Pharmaco/Toxicogenomics Studies to Facilitate the Understanding of Drug Metabolizing Enzymes

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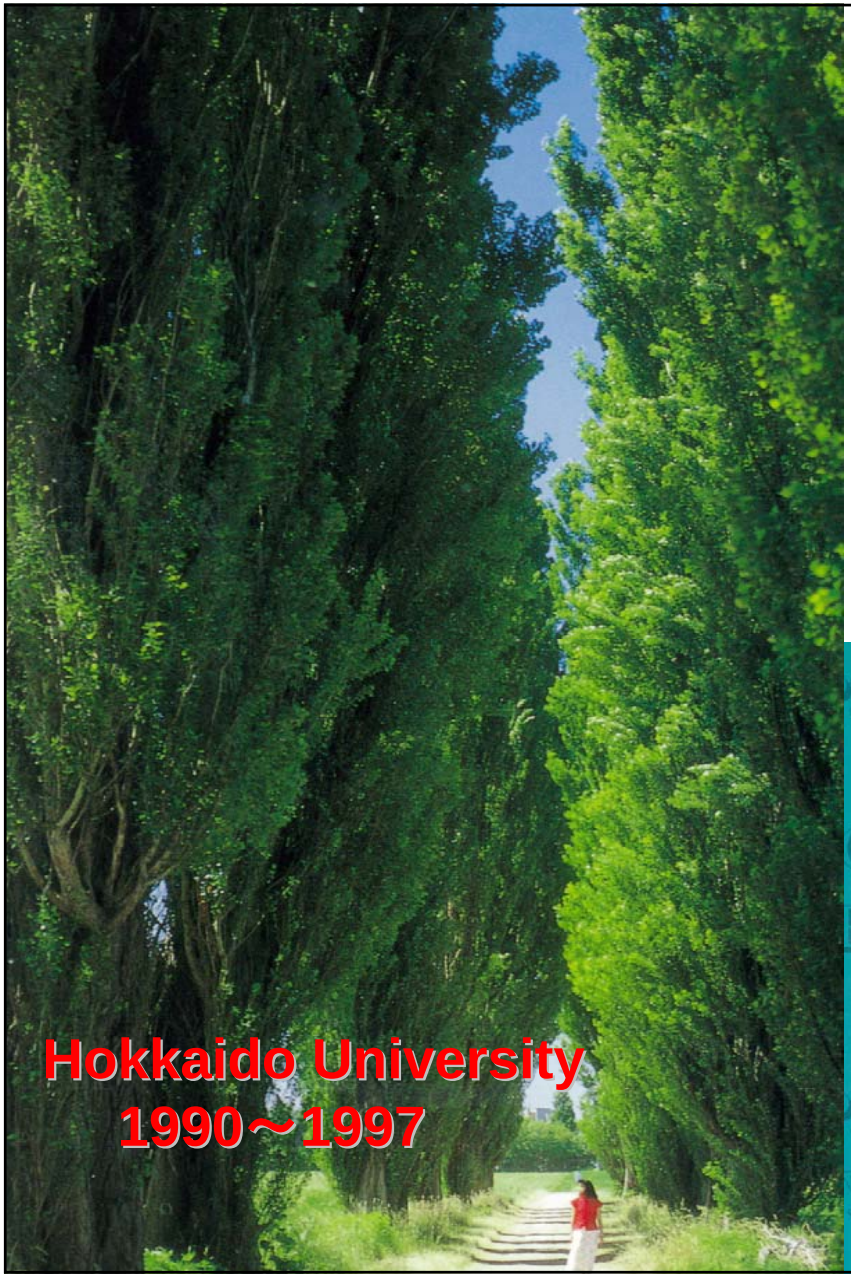
Drug Metabolism and Toxicology
Kanazawa University

24th JSSX Annual Meeting, Kyoto, Nov 28, 2009



北海道大学薬学部

代謝分析学（薬品分析化学）講座

１９８５～１９９６年の歩み

Acknowledgments

Drug Metabolism and Toxicology
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Kanazawa University

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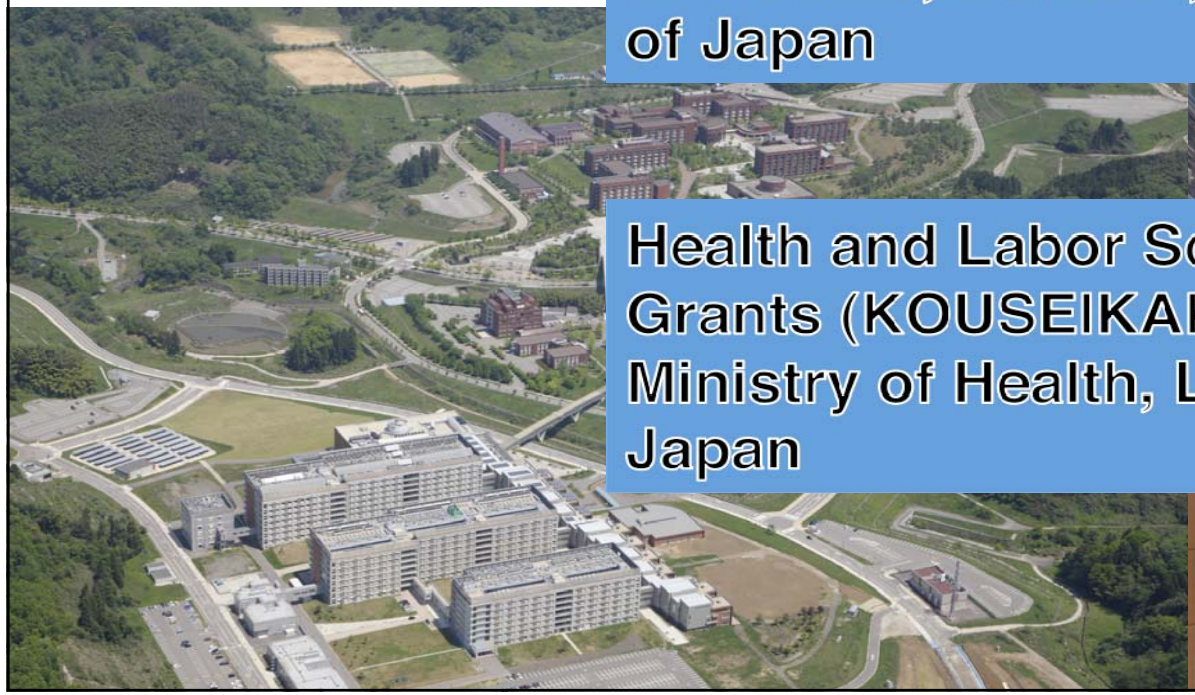
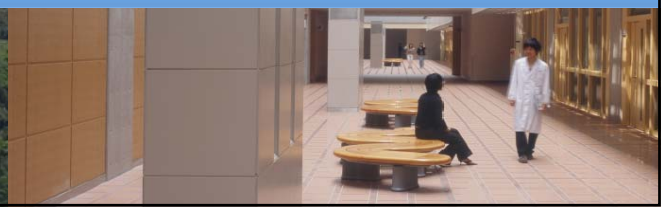




Main Research Interests

1. Pharmacokinetics of drugs and drug-drug interactions.
2. Interindividual and interethnic differences in drug metabolism.
3. Identification and characterization of drug metabolizing enzymes.
4. Regulation of drug metabolizing enzymes.
5. Metabolic activation of drugs and environmental compounds leading toxicity.
6. Development of experimental models to predict drug-induced liver injury in human.

1. Pharmacokinetics of drugs and drug-drug interactions. (CYP and UGT)
   Azelastine, Amiodarone, Nicotine, Cotinine, Troglitazone, Calcium antagonists, Phenytoin, Tegafur, Imipramine, Capecitabine, Pacritaxel, P-glycoprotein, Tranilast, Morphine

2. Interindividual and interethnic differences in drug metabolism.
   CYP2A6-nicotine, Azelastine, Amiodarone, Phenytoin, CES

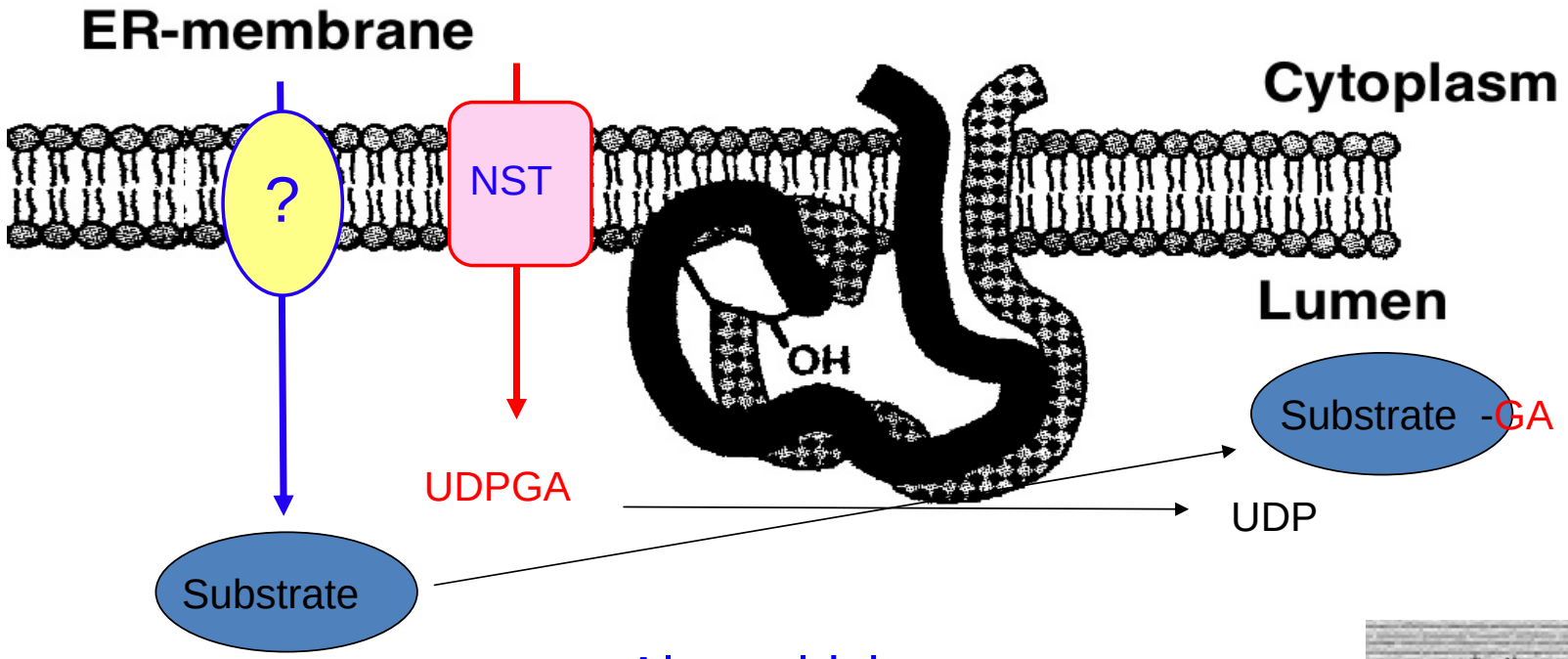
3. Identification and characterization of drug metabolizing enzymes.
   Troglitazone, Tegafur, Imipramine, Phenytoin, Etoposide, Capecitabine, Thyroxine, CES, AADAC

4. Regulation of drug metabolizing enzymes.
   CYP1B1, CYP2A6, CYP2A13, CYP2B6, UGT1A9, UGT2B7, CES, Chimeric mouse with humanized liver, Intestinal metabolism, CYP1B1, CYP3A4-PXR, CYP2E1, VDR, CYP24, HNF4α

5. Metabolic activation of drugs and environmental compounds leading toxicity.
   Troglitazone, Nitropyrenes, Benzophenone, Losartan, APAP, Flutamide, Leflnomide, Benzodiazepines, Halothane

6. Development of experimental models to predict drug-induced liver injury in human.
   Autoantibodies, DNA-chip, 2D-proteomics, Chimeric mouse, Adenovirus sh-RNA expression system, In vivo gene knockdown of GSH or SOD2, Adenovirus CYP-expression system, Immunotoxic system

Latency of UGT

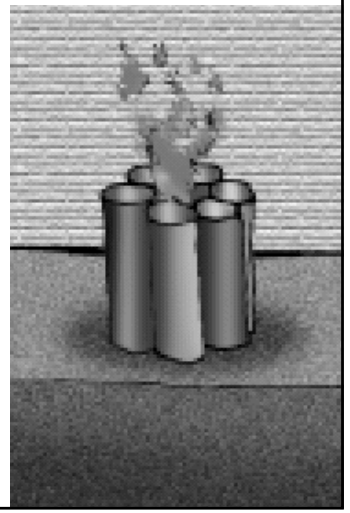


Alamethicin

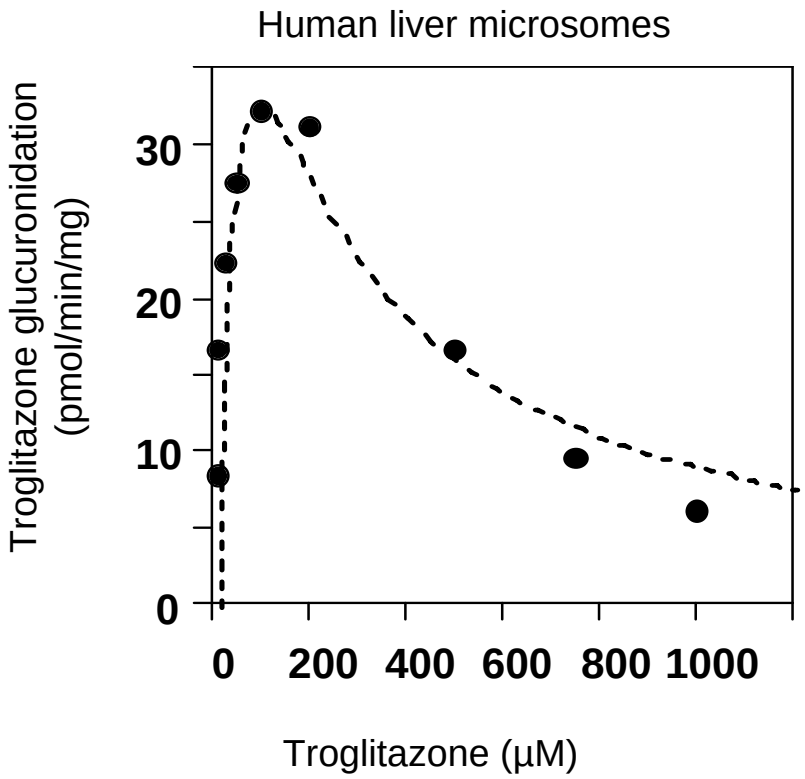
A peptide antibiotic, produced by trichoderma viride

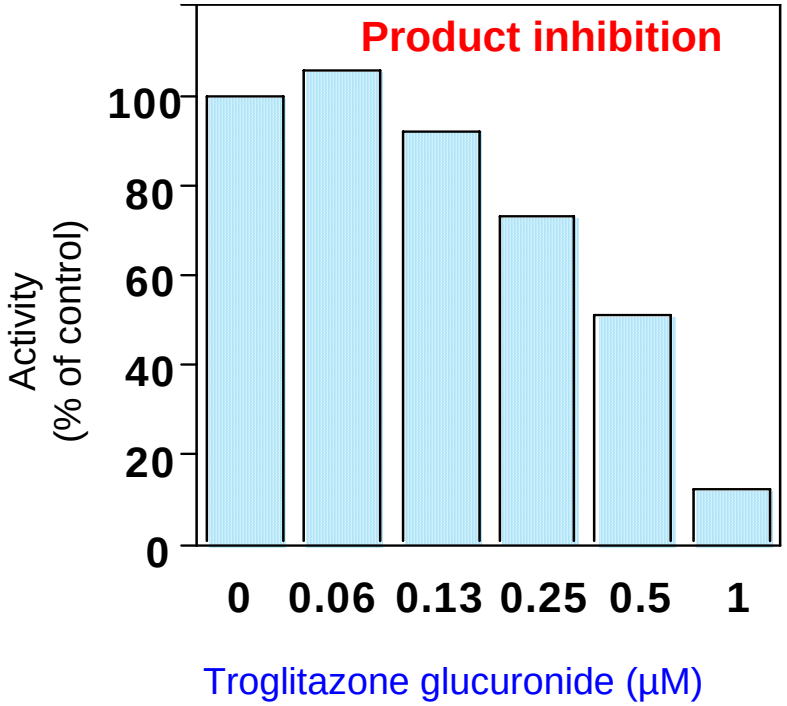
CH3CO-UPUAUAQUVUGLUPVUUEQF-OH

U = α-methylalanine, which strongly induces helical peptide structure



Troglitazone Glucuronidation is Inhibited by Troglitazone Glucuronide





Watanabe et al., *Drug Metab Dispos* 30: 1462-1469, 2002.

Glucuronidation of Typical Substrates by Human, Rat, and Mouse

Activity	Species	Liver Km or S50 μM	Liver Vmax nmol/min/mg	Liver n	Liver CL μL/min/mg	Intestine Km or S50 μM	Intestine Vmax nmol/min/mg	Intestine n	Intestine CL μL/min/mg
Estradiol 1A1,8,10	Human	17.0	0.4	1.8	11.8	30.7	0.8	-	26.1
	Rat	15.9	6.1	1.8	385.0	29.4	1.2	1.1	41.6
	Mouse	17.3	6.1	2.4	353.6	41.6	2.2	1.6	51.9
Imipramine 1A3, 4	Human	97.2	0.3	-	3.0	No data			
	Rat	Not detectable				Not detectable			
	Mouse	Not detectable				Not detectable			
TFP 1A4	Human	61.0	1.0	-	15.8	No data			
	Rat	Not detectable				Not detectable			
	Mouse	Not detectable				Not detectable			

Unpublished data.

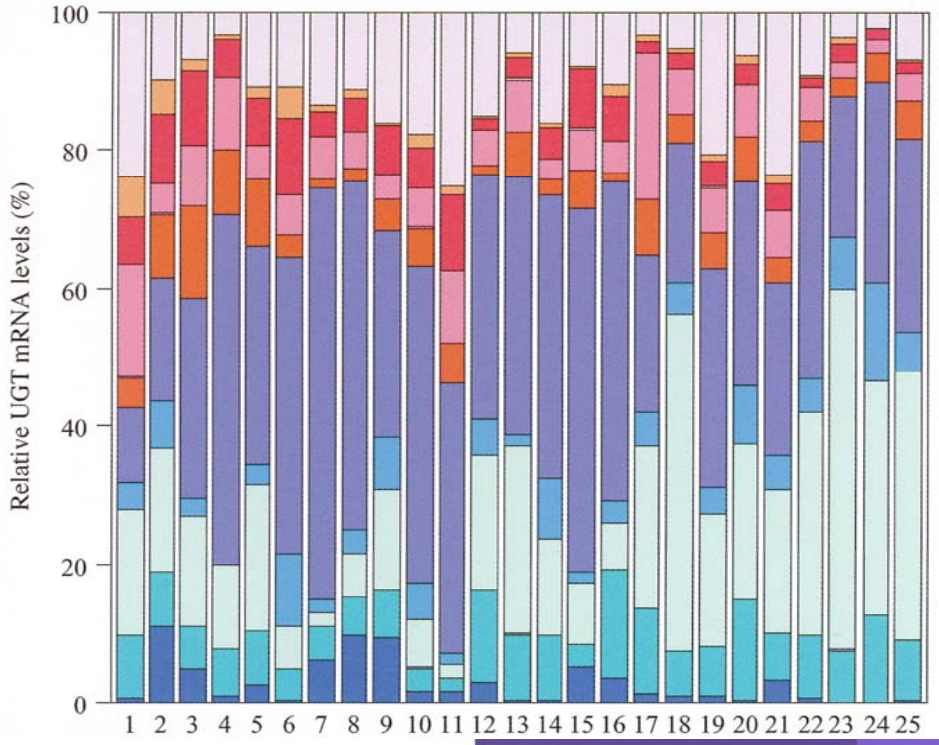
Imipramine	Rabbit UGT1A4	1.8 pmol/min/mg (100 μM)
Amitriptyline	Rabbit UGT1A4	0.7 pmol/min/mg (100 μM)

Shiratani et al., *Drug Metab Dispos,* 36: 1745-1752, 2008

Quantitative Analysis of UGT1A and UGT2B Expression in Human Livers.

Volume 37 ▪ Number 8 ▪ August 2009 ▪ ISSN 0090-9556

DRUG METABOLISM AND DISPOSITION

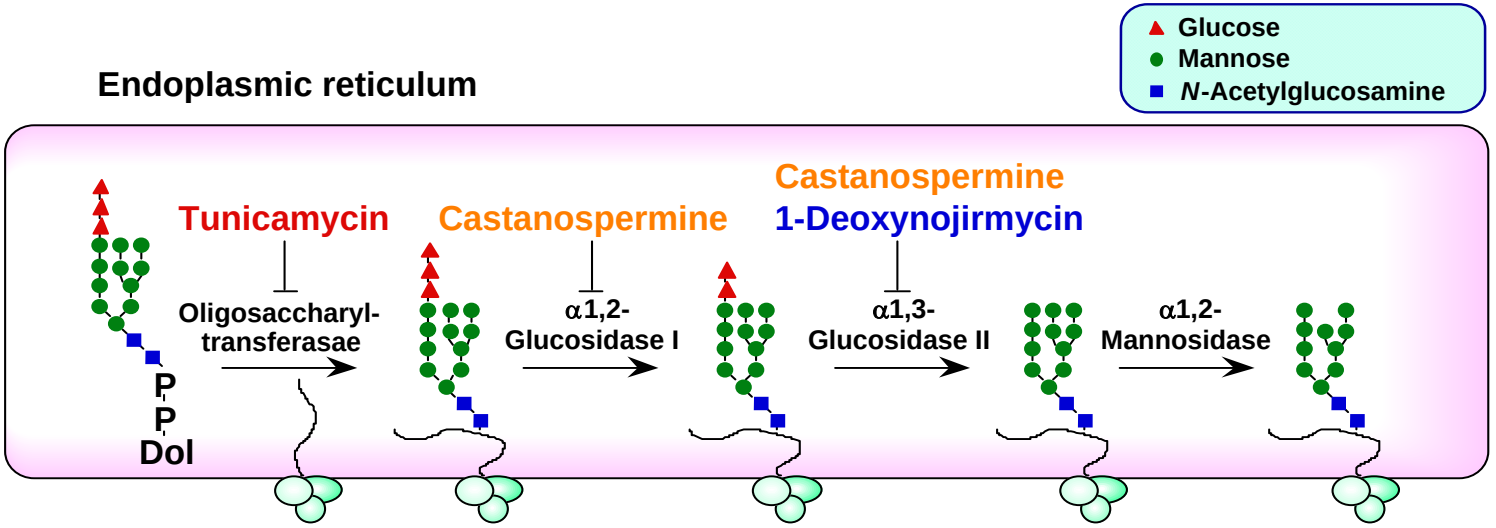


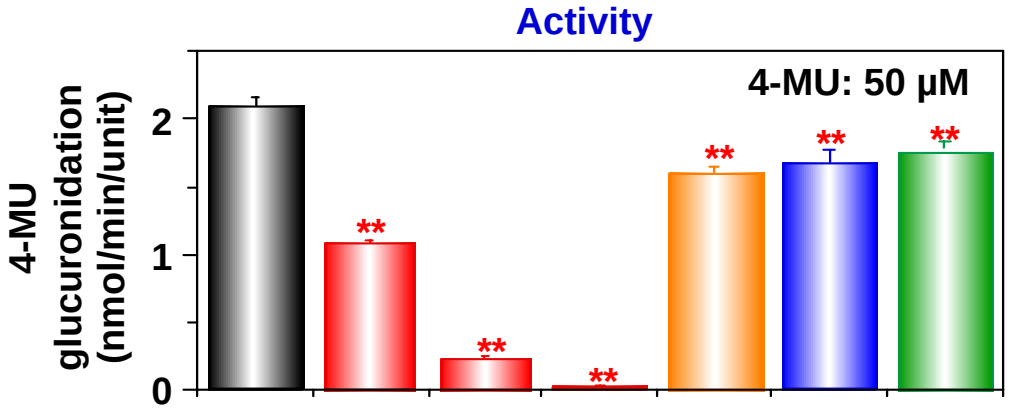
Isoforms	Percentage	(range)
UGT1A1	11.3 ± 6.8	(2.2 - 25.1)
UGT1A3	1.4 ± 1.4	(0.2 - 5.9)
UGT1A4	5.5 ± 3.2	(1.4 - 11.2)
UGT1A5	0.0 ± 0.1	(ND - 0.3)
UGT1A6	6.8 ± 4.3	(1.8 - 21.6)
UGT1A7	0.0 ± 0.0	(ND - 0.0)
UGT1A8	0.0 ± 0.0	(ND - 0.0)
UGT1A9	5.1 ± 3.1	(1.2 - 13.4)
UGT1A10	0.0 ± 0.0	(ND - 0.0)
UGT2B4	34.5 ± 12.1	(6.5 - 59.7)
UGT2B7	5.1 ± 3.2	(0.1 - 14.4)
UGT2B10	19.6 ± 13.4	(2.0 - 52.3)
UGT2B11	0.1 ± 0.1	(ND - 0.2)
UGT2B15	8.0 ± 3.5	(2.2 - 15.5)
UGT2B17	2.8 ± 3.3	(0.1 - 16.8)
UGT2B28	0.0 ± 0.0	(ND - 0.1)

A Publication of the American Society for Pharmacology and Experimental Therapeutics

Izukawa et al., *Drug Metab Dispos*, 37: 1759-1768, 2009.

Effects of Deglycosylation of Human UGT1A9 on Enzymatic Acitvity





Data are mean ± SD (n = 3).
** *P* < 0.005 compared with control.

Nakajima et al., *Biochemical Pha*
in press

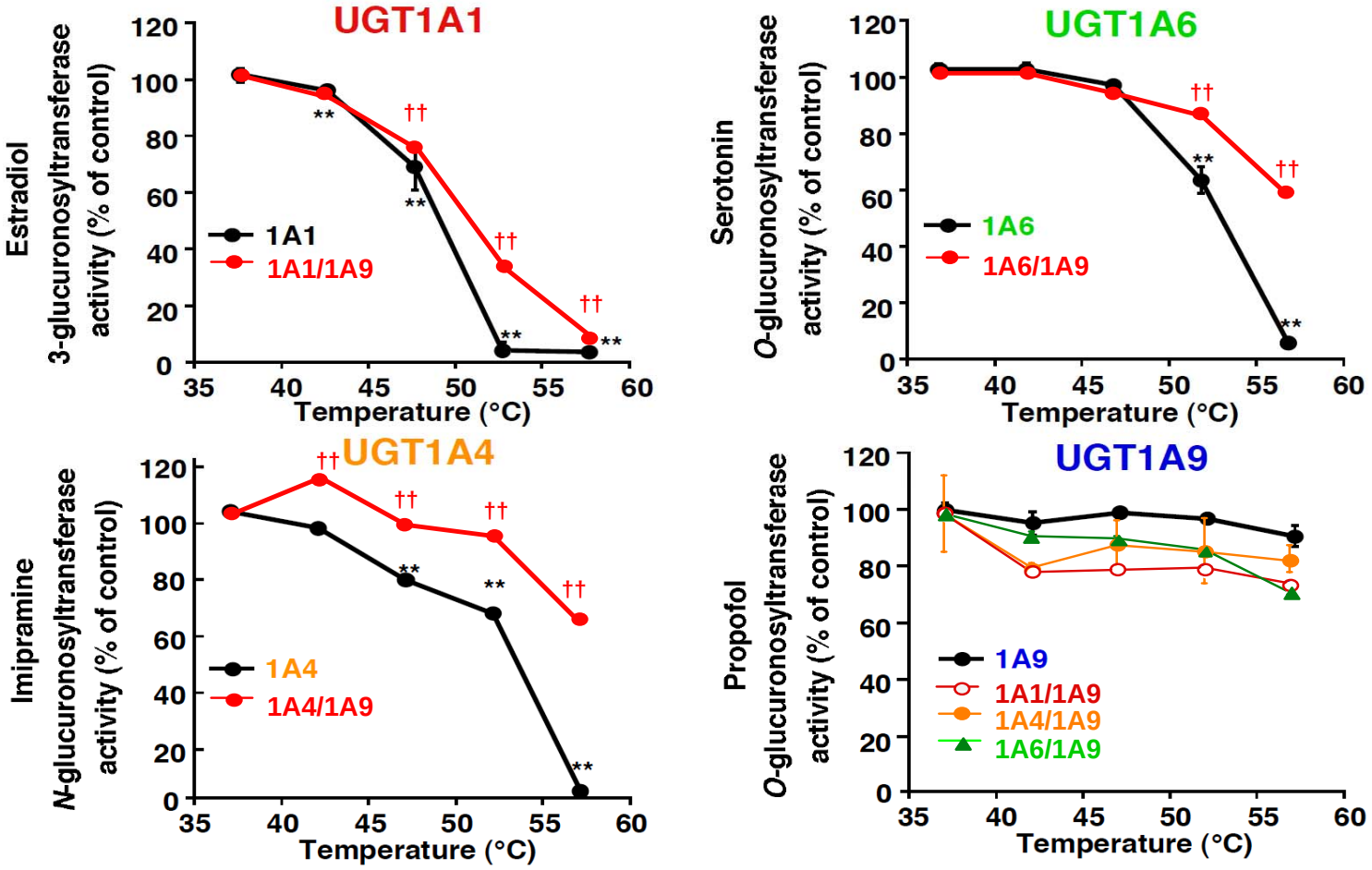
Effects of Coexpression of Other UGT1A on UGT1A1, UGT1A4, UGT1A6, and UGT1A9 Activities

Substrate (isoform)	Coexpression of UGT1A1	UGT1A4	UGT1A6	UGT1A9
Estradiol (1A1)	——	$S_{50}\downarrow$ $V_{max}\downarrow$	no change	$V_{max}\downarrow$
Bilirubin (1A1)	——	$S_{50}\downarrow$	$S_{50}\downarrow$	$S_{50}\downarrow$ $V_{max}\uparrow$
Imipramine (1A4)	no change	——	$V_{max}\uparrow$	$K_m\uparrow$ $V_{max}\uparrow$
Trifluoperazine (1A4)	no change	——	$K_m\uparrow$ $V_{max}\uparrow$	$V_{max}\uparrow$
Serotonin (1A6)	no change	$V_{max}\uparrow$	——	$V_{max}\downarrow$
Diclofenac (1A6)	$V_{max}\uparrow$	$S_{50}\uparrow$ n $\uparrow$	——	no change
Propofol (1A9)	$K_m\uparrow$ $V_{max}\downarrow$	no change	no change	——

Fujiwara et al., *Drug Metab Dispos*, 35: 747-757, 2007.

Fujiwara et al., *Drug Metab Dispos*, 35: 1781-1787, 2007.

Thermal Stability of UGT1A1, UGT1A4, UGT1A6, and UGT1A9

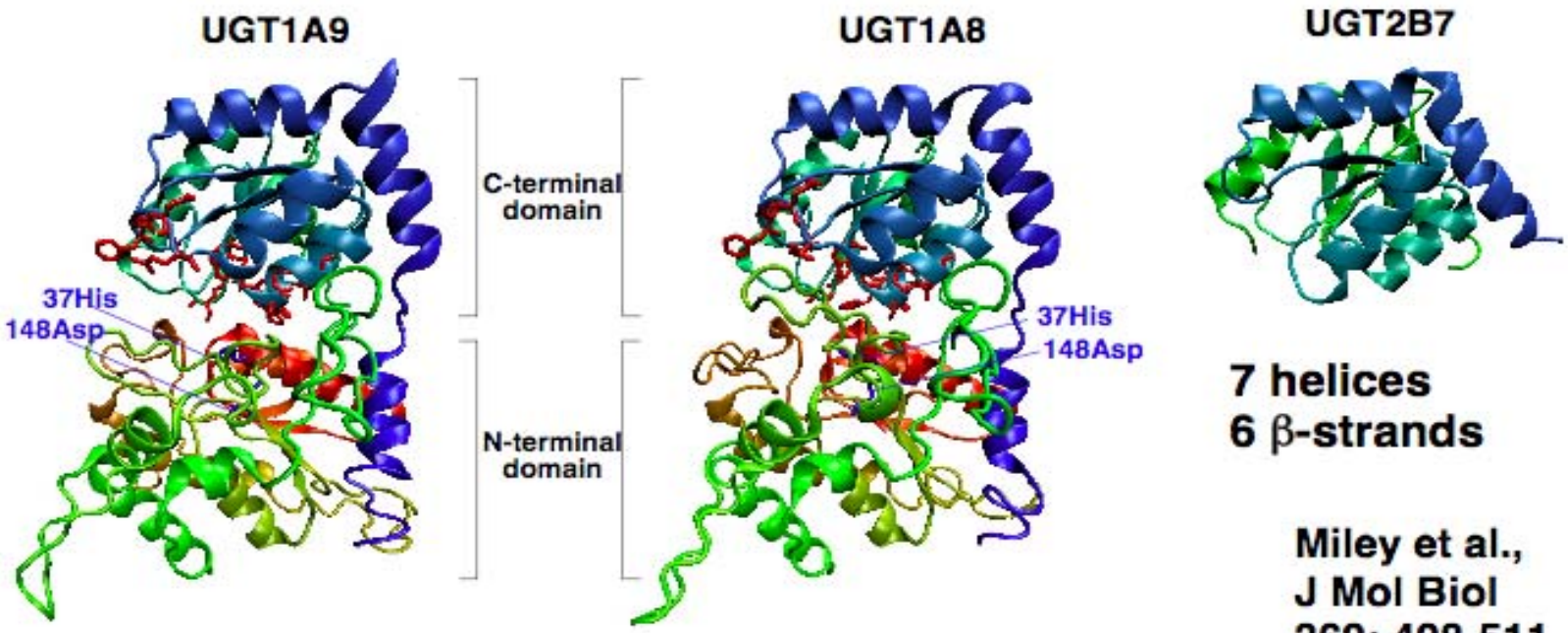


Data are mean ± SD (n = 3).

** *P* < 0.01 compared with the activities incubated at 37 °C.

†† *P* < 0.01 compared with the activities of single expression at each temperature.

Homology-Modeled Structures of Human UGT1A9 and UGT1A8

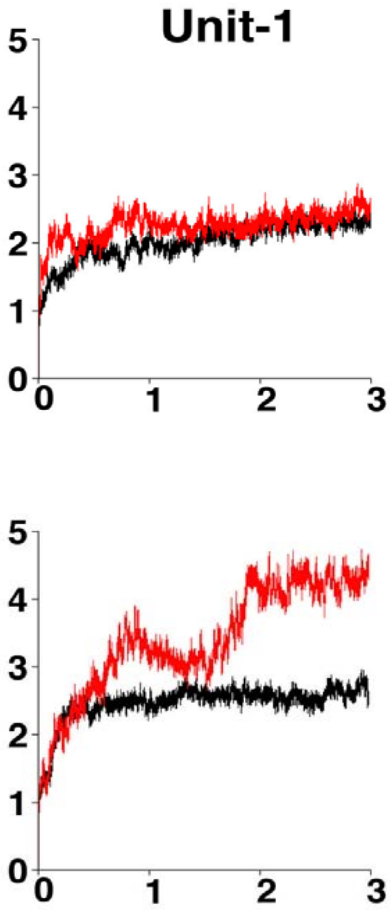


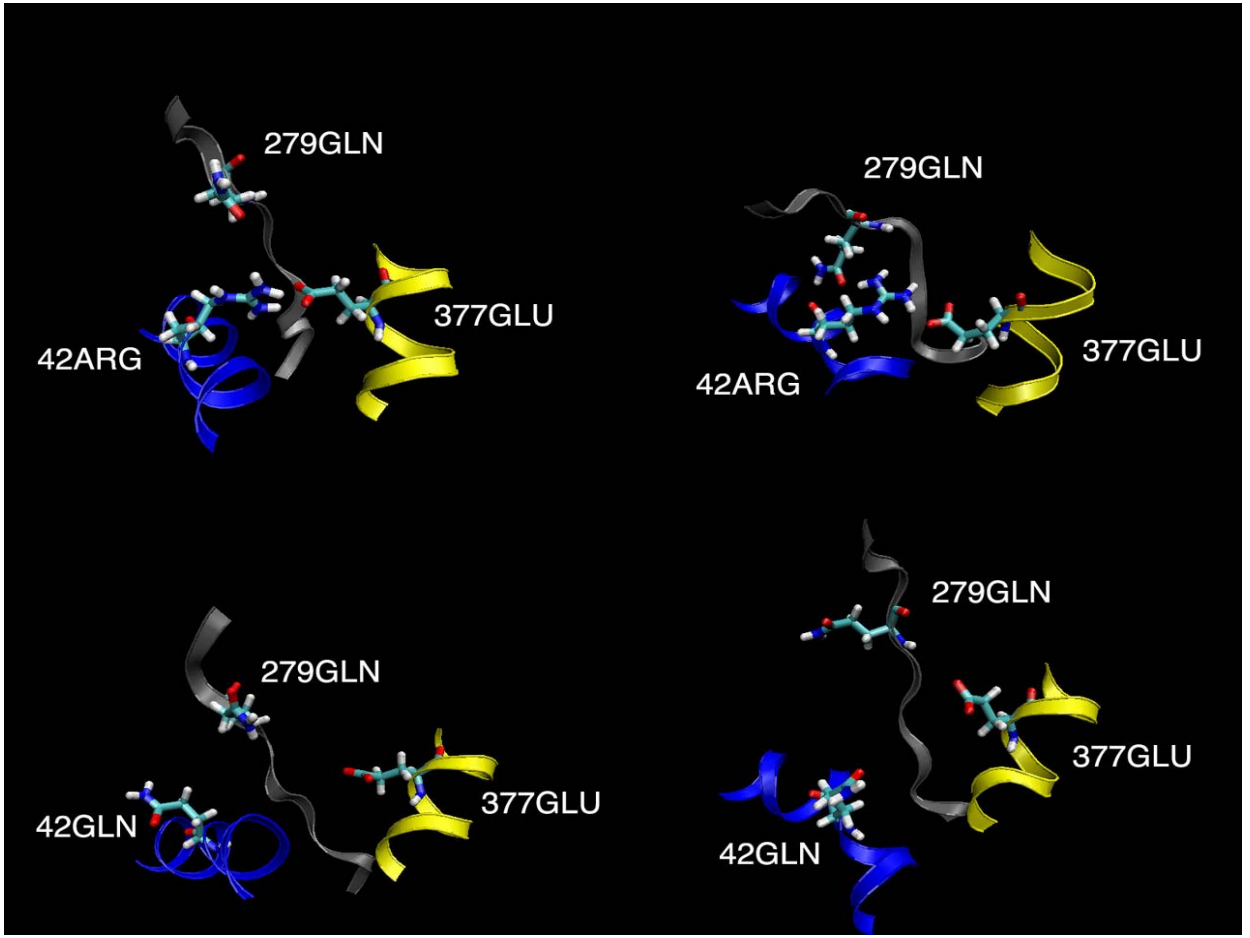
7 helices
6 β-strands

Miley et al.,
J Mol Biol
369: 498-511
(2007)

Structure of Domain 1 of UGT1A9 and UGT1A8 at 3.0 ns MD-Simulation

Domain 1





310K 360K

***Drug Metab Pharmacokinet*, 24: 226-234, 2009.**

Future UGT Studies in Our Fields

- **Species difference**

  To clarify the substrate specificity in experimental animals, organ specific expression of isoforms, inhibitors, etc,,,,

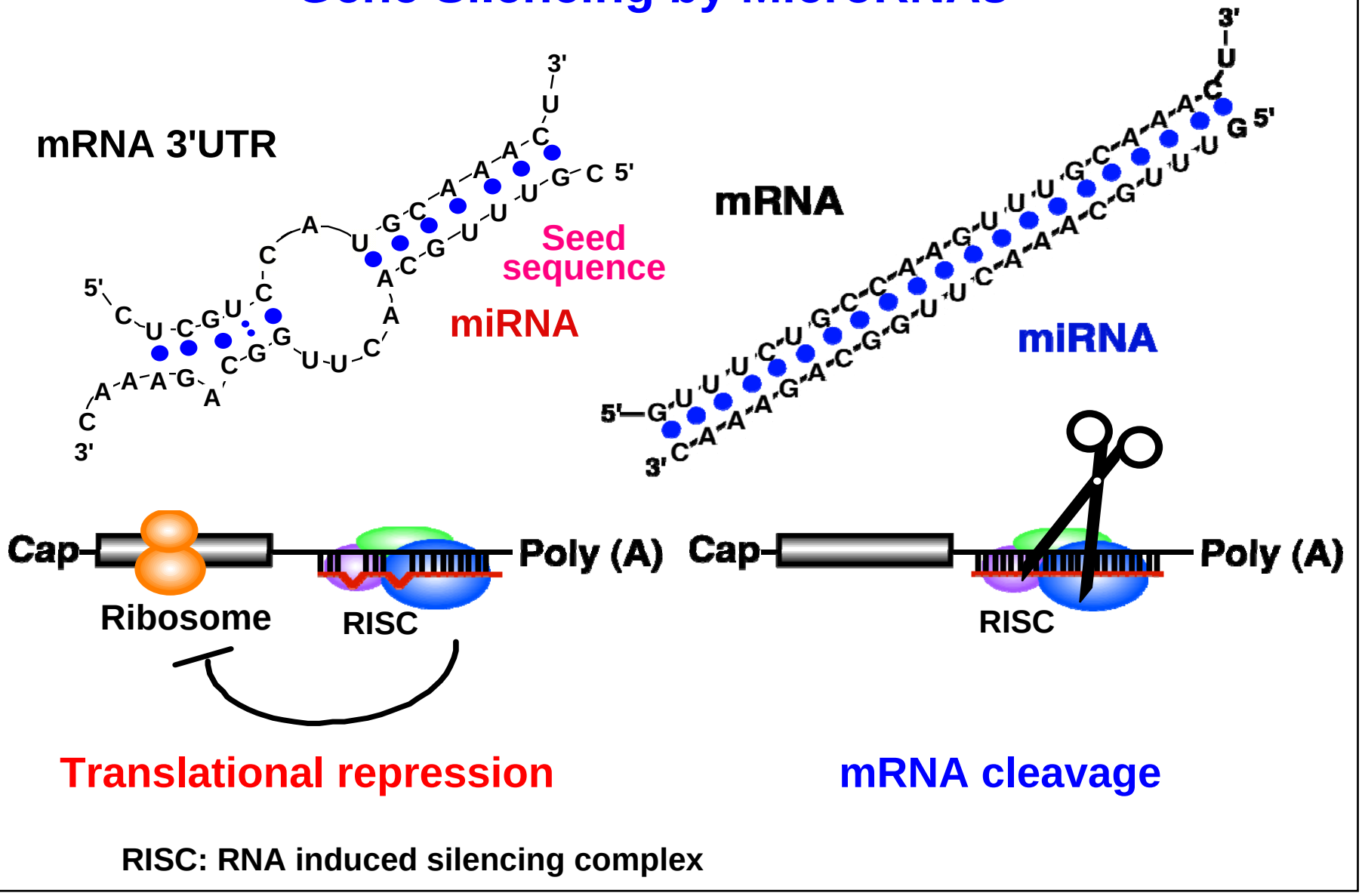
- **Evaluation of in vivo and in vitro activities**

  To clarify the regulation mechanism, protein interactions, **in vivo extrapolation** etc.,,, leads to establish RAF method.

- **Genetic polymorphisms and phenotyping**

  To establish the isoform specific phenotyping method.

Gene Silencing by MicroRNAs



Translational repression

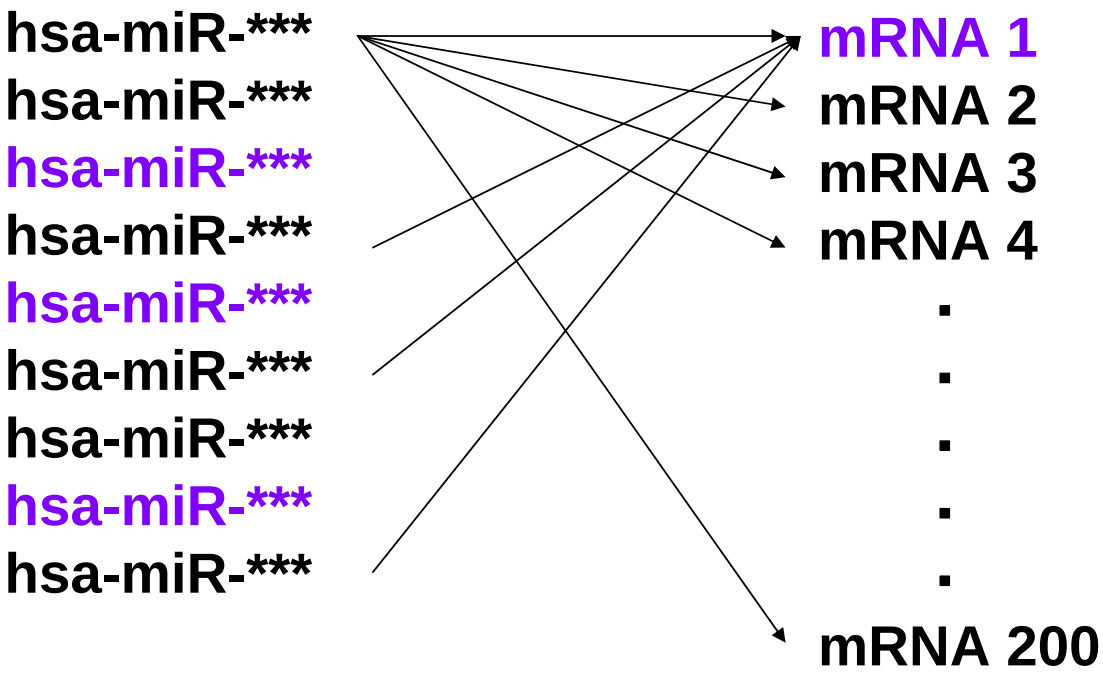
mRNA cleavage

RISC: RNA induced silencing complex

Because of imperfect base-pairing of miRNA with mRNA…

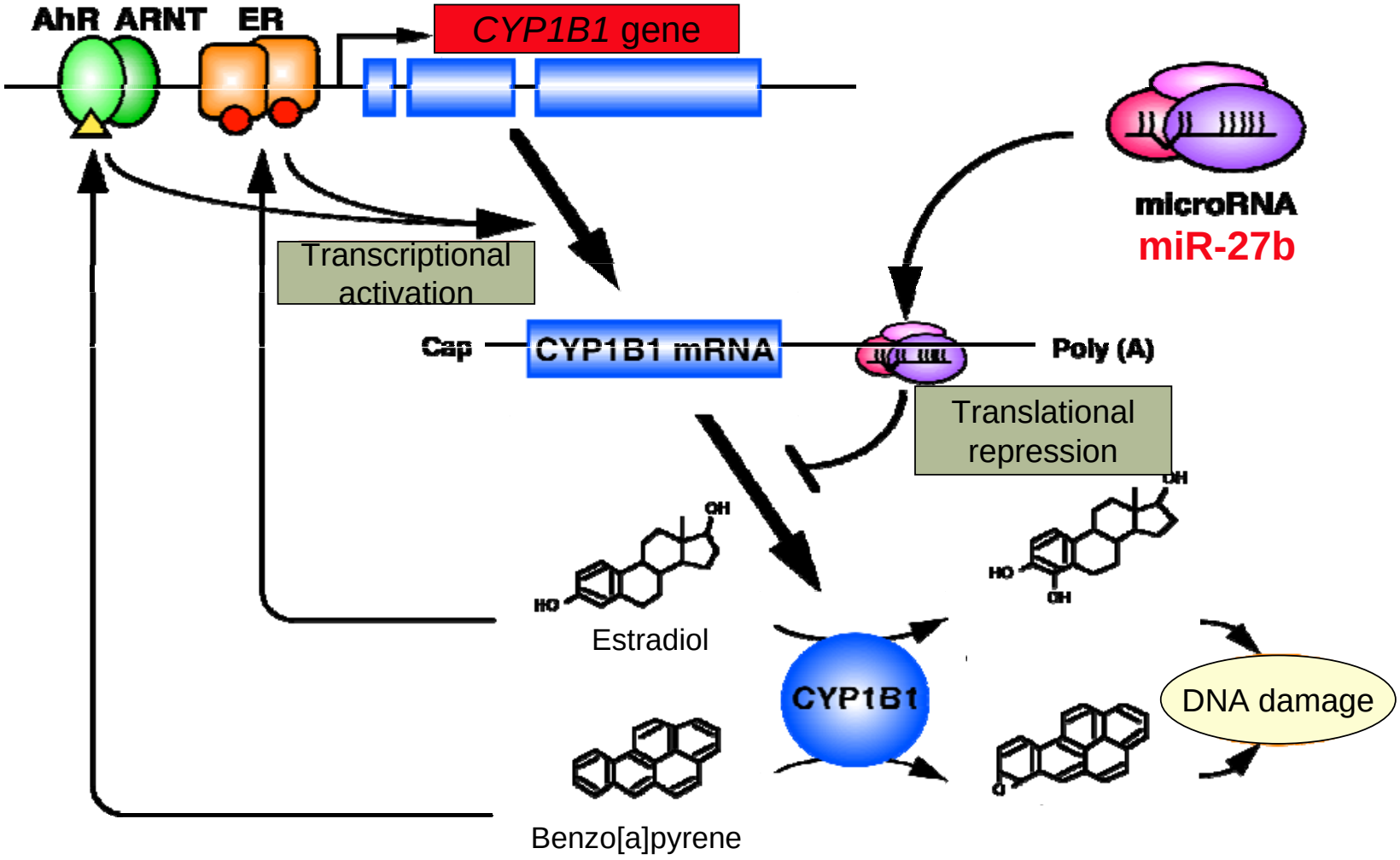
A single miRNA may target >200 transcripts

A single mRNA is regulated by multiple miRNAs



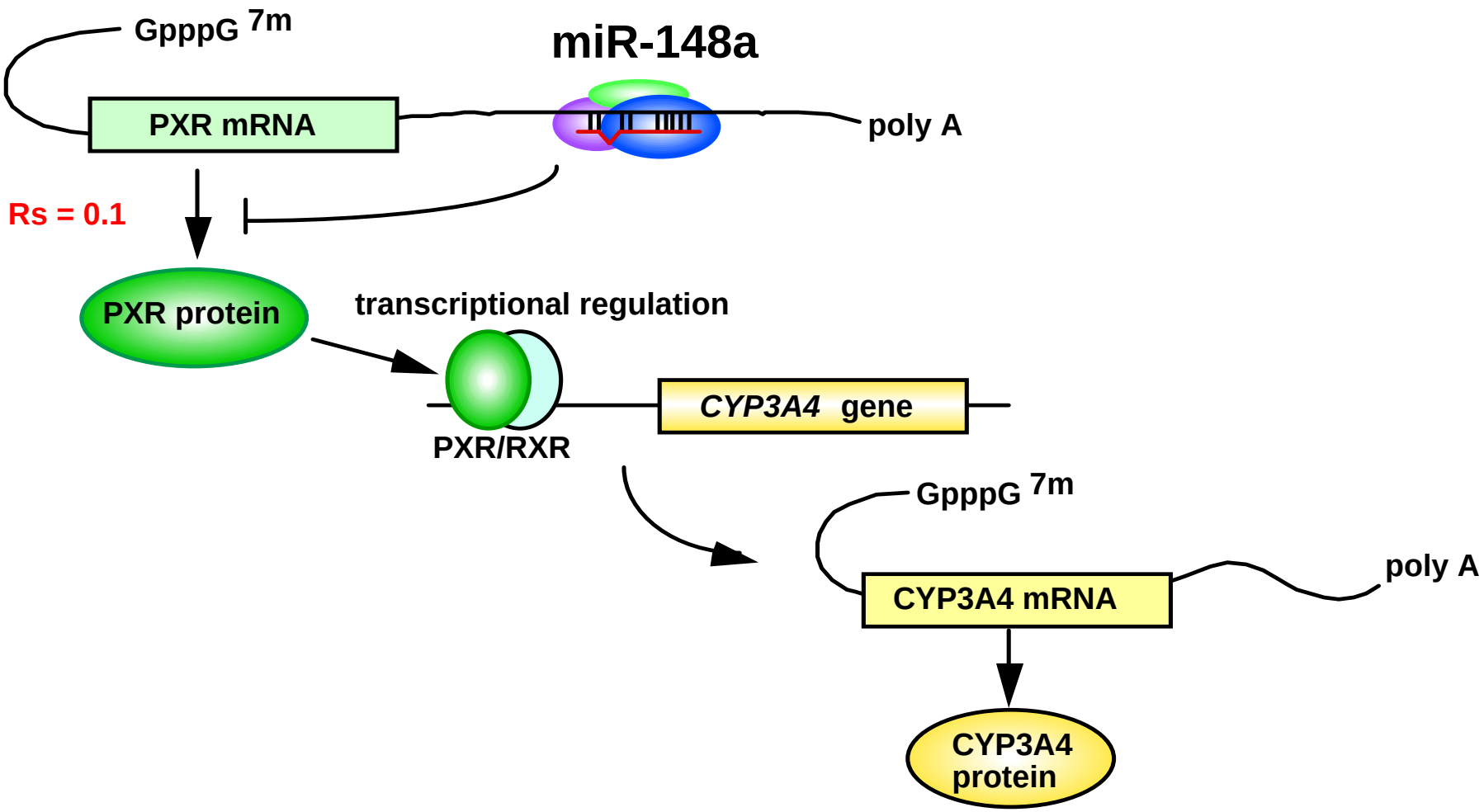
…targets of miRNA are inherently difficult to identify.

One third of human mRNAs are predicted to be regulated by miRNA, but few miRNA targets are experimentally confirmed.



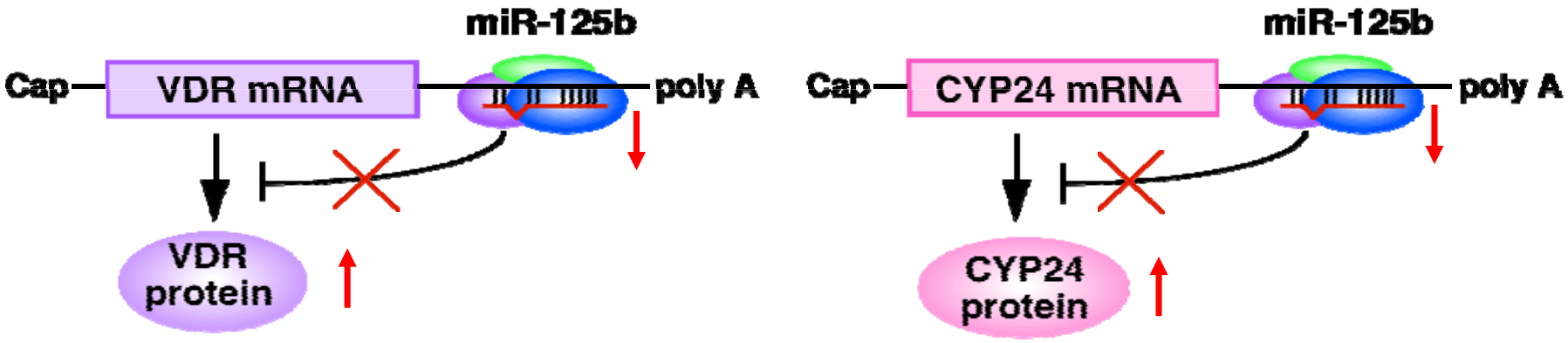
Tsuchiya et al., *Cancer Res.*, 66: 9090-9098, 2006.

Relationship between the expression of miR148a, PXR, and CYP3A4 in human liver.



Takagi et al., *J Biol Chem*, 283: 9674-80, 2008.

miR-125b Regulates Human Vitamin D Receptor and CYP24



Transactivation of target genes (CYP24)

Degradation of vitamin D_3

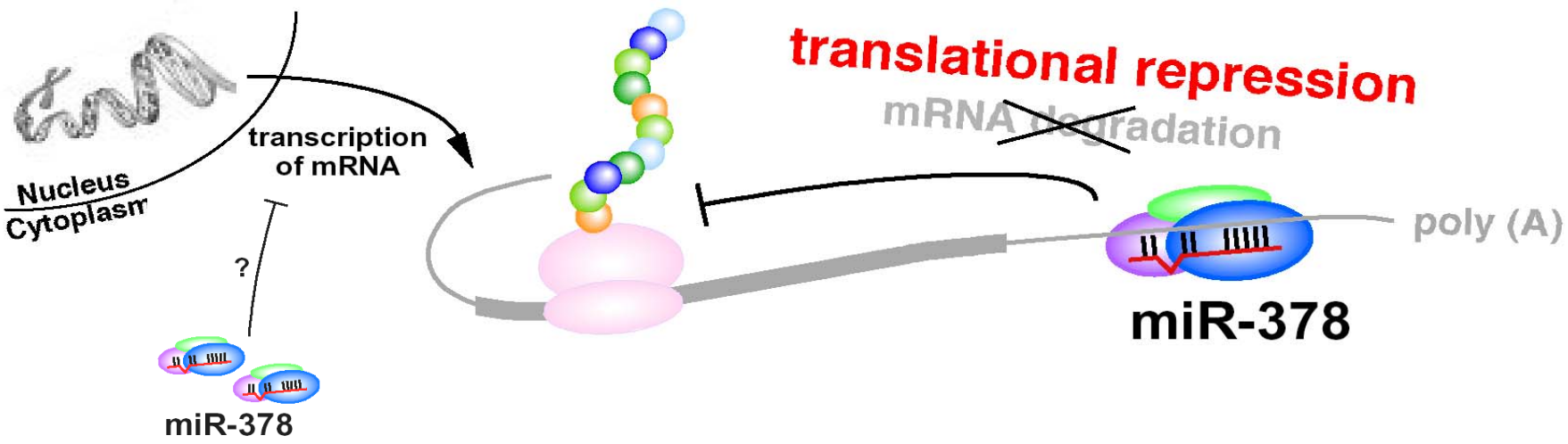
paradoxically

Anti-tumor effects of vitamin D_3

Komagata S. et al., *Mol Pharmacol*, 76: 702-709, 2009
Mohri T. et al., *Int J Cancer*, 125: 1328-1333, 2009

1-B1-15-2

Human CYP2E1 expression is regulated by miR-378, mainly via translational repression, not mRNA degradation.

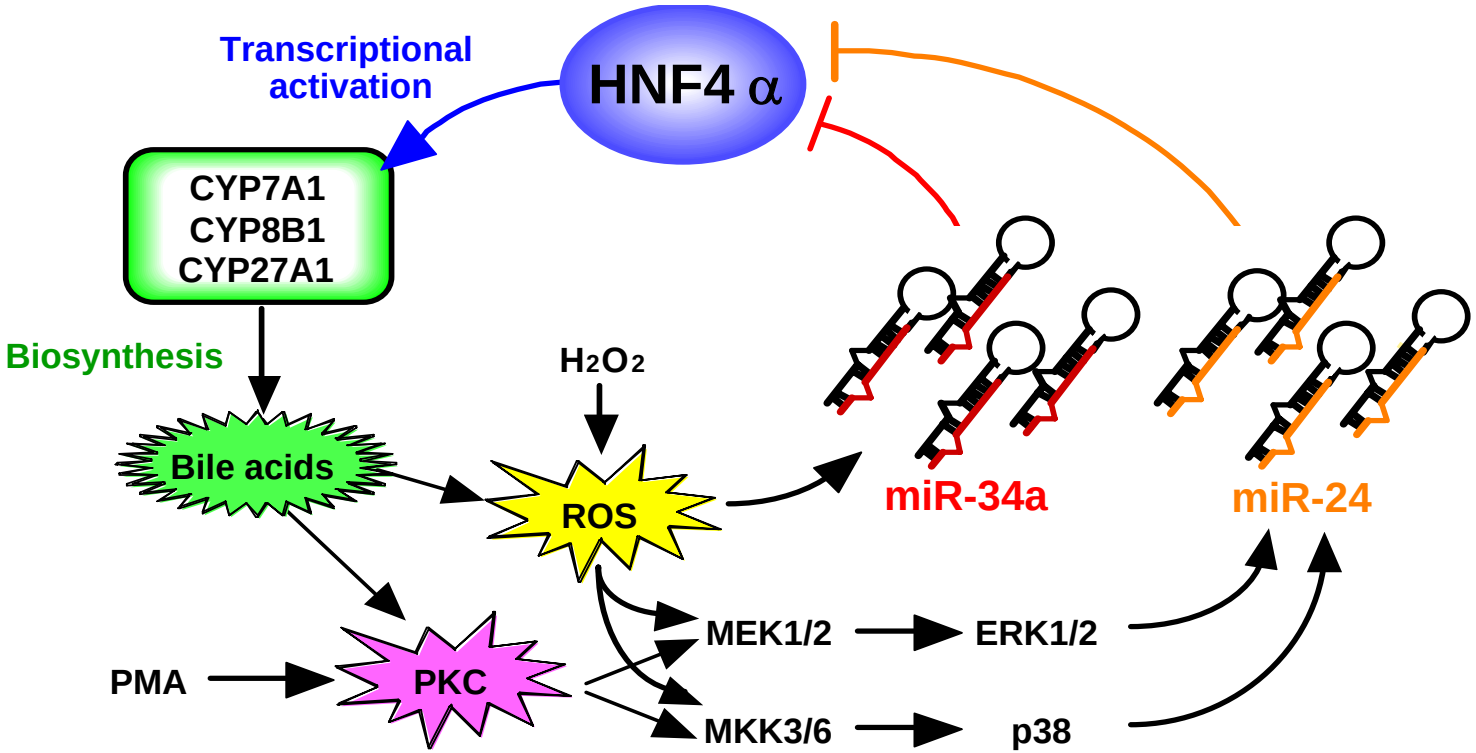


The miRNA-dependent regulation would be one of the factors of individual differences in the expression of CYP2E1.

Mohri T. et al., *Biochem Pharmacol*, in press

1-B1-15-3

Effect of miRNAs-mediated Regulation of HNF4α Expression in Hepatic Function

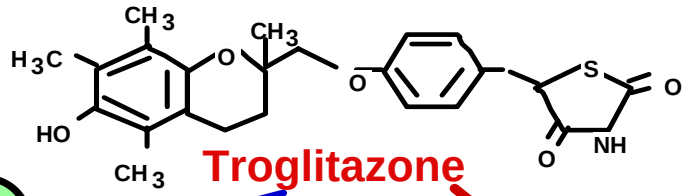


Human HNF4α is regulated by miR-24 and miR-34a, and this mechanism would affect hepatic function and development.

Future miRNA Studies in Our Fields

- **Xenobiotic and epigenetic** effects on the expression of miRNA.
- **Inter- and Intra-individual difference** of the expression of miRNA and effect on drug metabolism and disposition.
- **Genetic polymorphysim** of miRNAs and target mRNAs.
- **Circulating miRNAs**, potential biomarkers for drug-induced liver injury.

Reactive Metabolites and Immune Reaction in Drug InducedHepatotoxicity



Troglitazone

Detoxi-fication

Sulfation
Glucuronidation

CYP3A4

Reactive intermediate

GSH-conjugation

Covalent binding etc.

Cytotoxicity
Infulammation

Apoptosis

Th2

Production of antibody

Hapten, APC

Protein adduct

Hepatotoxicity

Induction of immune reaction

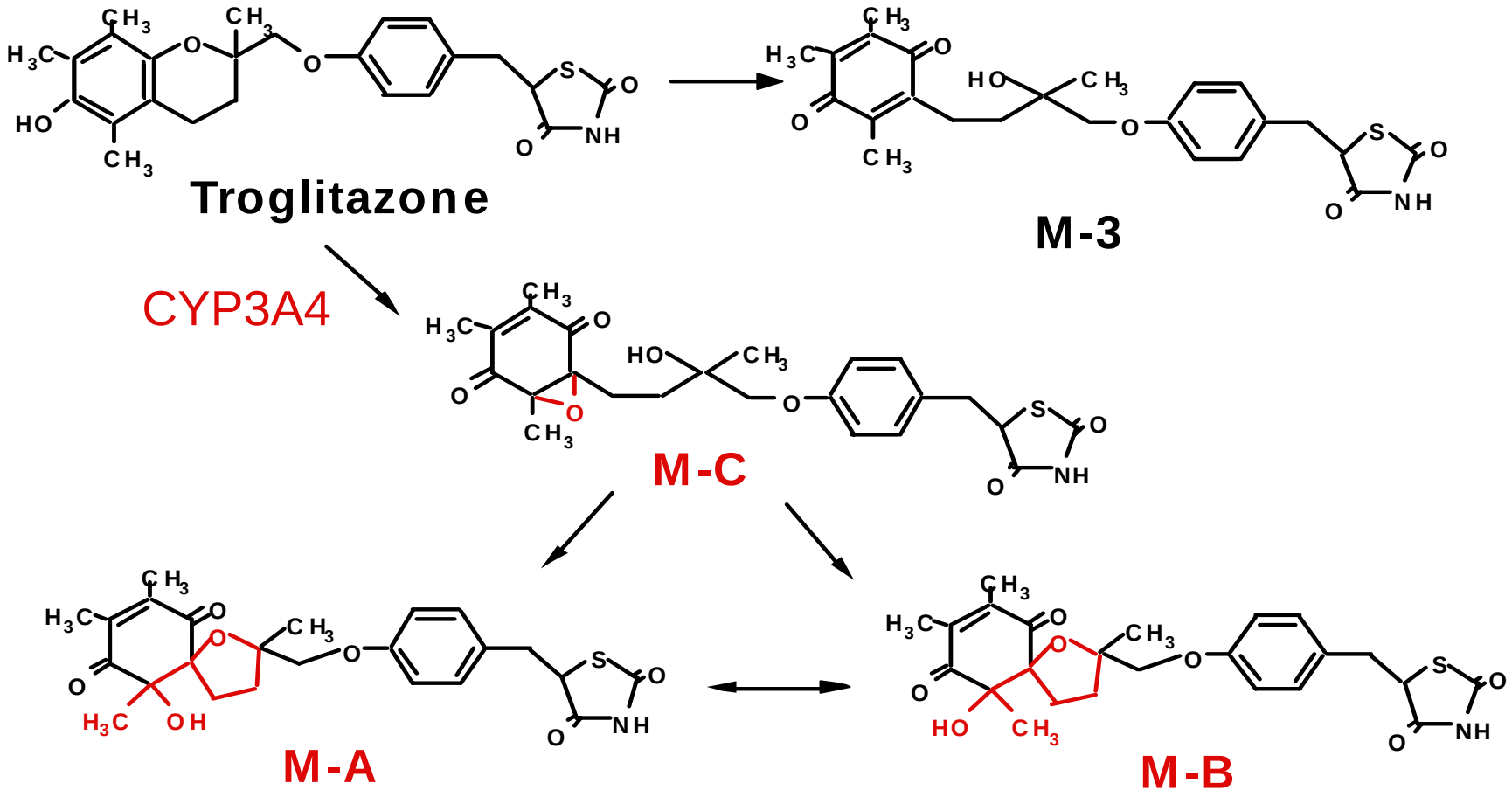
Loss of function

Th1

Activation of cytotoxic T lymphocyte

Danger signal

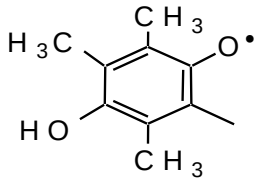
Proposed biotransformation pathways of the novel troglitazone metabolites.



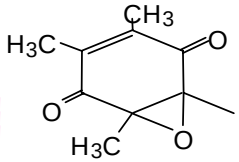
Yamamoto et al., *Drug Metab Dispos*, 30:155-160, 2002.

Active Metabolites of Troglitazone

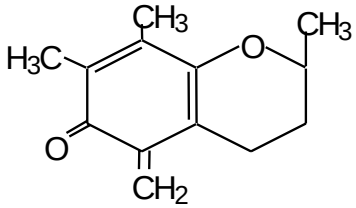
1. **Semiquinone redical of chroman ring**



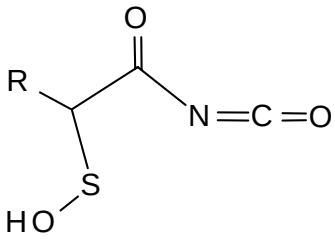
2. **Qinone-epoxide**



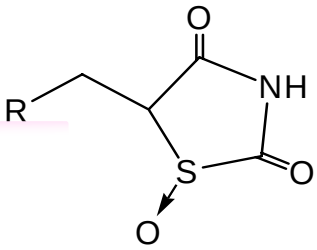
3. ***o*-Quinone-methyde**

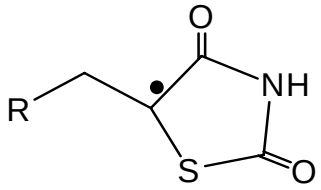


4. **α-Ketocyanic acid and sulfenic acid**



5. **Sulfoxide or radical**





All of those are catalyzed by CYP3A4

GSTT and GSTM deficient will be risk factors for troglitazone-induced liver injury in human.

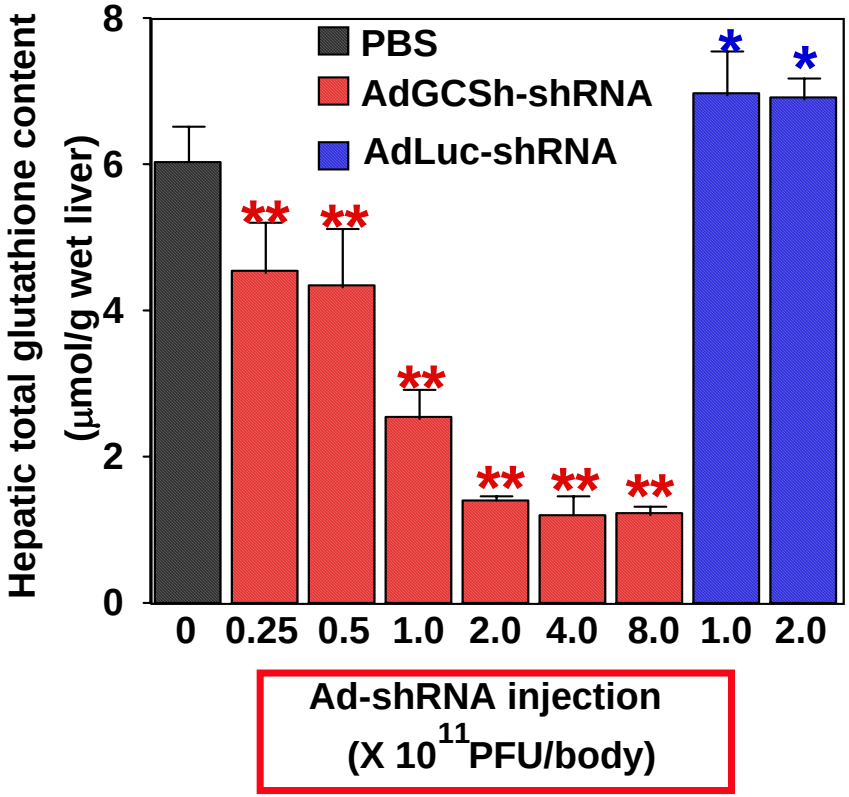
Glutathione conjugation ability in rodent is 5-10 folds higher compared with human.

↓

Generation of γ-glutamylcysteine synthetase heavy chain (GCSh) knockdown in vivo rat system

SOD2 knockdown in vivo rat system also established.

Dose Dependent Change of Total Glutathione in Rat Infected with Adenovirus



1 X PBS

ALT	10.1 ± 0.6	U/L
AST	26.0 ± 2.1	U/L
GSH	6.0 ± 0.5	μmol/g wet liver

AdLuc-shRNA 2.0 X 10^{11} PFU

ALT	13.6 ± 2.7	U/L
AST	36.2 ± 8.7	U/L
GSH	6.9 ± 0.3	μmol/g wet liver

AdGCSh-shRNA 2.0 X 10^{11} PFU

ALT	14.2 ± 1.7	U/L
AST	28.7 ± 4.1	U/L
GSH	1.4 ± 0.1	μmol/g wet liver

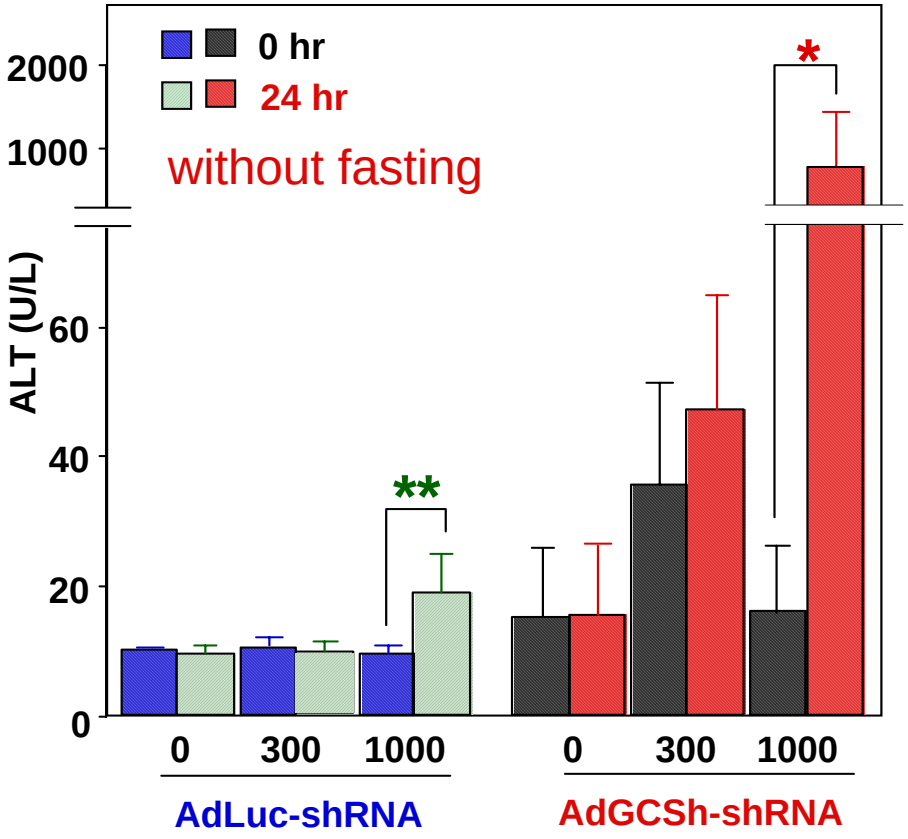
Data a re mean ± SD (n = 4 or 5).

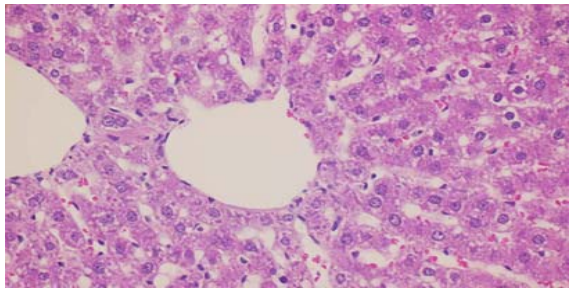
*P < 0.0 5 and ** P < 0.01 compared with control .

GSH: Total glutathione

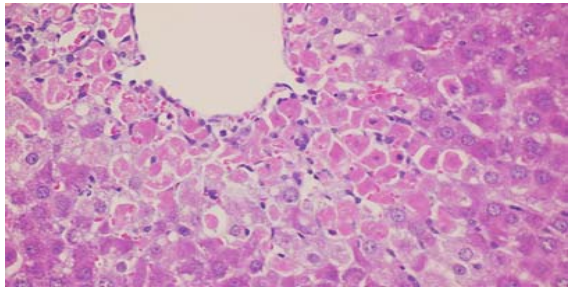
Akai et al., *J Biol Chem*, 282: 23996-24003,2007

Hepatotoxic Effect of APAP in AdGCSh-shRNA Infected Rat





AdLuc-shRNA 1000mg/kg APAP

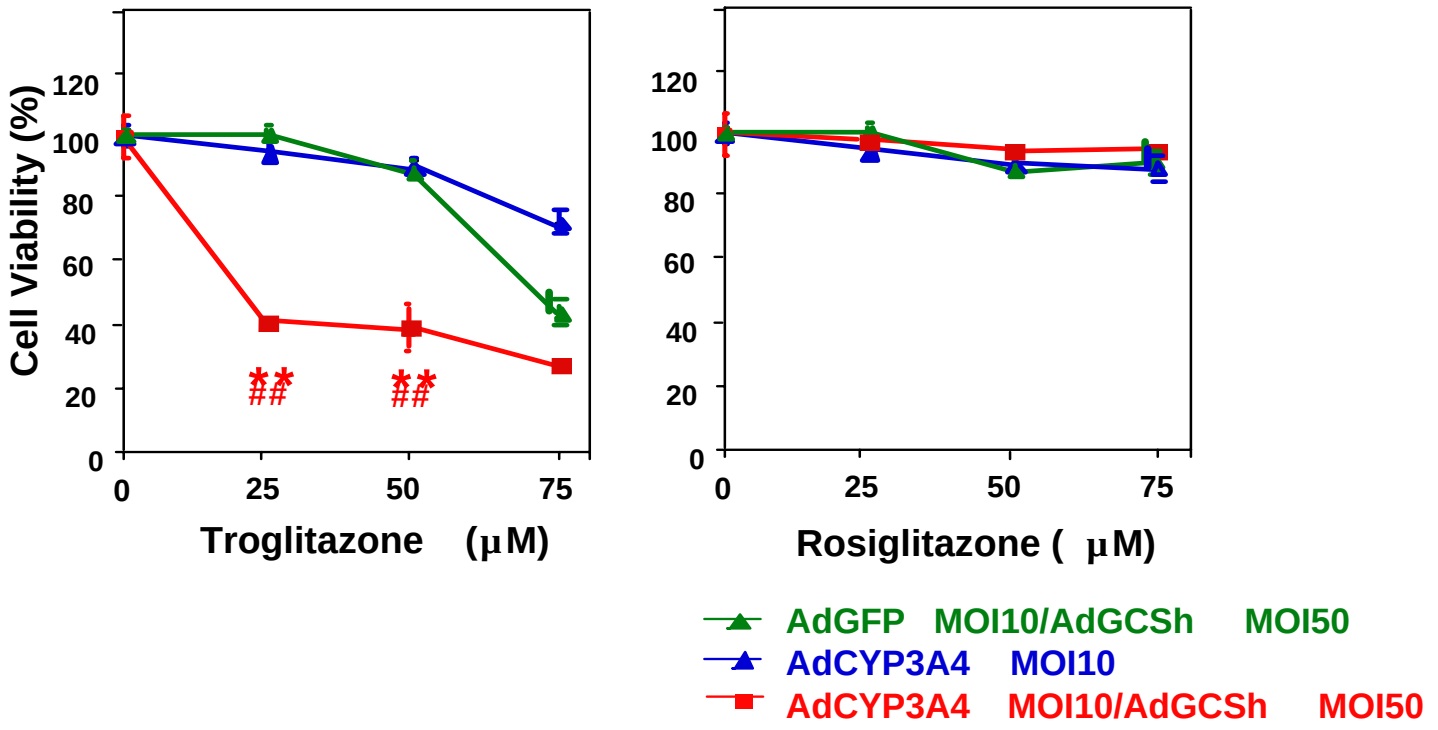


AdGCSh-shRNA 1000mg/kg APAP

Data a re mean ± SD (n = 4 or 5).
*P < 0.0 5 and ** P < 0.01 compared with control .

Akai et al., *J Biol Chem*, 282: 23996-24003,2007

MTT Assay in Adenovirus Infected H4IIE Cells After 24 hr Exposure to Troglitazone and Rosiglitazone



H4IIE cells were treated with troglitazone, acetaminophen, or flutamide for 2 4 hr after 2 days adenovirus infection. Each point represents the mean ± SD (n = 3) . * $P < 0.05$, ** $P < 0.01$, compared with AdCYP3A4 groups or # $P < 0.05$, ## $P < 0.01$, compared with AdGFP/AdGCSh groups by ANOVA followed by Dunnett test .

Hosomi et al., *Toxicol In Vitro*, in press

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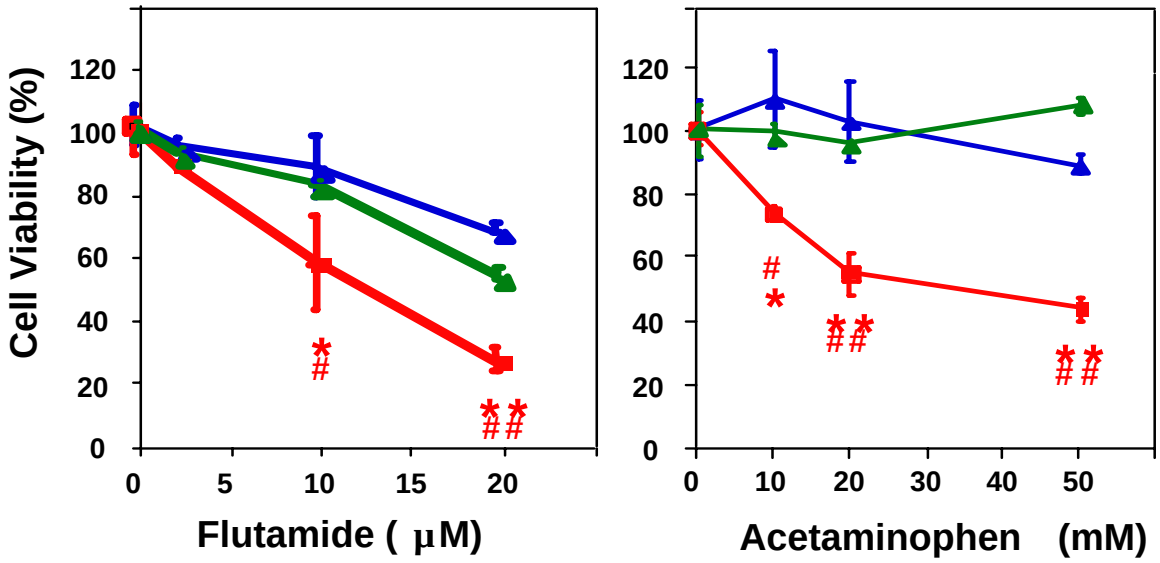

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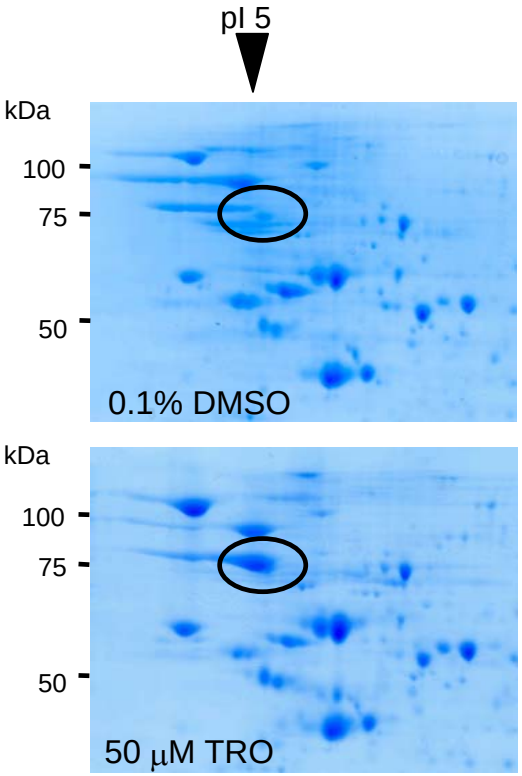
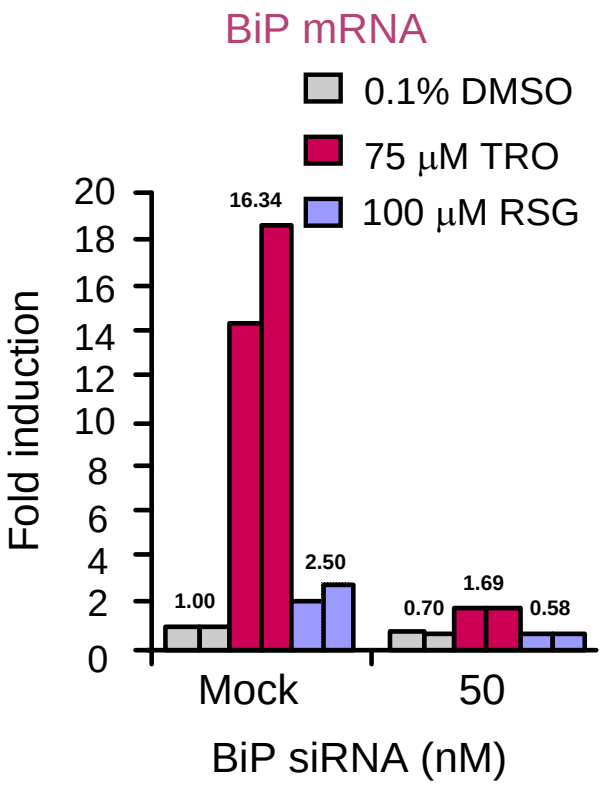
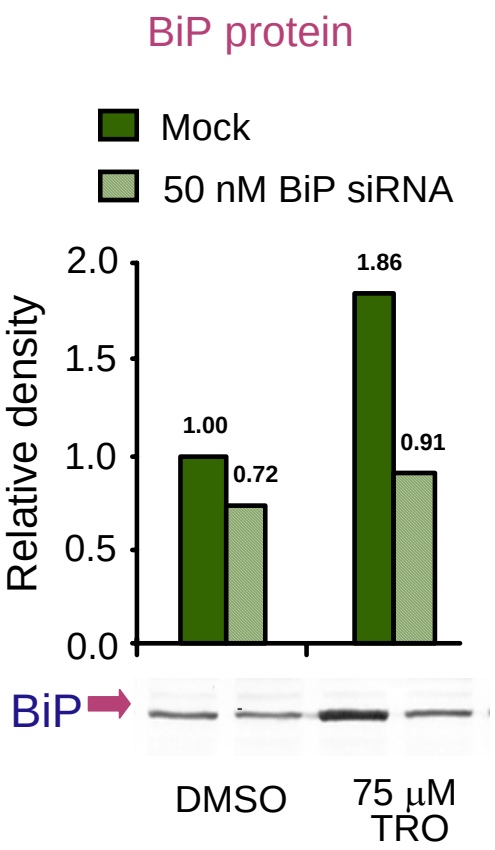
Chaperone Protein BiP Involved in Troglitazone-Induced Toxicity

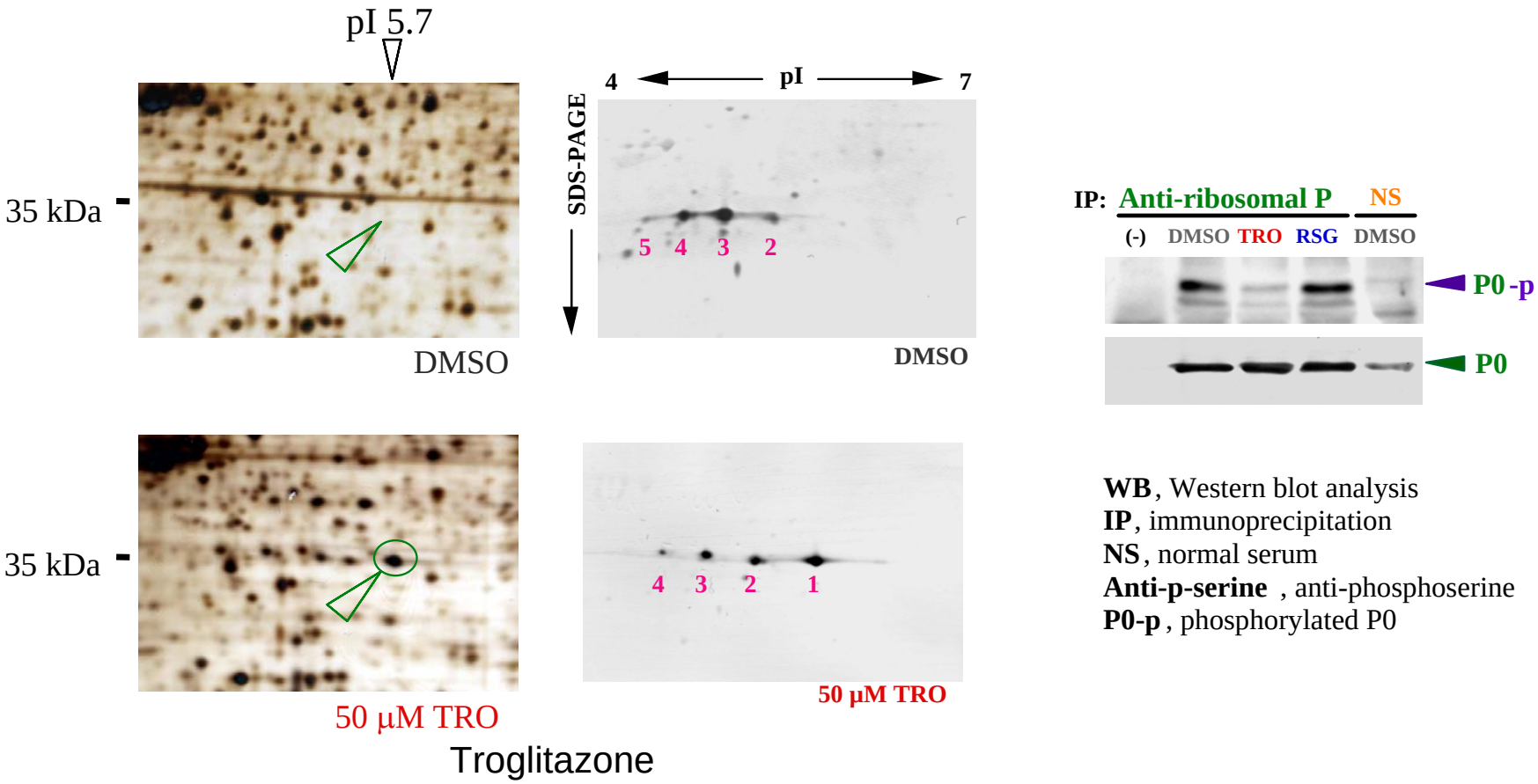






***Toxcol Sci*, 83: 293-302, 2005**

Dephosphorylation of Ribosomal Protein P0 in Response to Troglitazone-Induced Cytotoxicity

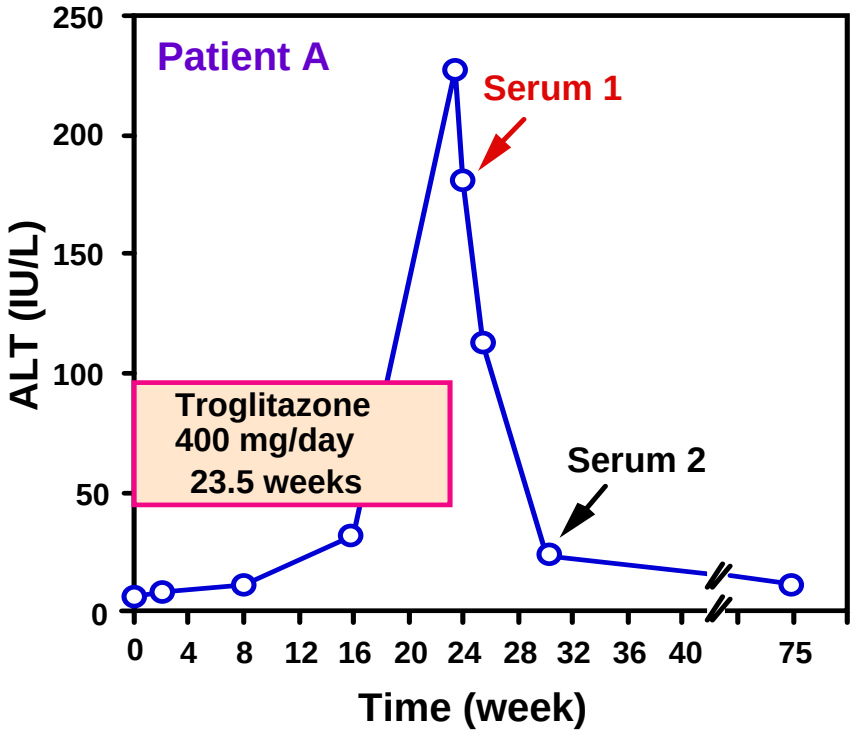


WB, Western blot analysis
IP, immunoprecipitation
NS, normal serum
Anti-p-serine , anti-phosphoserine
P0-p , phosphorylated P0

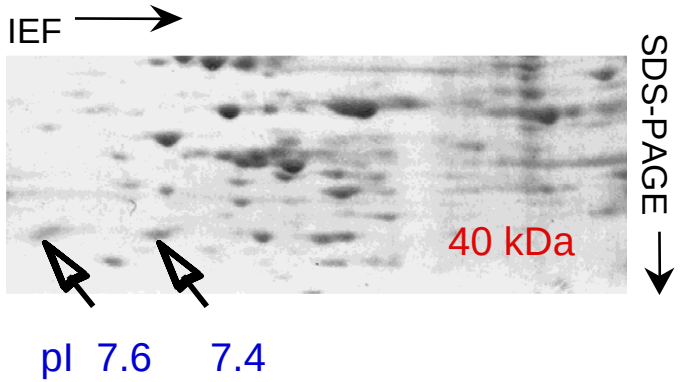
Maniratanachote et al., *Toxcol Let*, 166: 189-199, 2006.

Detection of Autoantibody to Aldolase B in Sera from Patients with Troglitazone-Induced Liver Dysfunction

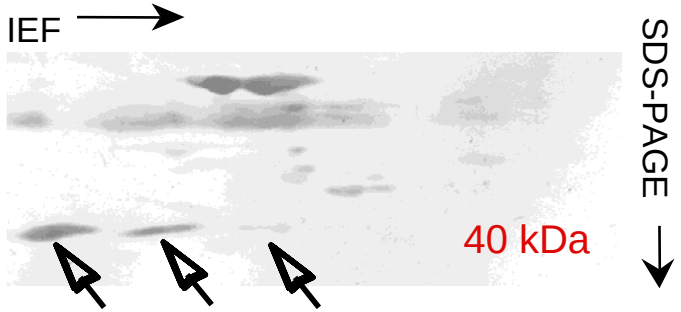
Female, 47-year old



Protein staining

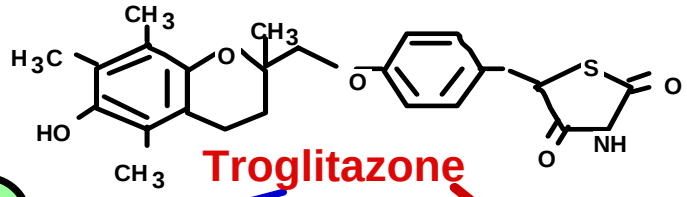


Patient A serum



Maniratanachote et al., *Toxcology*, 216: 15-23, 2005.

Reactive Metabolites and Immune Reaction in Drug InducedHepatotoxicity



Troglitazone

Detoxi-fication

Sulfation
Glucuronidation

CYP3A4

Reactive intermediate

GSH-conjugation

Covalent binding etc.

Cytotoxicity
Infulammation

Apoptosis

Th2
Production of antibody

Th1
Activation of cytotoxic T lymphocyte

Hapten, APC

Induction of immune reaction

Protein adduct

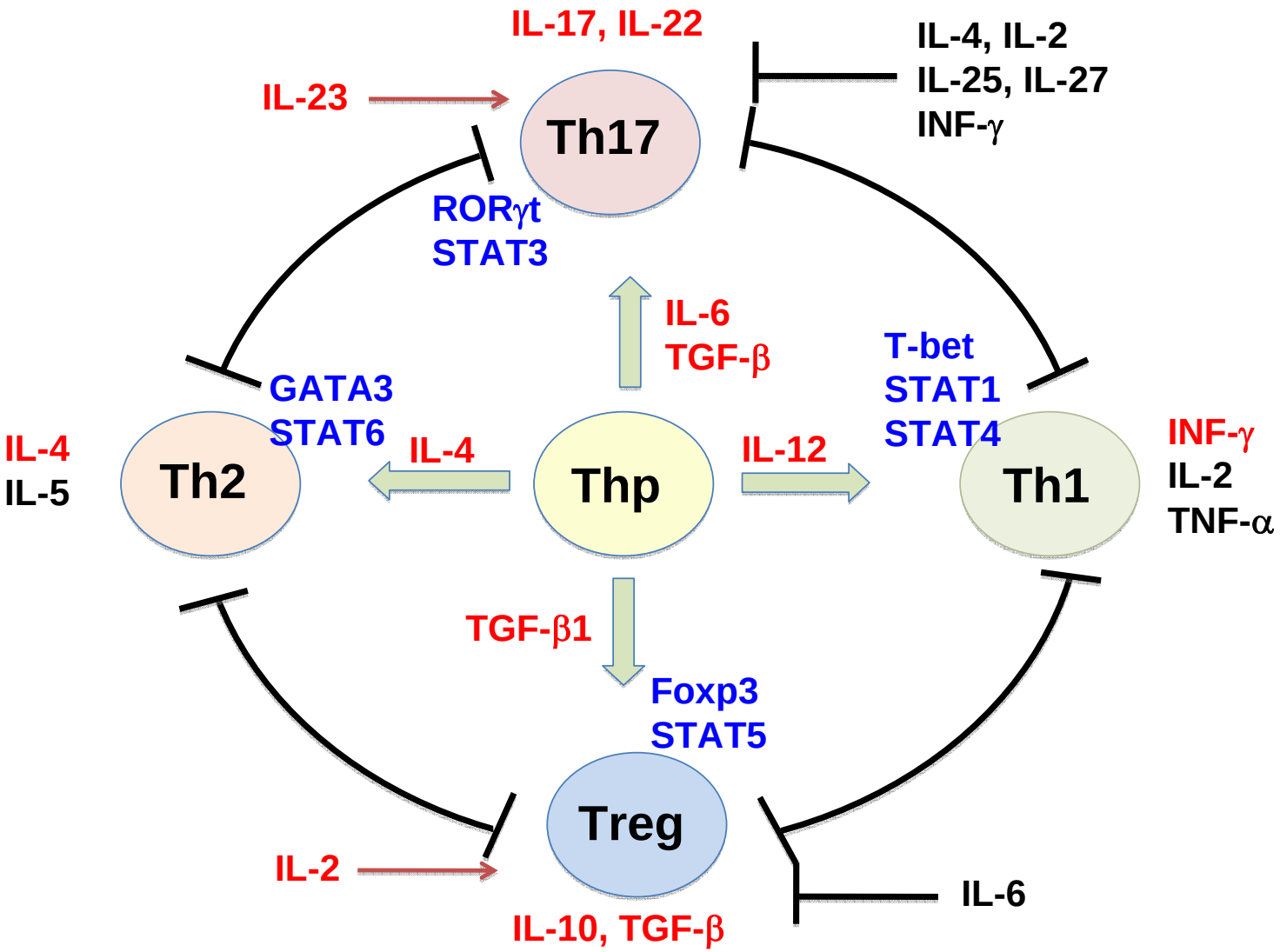
Loss of function

Hepatotoxicity

Danger signal

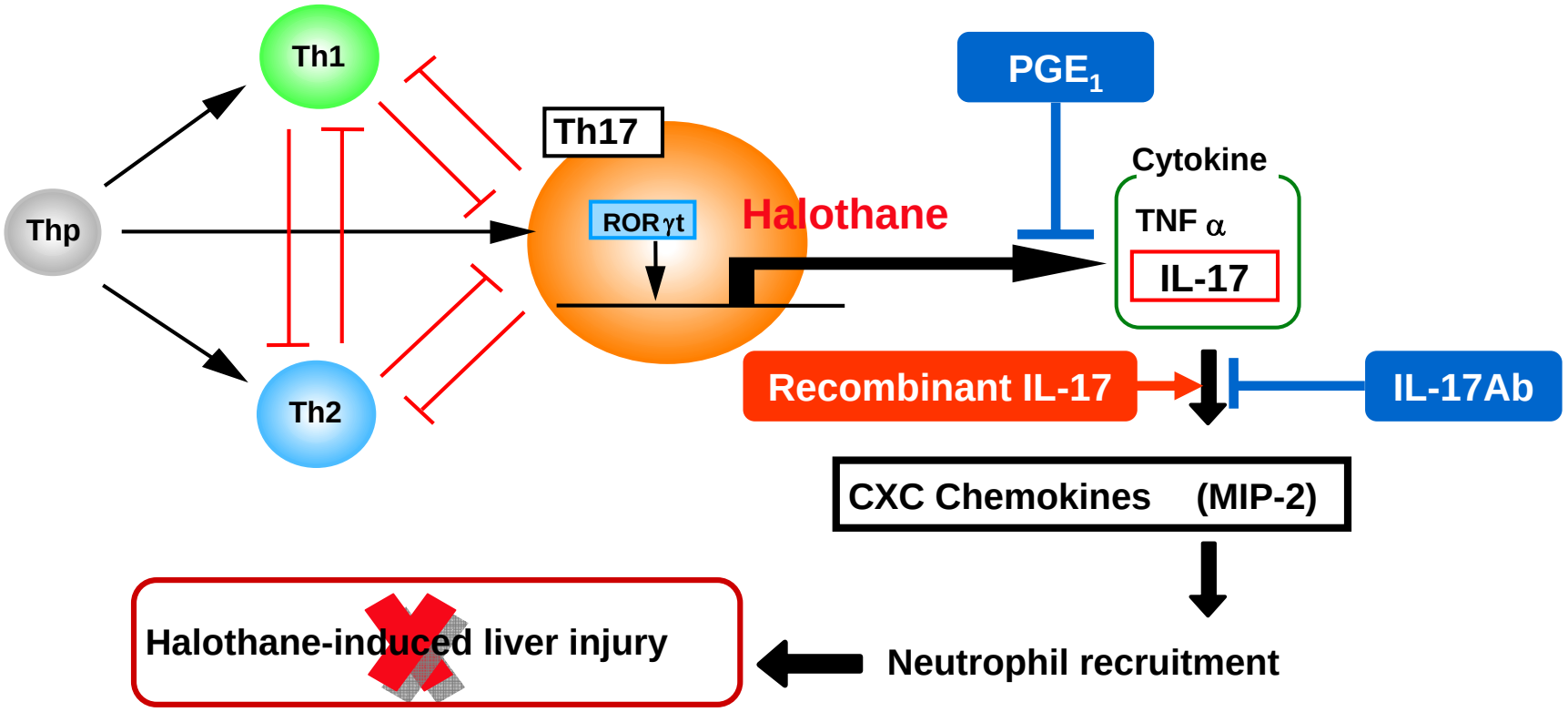
Hypersensitivity
Idiosyncrasy

Differentiation Mechanisms of CD4+ Cell Subsets



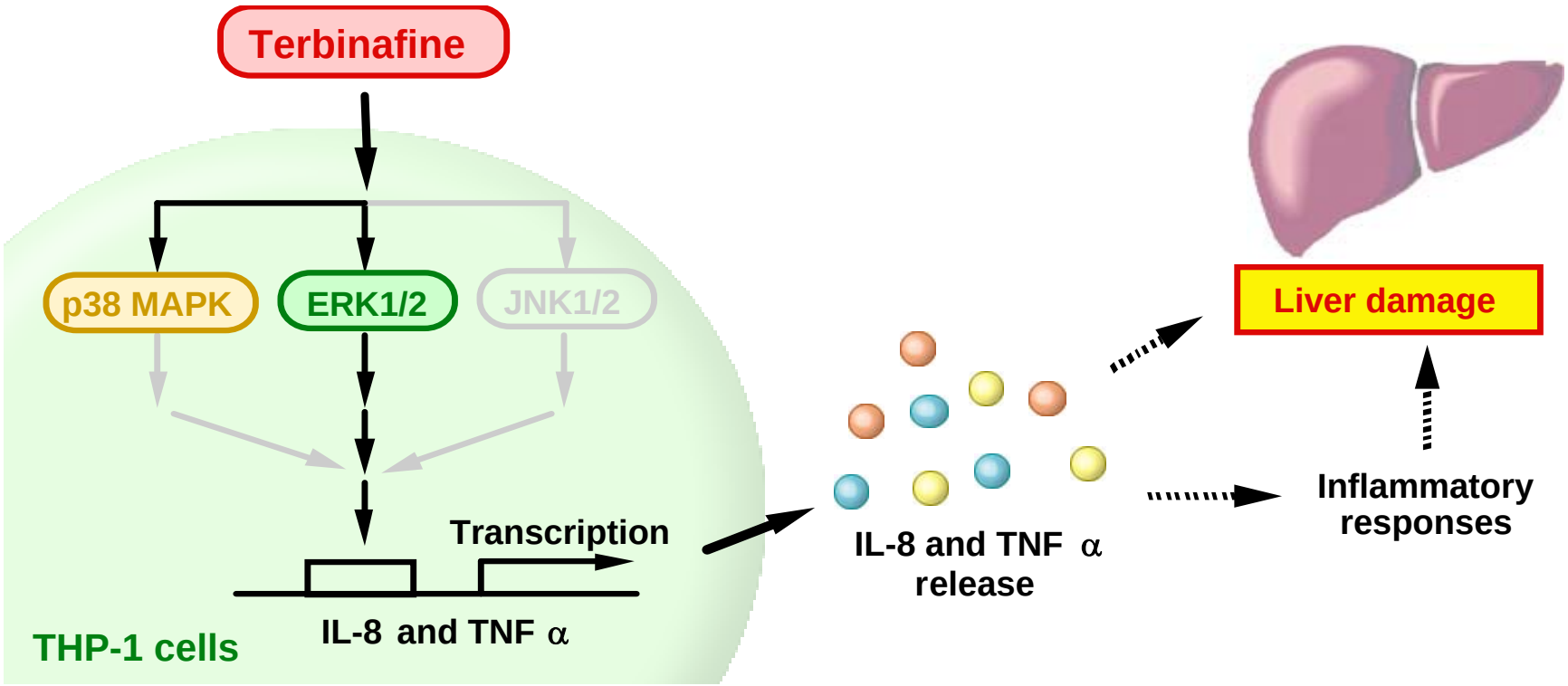
1-B1-17-1

Halothane-induced Liver Injury is Mediated by Interleukin-17 in Mice.



Kobayashi et al., *Toxcol Sci*, 111: 302-310, 2009

1-B2-9-5 Terbinafine Stimulate Inflammatory Cytokines Release in Human Monocytic Leukemia THP-1 Cells.

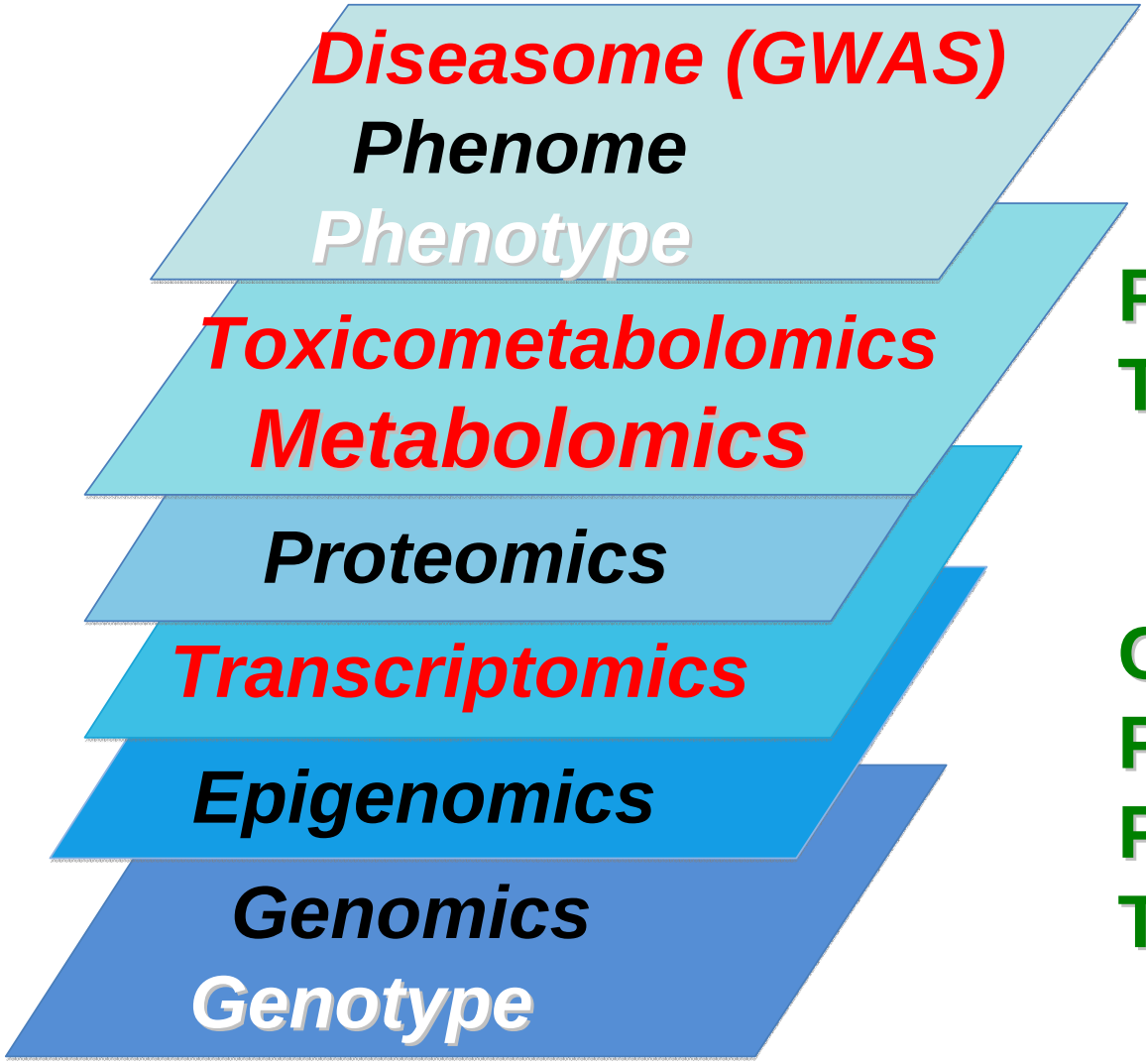


The activation of monocytic cells might be one of the mechanisms in immune-mediated liver injury of terbinafine.

Future Drug-induced Hepatic Injury and Immunohepatotoxicity Studies in Our Fields

- To establish **in vivo animal models** and **in vitro cell-based assay systems** to predict the drug-induced hepatic injury and immunohepatotoxic reactions.
- To clarify the involvement of metabolic activation **quantitatively**.
- To clarify the mechanisms of **idiosyncratic and hypersensitive** reactions.

Drug Development and Personalized Drug Therapy



Personalized Drug Therapy

Omics-based and Personalized Predictive Drug Therapy

ヒトに 社会に 医療に 役立つ基礎研究を

Today's Research leads Tomorrow's Happiness