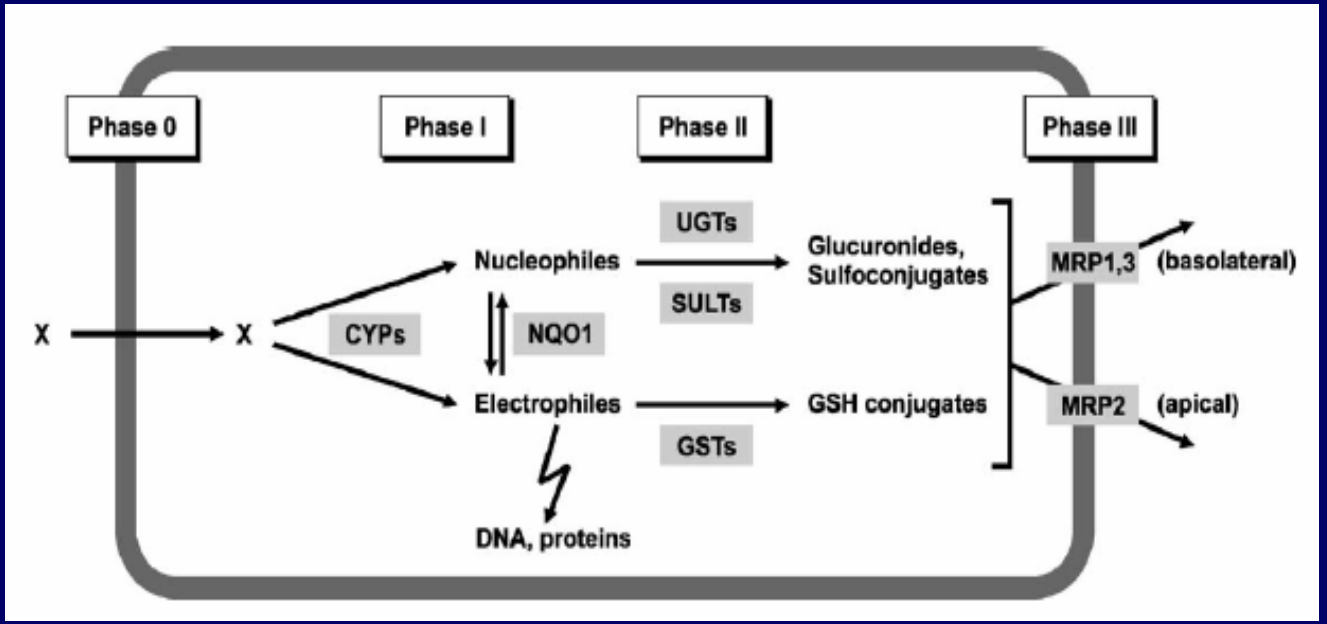
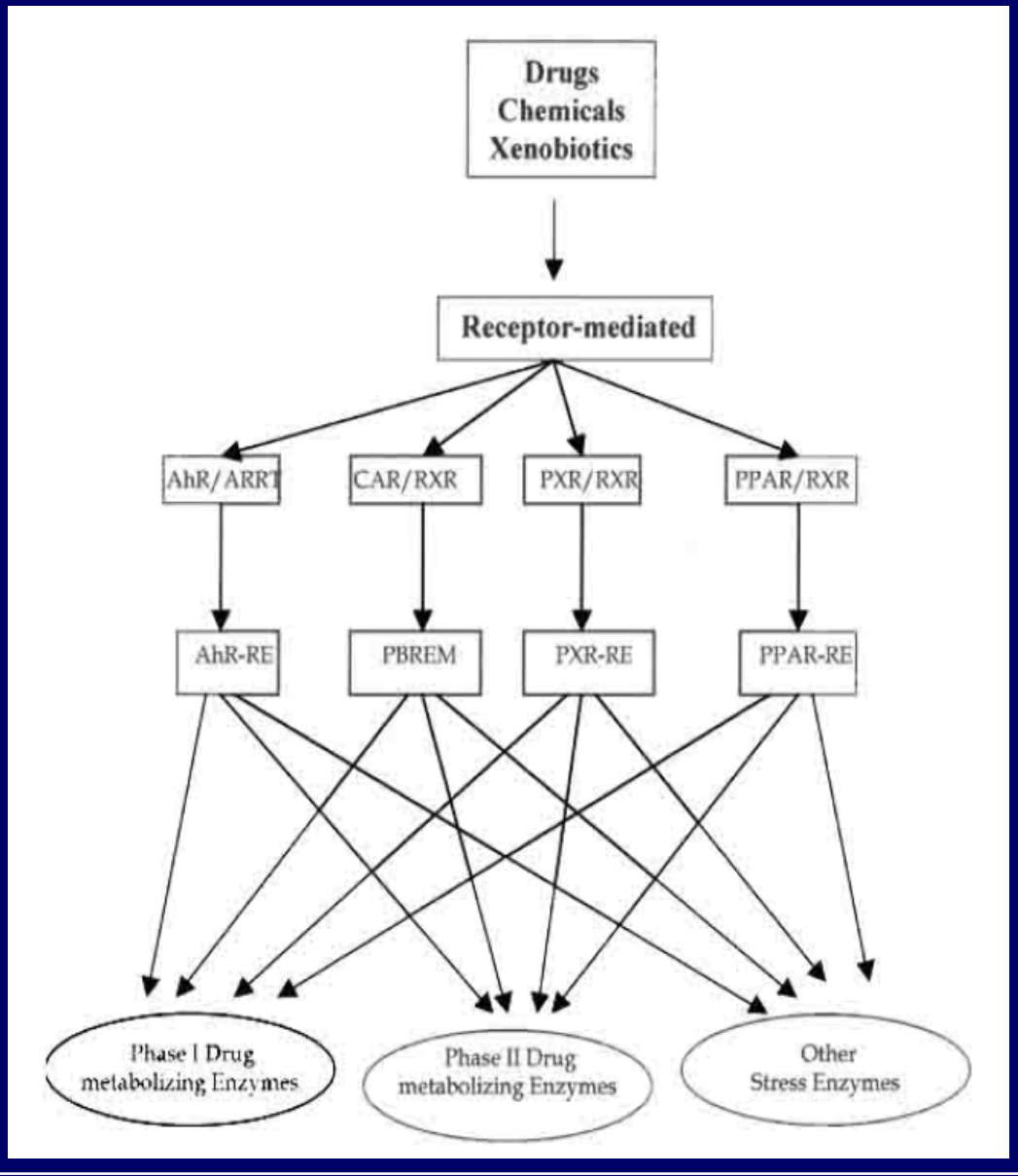
REGULACE BUNĚČNÝCH PROCESŮ

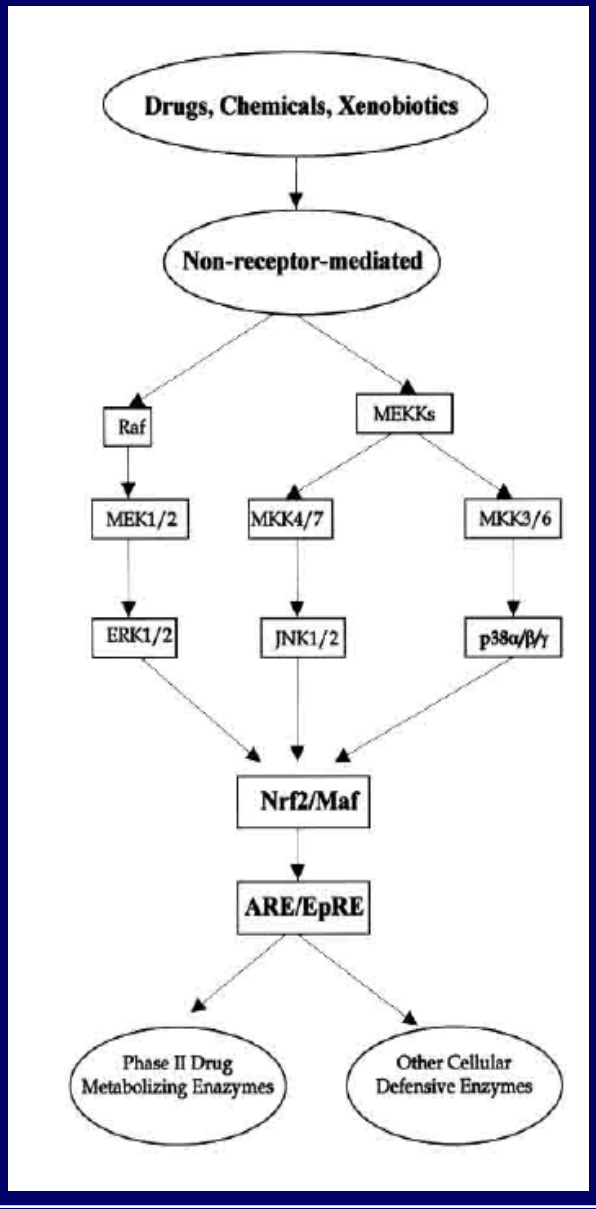
- Regulace tvorby aktivního enzymu: indukce/suprese **biosyntézy** (negat., pozitivní kontrola transkripce, mutace, specifické receptory), regulace enzymové **aktivity** (allosterická regulace, de/fosforylace, zpětnovazebná regulace produktem metabolismu), stabilizace a **degradace** proteinů, nespecifické mechanismy (ztráta energie - NAD(P)H, ATP).
- Typy **signalizace mezi buňkami** (endokrinní, parakrinní, autokrinní, přímé komunikace - GJIC, „tight junctions“, integriny aj.).
- **Intracelulární** signální transdukce (bun. povrchové receptory, aktivace enzymů - MAPK, lipázy, sekundární „messengery“, transkripční faktory).
- Extracelulární chemické stimuly: hormony, růstové faktory, cytokiny, xenobiotika, dietární PUFA atd.

ENZYMY METABOLISMU CHEMICKÝCH REGULÁTORŮ SIGNÁLNÍ TRANSDUKCE:

- Enzymy 1. fáze biotransformace xenobiotik, steroidních hormonů a mastných kyselin (CYP, AKR, FMO, COX, LOX, reduktázy, hydrolázy).
- 2. fáze biotransformace (GST, UDPGT, SULF aj.).
- Mitogen-aktivované proteinkinázy („moduly“ ERK, p38, JNK), fosfatázy; další enzymy biotransformace lipidů (fosfolipázy).
- Další enzymy signální transdukce...

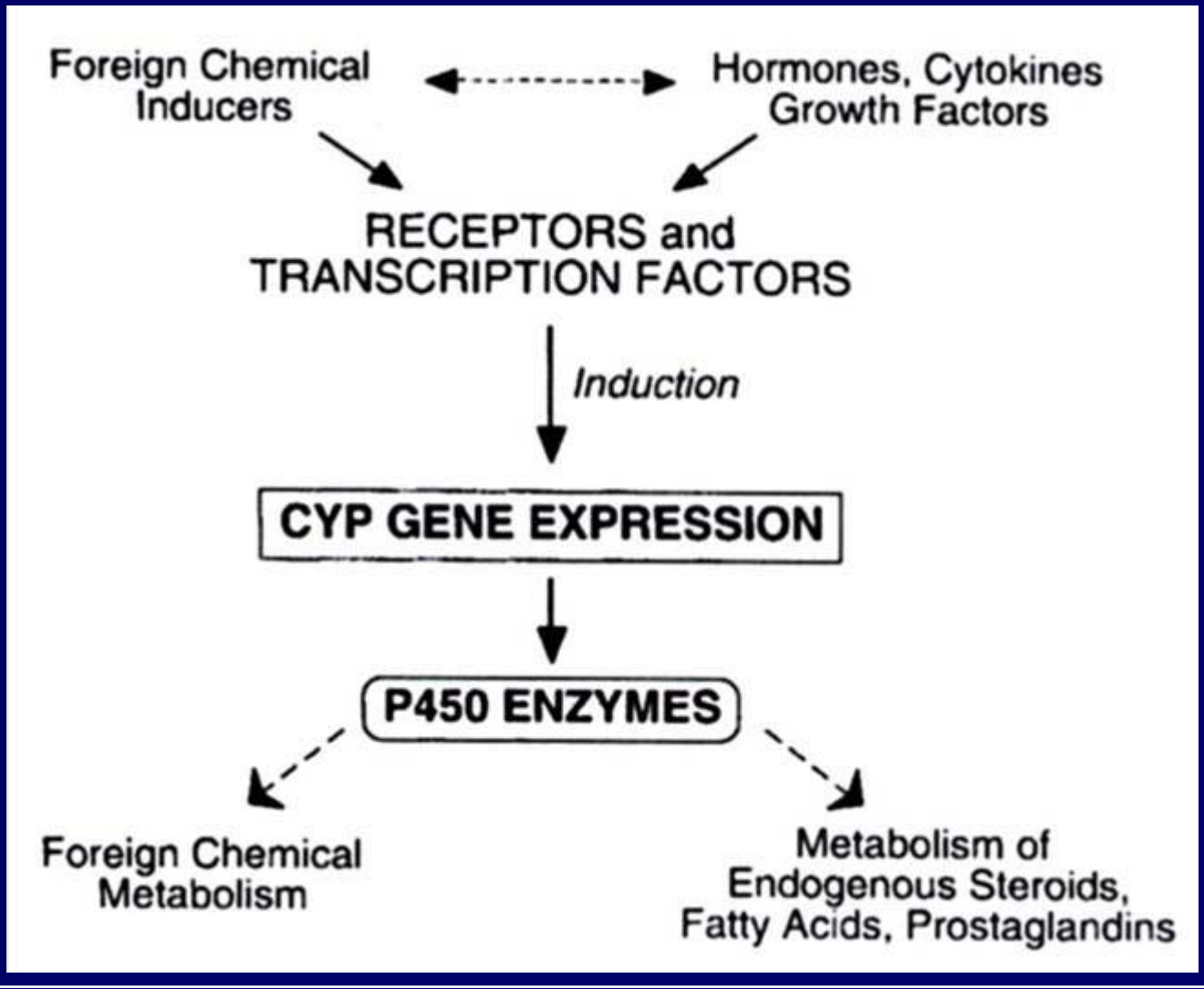

Phase 0
Phase I
Phase II
Phase III
X
X
CYPs
Nucleophiles
NQO1
Electrophiles
DNA, proteins
UGTs
SULTs
GSTs
Glucuronides,
Sulfoconjugates
GSH conjugates
MRP1,3
(basolateral)
MRP2
(apical)


Drugs
Chemicals
Xenobiotics
Receptor-mediated
AhR/ARRT
CAR/RXR
PXR/RXR
PPAR/RXR
AhR-RE
PBREM
PXR-RE
PPAR-RE
Phase I Drug metabolizing Enzymes
Phase II Drug metabolizing Enzymes
Other Stress Enzymes


Drugs, Chemicals, Xenobiotics
Non-receptor-mediated
Raf
MEKKs
MEK1/2
MKK4/7
MKK3/6
ERK1/2
JNK1/2
p38α/β/γ
Nrf2/Maf
ARE/EpRE
Phase II Drug Metabolizing Enazymes
Other Cellular Defensive Enzymes

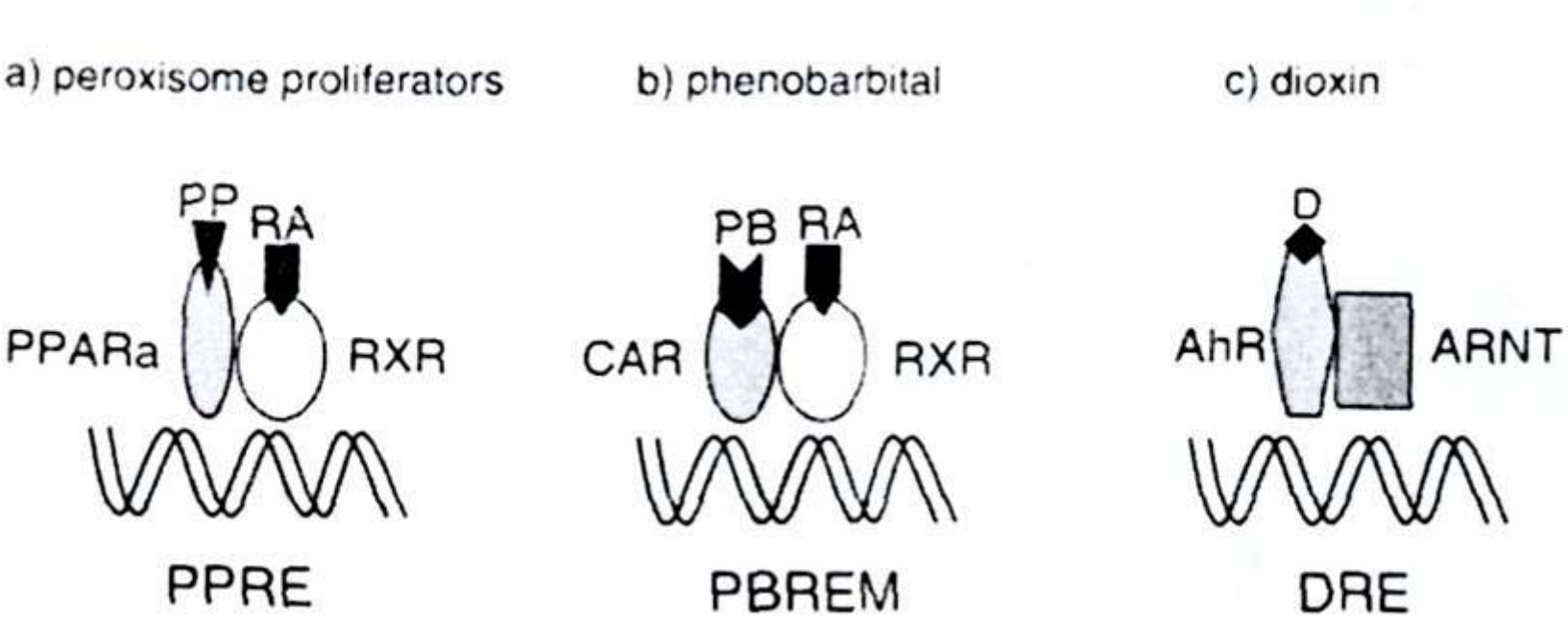
RECEPTORY REGULUJÍCÍ EXPRESI CYP ENZYMŮ / JADERNÉ RECEPTORY STEROIDŮ A LIPIDŮ

NUKLEÁRNÍ A CYTOSOLOVÉ RECEPTORY



NUKLEÁRNÍ A CYTOSOLOVÉ RECEPTORY

nukleární receptory **cytosolový receptor**



NUKLEÁRNÍ A CYTOSOLOVÉ RECEPTORY

CYP Induction Mediated by Nuclear Receptors

P450 inducing agents	Prototypic responsive rat liver CYPs	Receptor
Polycyclic aromatic hydrocarbons	1A1, 1A2, 1B1	Ah receptor[a]
Phenobarbital	2B1, 2B2	CAR
Dexamethasone	3A1, 3A2, 3A23	PXR
Fibrate drugs	4A1, 4A2, 4A3	PPARα
Cholesterol	7A1	LXRα
Bile acids[b]	7A1	FXR
Thyroid hormone	P450 reductase	TR

[a] PAS transcription family member, not a nuclear receptor.
[b] Inhibitors of CYP7A1 transcription.

Nuclear Receptors: Endogenous Ligands and DNA Response Elements in Target CYP Genes

Nuclear receptor	Representative endogenous ligands[a]	AGGTCA-based DNA response element[b]
CAR	Androstanol, androstenol	DR4
PXR	Pregnenolone, corticosterone	DR3, ER6
PPARα	Linoleic acid, arachidonic acid	DR1
LXRα	24(*S*)-hydroxycholesterol	DR4
FXR	Chenodeoxycholic acid	IR1
TR	Thyroid hormone	DR4

SEZNAM CYTOCHROMŮ P450 (CYP) ZODPOVĚDNÝCH ZA BIOSYNTÉZU / METABOLISMUS STEROIDŮ A METABOLISMUS XENOBIOTIK

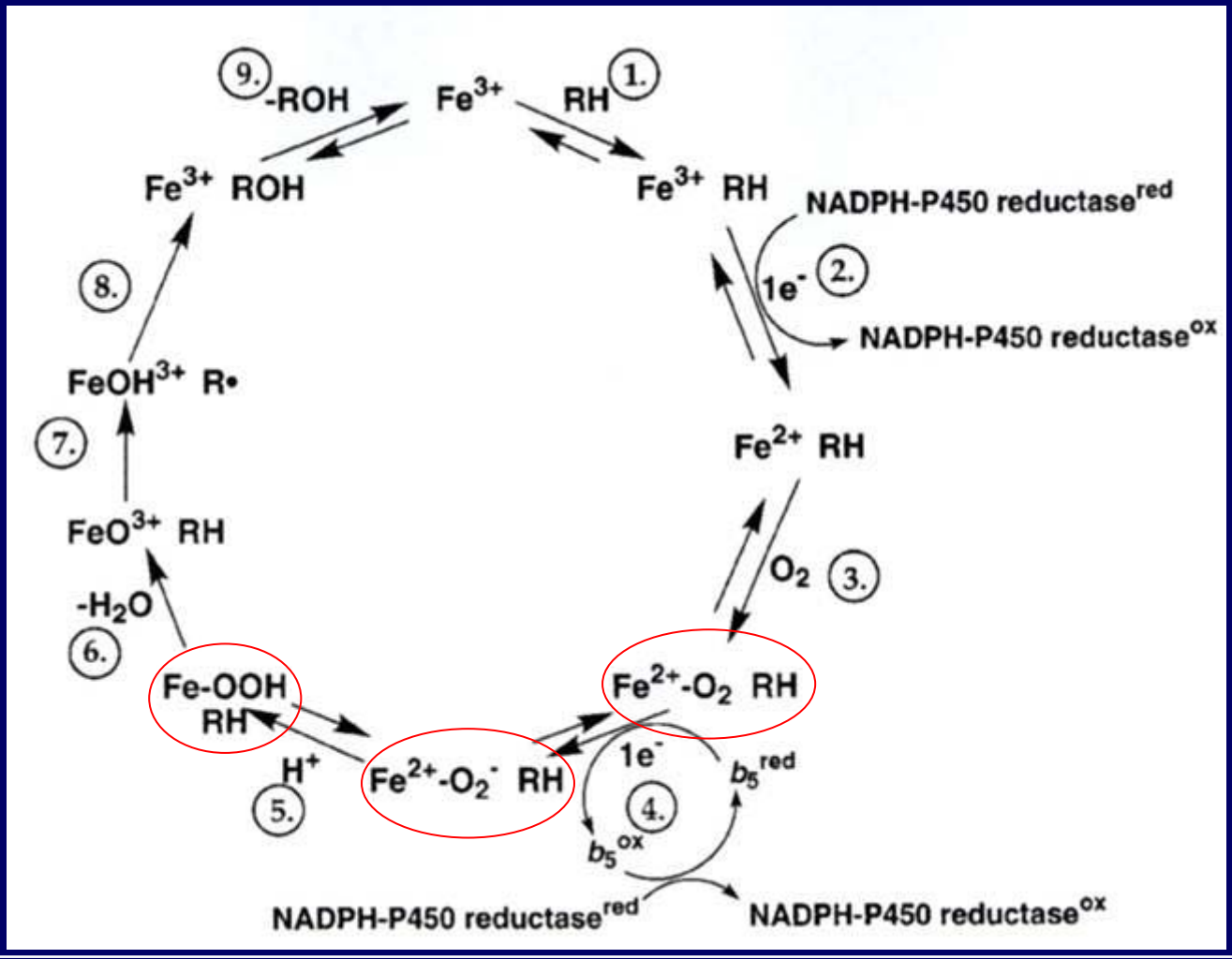
- CYP1A1, CYP1A2, CYP1B1: genová exprese regulována AhR (indukce ioxiny, PAH; endogenní ligandy?) monooxygenace PAH, estradiolu;
- CYP2B: exprese regulována GR/CAR, indukce steroidy, fenobarbitalem, onooxygenace velké řady xenobiotik, testosteronu aj.
- CYP2A, CYP2C, CYP2D
- CYP2E: indukce především stabilizací proteinu (indukují etanol, pyrazol aj.), onooxygenace etanolu, (ω-1)-hydroxylace mastných kyselin
- CYP3A: exprese regulována GR/PXR; indukce dexametazonem aj. steroidy, onooxygenace velké řady xenobiotik (nejdůležitější enzym biotranformace), β-hydroxylace testosteronu
- CYP4A: exprese regulována PPARα, indukce peroxisomálními proliferátory clofibrate, dialkylestery kyseliny ftalové), ω-hydroxylace mastných kyselin
- CYP7A, CYP11A, CYP17, CYP19 (aromatáza): enzymy steroidogeneze
- další důležité CYP metabolismu vitamínu D3 a kyseliny retinové

CYKLUS CYTOCHROMU P450: MONOOXYGENACE A AKTIVACE KYSLÍKU

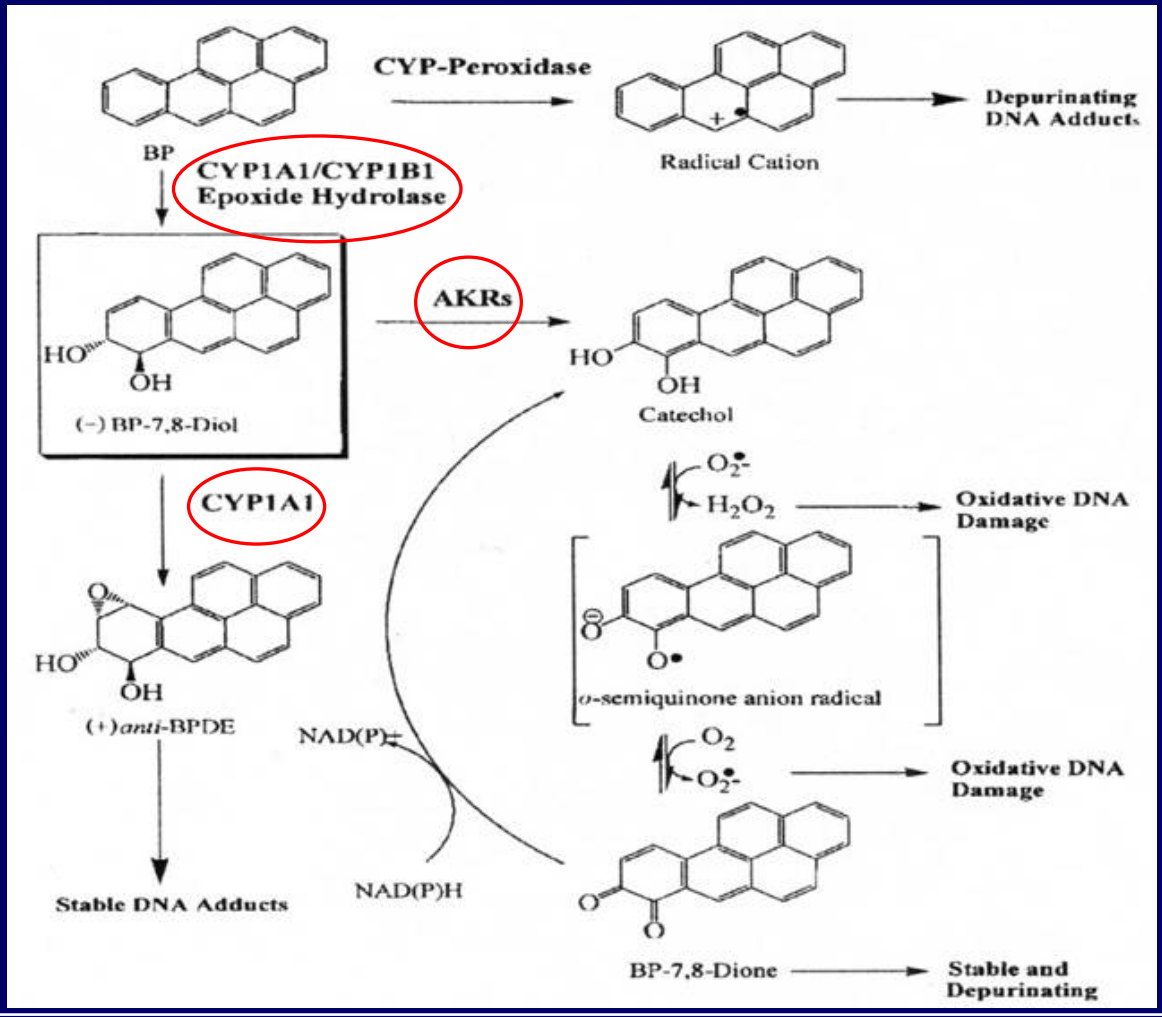
Přenos elektronů:
NADPH
↓
reduktáza
↓
cytochrom P450

substrát RH,
produkt ROH,
produkce ROS)

Aktivace kyslíku:
ROS = vedlejší
produkty



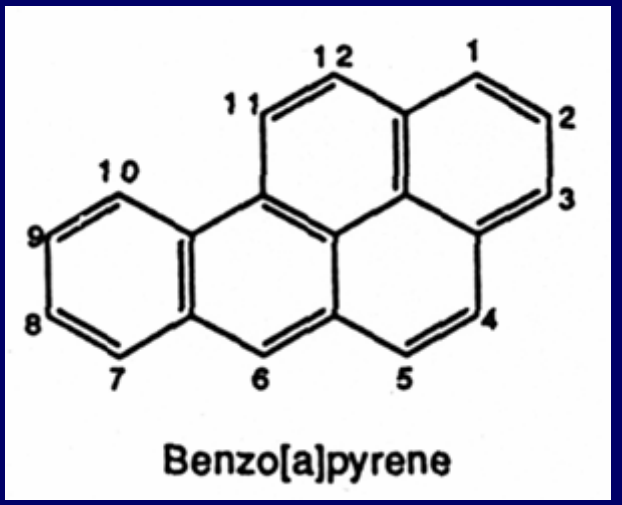
Příklad monooxygenázové reakce: účast CYP1A1/1A2/1B1, EH a AKR



SPECIFICKÉ SUBSTRÁTY CYP1 ENZYMŮ

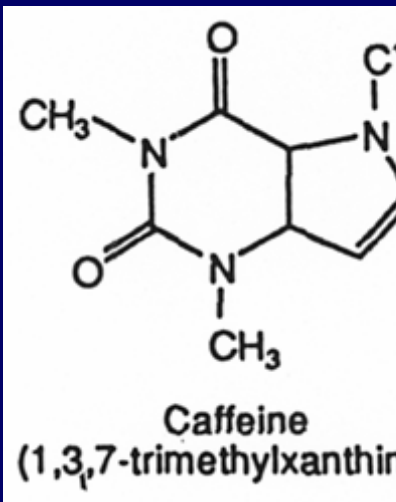
Alkylresorufins
(alkoxyphenoxazones)

EROD / MROD



Benzo[a]pyrene

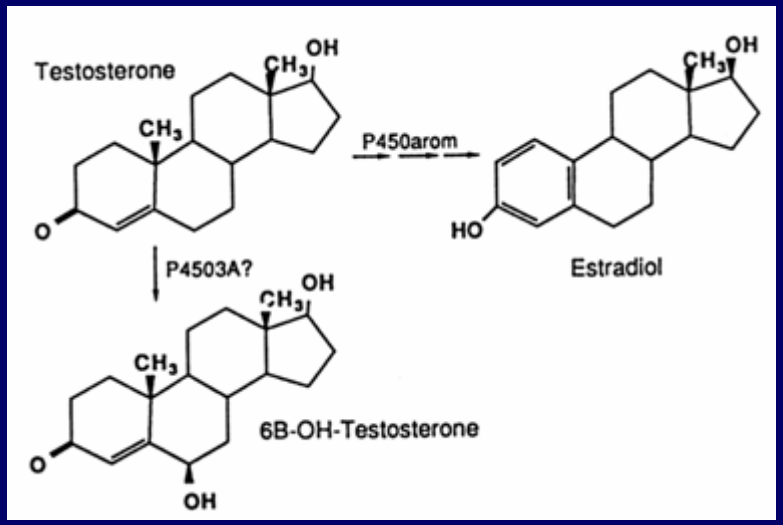
BaP-hydroxyláza

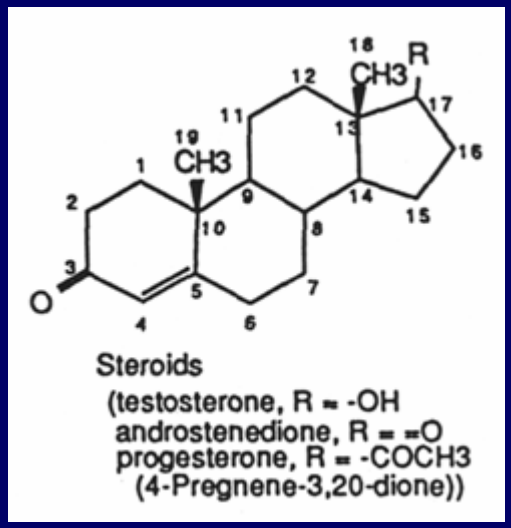


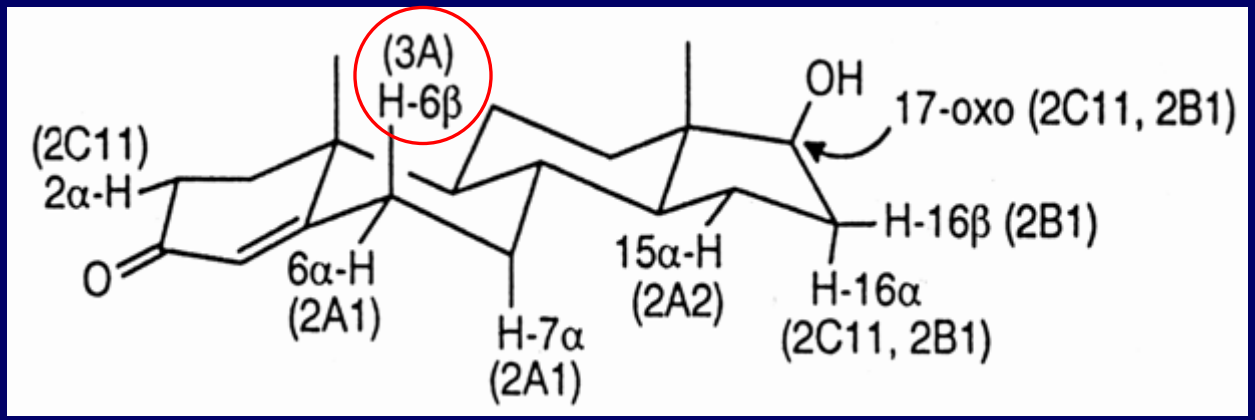
Caffeine
(1,3,7-trimethylxanthin

Kofeindemethylázy

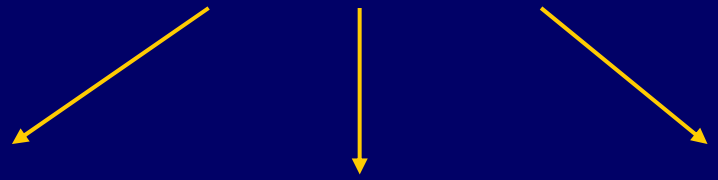
SPECIFICKÉ SUBSTRÁTY CYP ENZYMŮ







CYTOSOLOVÉ RECEPTORY: AhR (Aryl hydrocarbon receptor)



CYP1A1/1A2/1B1, UGT, GST, NQO1

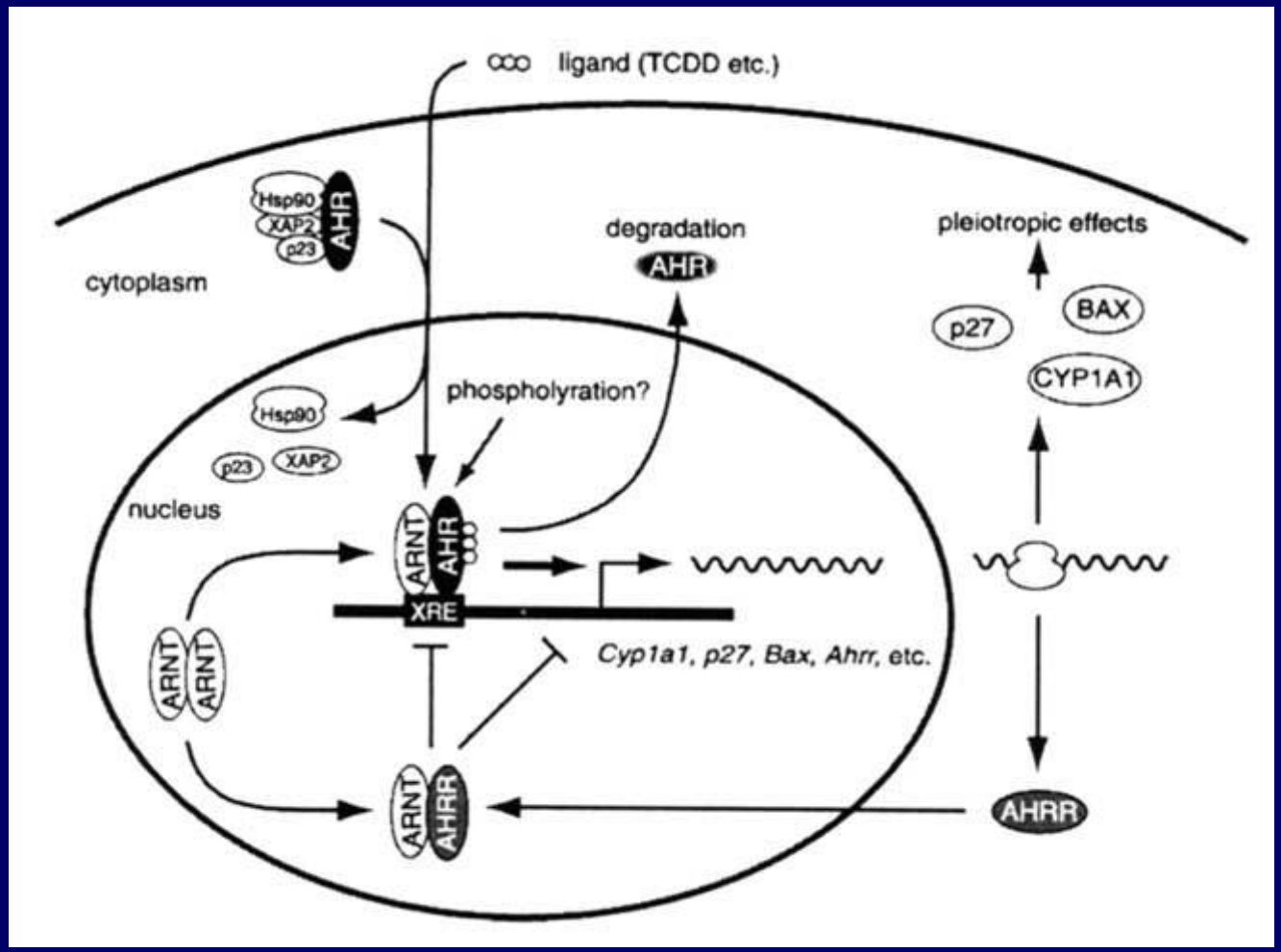
p27, Bax, ...

přímé interakce (ER?,..)

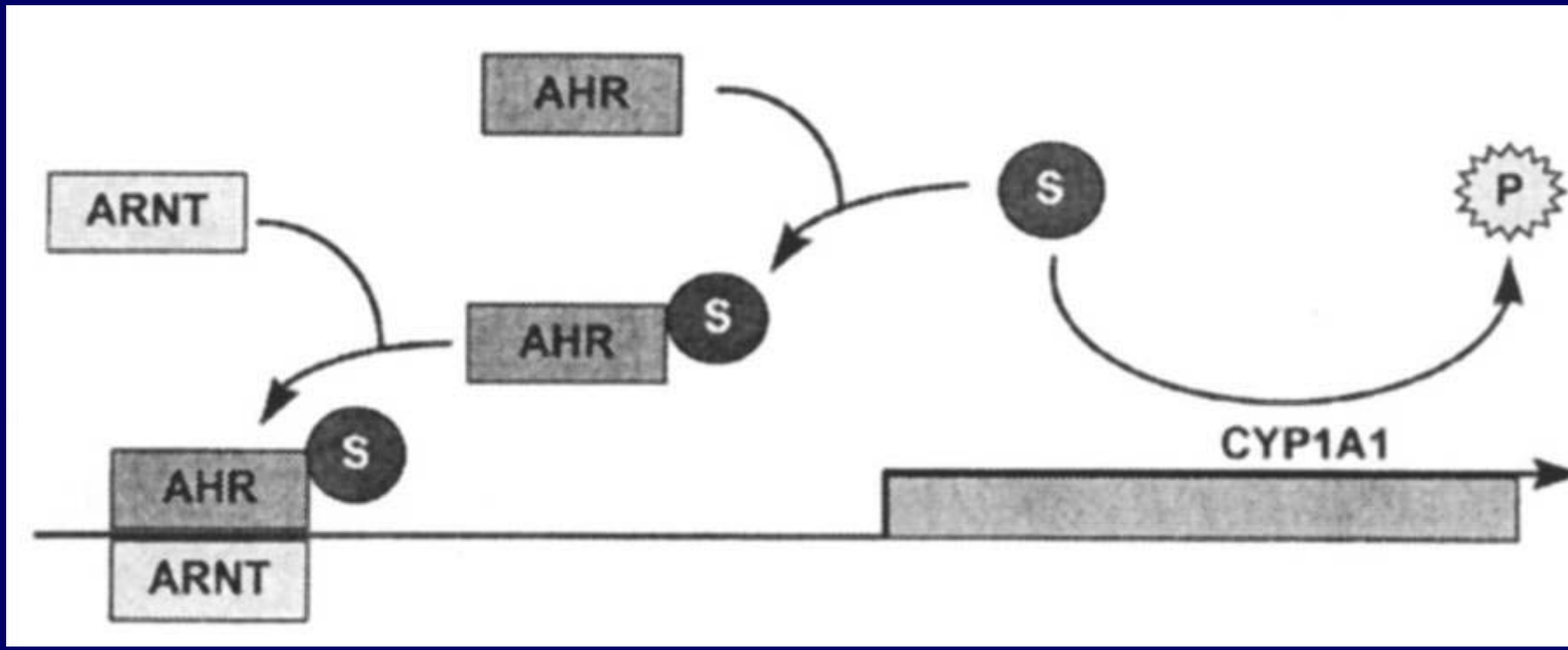
AKTIVACE Ah RECEPTORU / AhR-DEPENDENTNÍ GENOVÁ EXPRESE

rodukty genové
xprese:
letox./bioaktiv.
nzymy;
apoptóza;
bun. cyklus;

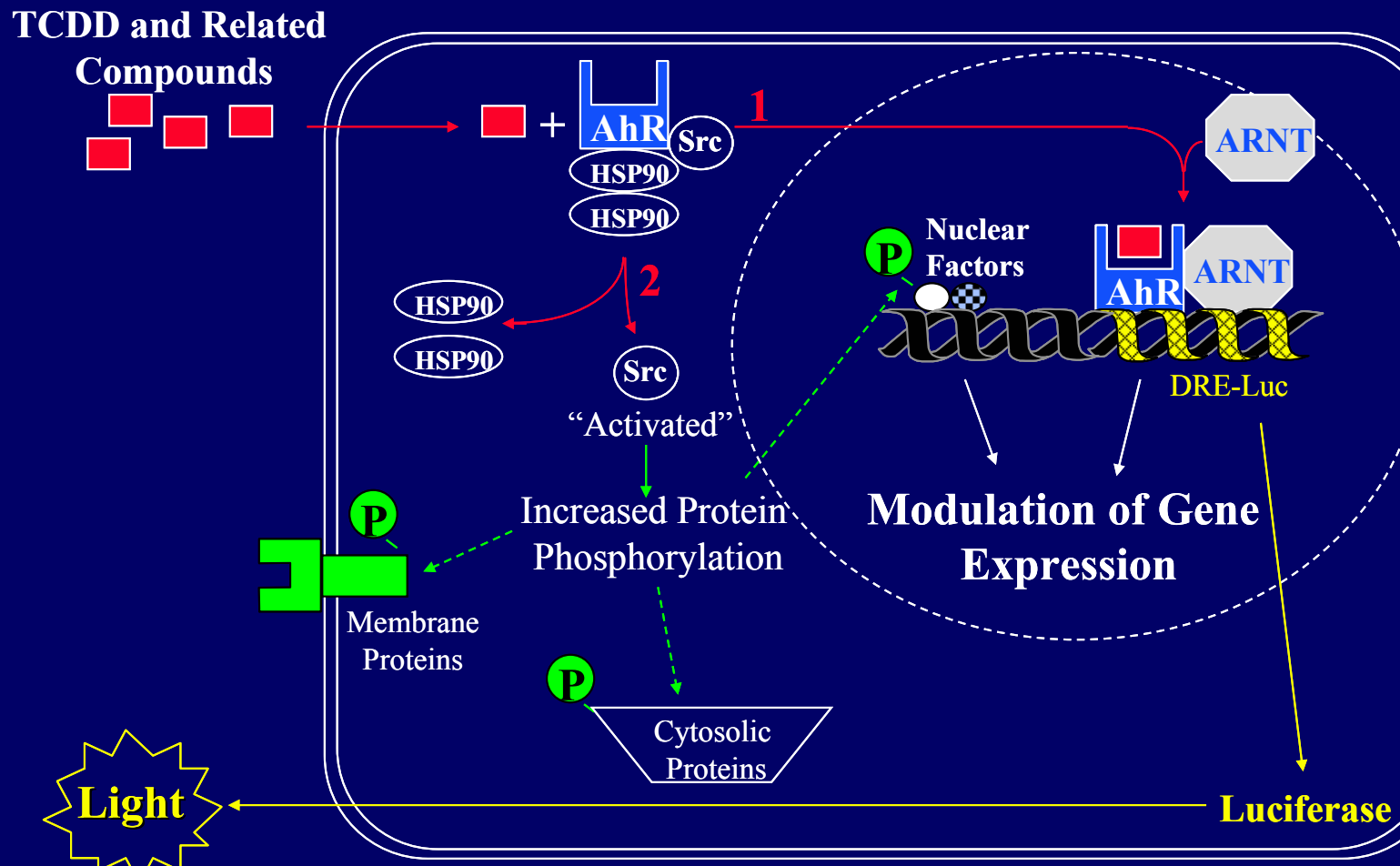
římé interakce
hR:
un. cyklus;
poptóza;
levelop. procesy



AKTIVACE Ah RECEPTORU A INDUKCE CYP1A1 SUBSTRÁTEM CYP1A



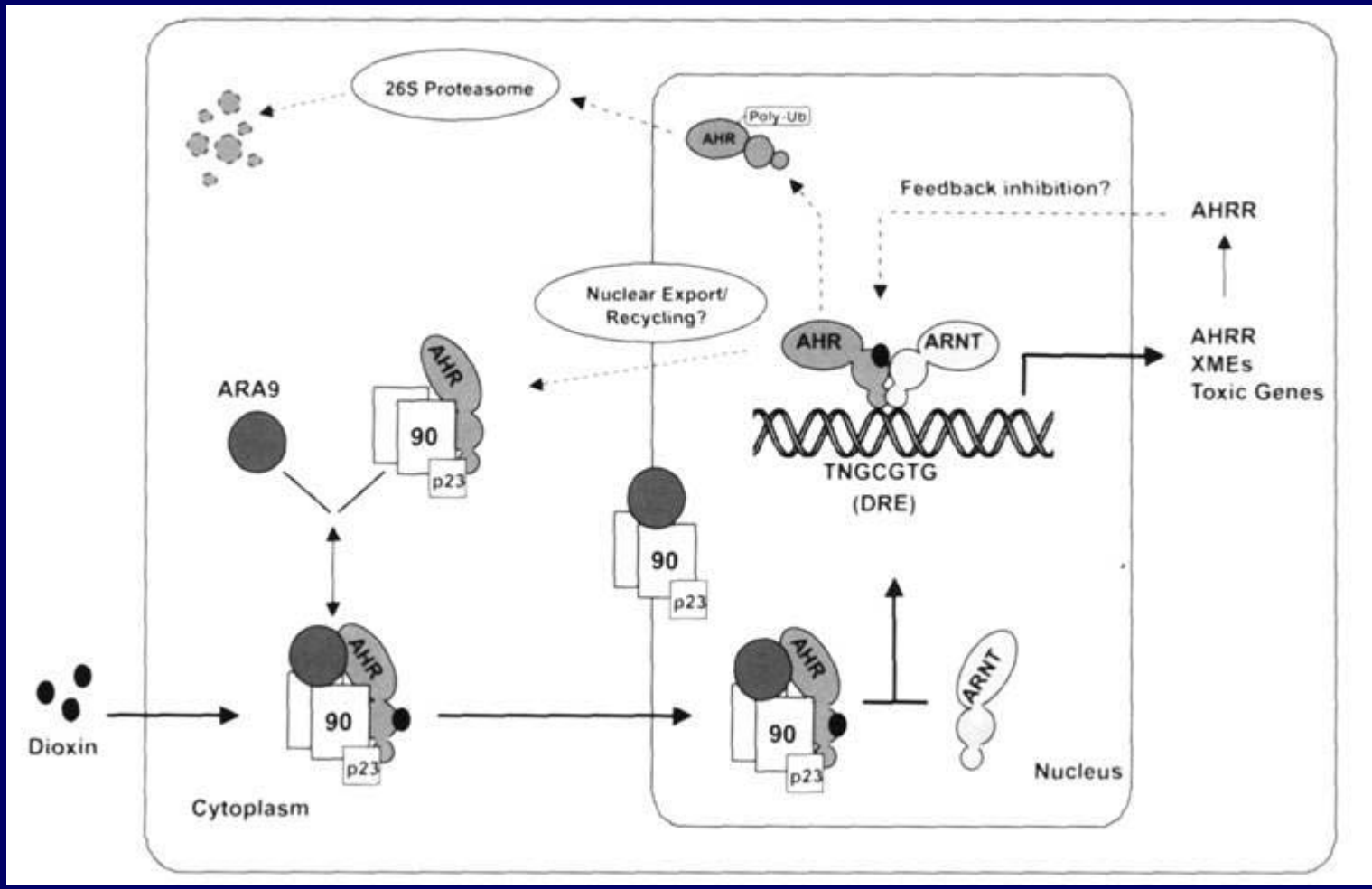
AKTIVACE Ah RECEPTORU
(transgenní model - luciferázový reportérový gen)



Adapted from Blankenship (1994)

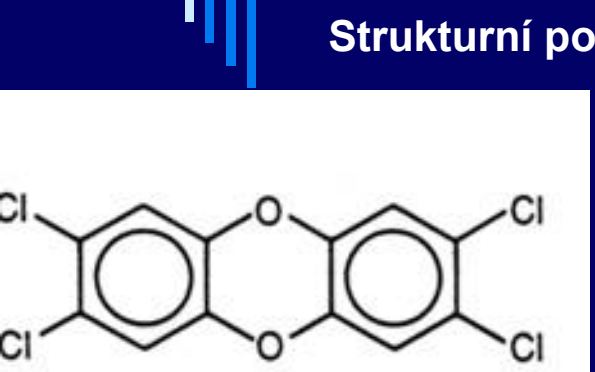
ARYL HYDROCARBON RECEPTOR

„Cyklus“ AhR:
AhR represor,
degradace

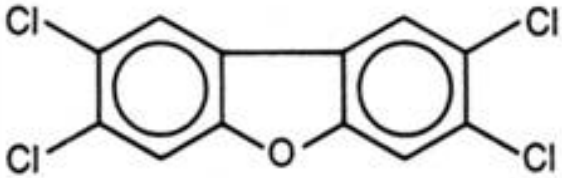


LIGANDY AhR

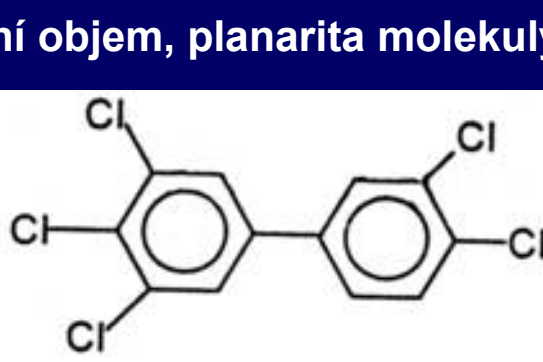
Strukturní podobnost (šířka, výška, molekulární objem, planarita molekuly



,3,7,8-tetra-chlorodibenzodioxin
(TCDD)

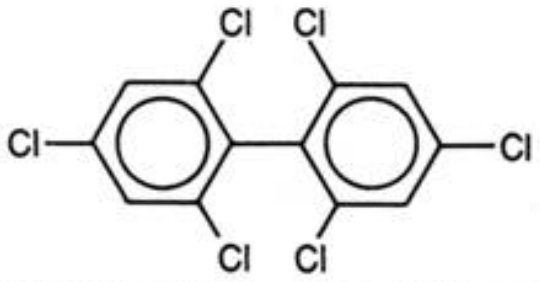


2,3,7,8-tetra-chlorodibenzofuran
(TCDF)



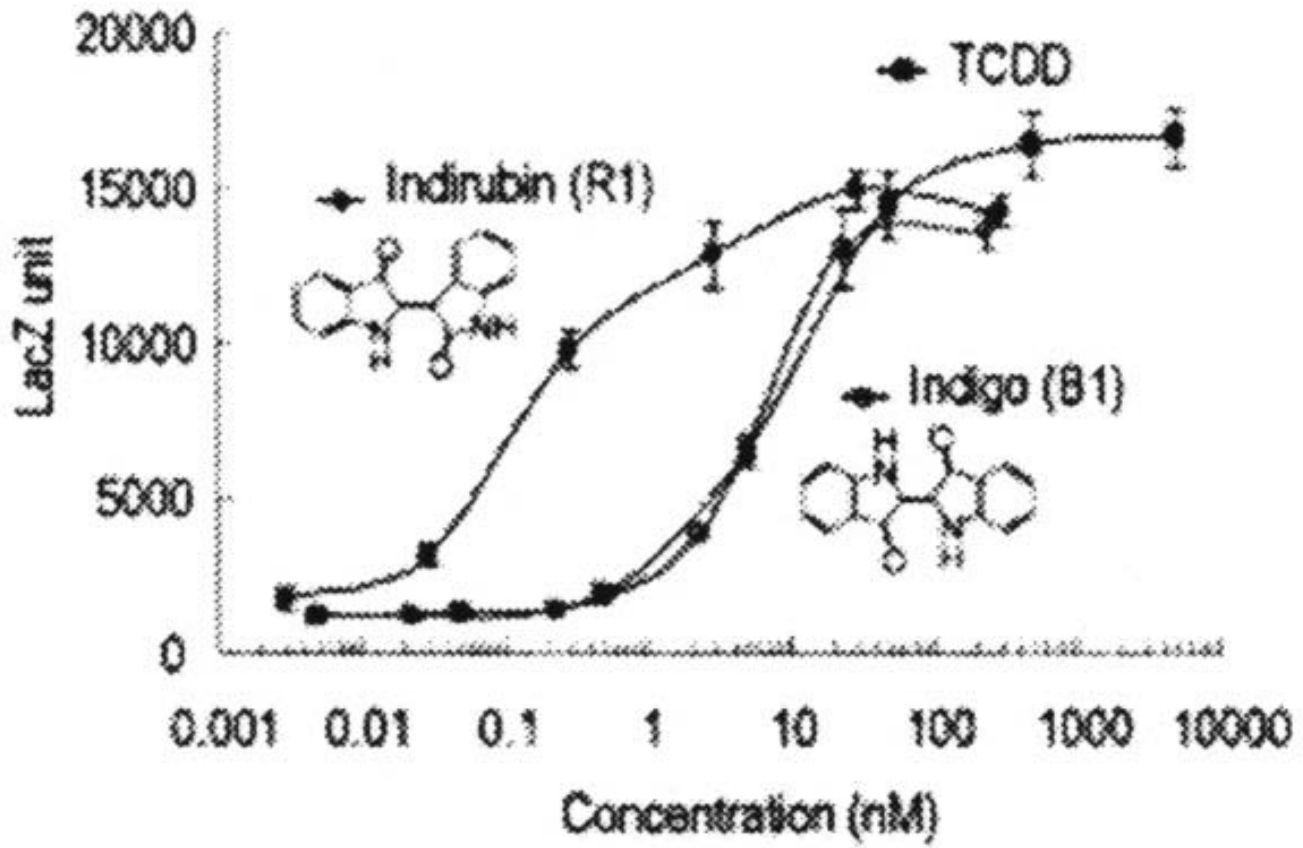
3,4,5,3',4'-pentachlorbifenyl
(PCB)

Nekoplanární látky (např. PCB se dvěma chlóry v pozici ortho) se nevyskytují v planární pozici aromatických jader a nejsou agonisty AhR



2,2',4,4',6,6'-Hexachlorobiphenyl
(Not coplanar)

ENDOGENNÍ AGONISTÉ AhR

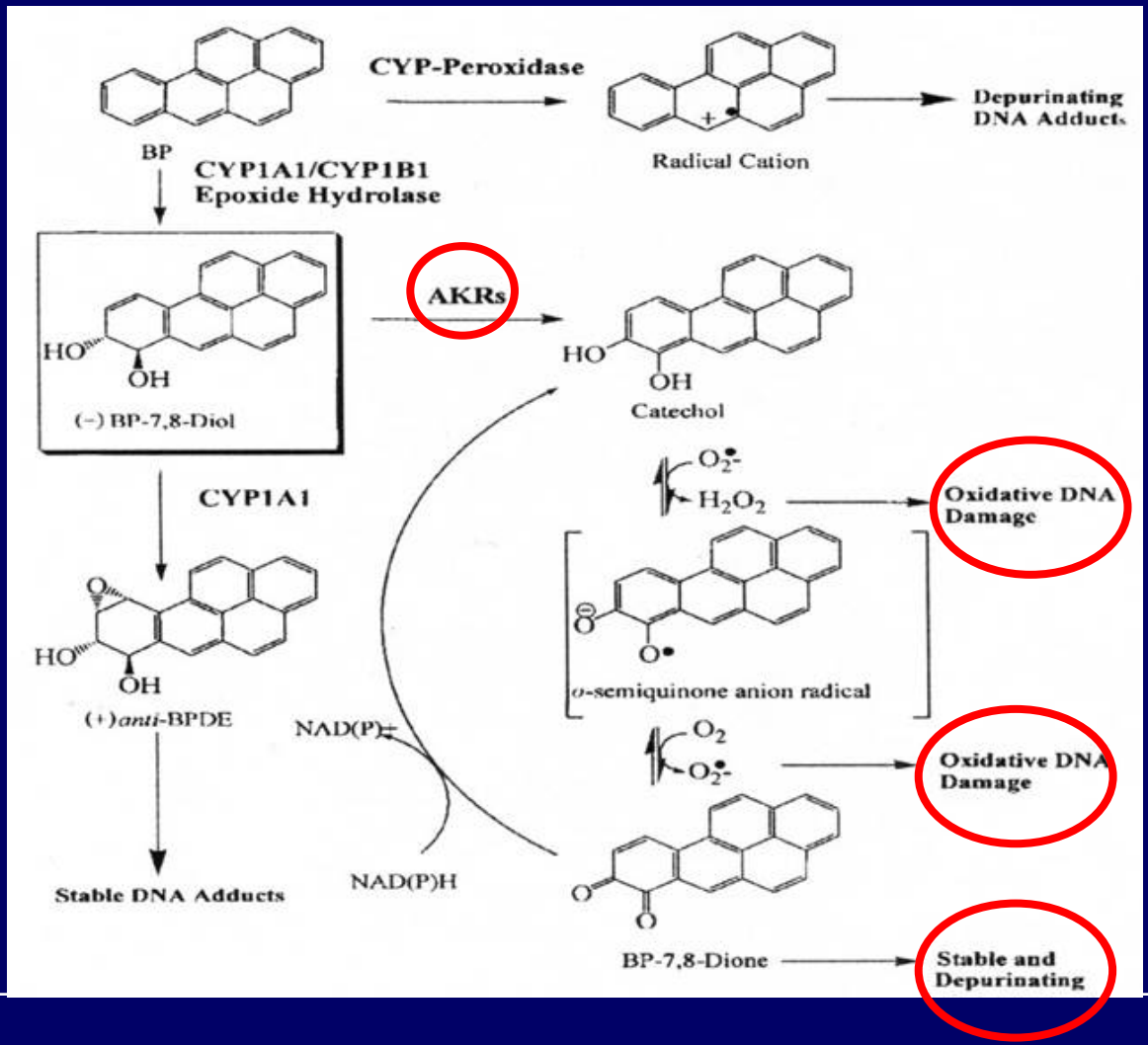


ALDOKETOREDUKTÁZY

-většinou monomerické NAD(P)(H)-dependentní oxidoreduktáz
- konvertují karbonyl $\rightleftharpoons$ alkohol
- dosud 115 enzymů ve 14 „genových rodinách“
- substrátová specifita: cukerné aldehydy; steroidní hormony; prostaglandiny a lipidové aldehydy; chemické karcinogeny (NNK, PAH-*trans*-dihydrodioly, aflatoxindialdehyd)

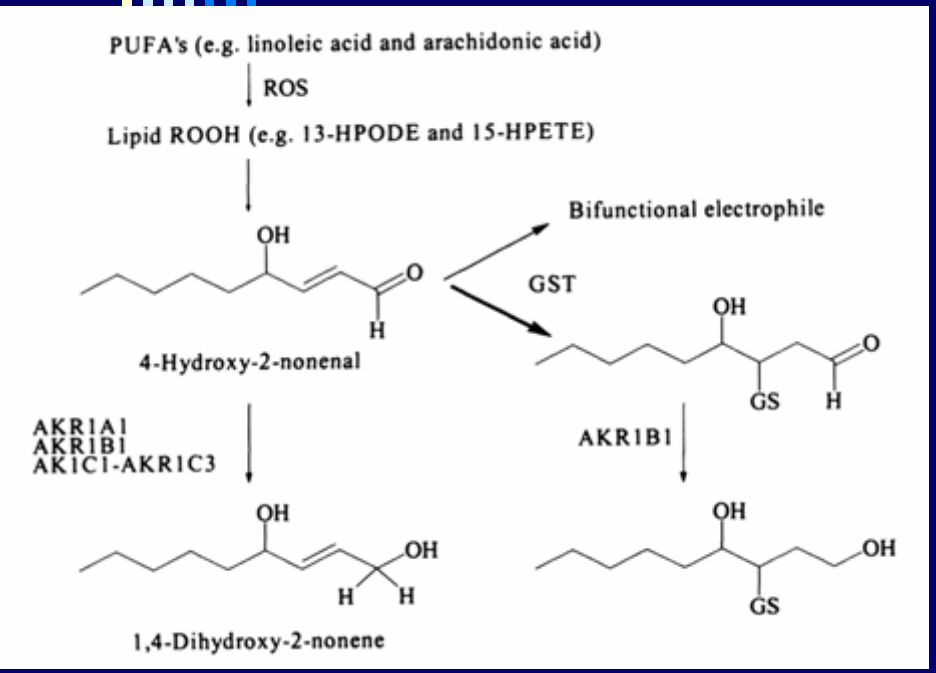
Příklady lidských AKR:
AKR1A1 (aldehydreduktáza)
AKR1B1 (aldosareduktáza)
AKR1C1-1C4 (20α-, 3α-, 3α/17α-, 3α-HSD)
AKR1D1 (5β-reduktáza)

Příklad monooxygenázové reakce: účast CYP1A1/1A2/1B1, EH a AKR

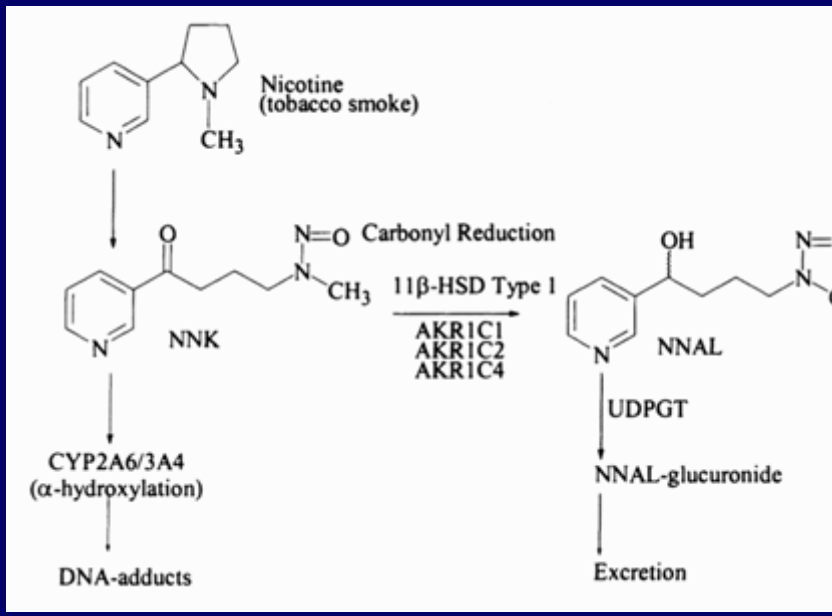


katecholy =
- metabolity PAH
-metabolity steroidů (E2)

ALDOKETOREDUKTÁZY

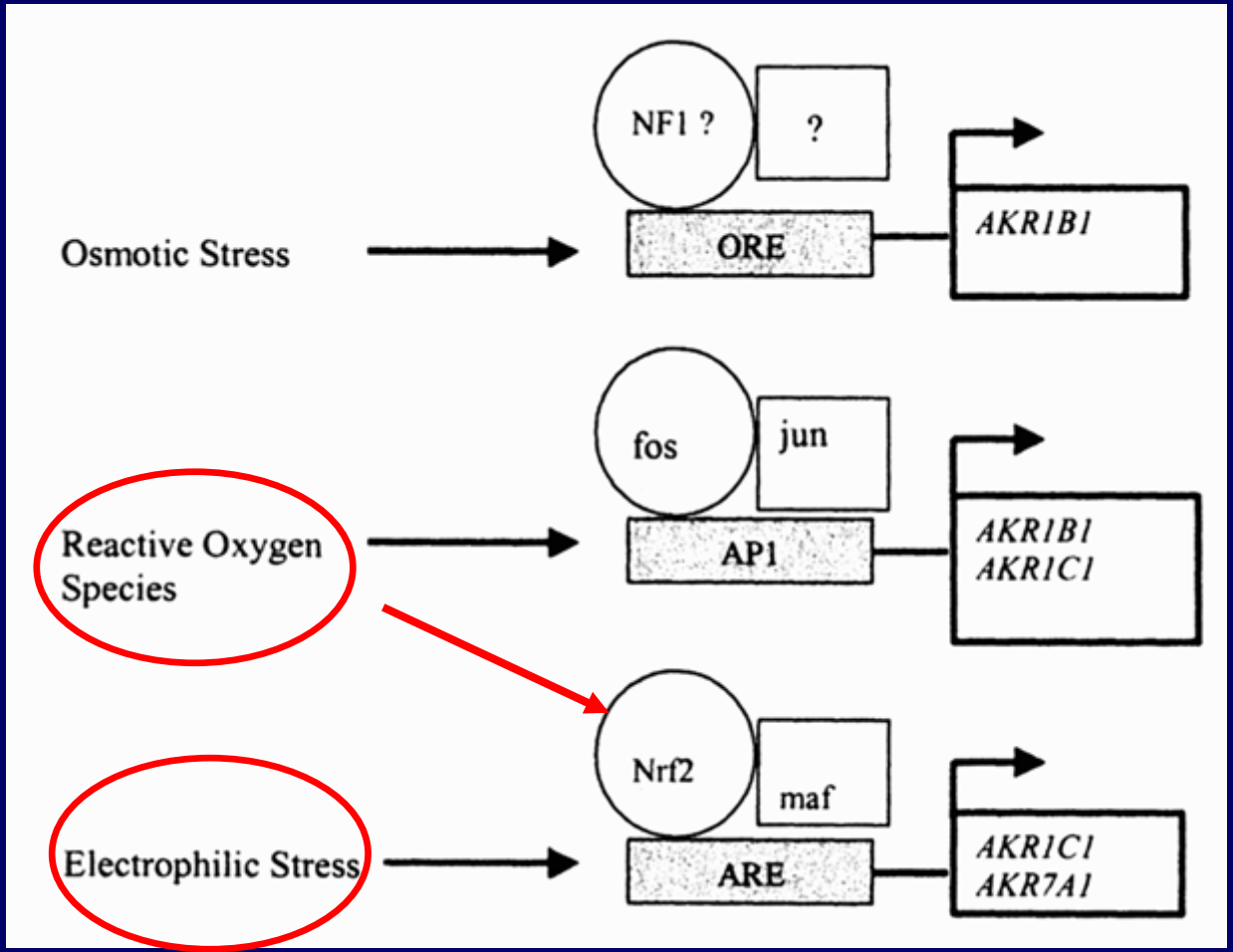


Biotransformace aldehydů
lipidní peroxidace

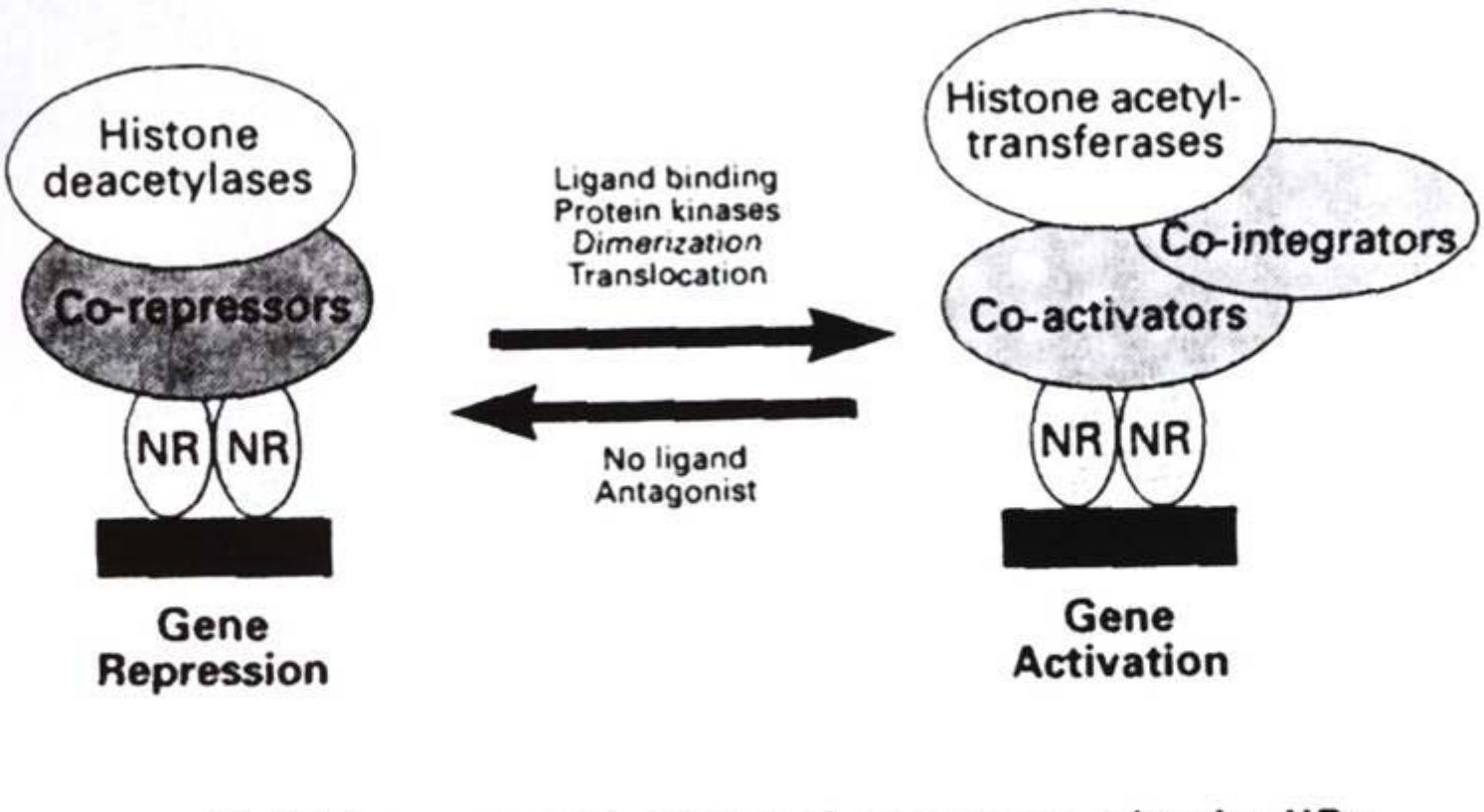


Detoxikace NNK

REGULACE ALDOKETOREDUKTÁZ

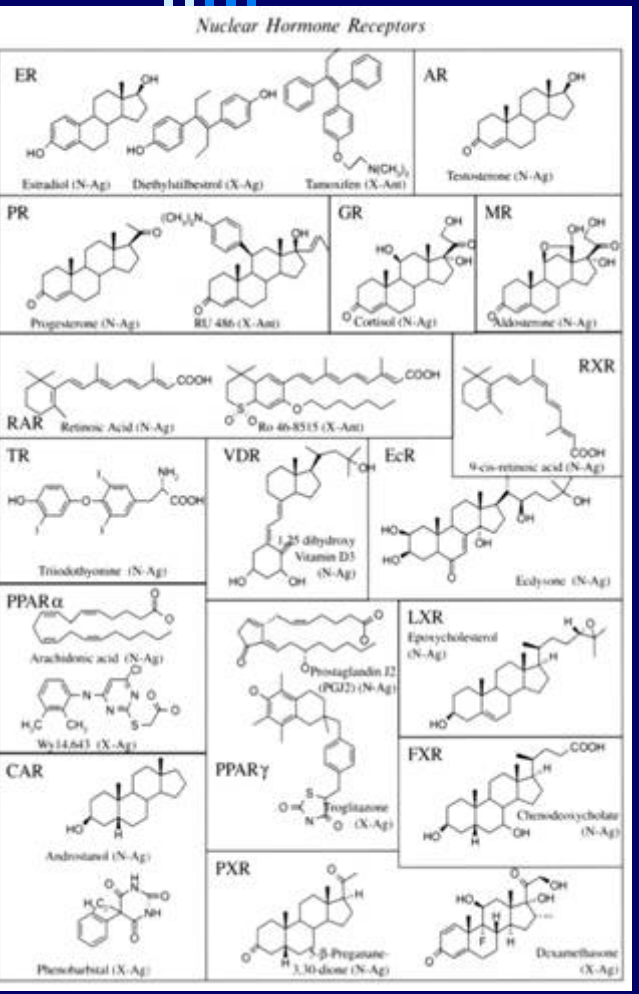


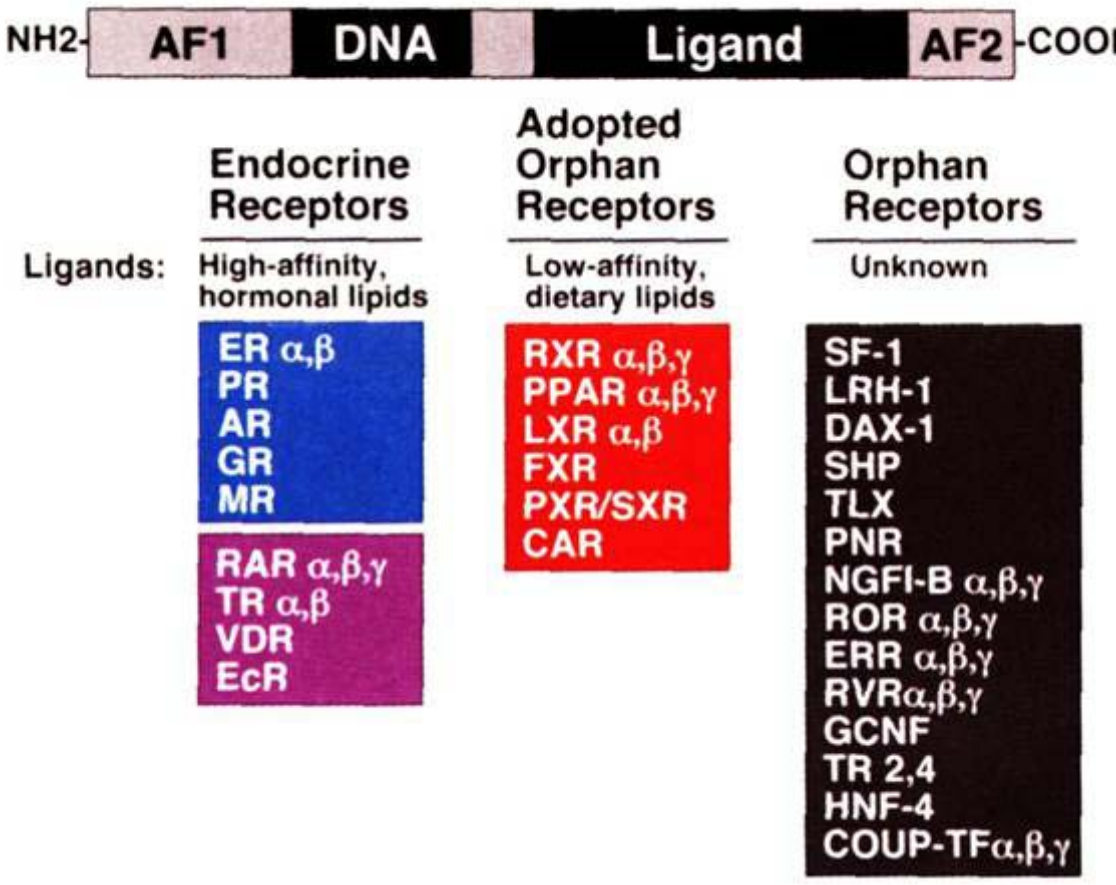
NUKLEÁRNÍ RECEPTORY



Model for gene activation and gene repression by NRs

NUKLEÁRNÍ RECEPTORY

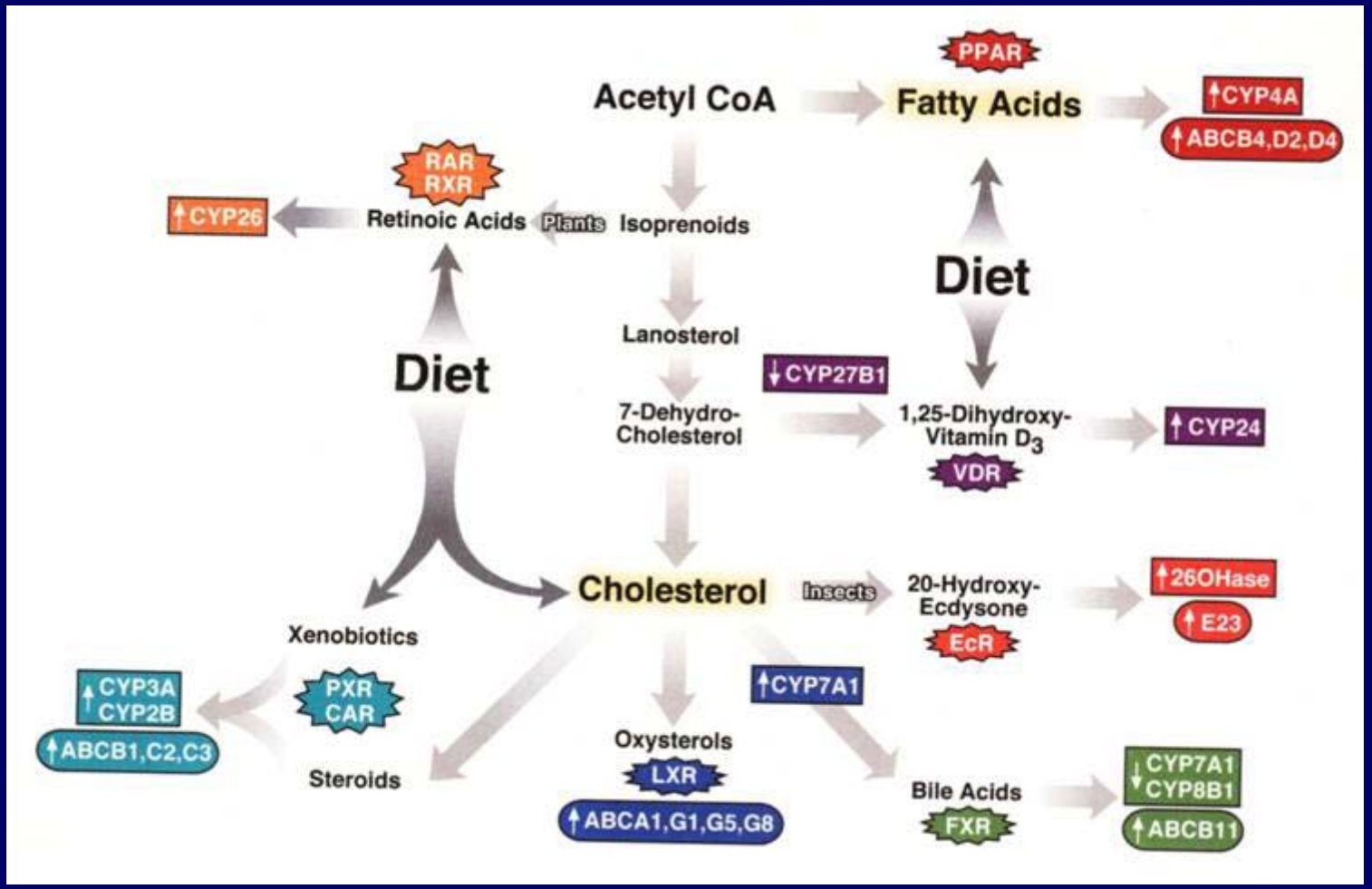




NUKLEÁRNÍ RECEPTORY

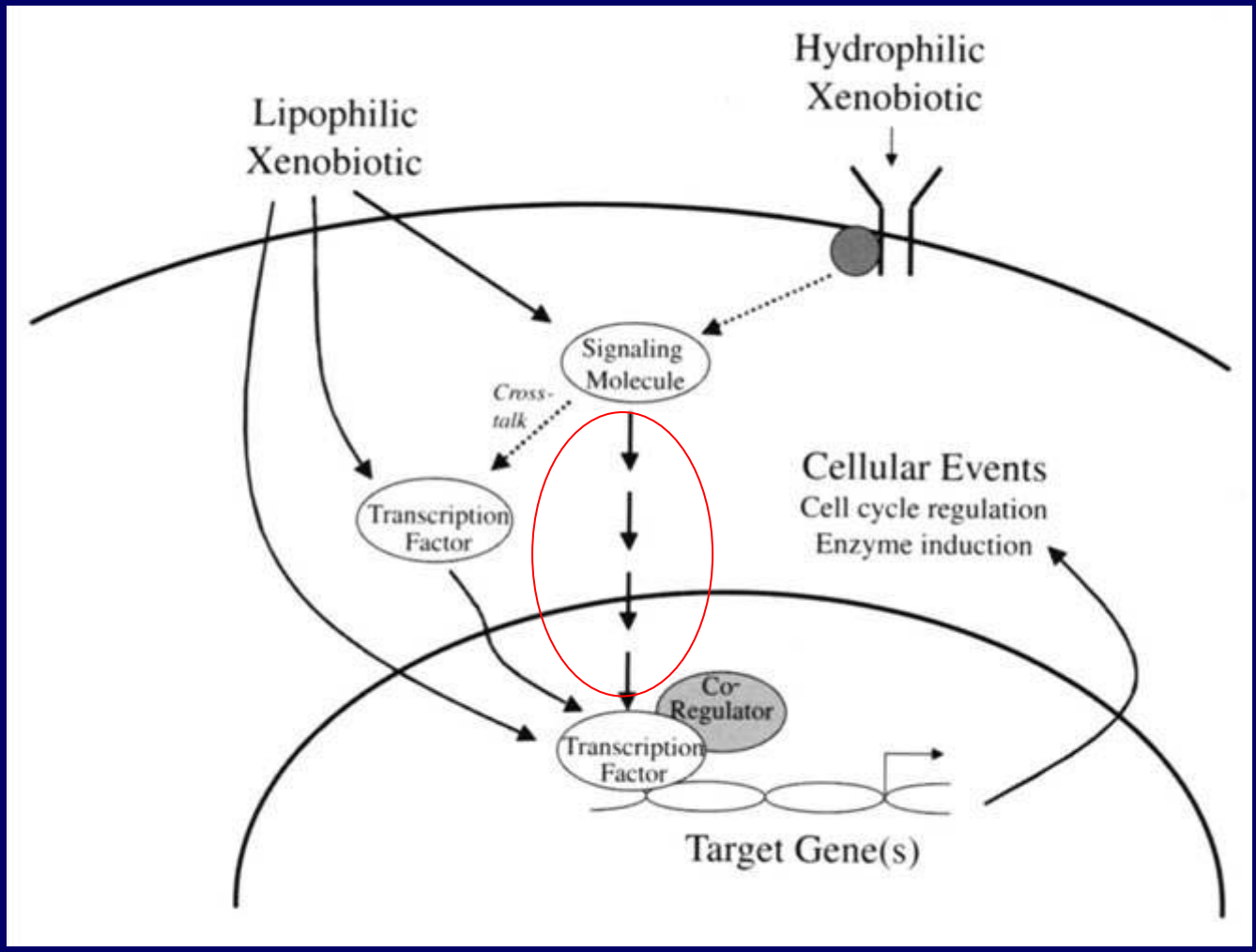
Nuclear receptor		Ligand	CYP enzyme	Cytosolic binding *protein*	ABC transporter
Retinoid X receptors*	RXRα,β,γ	9-*cis* Retinoic acid	–	–	–
Peroxisome proliferator-activated receptors	PPARα	Fatty acids Fibrates	↑ CYP4A1 ↑ CYP4A3	↑ L-FABP	↑ ABCD2, ABCD3 ↑ ABCB4
	PPARδ	Fatty acids Carboprostacyclin	(?)	(?)	(?)
	PPARγ	Fatty acids Eicosanoids Thiazolidinediones	↑ CYP4B1	↑ ALBP/aP2 ↑ H-FABP	(?)
Liver X receptors	LXRα,β	Oxysterols	↑ CYP7A1	OSBPs?	↑ ABCA1, ↑ ABCG1, ABCG4 ↑ ABCG5, ABCG8
Farnesoid X receptor	FXR	Bile acids	↓ CYP7A1 ↓ CYP8B1	↑ IBABP	↑ ABCB11
Xenobiotic receptors	SXR/PXR	Xenobiotics Steroids	↑ CYP3A ↑ CYP2C	(?)	↑ ABCB1, ABCC2
	CAR	Xenobiotics Phenobarbital	↑ CYP2B ↑ CYP2C	(?)	↑ ABCC3
Ecdysone receptor	EcR	20(OH)-ecdysone	↑ 26-(OH)ase	Hexamerins	↑ E23
Retinoic acid receptors	RARα,β,γ	Retinoic acids	↑ CYP26A1	↑ CRABPII ↑ CRBPI	(?)
Vitamin D receptor	VDR	1,25(OH)$_2$-vitamin D$_3$	↑ CYP24 ↓ CYP27B1	(?)	(?)

NUKLEÁRNÍ RECEPTORY

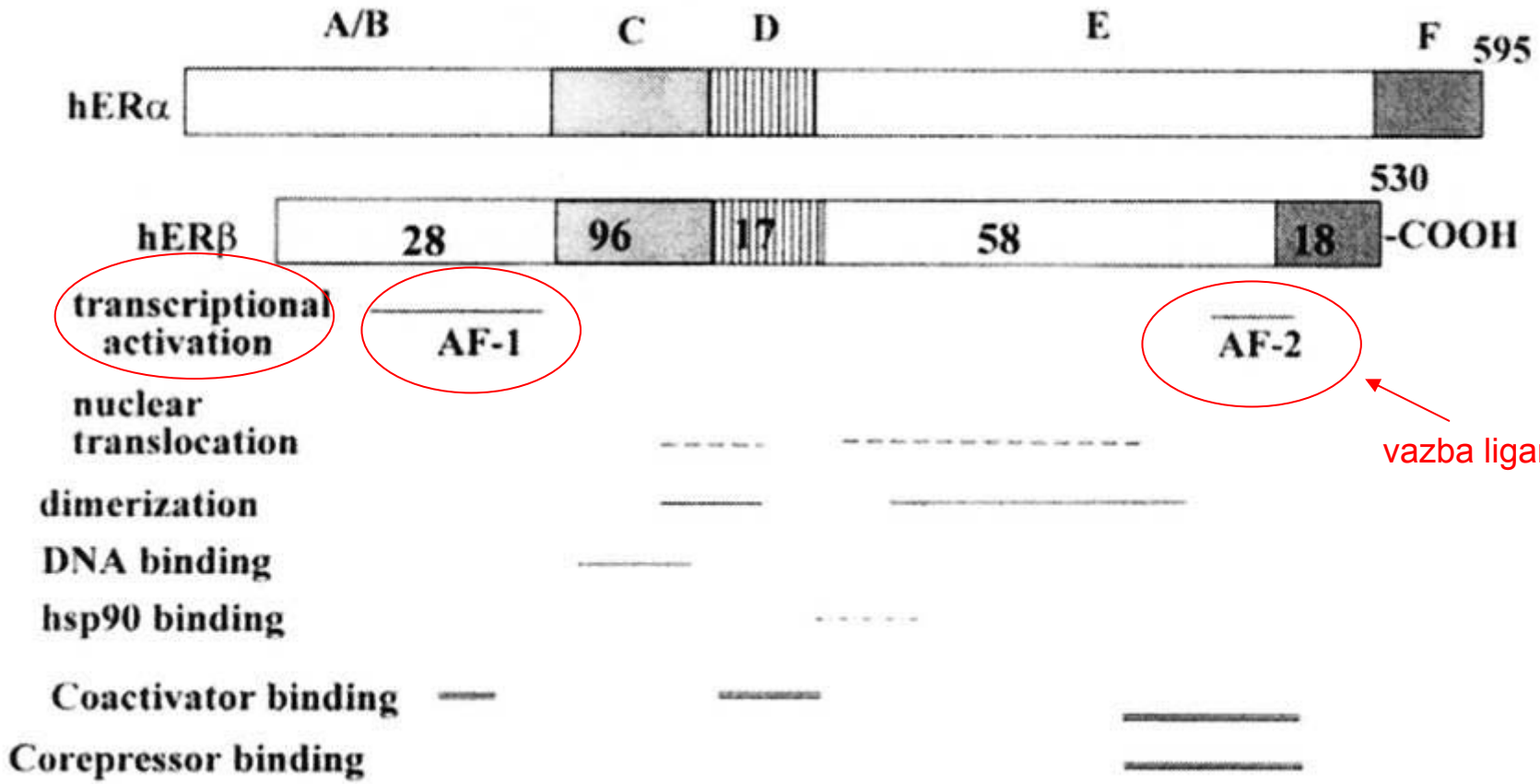


NUKLEÁRNÍ RECEPTORY

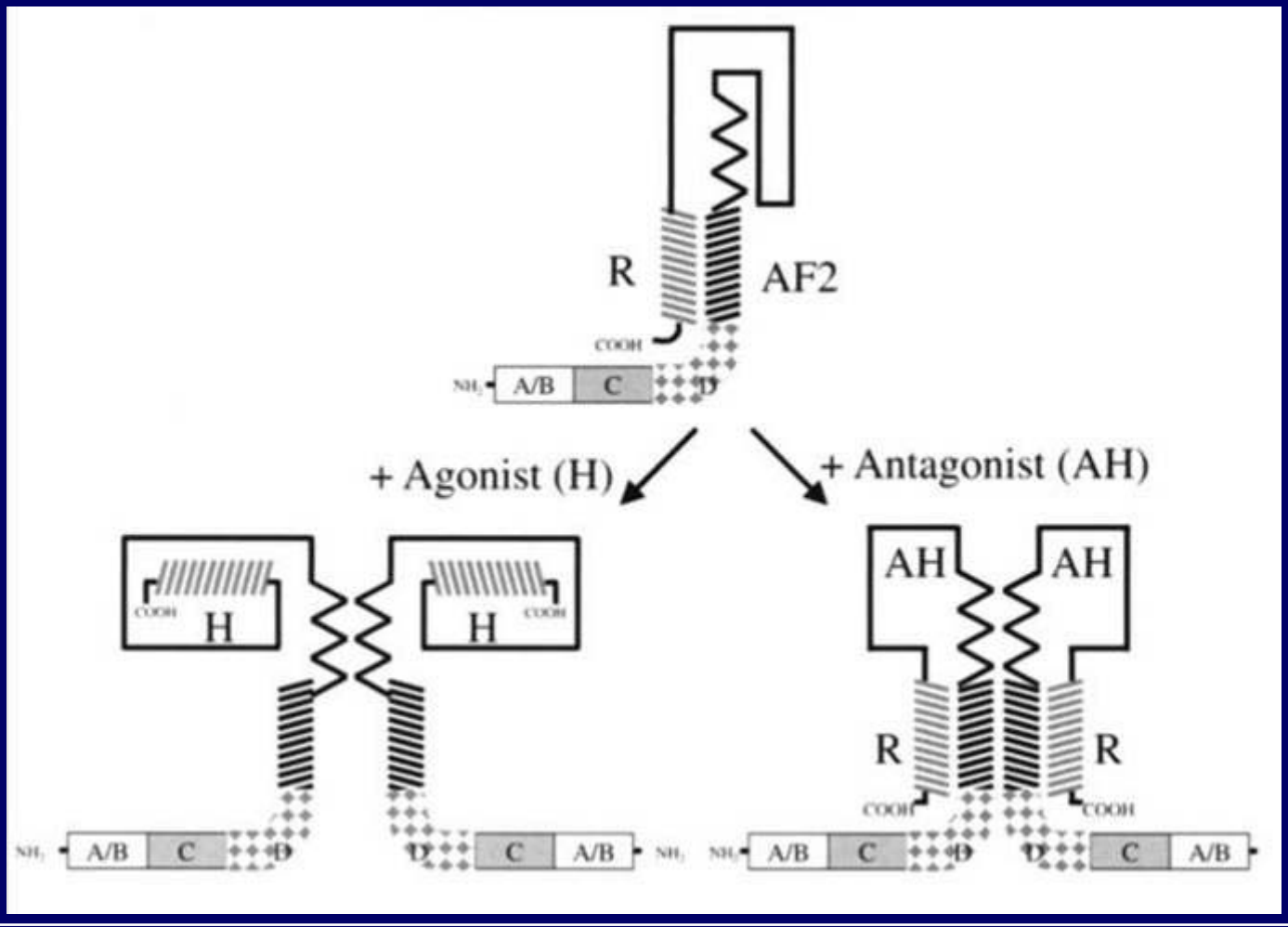
Ligand-independentní modulace transkripčního faktoru



NUKLEÁRNÍ RECEPTORY: struktura domén receptorů



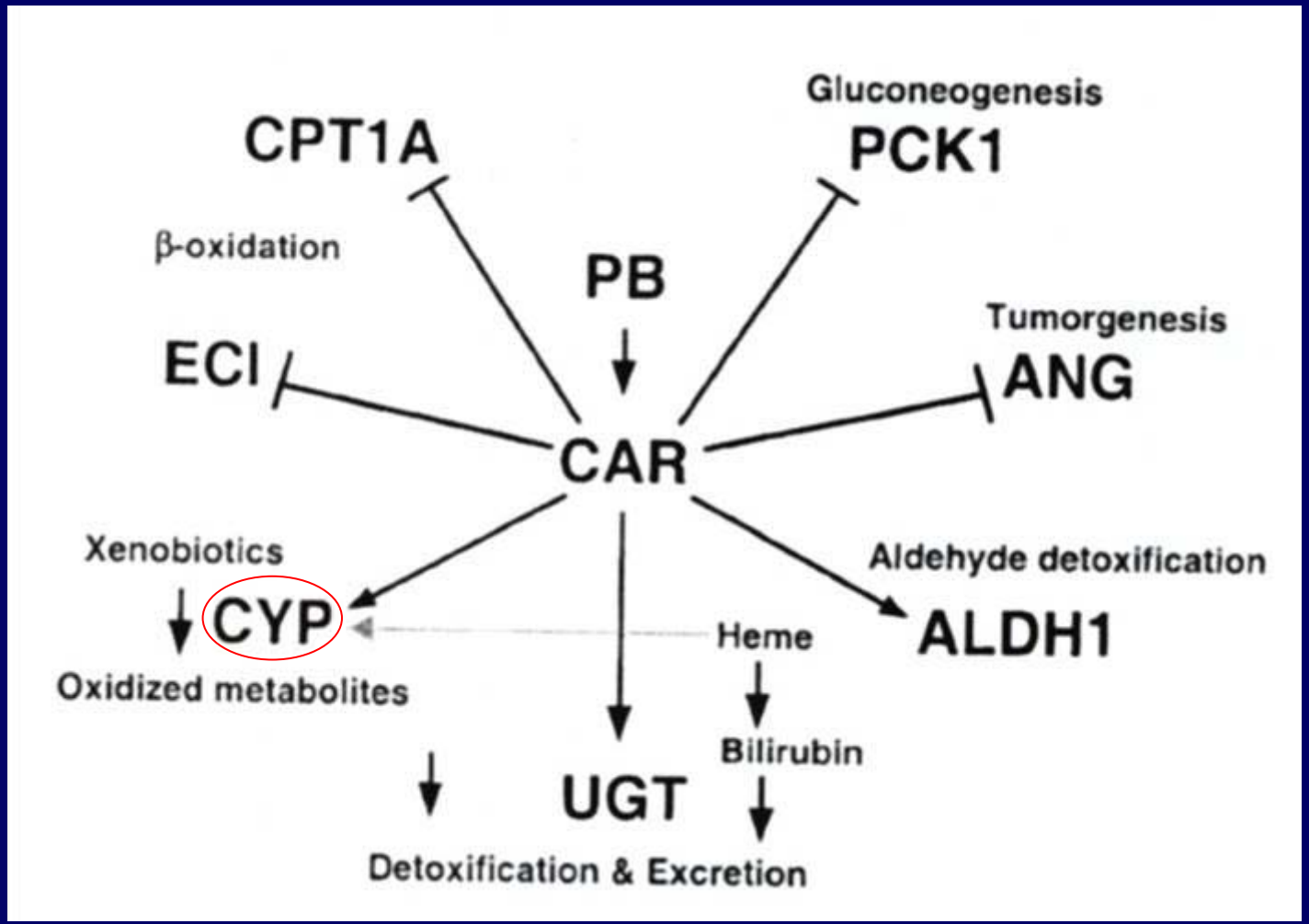
NUKLEÁRNÍ RECEPTORY:
vazba antagonistického a agonistického ligandu



KOMPLEXNÍ INTERAKCE RECEPTORU CAR

ndukce
CYP2B

zvýšení
oxidativního
metabolismu
xenobiotik

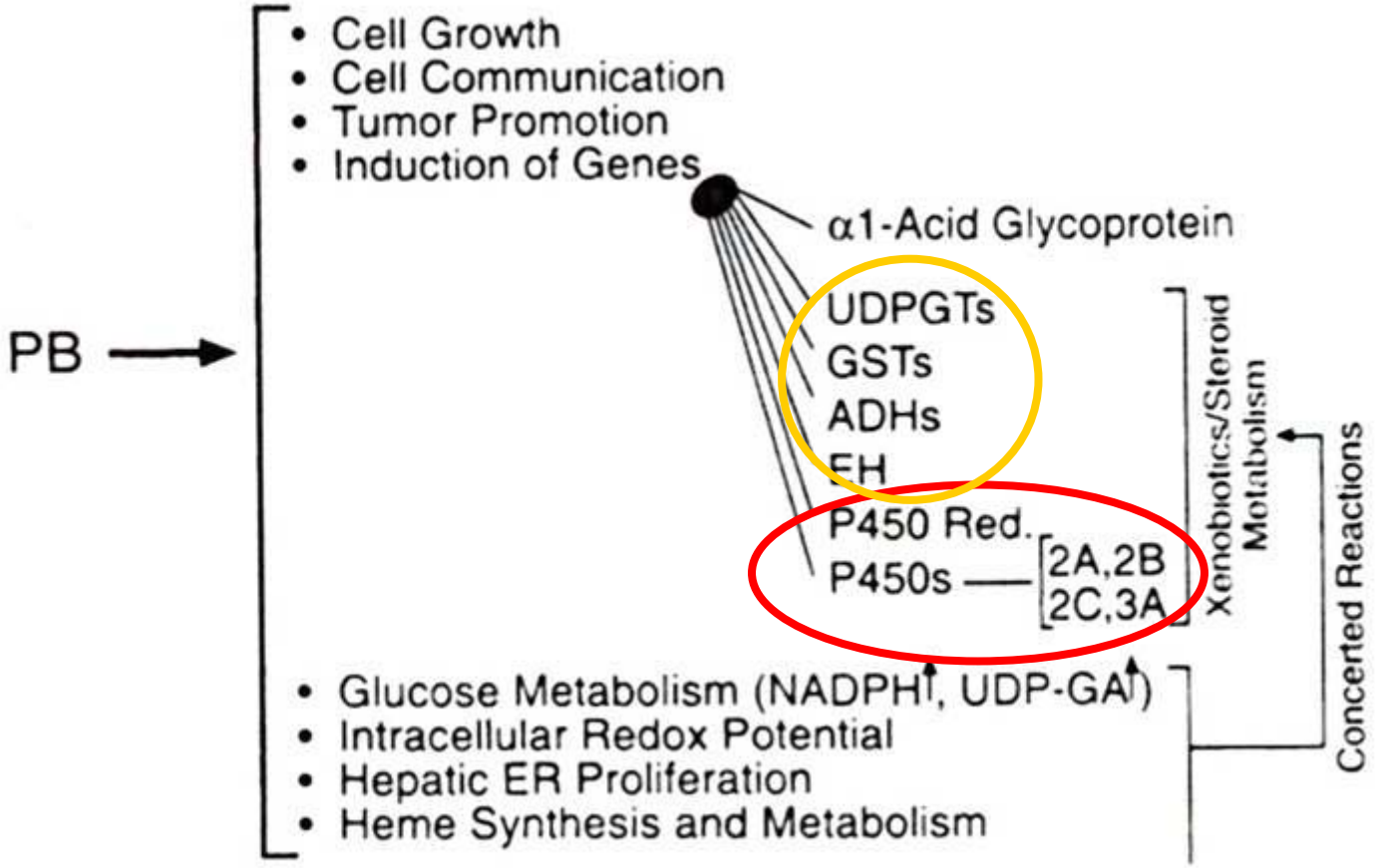


FENOBARBITAL (PB) – AGONISTA CAR:

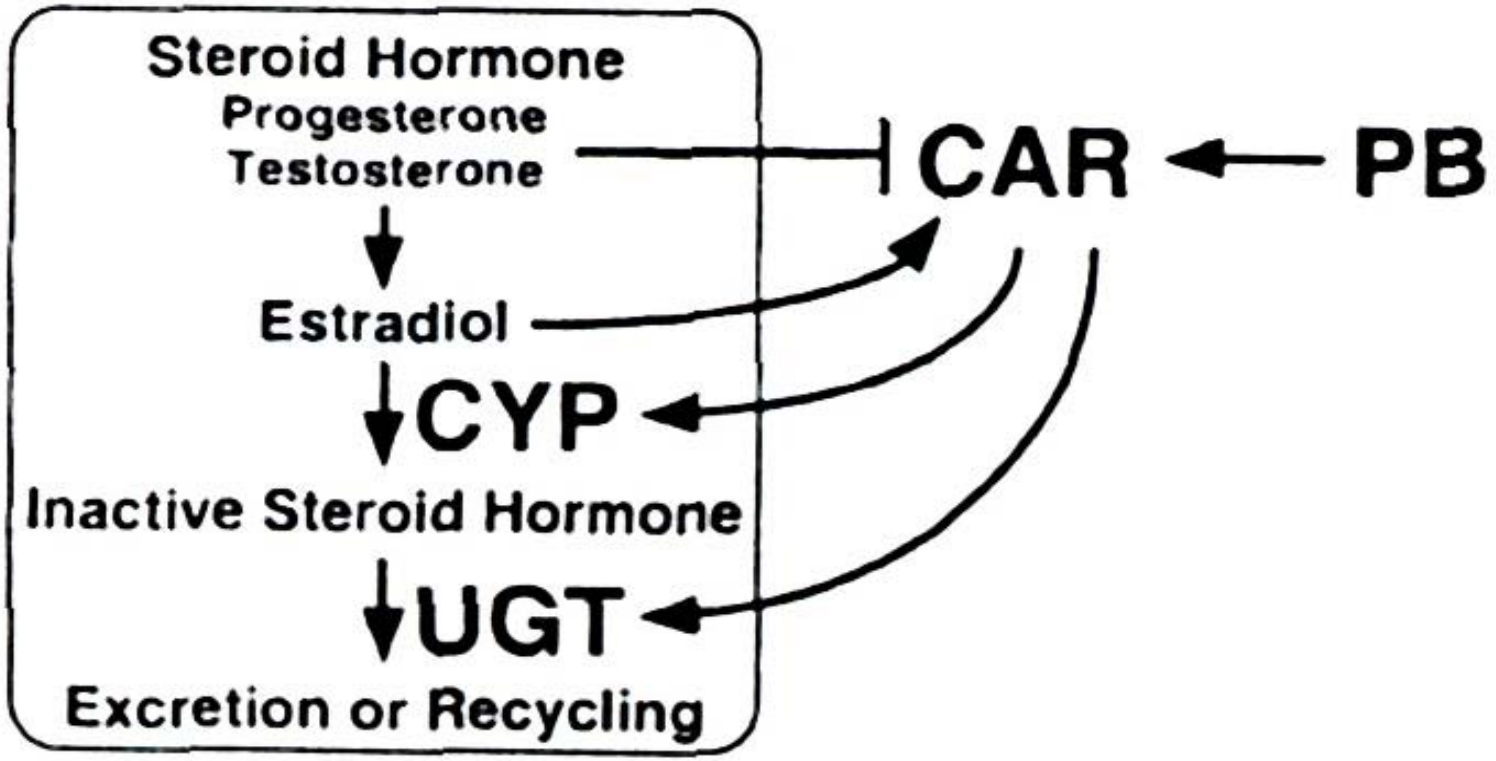
iotransformace:

nzymy 1. fáze

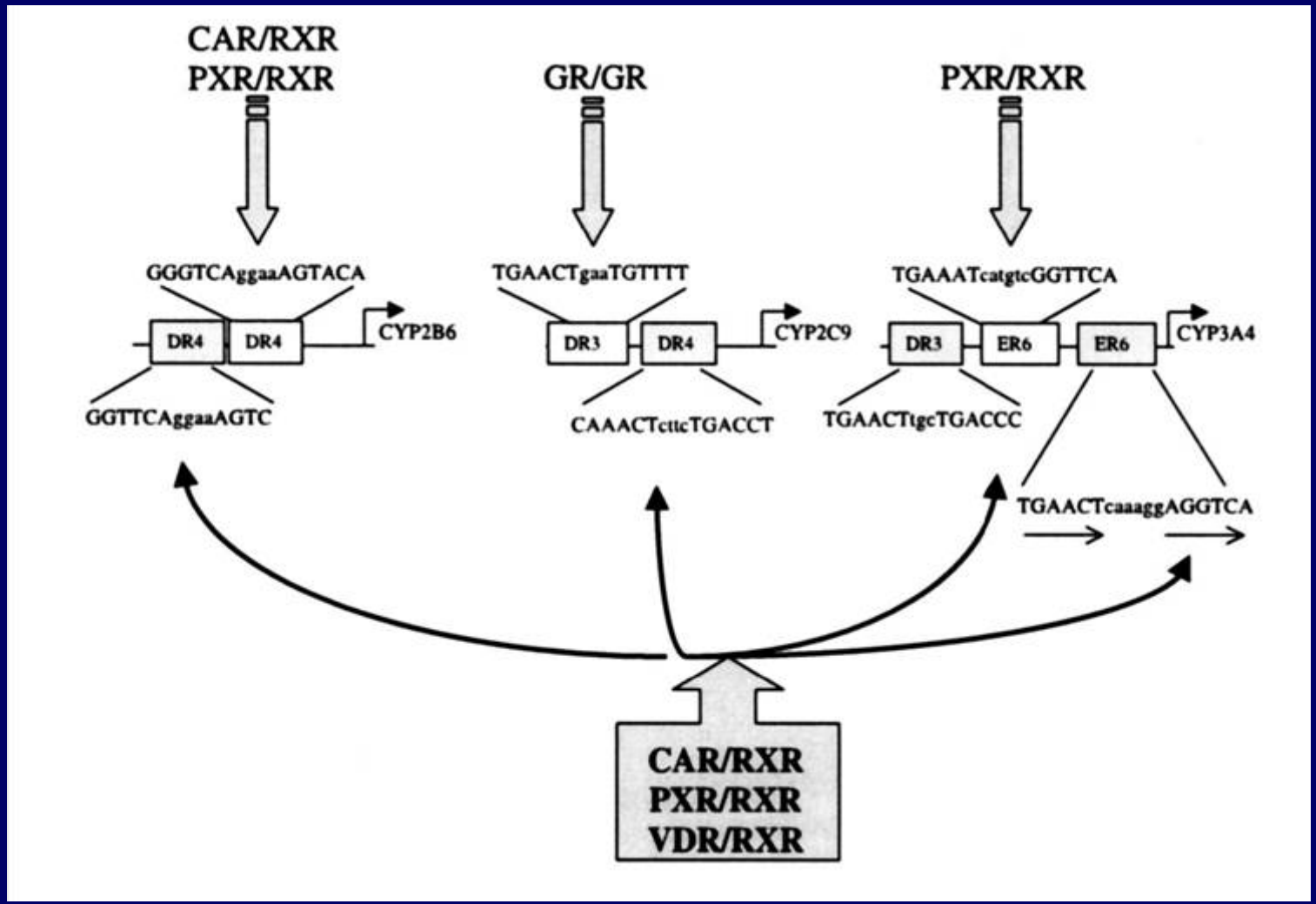
nzymy 2. fáze



REGULACE CAR STEROIDNÍMI HORMONY

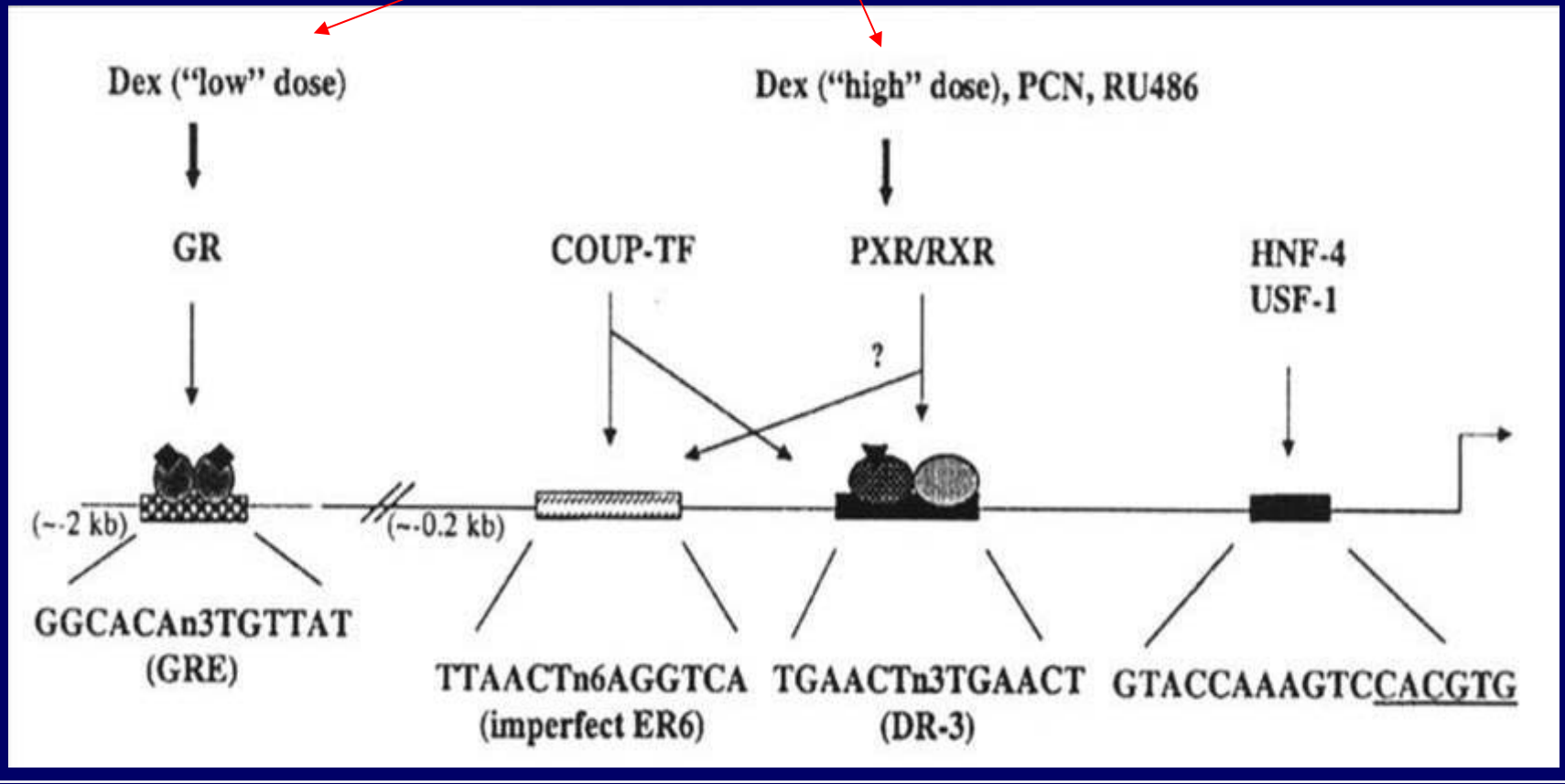


REGULACE GR / PXR / CAR



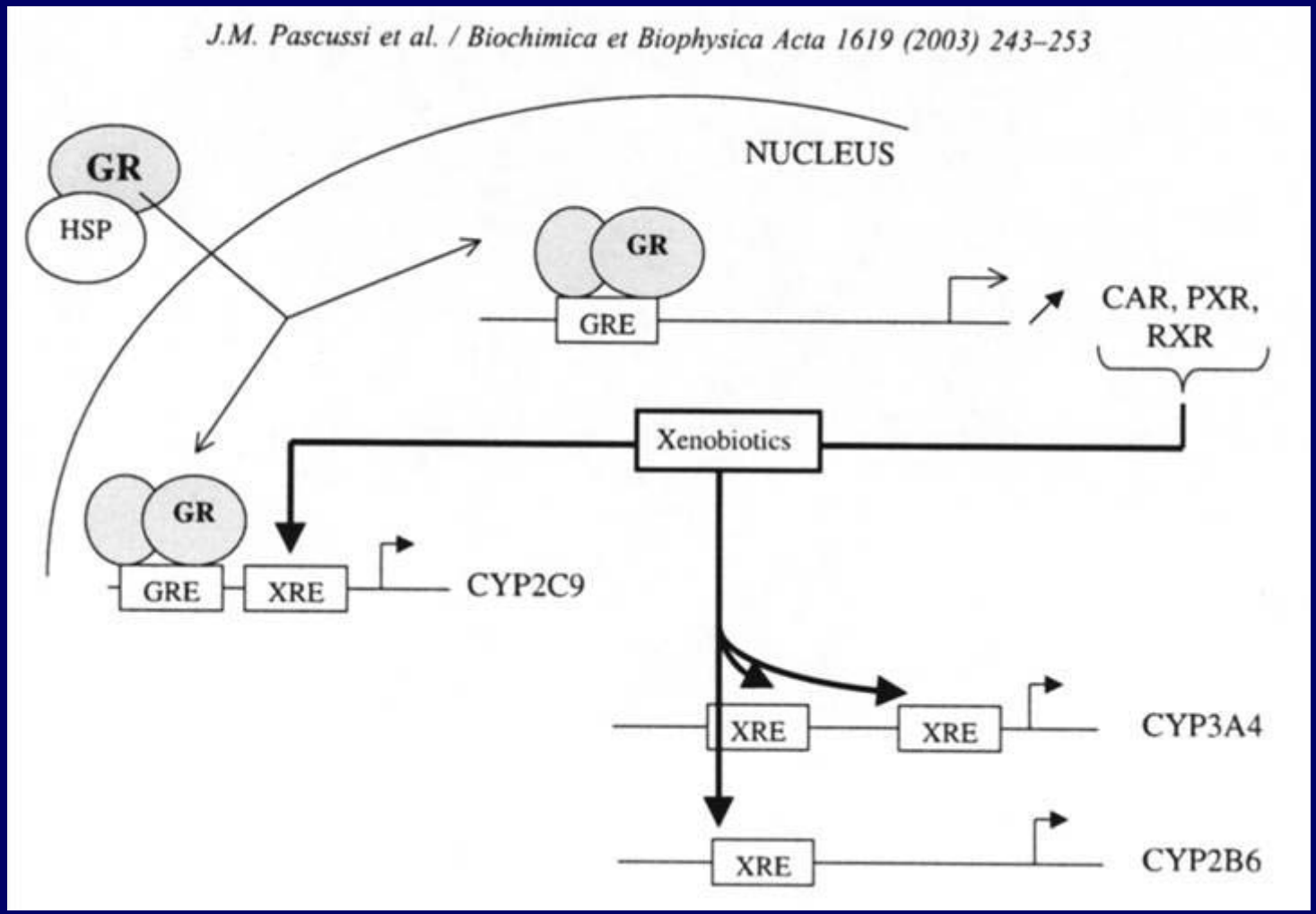
REGULACE GR / PXR / CAR

aktivace nízkou vs. vysokou koncentrací ligandu



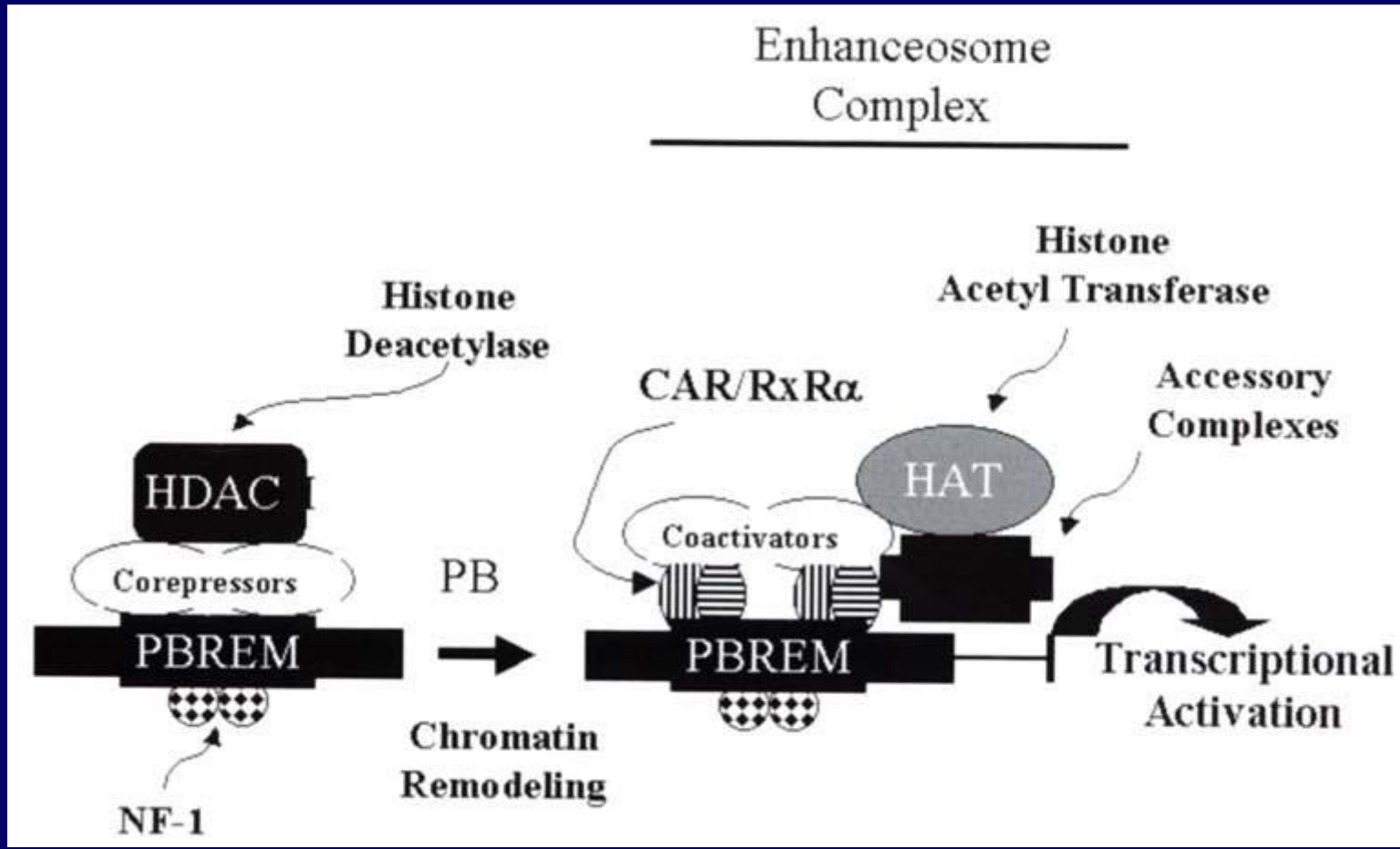
REGULACE GR / PXR / CAR

Více úrovní regulace



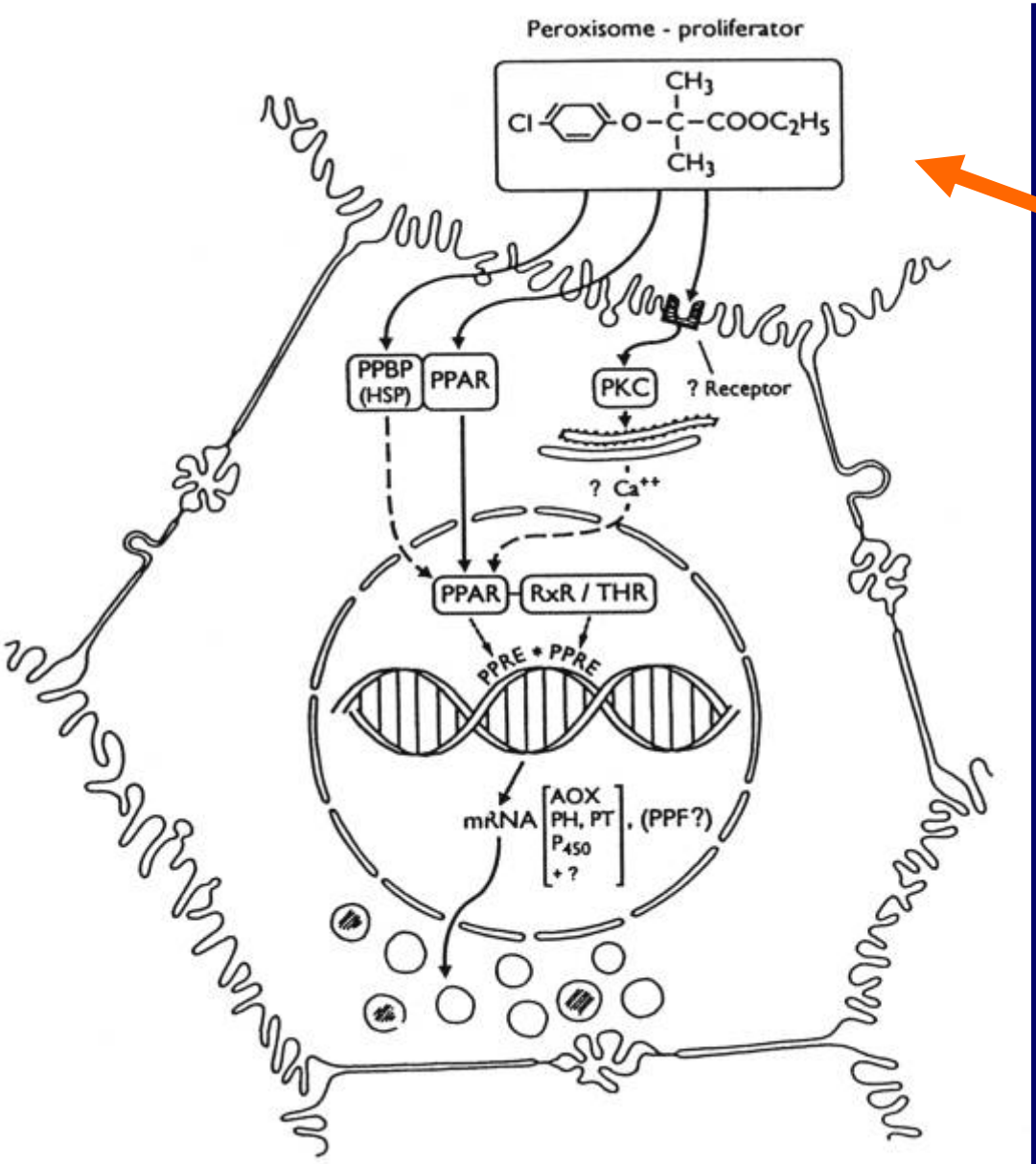
REGULACE GR / PXR / CAR

Další faktory modulují aktivaci transkripce



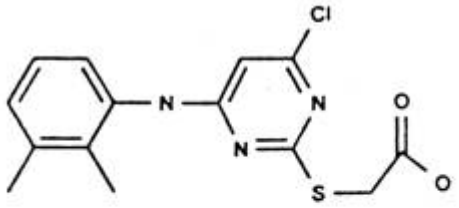
PPARα

(Peroxisomal proliferator-activated receptor alpha)

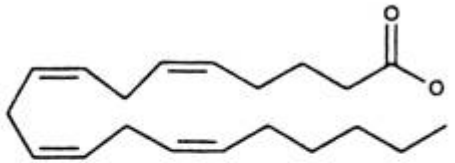


Induktory: mastné kyseliny, hypo-lipidemika (fibráty), environ. látky (ftaláty)

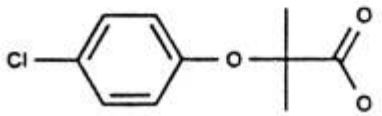
LIGANDY PPARα



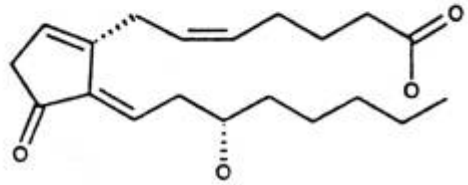
Wy 14,643



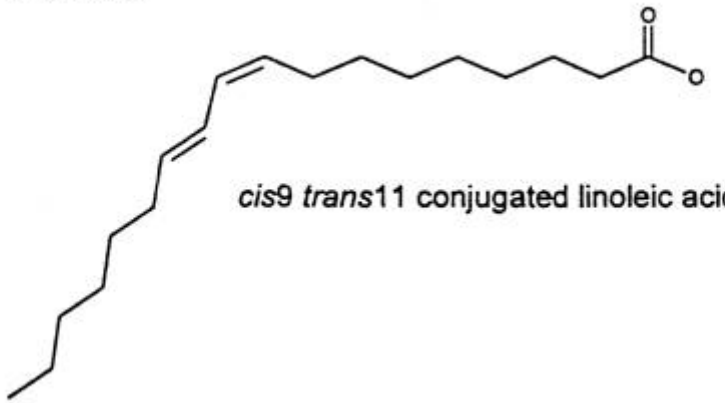
Arachidonic Acid



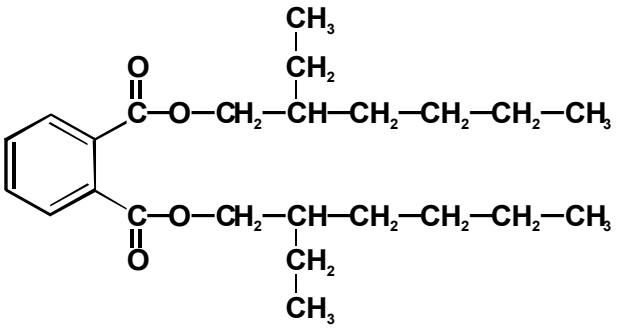
Clofibrate



Prostaglandin J2



*cis*9 *trans*11 conjugated linoleic acid



Di(2-ethylhexyl)ftalát (DEHP)

DŮSLEDKY AKTIVACE PPARα

Nerovnoměrná indukce CYP4A (produkce H_2O_2) a dalších enzymů (např. CAT) dependentních na PPARalfa