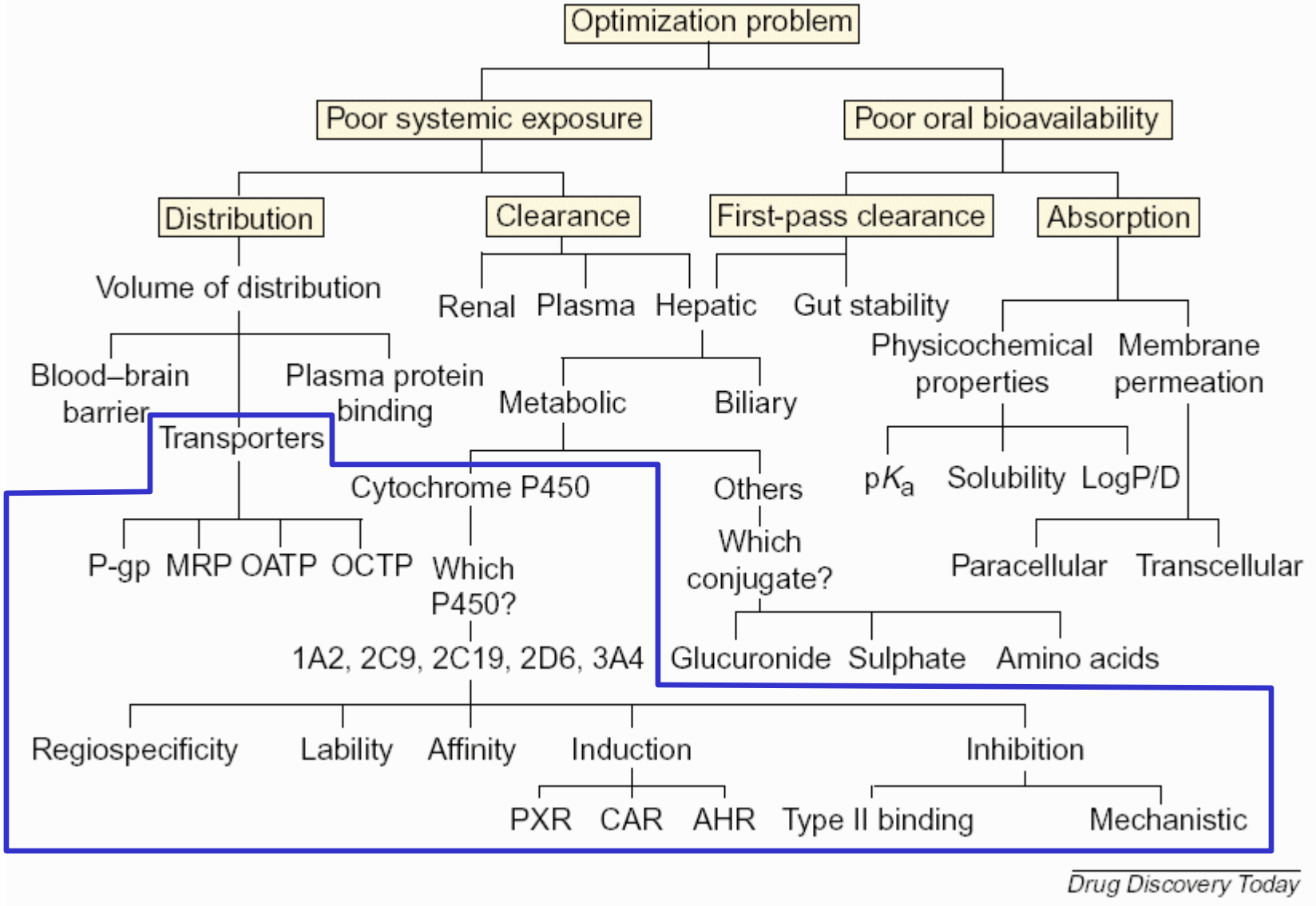
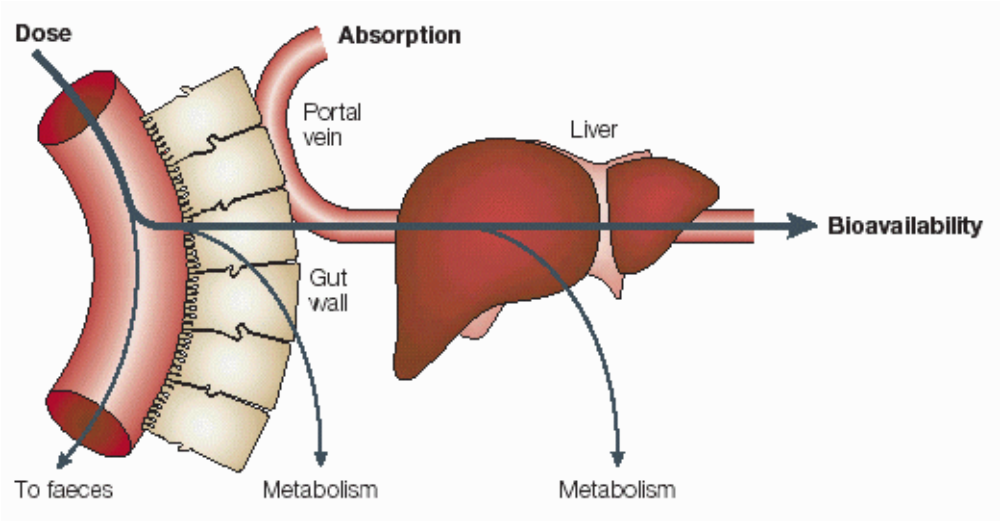
Cytochrome P450, Polymorphism, Transporters



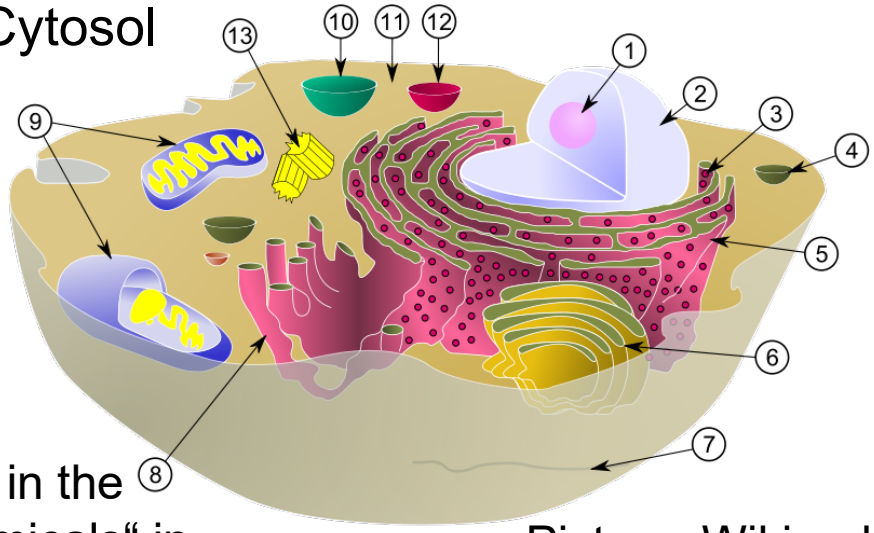
Absorption and Metabolism

Nutricients as wells as xenobiotics enter the blood circulation via the portal vein from the small intestine and reach the liver. Here, a variety of biochemical conversions of all substances is carried out.



Enzyme Systems That Metabolize Xenobiotics

Enzymatic System	Main Site of Location
Cytochrome P450	Endoplasmatic Reticulum (5, 8)
FAD-Monooxygenase	Endoplasmatic Reticulum
Monoamine Oxidase	Mitochondria (9)
Alcohol/Aldehyde Dehydrogenase	Cytosol
Epoxide Hydrolase	Endoplasmatic Reticulum
Gluthathione S-Transferase	Cytosol
Sulfotransferase	Cytosol
Acetyltransferase	Cytosol
Methyltransferase	Cytosol
Oxidoreductase	Cytosol
Xanthine Oxidase	Cytosol



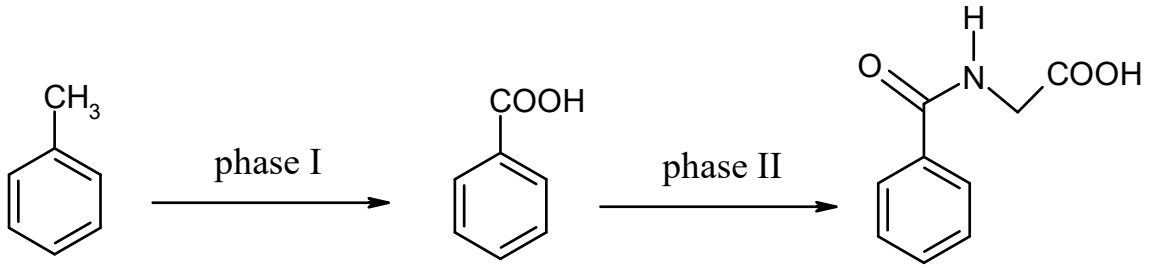
Picture: Wikipedia

Lit: C. Ioannides „Cytochromes P450 in the Metabolism and Bioactivation of Chemicals“ in *Chemistry and Molecular Aspects of Drug Design and Action*, Eds. E.A. Rekka, P.N. Kourounakis, CRC Press, Boca Raton, FL, 2008.

Cytochrome P450 Metabolism (I)

First reactions: *First pass effect*

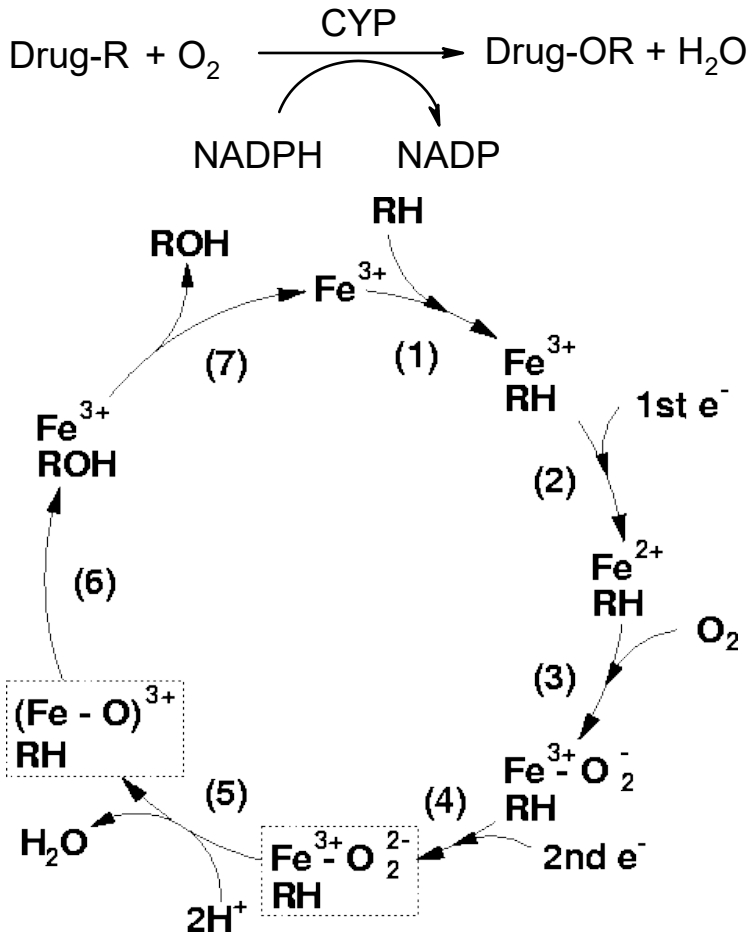
predominately lipophilic or heavy (MW >500) compounds are metabolized eccessively, whereby they become more hydrophilic and thus easier to excret.

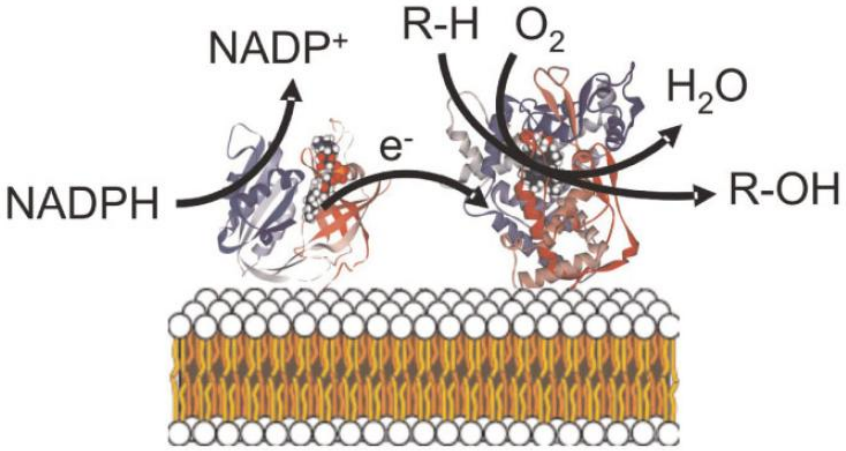


For the reactions comprising Phase I mainly the group of Cytochrome P450 enzymes (CYP) is responsible.
Usually substances are oxidized (formal addition of oxygen; redox reaction), however reduction and further chemical reactions (depending on the substrate) have been observed.
→ difficult to predict!

Cytochrome P450 Metabolism (II)

This mono-oxygenation of the substrates occurs in a catalytic cycle mediated by a hemoglobin-iron (Fe)





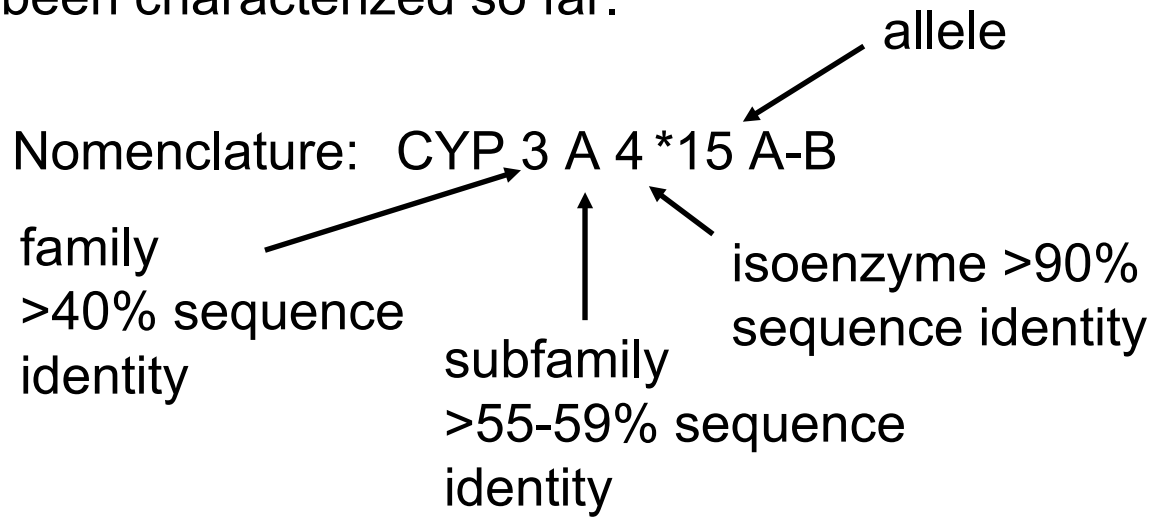
The electrons are provided by the cytochrome reductase

Cytochrome P450 Metabolism (III)

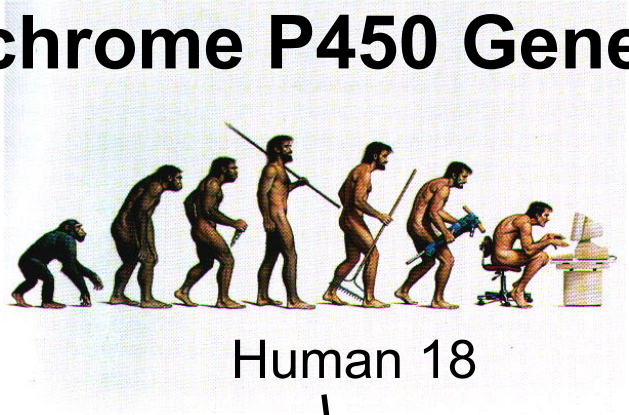
The cytochrome enzymes that account for the metabolism are predominately mono-oxygenases that evolved from enzymes for steroid and fatty acid synthesis. → cellular membranes

Sequence conservation is pretty low among CYPs in comparsion to other enzymes.

In human 18 CYP-families containing about 58 isoforms have been characterized so far.



Cytochrome P450 Gene families

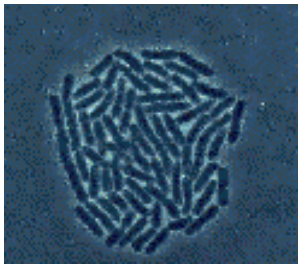


Human 18

Molluscs 1

CYP450

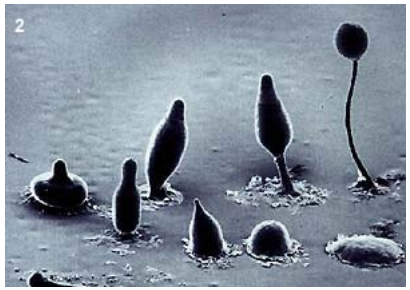
Plants 22



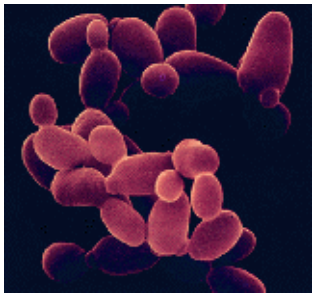
Bacteria 18

Insects 3

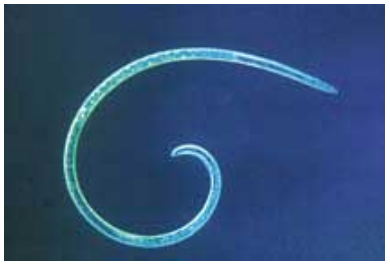
Fungi 11



Yeasts 2



Nematodes 3



Human cytochrome P450 family

From the super-familiy of the cytochromes, the following families have been found in human:

CYP 1-5, 7, 8, 11, 17, 19-21, 24, 26, 27, 39, 46, 51

CYP 1, 2A, 2B, 2C, 2D, 2E, 2F, 3 metabolisms of xenobiotics

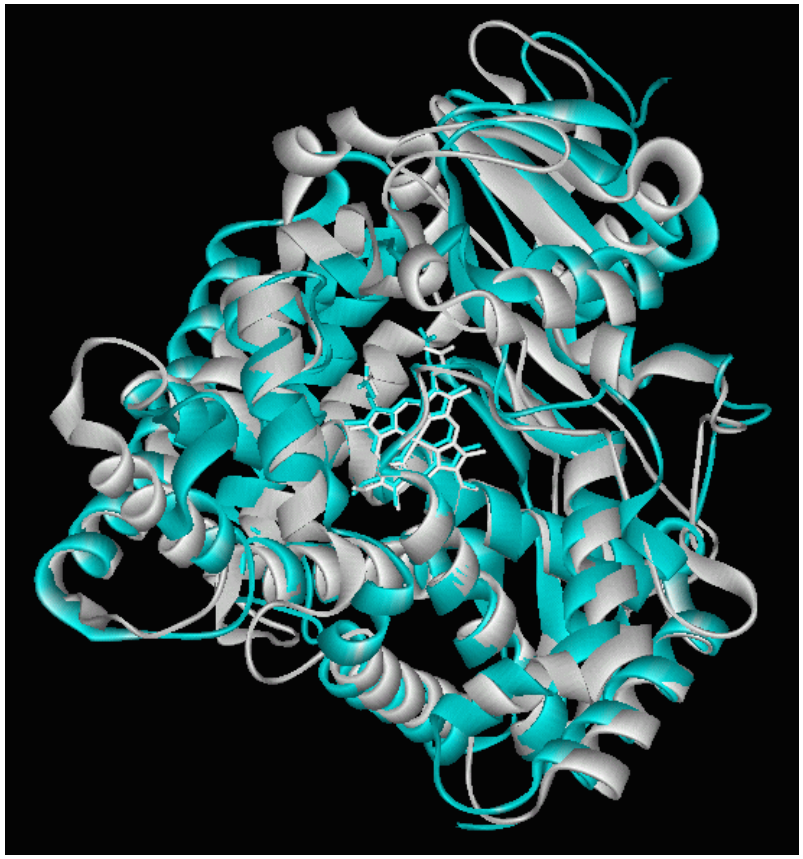
CYP 2G1, 7, 8B1, 11, 17, 19, 21, 27A1, 46, 51 steroid metabolisms

CYP 2J2, 4, 5, 8A1 fatty acids metabolisms

CYP 24 (vitamine D), 26 (retinoic acid), 27B1 (vitamine D), ... synthesis

Cytochrome P450 Enzymes (I)

Despite the low sequence identity of CYPs from different species, the oveall tertiary structure is conserved, esp. in the active center. In the outer regions, however, strong deviations occur. Nevertheless, substrate and product specificity is governed by mutations in the binding pocket.



Superposition of human *h*CYP 2C9 (1OG5.pdb) and CYP 450 BM3 (2BMH.pdb) *Bacillus megaterium*

In contrast to bacterial CYPs, *mammalian* CYPs are typically partially embedded in the membrane. Lipophilic substrates can diffuse within the membrane to the CYP.

Cytochrome P450 Enzymes (II)

flavin monooxygenase isoenzyme (FMO)

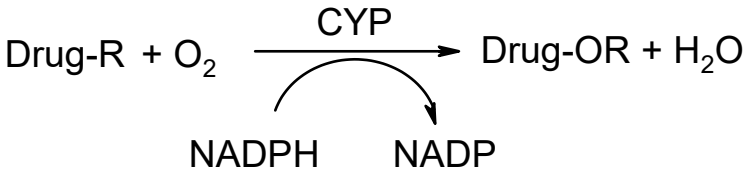
monoamine dehydrogenase (MAO)

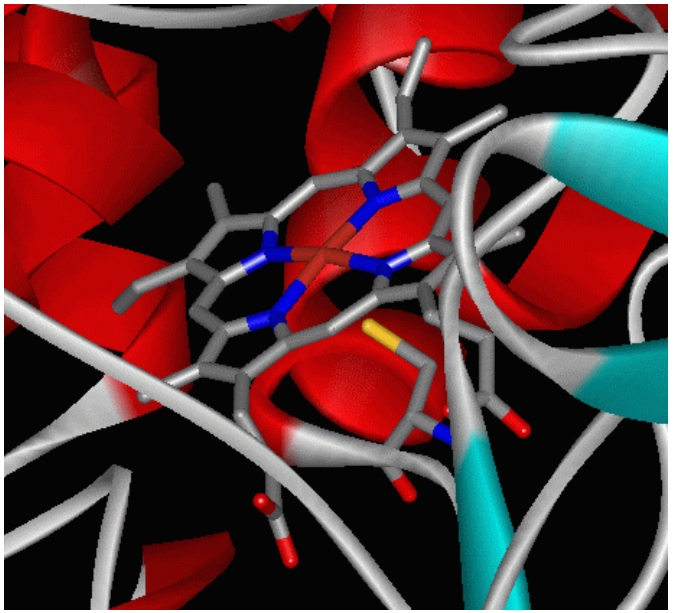
aldo-keto reductase (AKR)

alcohol dehydrogenase

aldehyde oxidase

} Further phase I enzymes

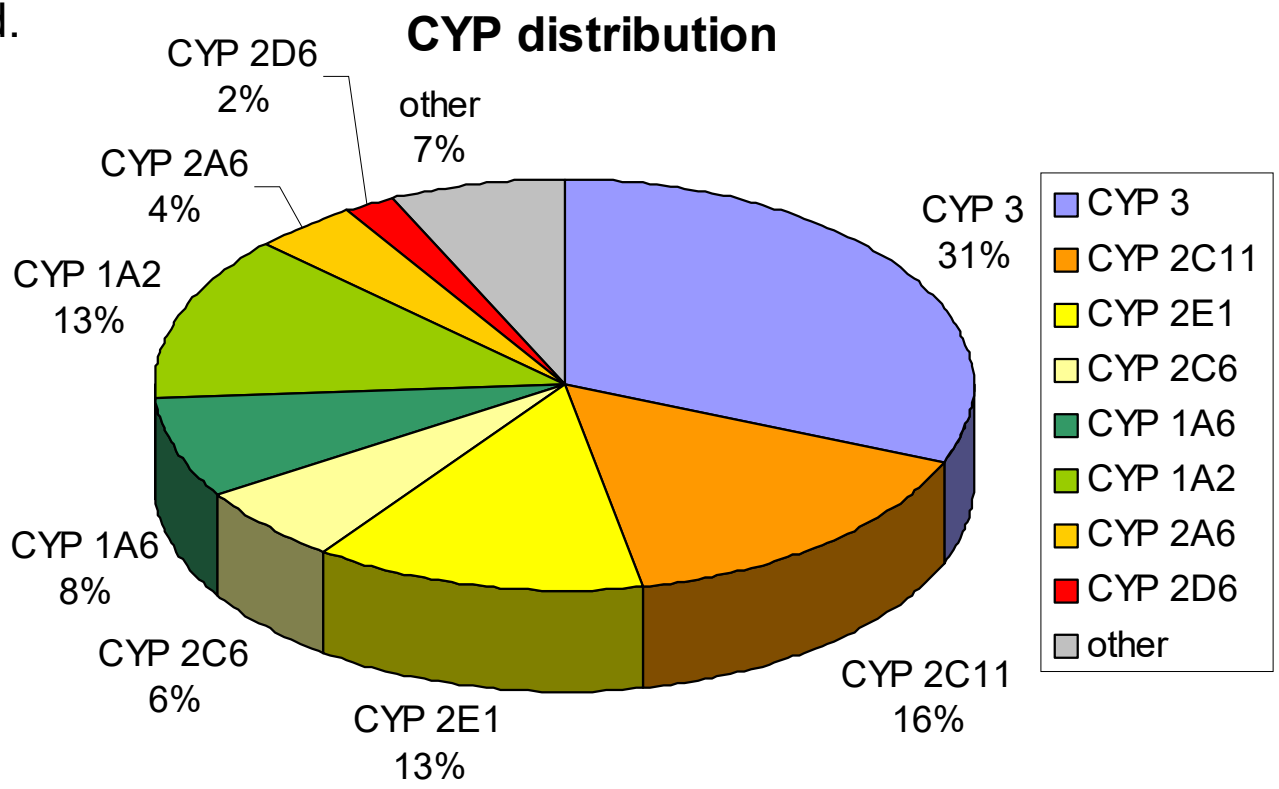




Cytochrome P450 Enzymes (III)

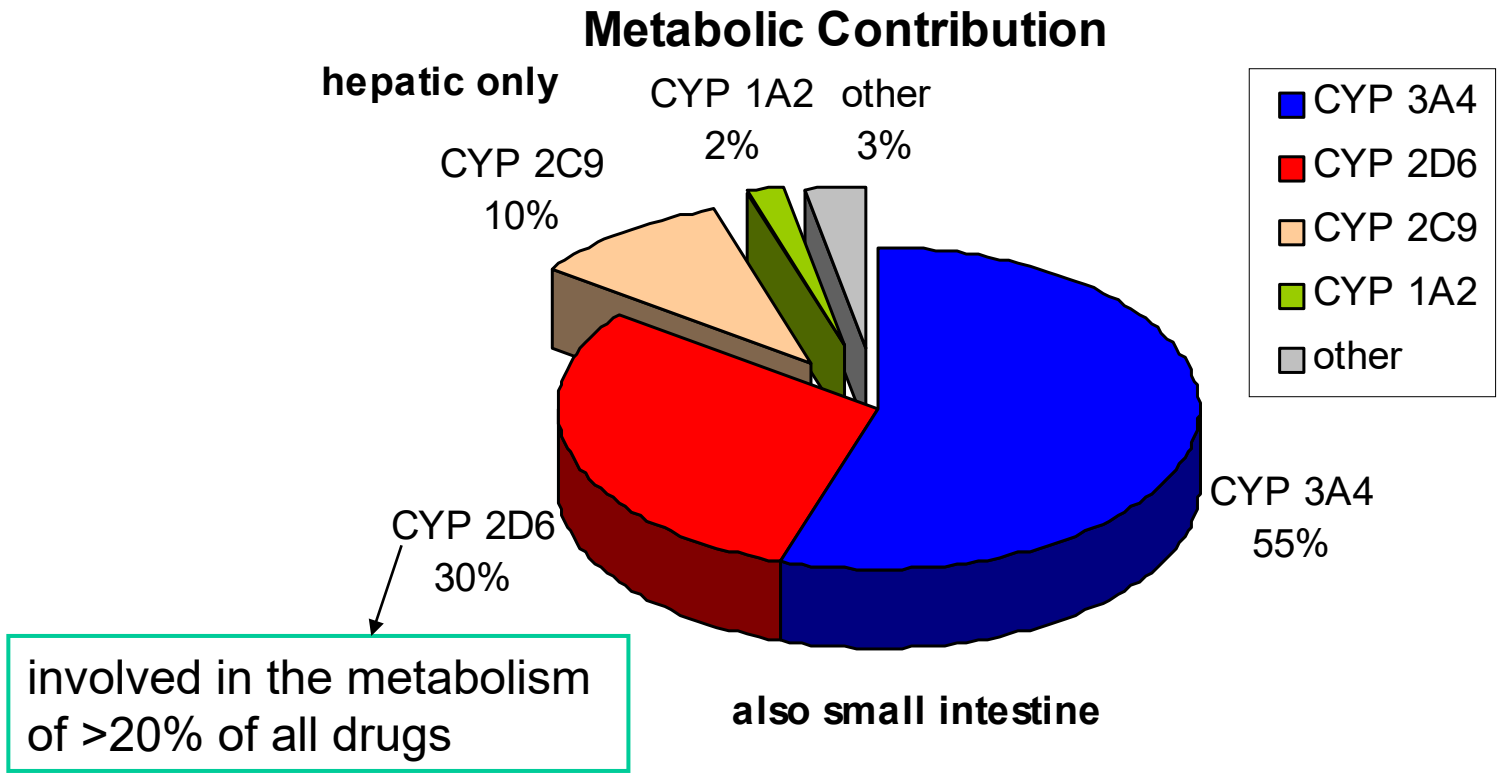
The prevailing amount of CYPs is present in the liver, however, certain CYPs are also expressed in cells of the instestine wall, lung, brain, heart, eye, mammal gland, and adrenal cortex.

The *mammalian* CYPs are closely attached to the membrane of the endoplasmatic reticulum via a membrane anchor, or are partially embedded.



Cytochrome P450 Enzymes (IV)

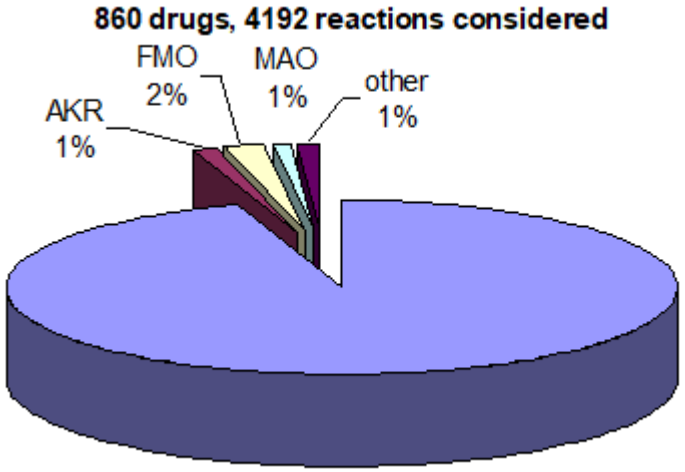
The metabolism of endogenous substances (xenobiotics) is carried out predominately by CYP 3A4, CYP 2D6, and CYP 2C9.

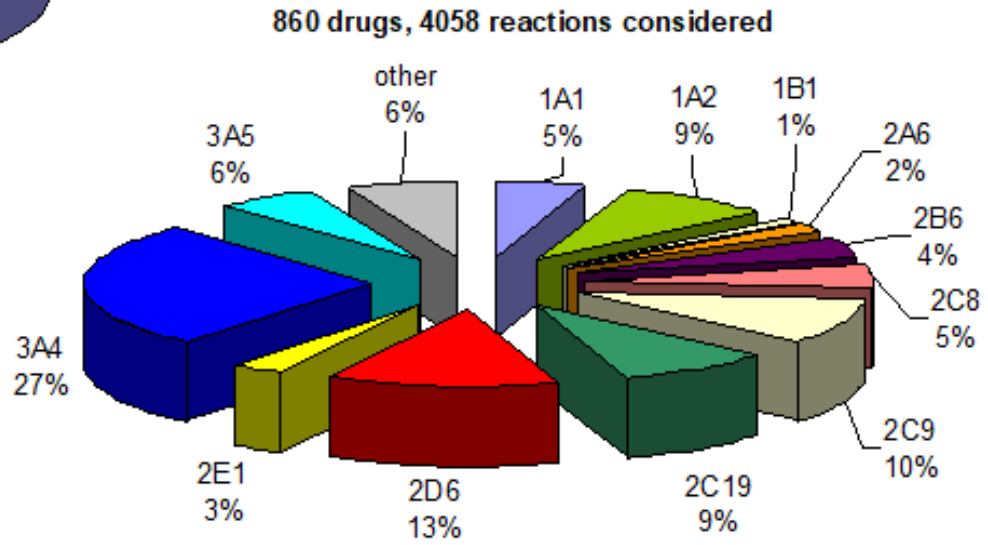


involved in the metabolism of >20% of all drugs

Cytochrome P450 Enzymes (V)

Human oxidoreductases participating in the metabolization of drugs according to the number of chemical reactions





Lit. Rendic & Guengerich *Chem.Res.Toxicol.* **28** (2015) 39

Substrate specificity of CYPs (I)

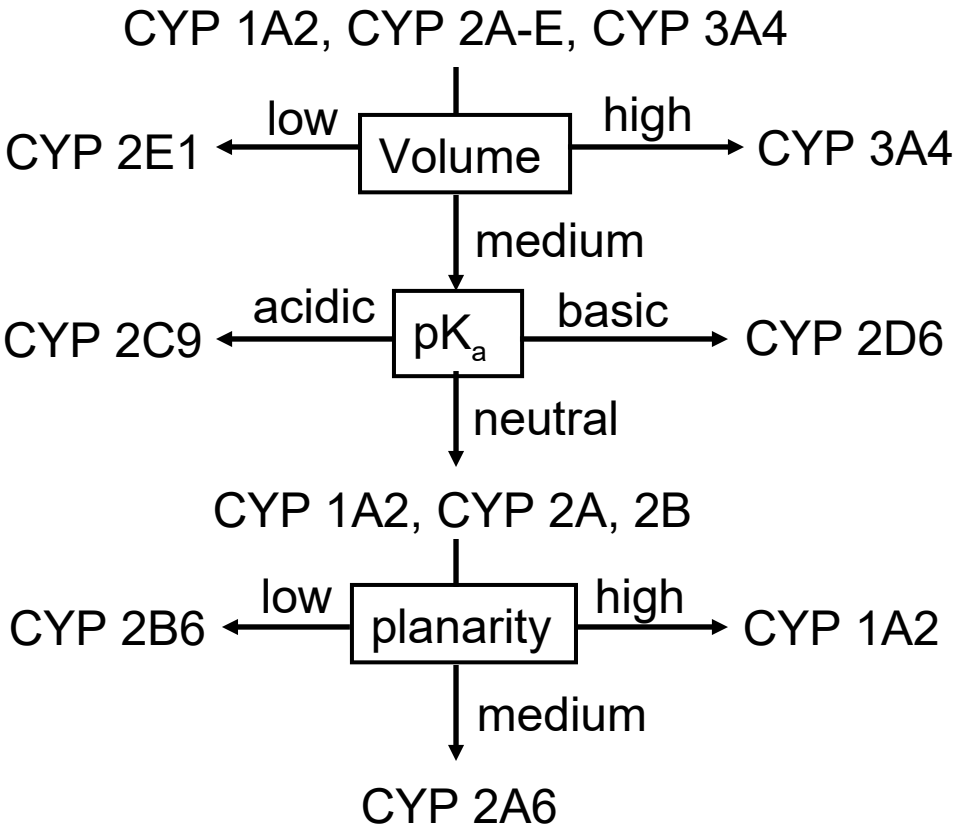
Specific substrates of certain human CYPs

CYP 1A2	verapamil, imipramine, amitryptiline, caffeine (arylamine *N*-oxidation)
CYP 2A6	nicotine
CYP 2B6	cyclophosphamid
CYP 2C9	diclofenac, naproxen, piroxicam, warfarin
CYP 2C19	diazepam, omeprazole, propanolol
CYP 2D6	amitryptiline, captopril, codeine, mianserin, chlorpromazine
CYP 2E1	dapsone, ethanol, halothane, paracetamol
CYP 3A4	alprazolam, cisapride, terfenadine, ...

Often substances are metabolized by multiple CYPs

Substrate specificity of CYPs (II)

„hand made“ decision tree for human P450 substrates

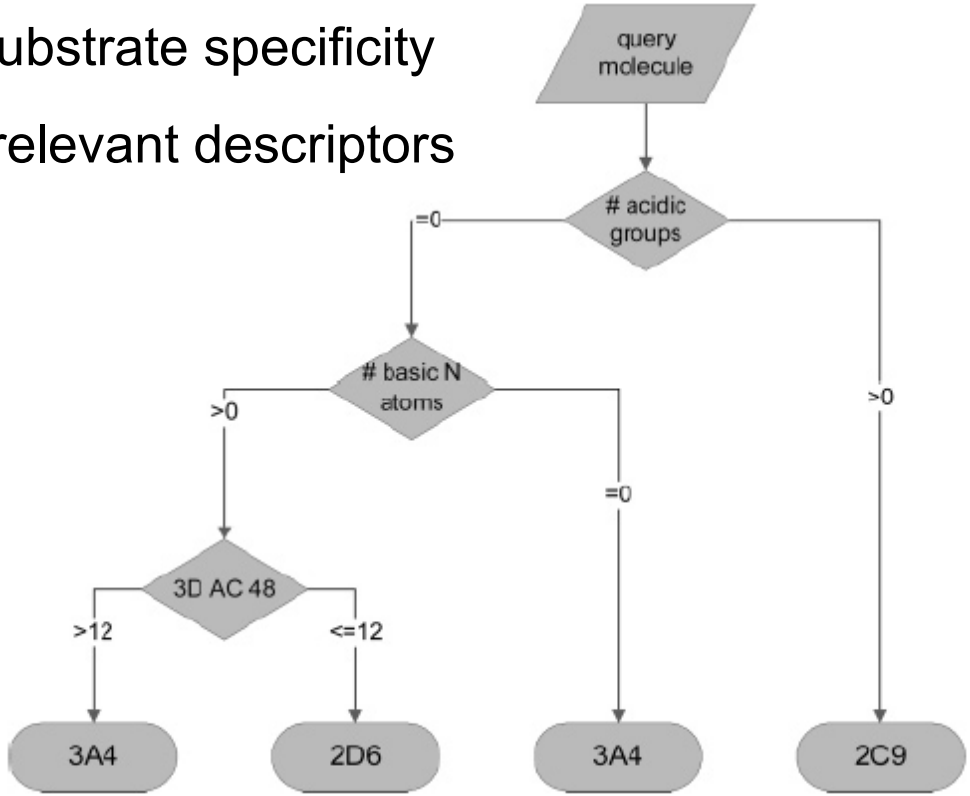


Lit: D.F.V. Lewis *Biochem. Pharmacol.* **60** (2000) 293

Prediction Models for Cytochrome P450 Metabolism (I)

Decision Tree for substrate specificity

→ Identification of relevant descriptors



Lit. L.Terfloth et al. *J.Chem.Inf.Model.* **47** (2007) 1688-1701.

Major public source of experimental data:
S.Rendic *Drug Metabol.Rev.* **34** (2002) 83-448.

Prediction Models for Cytochrome P450 Metabolism (II)

Qualitative prediction of metabolism for specific CYPs:

Binary classification into substrates / non-substrates

inhibitors / non-inhibitors

Problems: partial overlap of inhibitors and non-substrates

variability of data sets (how much of a non-substrate is metabolized?), unbalanced data sets (one class dominating)

Used machine learning algorithms: decision trees, neural networks, support vector machines, *k*-nearest neighbor, naïve Bayes

Lit. C.W.Yap & Y.Z.Chen *J.Chem.Inf.Model.* **45** (2005) 982-992.
J.M.Kriegl et al. *QSAR Comb.Sci.* **24** (2005) 491-502.
P.S.Bazeley et al. *J.Chem.Inf.Model.* **46** (2006) 2698-2708.
B.F.Jensen et al. *J.Med.Chem.* **50** (2007) 501-511.
M.Carbon-Mangels & M.C.Hutter. *J.Mol.Inf.* **30** (2011) 885-895.

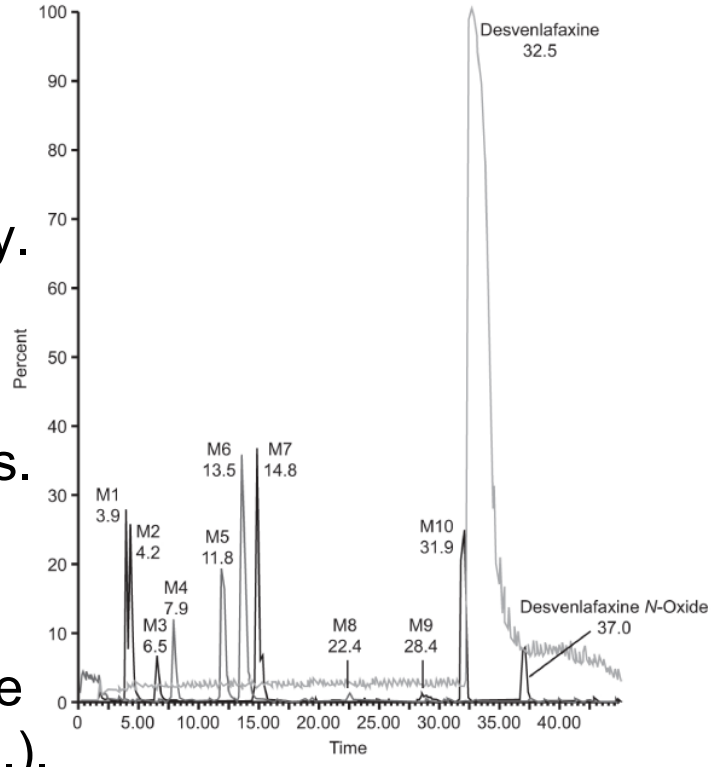
Cytochrome P450 Metabolism (IV)

During pre-clinical development it is of importance to characterize also the metabolic products of drugs since these might be toxic themselves.

Experimentally, the according (human) CYP-enzymes are expressed in *E. coli*, and the conversion is monitored by gas chromatography and mass spectroscopy.

This allows the selective determination of metabolites by single cytochrome P450 enzymes and their genetic variants.

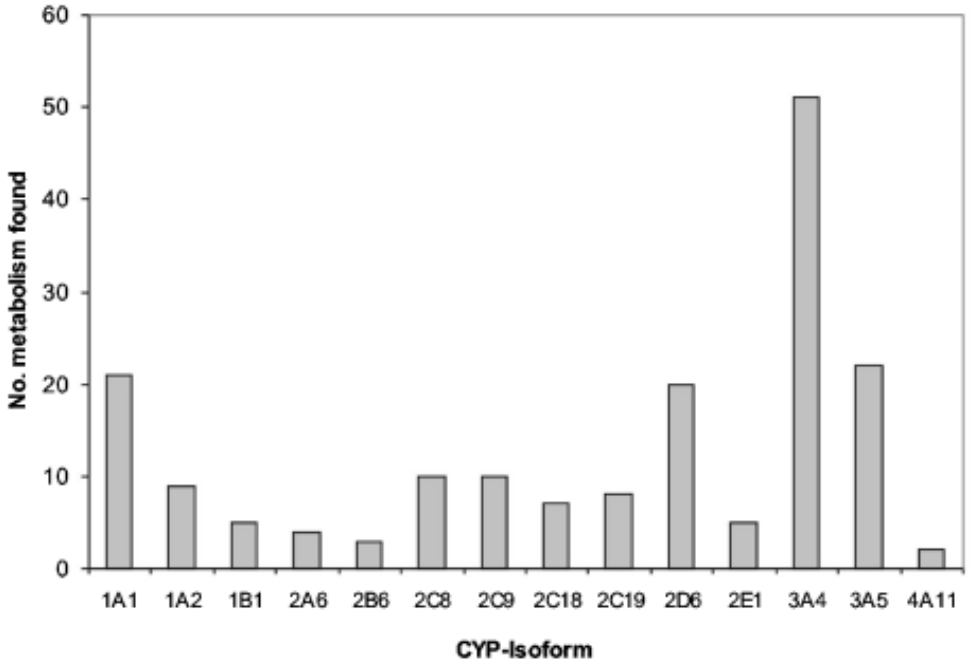
The results are compare to corresponding *in vivo* results from animals in order to chose the appropriate animal model (mouse, dog, guinea pig,...).



Lit. K.Schroer, M.Kittelmann, S.Lütz *Biotechnol. & Bioengin.* **106** (2010) 699.

Cytochrome P450 Metabolism (V)

The most prominent CYP-Enzymes during pre-clinical development used for generating metabolites



Number of metabolism events found (60 compounds tested)

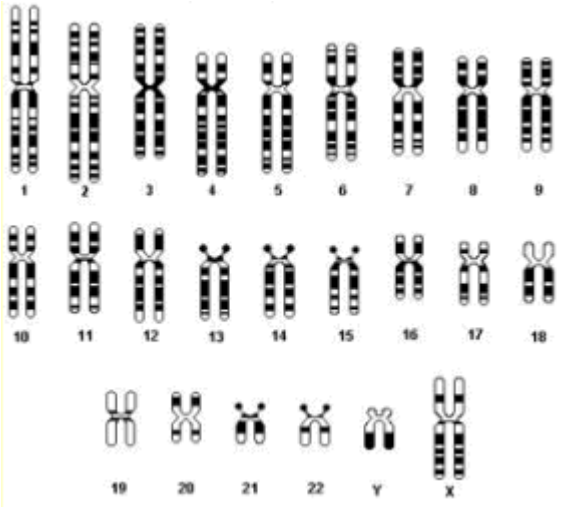
Lit. K.Schroer, M.Kittelmann, S.Lütz *Biotechnol. & Bioengin.* **106** (2010) 699.

Cytochrome P450 polymorphism

„Every human differs (more or less)“

The phenotype can be distinguished by the actual activity or the amount of the expressed CYP enzyme.

The genotype, however, is determined by the individual DNA sequence. Human: two sets of chromosomes (diploid)



That means: The same genotype enables different phenotypes

Depending on the metabolic activity, three major cathegories of metabolizers are separated: *extensive metabolizer* (normal), *poor metabolizer*, and *ultra-rapid metabolizer* (increased metabolism of xenobiotics)

Lit: K. Nagata et al. *Drug Metabol. Pharmacokin* **3** (2002) 167

Single Nucleotide Polymorphism (SNP)

SNPs are differences of single bases in the DNA that can be observed between individuals in a population.

Alleles occuring in at least 1% of the population are defined as polymorphism, which means that these genotypes occur regularly without causing diseases.

Conversely, differences in the genom that occur in less than 1% are refered to as mutations.

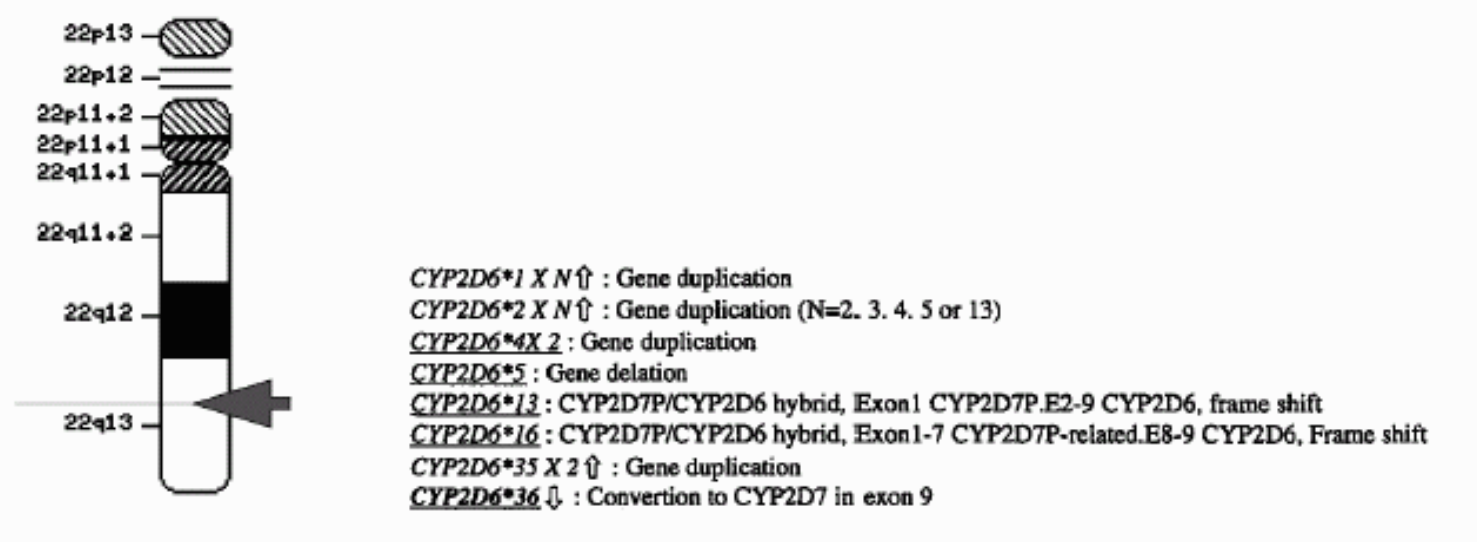
In the case of rare inhereted diseases, typically mutations in the coding region of DNA sequences are observed.

Lit: A.D. Rose *Nature* **405** (2000) 857.

CYP 2D6 Polymorphism (I)

The polymorphismus of CYP 2D6 (debrisoquine 4-hydroxylase) has been studied in great detail, as metabolic differences have first been described for debrisoquine and sparteine (antipsychotics).

See: D.B.Goldstein et al. *Nature Rev. Genetics* **4** (2003) 937.



localized on chromosome 22

Of the 75 allels, 26 exprime CYP2D6 proteines

see http://www.imm.ki.se/CYPalleles/cyp2d6.htm

CYP 2D6 Polymorphisms (II)

Designation	Characteristic mutation(s)	Enzyme activity	Allelic frequency (%)
*CYP2D6*1*	Wild type	Normal	
*CYP2D6*2*	$G_{1749}C$, $C_{2938}T$, $G_{4268}C$ substitutions	Normal	30
*CYP2D6*3*	A_{2637} deletion	Deficient	2
*CYP2D6*4*	$G_{1934}A$ substitution	Deficient	22
*CYP2D6*5*	Gene deletion	Deficient	2
*CYP2D6*6*	T_{1795} deletion	Deficient	2
*CYP2D6*7*	$A_{3023}C$ substitution	Deficient	0·1
*CYP2D6*8*	$G_{1846}T$ substitution	Deficient	0·1
*CYP2D6*9*	(A_{2701}–A_{2703}) or (G_{2702}–A_{2704}) deletion	Decreased	1·5
*CYP2D6*10*	$C_{188}T$, $G_{1749}C$, $G_{4268}C$ substitutions	Decreased	1·5
*CYP2D6*11*	$G_{971}C$ substitution	Deficient	0·1
*CYP2D6*12*	$G_{212}A$ substitution	Deficient	0·1
*CYP2D6*13*	Hybrid: *2D7* exon 1, *2D6* exons 2–9	Deficient	0·1
*CYP2D6*14*	$G_{1846}A$ substitution	Deficient	0·1
*CYP2D6*15*	T_{226} insertion	Deficient	0·1
*CYP2D6*16*	Hybrid: *2D7* exons 1–7, *2D6* exons 8–9	Deficient	0·1
*CYP2D6*1* × 2	Gene duplication	Increased	1
*CYP2D6*2* × 2	Gene duplication	Increased	1·5
*CYP2D6*4* × 2	Gene duplication	Deficient	0·5

Lit: J. van der Weide et al. *Ann. Clin. Biochem* **36** (1999) 722

CYP 2D6 Polymorphismus (III)

MGLEALVPLAVIVAIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQ

poor debrisoquine metabolism S

R impaired mechanism of sparteine

LRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFGPRSQGVF

poor debrisoquine metabolism I

LARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDK

poor debrisoquine metabolism R

AVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAVPVLLHIPALAGKV

LRFQKAFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNDENLRIVVA

missing in CYP2D6*9 allele

DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVI

P loss of activity in CYP2D6*7

HEVQRFGDIVPLGMTHMTSRDIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFRFHPEHF

LDAQGHFVKPEAFLPFSAGRRACLGEPLARMELFLFFTSLLQHFSFSVPTGQPRPSHHGV

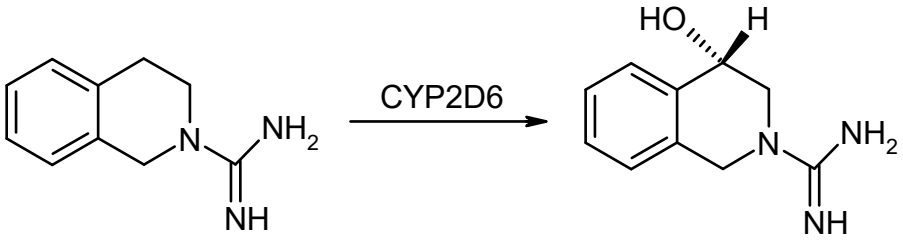
FAFLVSPSPYELCAVPR

T impaired metabolism of sparteine in alleles 2, 10, 12, 14 and 17 of CYP2D6

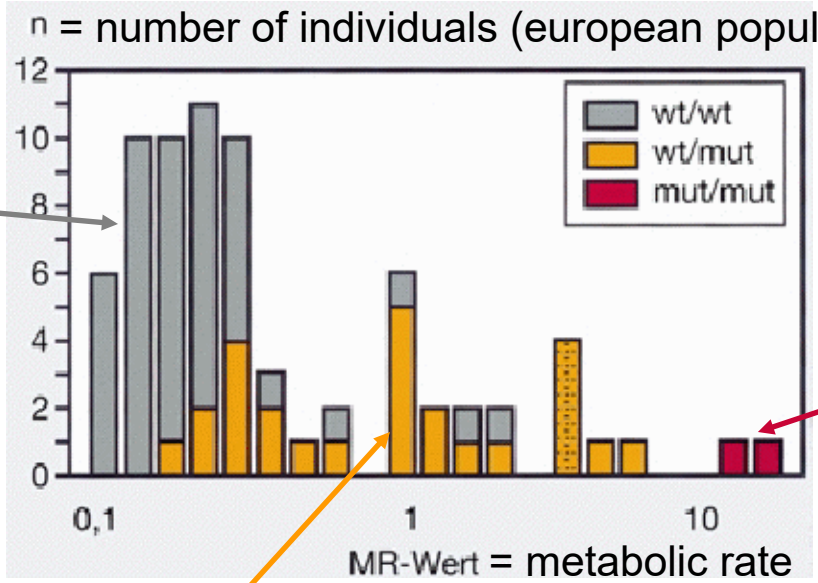
see http://www.expasy.org/cgi-bin/niceprot.pl?P10635

CYP 2D6 Polymorphism (IV)

variability of debrisoquine-4-hydroxylation



homocygote extensive metabolizers



homocygote poor metabolizers

heterocygote extensive metabolizers

Lit: T. Winkler *Deutsche Apothekerzeitung* **140** (2000) 38

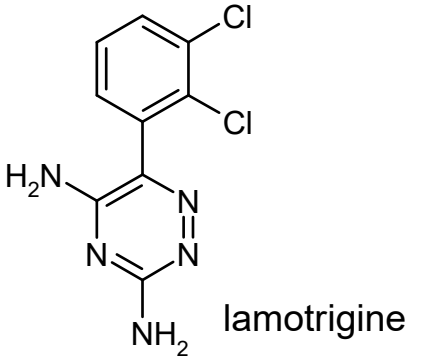
CYP 2D6 Polymorphismus (V)

the *poor metabolizer* phenotyp has consequences for the metabolism of more than 25% of all common drugs, since it causes an increased concentration of xenobiotics that are not metabolized.

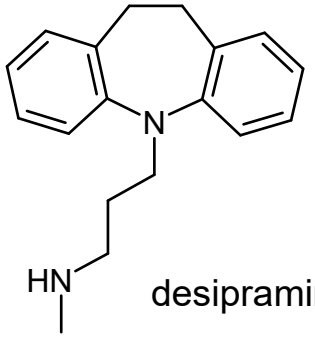
Lit: H.K.Kroemer & M.Eichelbaum. *Life Sci.* **56** (1995) 2285.

Thus, CYP2D6 genotyping is already applied to select appropriate test candiates in phase II of clinical tests:

lamotrigine, desipramine (Antidepressants)



lamotrigine



desipramine

Lit: M.P.Murphy et al. *Pharmacogenetics* **10** (2000) 583.

Polymorphism of further CYPs

CYP 1A1 ca. 16 isoforms (lung)

CYP 1A2 individual; strong, medium, and slow conversion of caffeine

CYP 2B6 absent in 3-4 % of the caucasian population

CYP 2C9 deficit in 1-3 % of the caucasian population

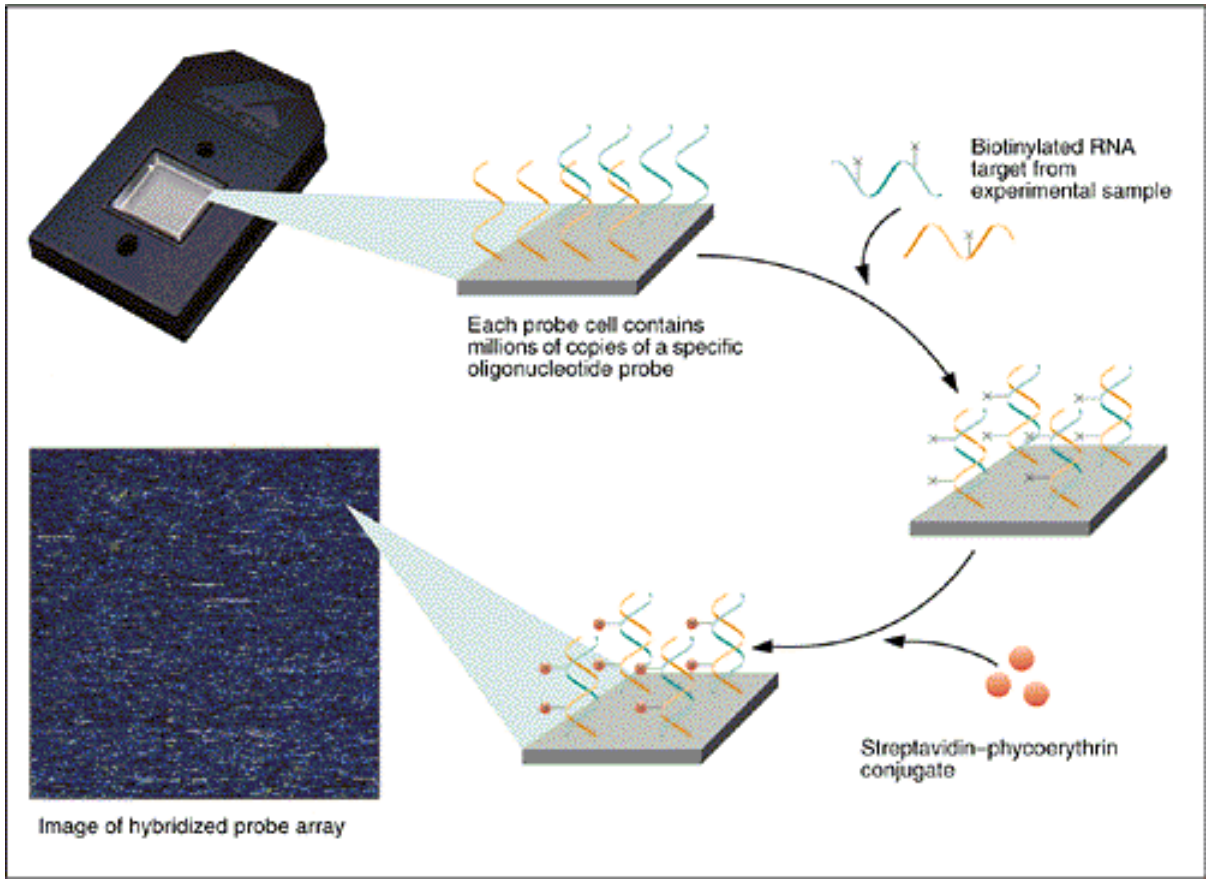
CYP 2C19 individues with inactive enzyme (3-6 % of the caucasian and 15-20 % of the asian population)

CYP 2D6 poor metabolizers in 5-8 % of the european, 10 % of the caucasian and <1% in the japanese population. Overexpression (gene duplication) in parts of the african and oriental population

CYP 3A4 ca. 56 isoforms (liver)

Genotyping for P450 alleles

Affymetrix (US) has developped microarrays (gene chips) using immobilized synthetic copies of P450 nucleotides, that allow the identification of all clinically relevant allelic variants.



Induction and regulation of CYP3A (I)

A series of xenobiotics have been identified that lead to increased expression of enzymes of the CYP3A family.

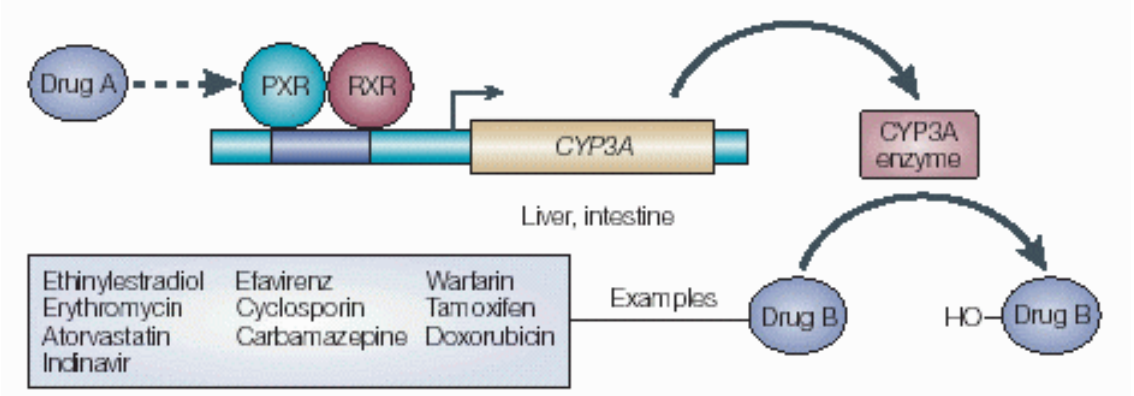
indinavir	antiviral
efavirenz	antiviral
cyclosporine	immuno-suppressant
carbamazepine	antipsychotic
atorvastatin	HMG CoA reductase inhibitor
tamoxifen	anti-hormone

These bind to the *pregnane X receptor* (PXR) which is the transcription factor for the regulation of the CYP3A gene expression.

Lit: T.M. Wilson et al. *Nature Rev. Drug Disc.* **1** (2002) 259

Induction and regulation of CYP3A (II)

The PXR receptor operates together with the *retinoid X receptor* (RXR) as a heterodimer.



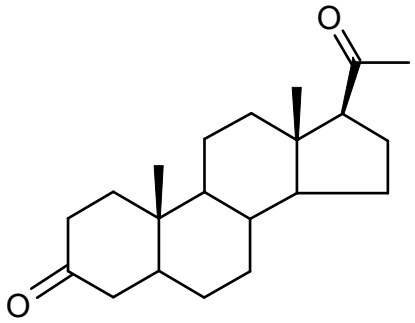
CYP3A induction leads to an increased metabolism of the administered substance due to upregulated enzymes. This can cause adverse reactions, such as inflammation of the liver (hepatitis).

Lit: T.M. Wilson et al. *Nature Rev. Drug Disc.* **1** (2002) 259

RXR and other nuclear receptors (I)

As a specific, endogen activator of RXR, 5β-pregnane-3,20-dione has been identified.

In contrast, PXR is much less specific and is activated by glucocorticoids as well as by anti-glucocorticoids.



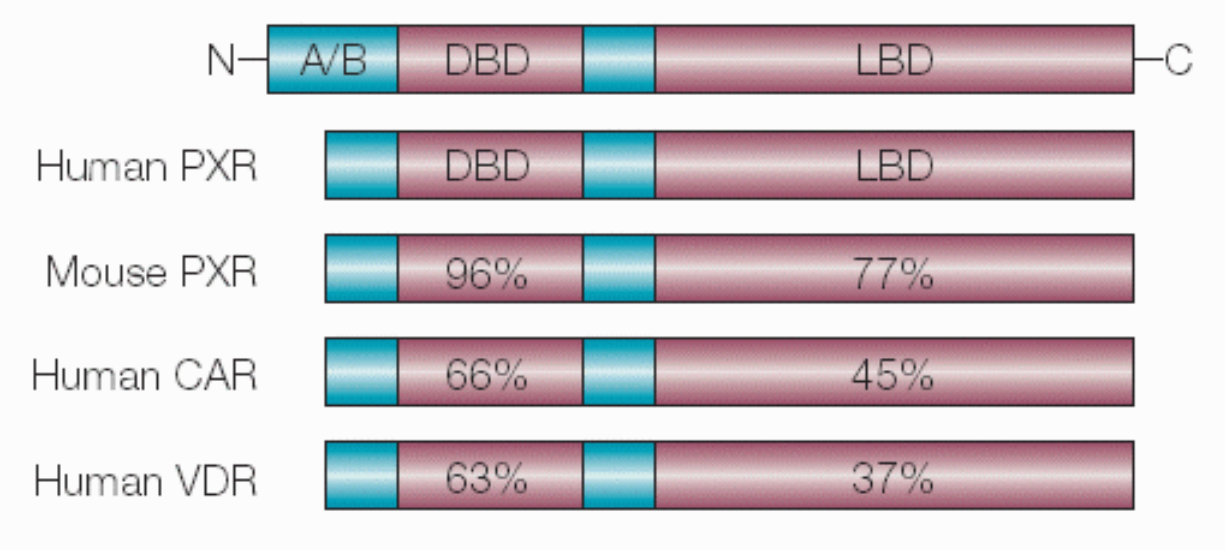
5β-pregane-3,20-dione

Conversely, the unspecific *constitutive androgen receptor* (CAR) is found in the cytoplasm and dimerizes with PXR in the nucleus. Analog to PXR, the CYP2B gene is regulated.

Likewise high sequence homology has been found for the *vitamine D receptor* (VDR) that regulates CYP27, and for the *arylhydrocarbon receptor* (AHR) (dioxin receptor).

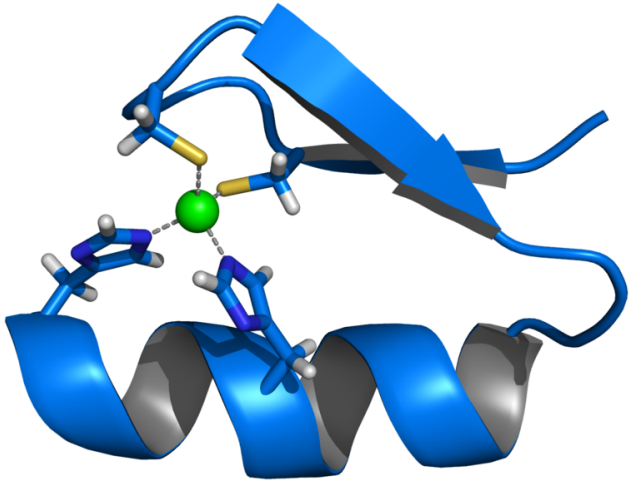
RXR and other nuclear receptors (II)

These nuclear receptors all belong to a family of transcription factors. Each one possess a double zinc-finger DNA-binding domain (DBD), and a larger ligand binding domain (LBD) which is located at the C-terminus.



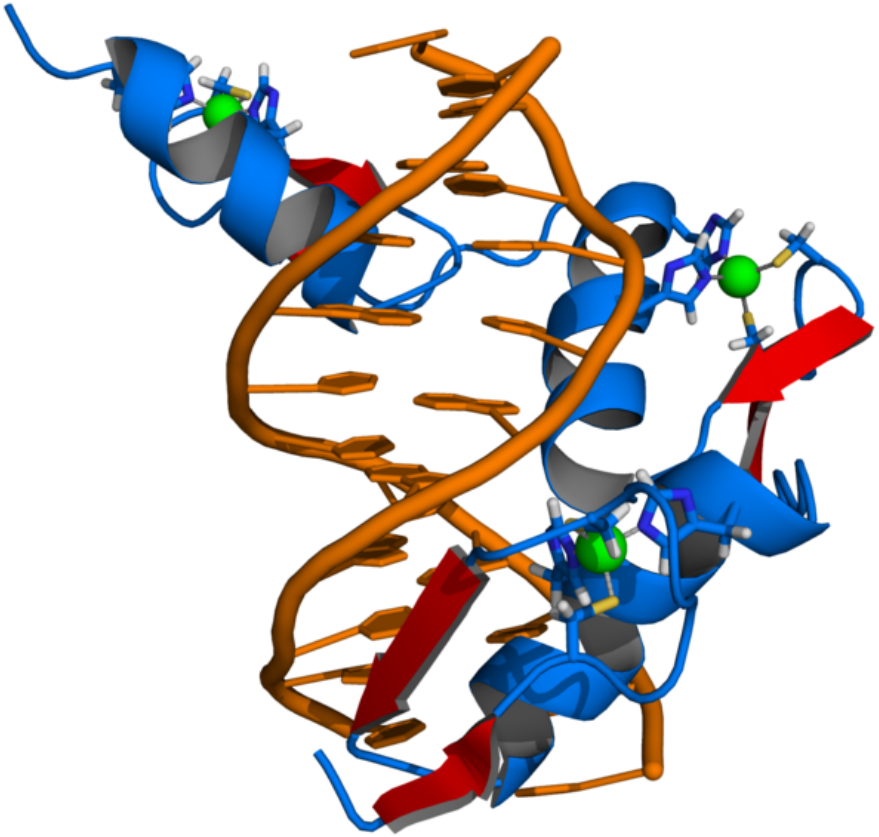
They have been initally called *orphan nuclear receptors* as their ligands have been found later.

Zinc finger motiv in DNA-binding motifs



The zinc ion is coordinated by two cysteines and two histidines.

Source: Wikipedia



The protein Zif268 contains three zinc fingers motives in complex with the DNA

Human Orphan Nuclear Receptors

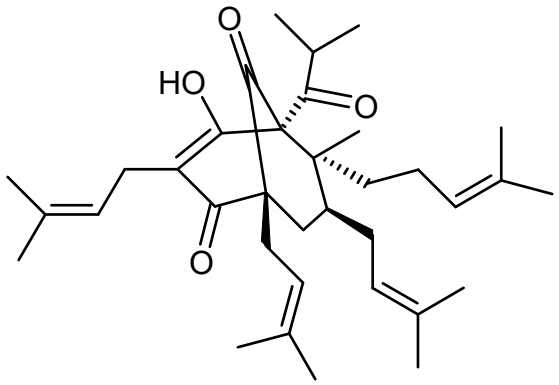
receptor (gene ID)	natural ligand / synthetic ligand
CAR (NR1I3)	3α,5α-androstanol
COUP (NR2F1)	ß-napthoflavone
ERR (NR3B1)	isoflavones, 4-hydroxytamoxifen (=afimoxifene)
FXR (NR1H4)	chenodeoxycholic acid (a bile acid)
HNF4 (NR2A1)	palmitic acid
LRH (NR5A2)	phospolipids, GSK8470
PPAR (NR1C1)	eicosapentaenoic acid, hypolipidemic drugs, e.g. saroglitazar
PXR (NR1I2)	5β-pregnane-3,20-dione, rifampicin
ROR (NR1F1)	all-*trans* retinoic acid, melatonin
RXR (NR2B1)	9-*cis*-RNA, adapalene, etodolac

Selection only, for more see references:

Lit: T.M.Wilson & J.T.Moore *Mol. Endocrin.* **16** (2002) 1135.
I.M.S.de Vera *ACS Pharmacol.Transl.Sci.* **1** (2018) 134.

Additions from corresponding UniProt entries.

Induction and regulation of CYP3A (III)



hyperforin, a natural ingredient of St. John‘s wort (Johanniskraut, *Hypericum performatum*) exhibits the highest measured affinity to PXR (K_d = 27 nM) so far.

Application: remedy against cholestasis [Gallestauung], mild antidepressant (heavily debated if available concentration in preparations of St. John‘s wort is sufficiently high)

Induction and regulation of CYP3A (IV)

X-ray structure of PXR complexed with hyperforin (1M13.pdb)

Lit: R.E. Watkins et al. *Biochemistry* **42** (2003) 1430

Induction of further CYPs

CYP 1A2	omeprazole, insulin, aromatic hydrocarbons (cigarette smoking, charbroiled meat)

causes increased caffeine level in the plasma,
if you quit smoking.

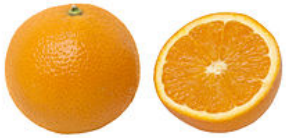
CYP 2C9	rifampicin, secobarbital
CYP 2C19	carbamazepine, prednisone
CYP 2D6	dexamethason
CYP 2E1	ethanol, isoniazid
CYP 3A4	glucocorticoides, phenobarbitone, rifampicin, nevirapine, sulfadimindine, nevirapine, sulfinpyrazone, troglitazone

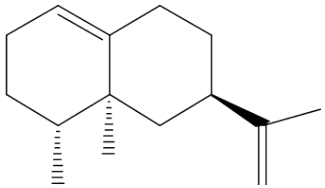
Typical inhibitors of various CYPs

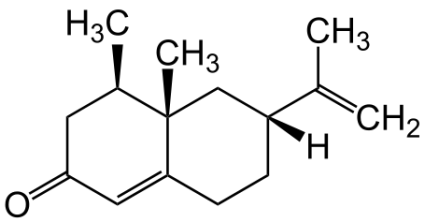
CYP 1A2	cimetidine, ciprofloxacine, enoxacine... grapefruit juice (naringin, 6‘,7‘-dihydroxy-bergamottin)
CYP 2C9	chloramphenicol, amiodarone, omeprazole,...
CYP 2C19	fluoxetine, fluvastatin, sertraline,...
CYP 2D6	fluoxetine, paroxetine, quinidine, haloperidol, ritonavir,...
CYP 2E1	disulfiram, cimetidine,...
CYP 3A4	cannabinoids, erythromycin, ritonavir, ketoconazole, grapefruit juice

Biotechnical Application of CYPs

Synthetic steps that cannot be carried out by conventional chemistry







valencene
from orange peels

(+)-nootkatone
0.5 vol.% in grapefruit oil; 1g: 118€)

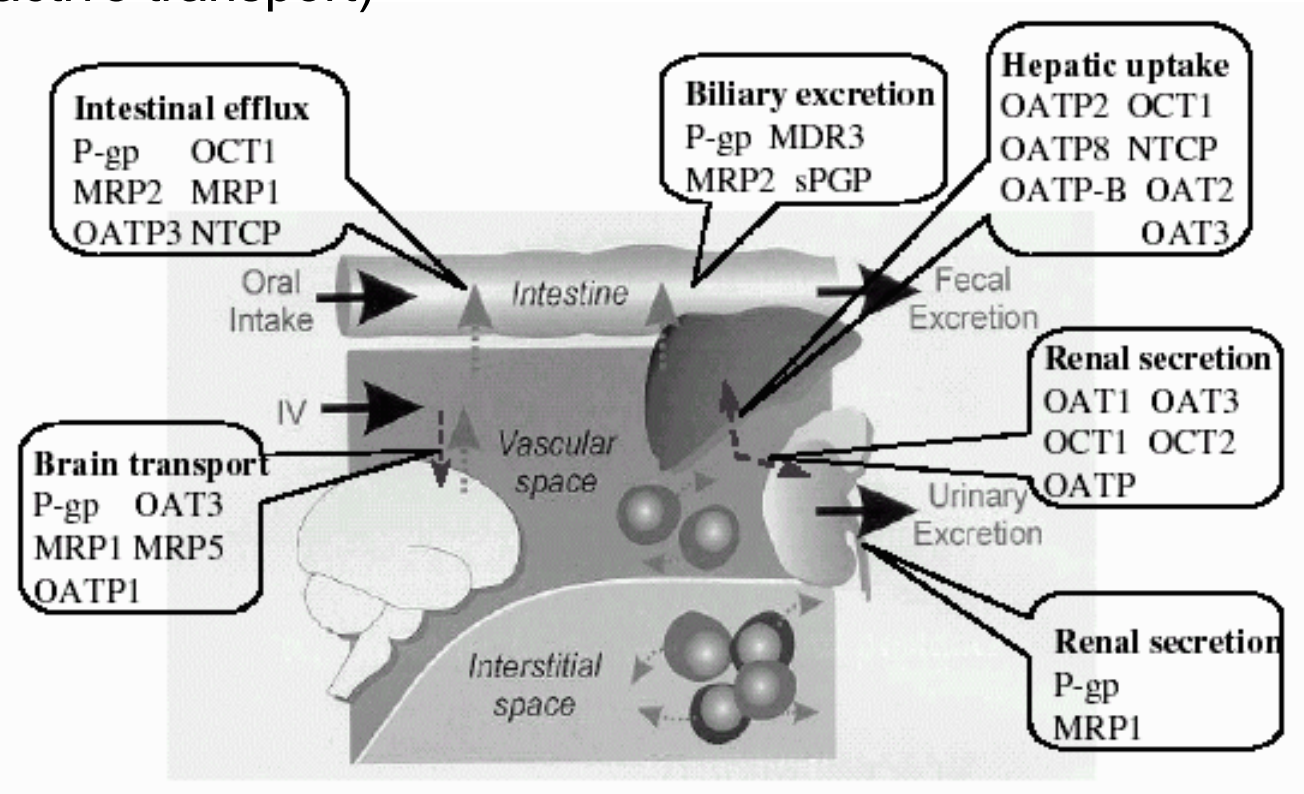
Synthesis of expensive aroma compounds (nootkatone, limonene, citral, ionone) from cheaper natural products.

Synthesis of drug metabolites as test standards.

Picture sources: wikipedia.org

Transporters (I)

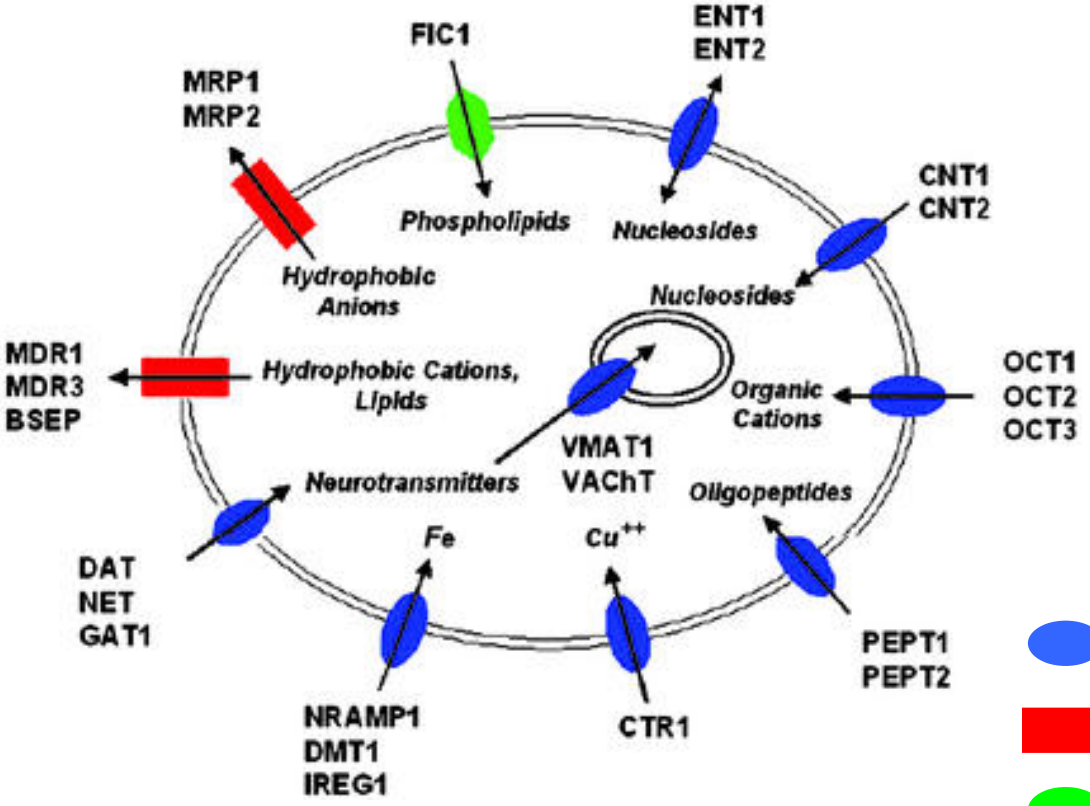
In contrast to the passive diffusion through membranes transporters cause increased *influx* into, or conversely *efflux* from compartments, whereby ATP is consumed. (active transport)



Lit: A.Ayrton et al. *Xenobiotica* **31** (2001) 469

Transporters (II)

Membrane bound transporters involved in the pharmacokinetic of endogenous substances

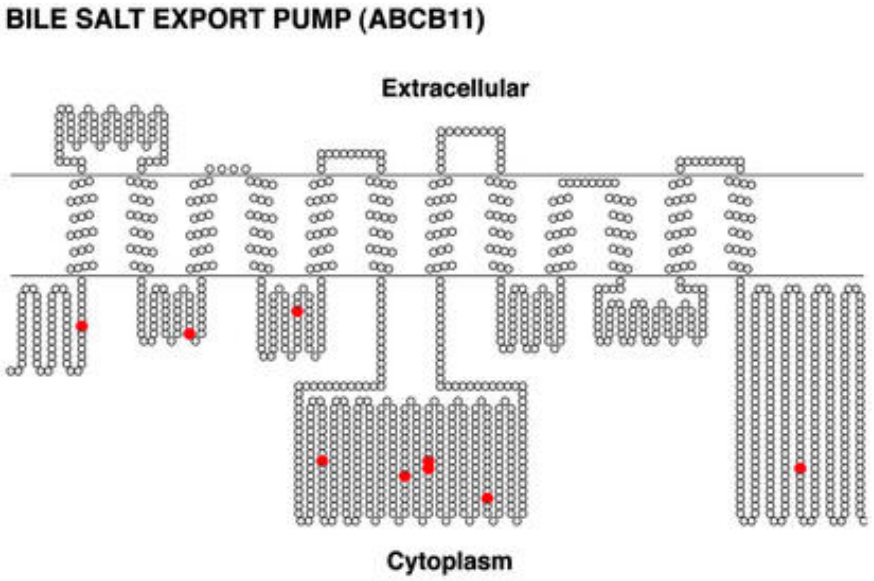


superfamilies:

- solute carriers (SLC)
- ATP-binding cassette (ABC)
- P-type ATPase

Lit: M.K.Leabman et al. *Proc.Nat.Acad.Sci.USA* **100** (2003) 5896

Structure of membrane-bound transporters

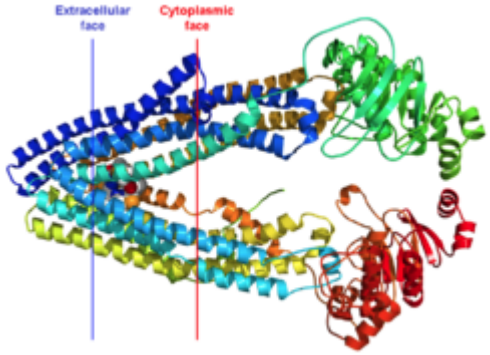


Membrane-bound transporters are proteins with up to 12 and more transmembrane helices that are connected by loops. So far no X-ray structure of a transporter has been achieved.

Lit: M.K.Leabman et al. *Proc.Nat.Acad.Sci.USA* **100** (2003) 5896

P-glycoprotein (P-gp pump, MDR1)

P-gp belongs to the group of *multidrug resistant proteins* (MDR) and is encoded by the ABCB1 gene.



It is an ATP-dependent efflux pump and transmembrane protein.
Especially the bioavailability of antipsychotics is limited by the mediated efflux from the brain and central nervous system back into the system blood circulation.

Likewise transport of substances from the liver into the gastrointestine (*bilary excretion)* e.g. of indinavir

Overexpression of P-gp in cancer cells leads to resistance against antineoplastics.

Lit: A.Ayrton et al. *Xenobiotica* **31** (2001) 469.
A.Seelig *Eur.J.Biochem.* **251** (1998) 252.
picture source: wikipedia.org

Transporter proteins for organic ions

Comprising the families of the

Organic Anion Transporters (OAT) and the

Organic Cation Transporters (hOCT1-3, hOCTN1-2)

The contribute in particular to the hepatic uptake and renal excretion of hydrophilic metabolites and katabolites.

Lit: A.Ayrton et al. *Xenobiotica* **31** (2001) 469.

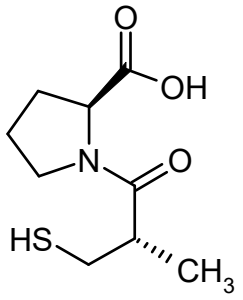
H.Koepsell *Pharmacol. Rev.* **72** (2020) 253.

Transporter proteins for *influx*

There are also transporters that mediate the active uptake of substance from the intestine

PepT1 (intestinal peptide transporter 1, SLC15A1) transmembrane protein possesing 12 TM-helices
Responsible for the uptake of nitrogen!

substrates: small peptides (di- and tripeptide, as well as compounds exhibiting peptide-like features, e.g. captopril)



Polymorphisms of transporters

Also transporters show considerable genetic variations:

gene	protein / function
ABCB1	(ATP-binding cassette subfamily B member 1) P-gp efflux
SLC6A3	(dopamine transporter) neurotransmitter
SLC6A4	(serotonin transporter) neurotransmitter
ADRB2	(β-adrenergic receptor) receptor for β-blockers
ALOX5	(arachidonate 5-lipoxygenase) biosynthesis of leukotrienes

See: D.B.Goldstein et al. *Nature Rev. Genetics* **4** (2003) 937.