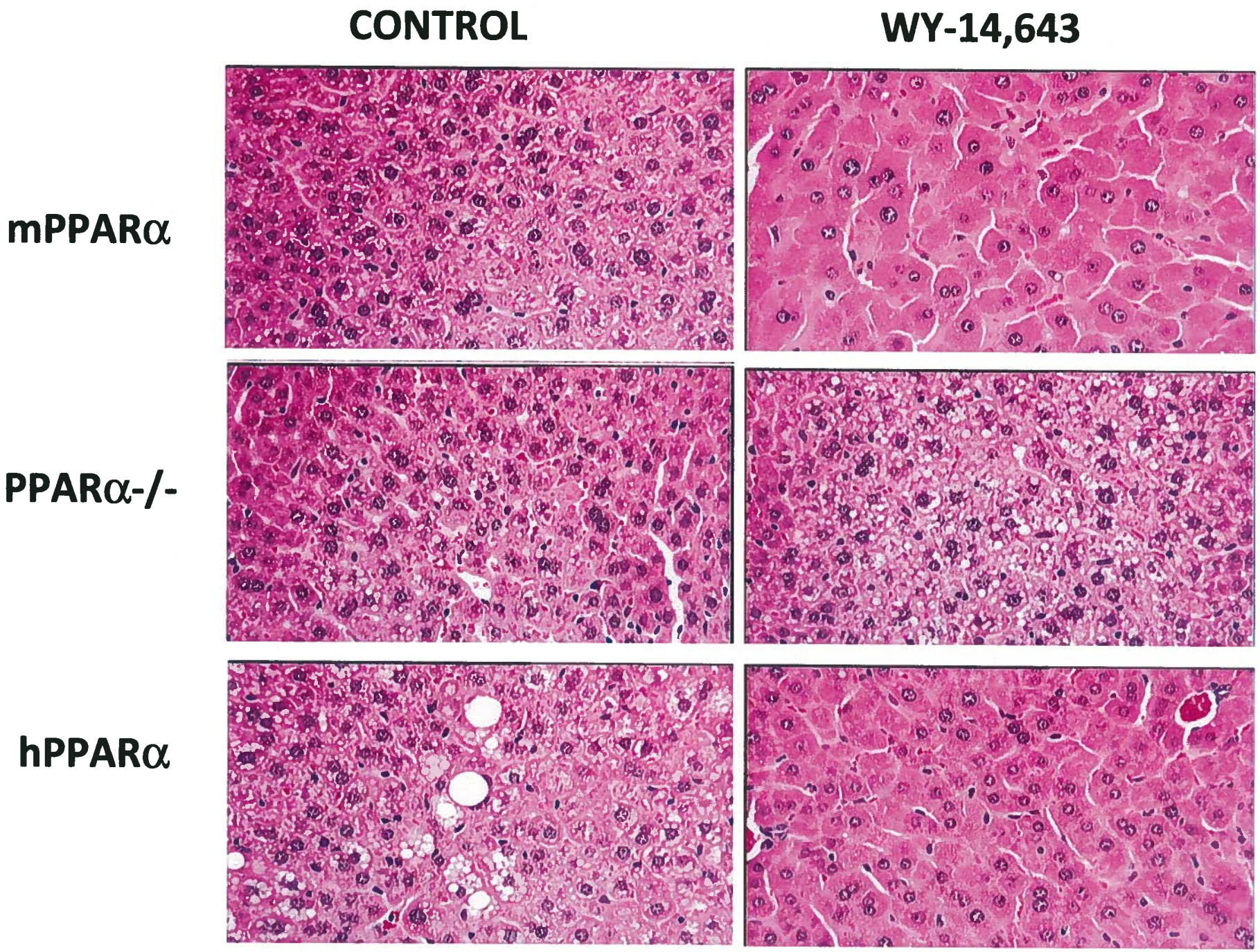


Serum Lipids

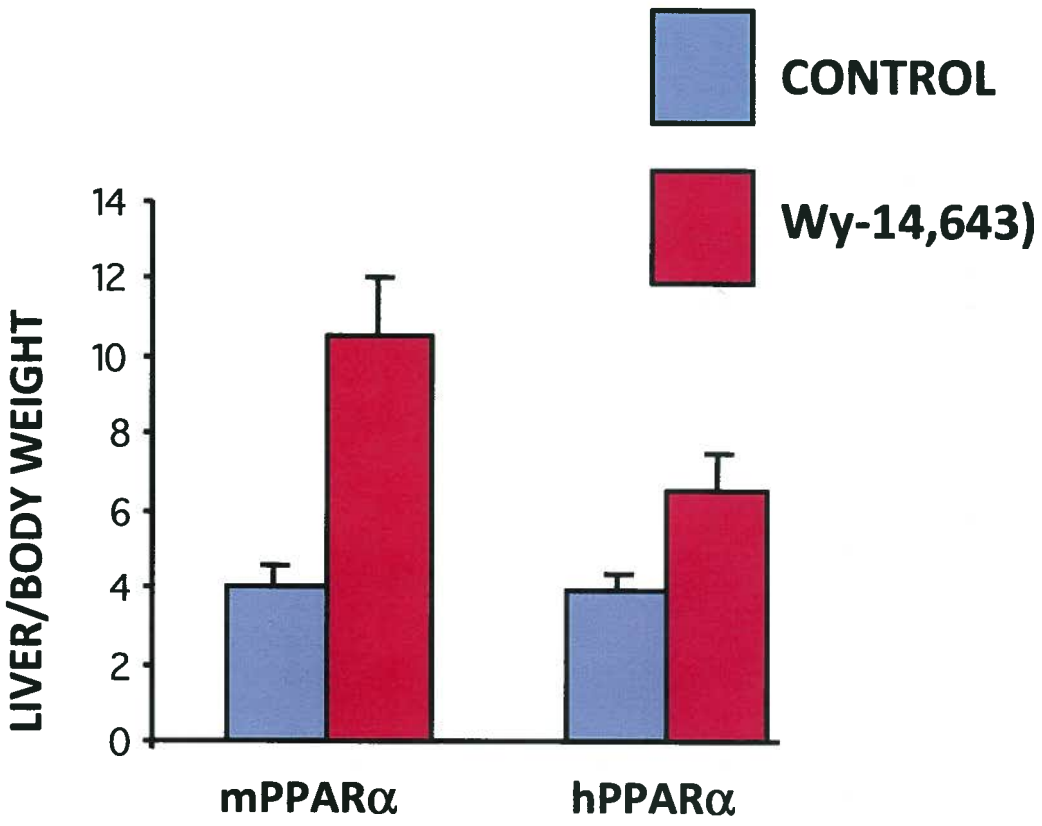


Expression of PPARα target genes

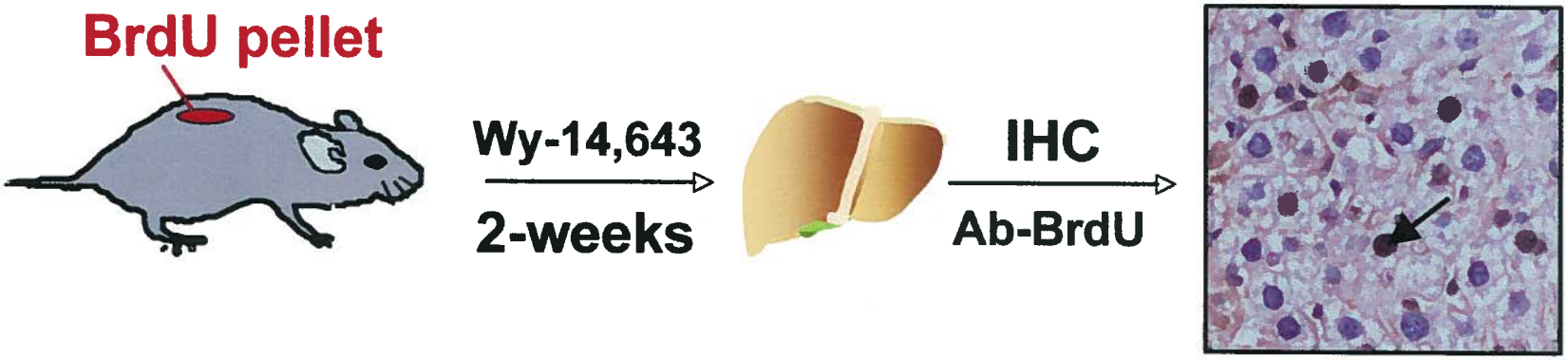


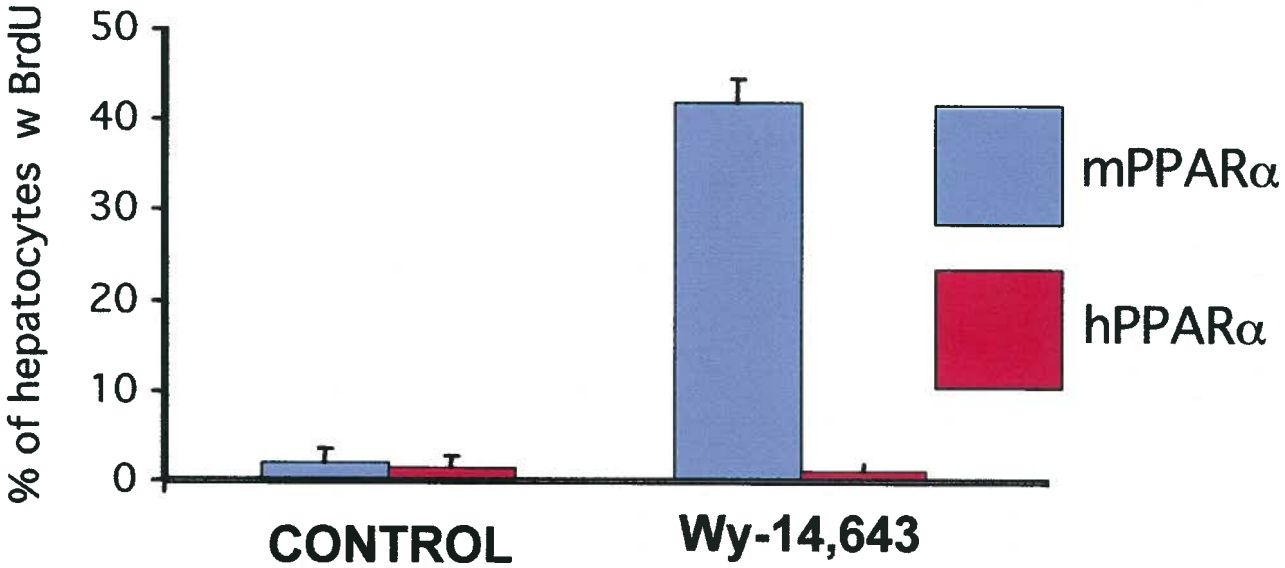

CONTROL
WY-14,643
mPPARα
PPARα-/-
hPPARα

Liver/Body Wt in Mice Treated With Wy-14,643



Wy-14,643-induced BrdU Hepatocyte Labeling Index





Conclusions

1. **Stable expression of a PAC transgene in appropriate target tissues**

2. **Human PPARα and mouse PPARα exhibit differences in response to ligand**

   Mouse – lipid lowering, hepatomagaly, liver cancer
   Human – lipid lowering

3. **Mechanism: Differential expression of an oncogenic MicroRNA**

   Mol Cell Biol. 27:4238-47, 2007

Laboratory of Metabolism, CCR

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Kris Krausz