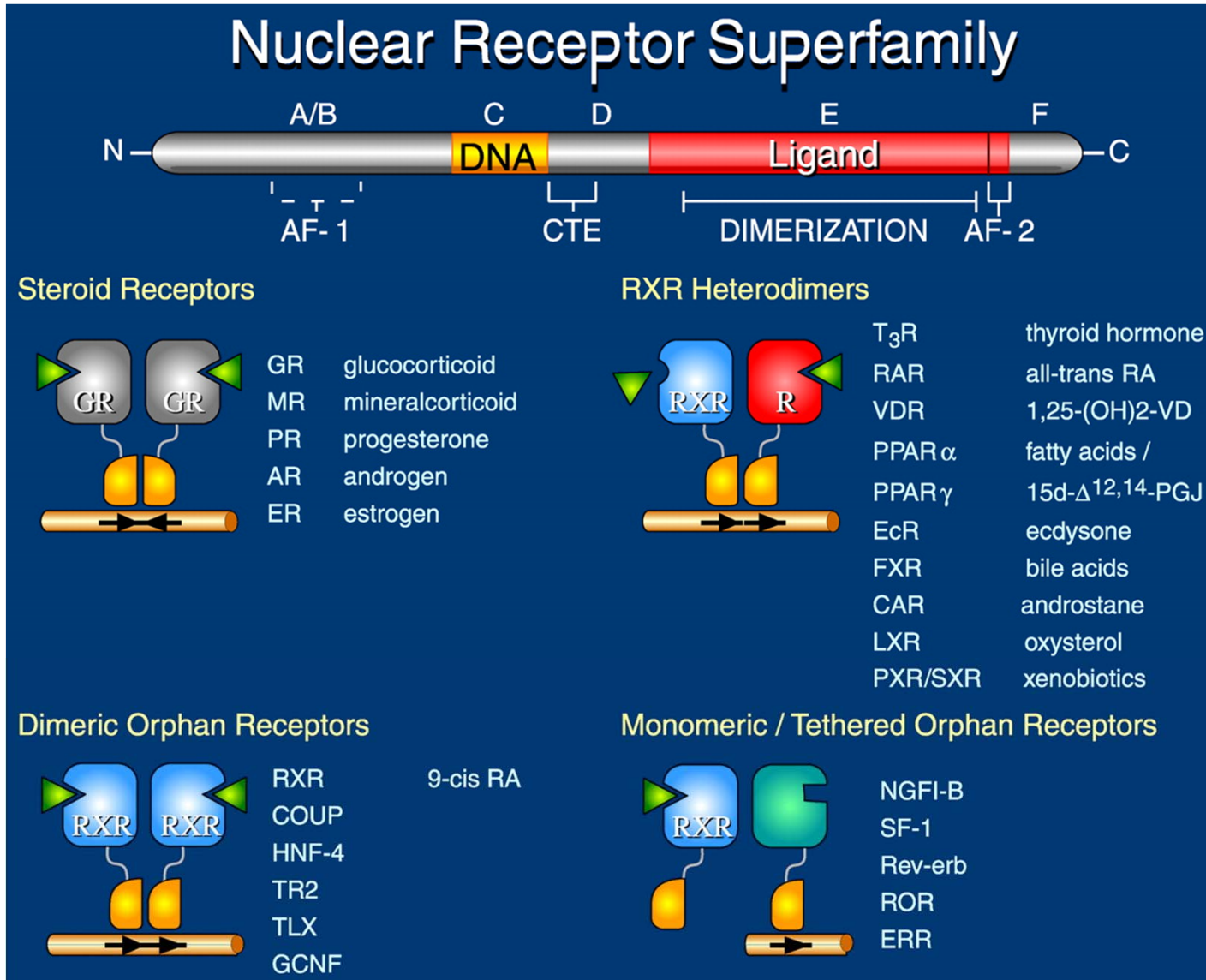
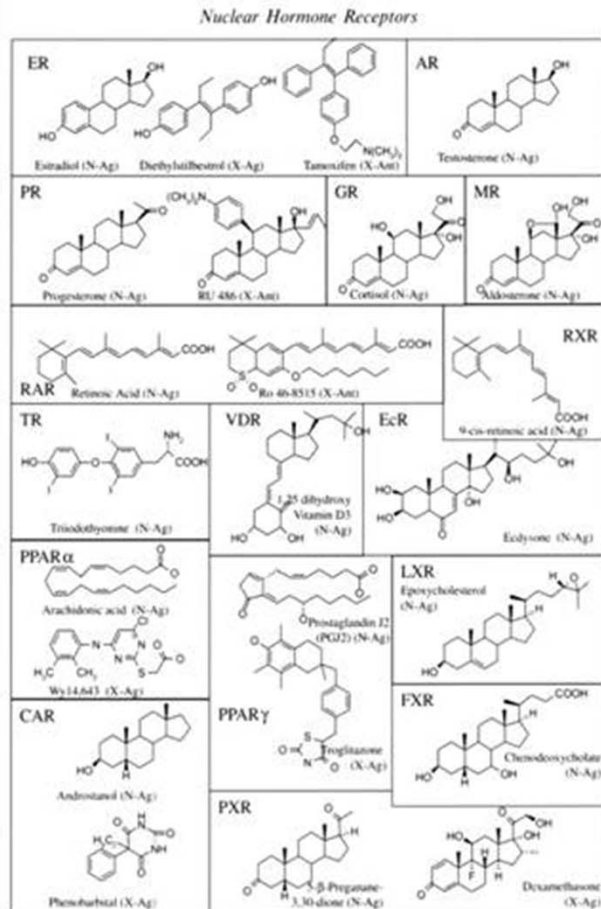


## Modelové interakce jaderných receptorů a enzymových systémů

# JADERNÉ RECEPTORY



# JADERNÉ RECEPTORY



Ligands:

## Endocrine Receptors

High-affinity, hormonal lipids

ER  $\alpha, \beta$   
PR  
AR  
GR  
MR

RAR  $\alpha, \beta, \gamma$   
TR  $\alpha, \beta$   
VDR  
EcR

## Adopted Orphan Receptors

Low-affinity, dietary lipids

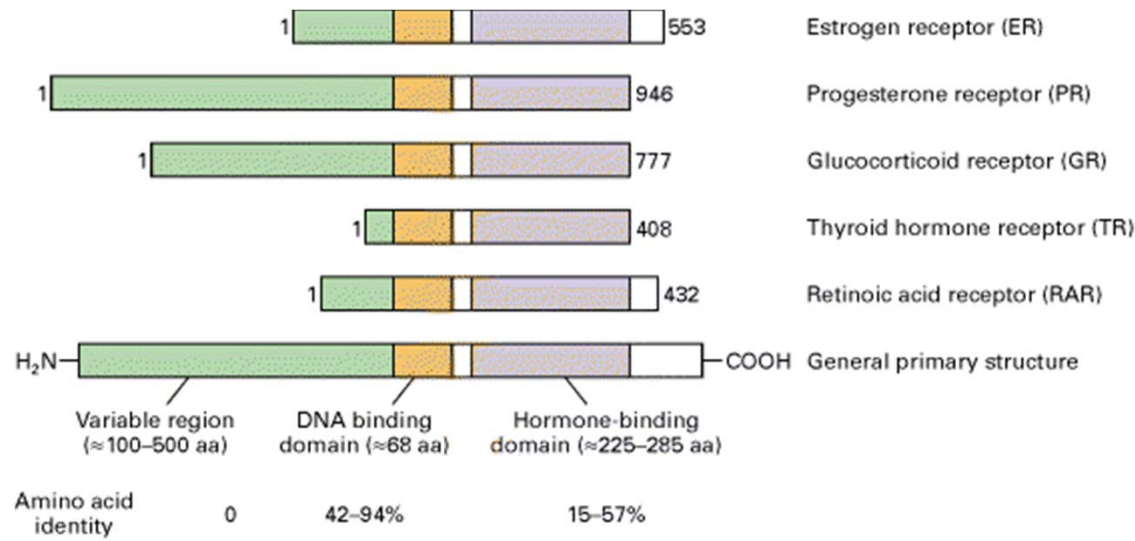
RXR  $\alpha, \beta, \gamma$   
PPAR  $\alpha, \beta, \gamma$   
LXR  $\alpha, \beta$   
FXR  
PXR/SXR  
CAR

## Orphan Receptors

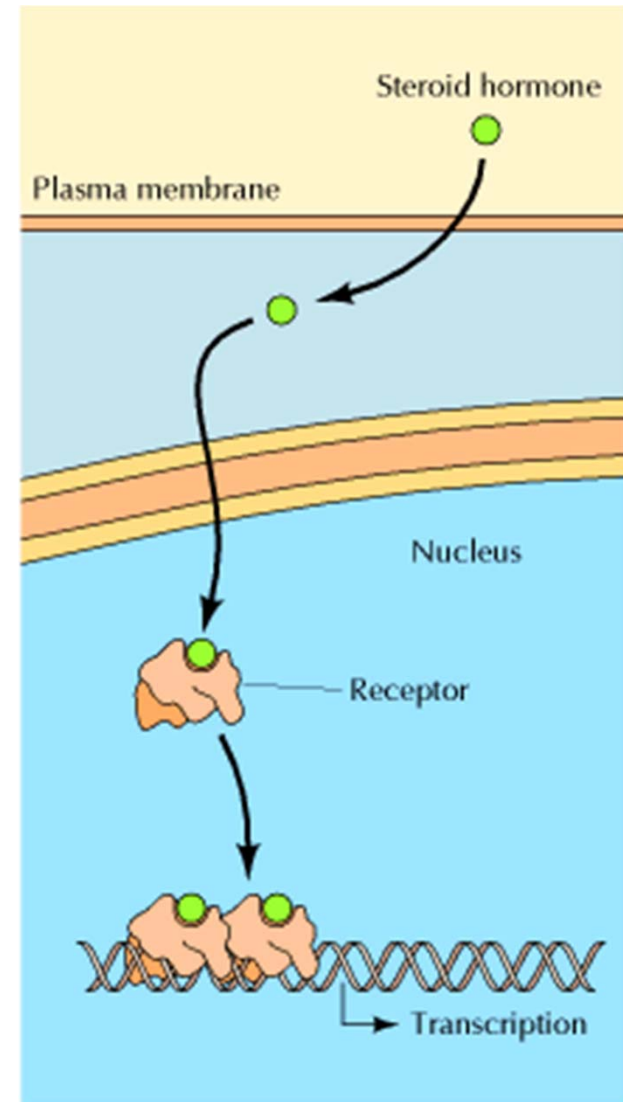
Unknown

SF-1  
LRH-1  
DAX-1  
SHP  
TLX  
PNR  
NGFI-B  $\alpha, \beta, \gamma$   
ROR  $\alpha, \beta, \gamma$   
ERR  $\alpha, \beta, \gamma$   
RVR  $\alpha, \beta, \gamma$   
GCNF  
TR 2,4  
HNF-4  
COUP-TF  $\alpha, \beta, \gamma$

<http://www.ens-lyon.fr/LBMC/laudet/nurebase/nurebase.html>

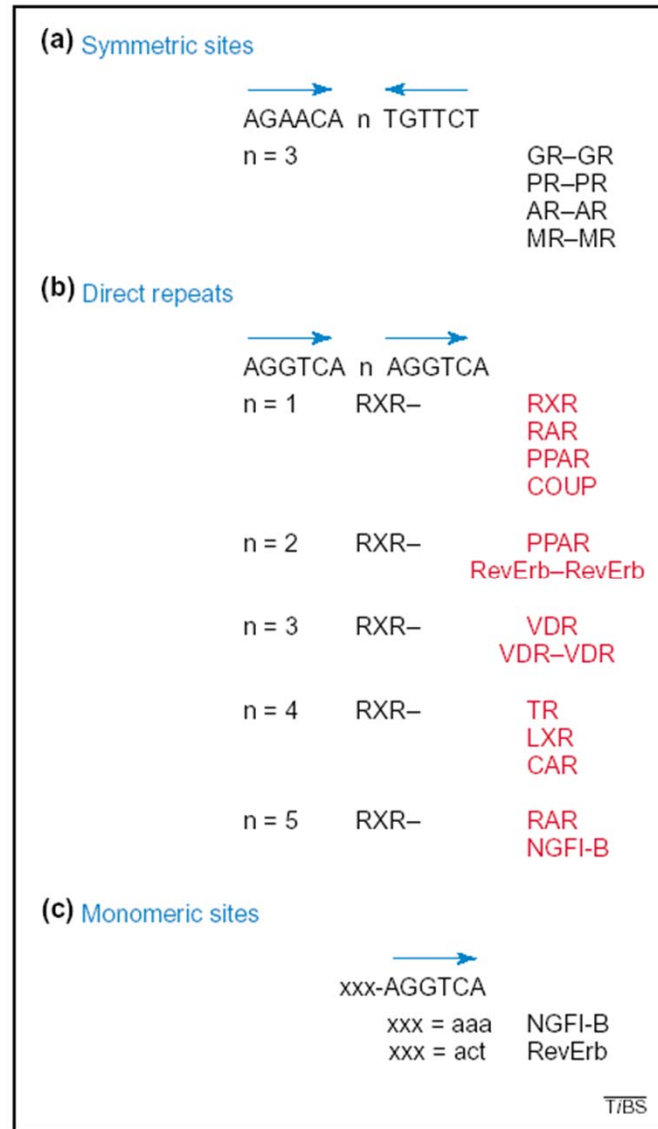


General design of transcription factors in nuclear-receptor superfamily. The centrally located DNA-binding domain exhibits considerable sequence homology among different receptors and has the C4 zinc-finger motif. The C-terminal hormone-binding domain exhibits somewhat less homology. The N-terminal regions in various receptors vary in length, have unique sequences, and may contain one or more activation domains. This general pattern has been found in the estrogen receptor (553 amino acids [aa]), progesterone receptor (946 aa), glucocorticoid receptor (777 aa), thyroid hormone receptor (408 aa), and retinoic acid receptor (432 aa). [See R. M. Evans, 1988, *Science* 240:889.]

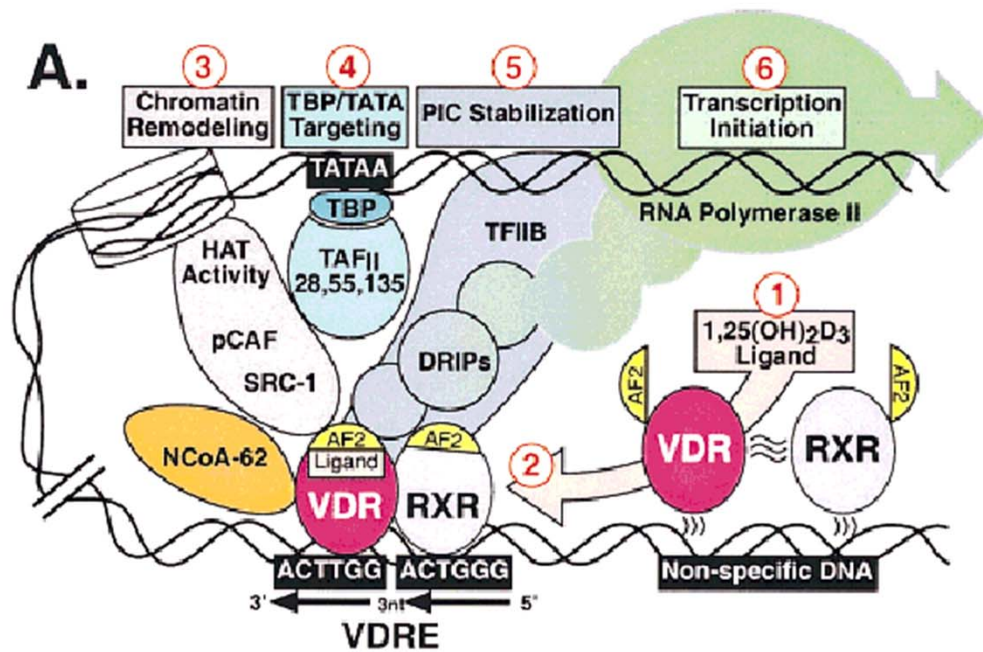


# Vazba na DNA:

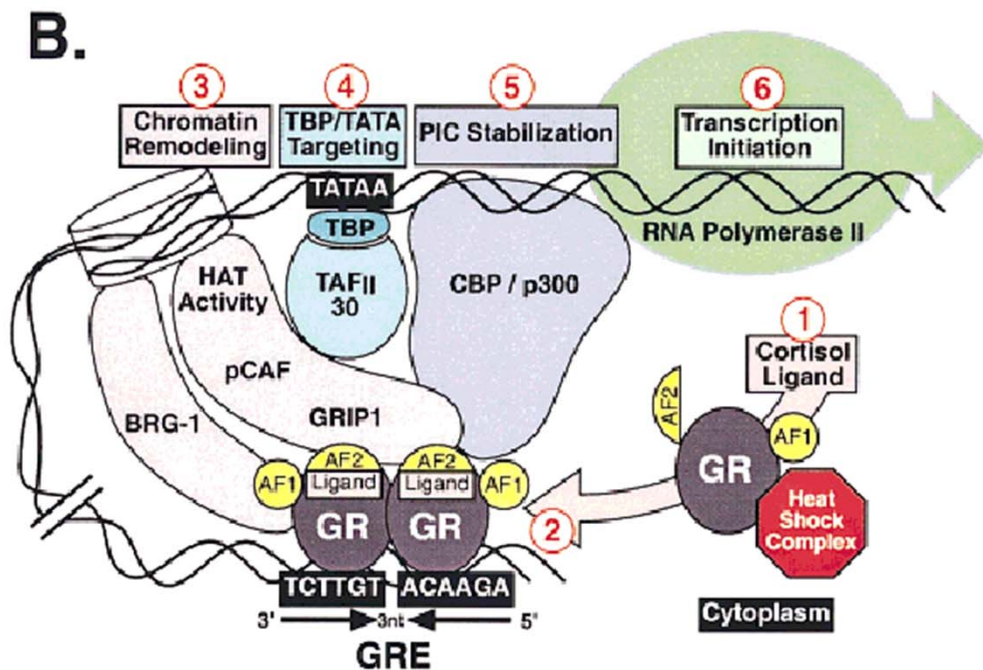
**Fig. 2.** The types of DNA-response elements used by nuclear receptors. (a) Symmetric repeats using the consensus half-site 5'-AGAACA-3' are used by the glucocorticoid receptor (GR), progesterone receptor (PR), androgen receptor (AR) and mineralocorticoid receptor (MR), each of which is a homodimer. The estrogen receptor (ER) binds similar symmetric sites but with consensus 5'-AGGTCA-3' half-sites. (b) A '1-5 rule' specifies the use of direct repeats with variable spacings by RXR and its many partners (depicted in red). Some receptors, such as the vitamin D receptor (VDR) or RevErb, can form homodimers as an alternative to heterodimers. The size of the inter-half-site spacing (n) can vary from one to five base-pairs. (c) Sites containing just one copy of 5'-AGGTCA-3' flanked with specific 5' sequences (xxx) are used by the nerve growth factor induced B (NGFI-B) receptor, RevErb and some other orphan receptors.



We can divide the receptors into subgroups on the basis of their pattern of dimerization. One group consists of the steroid receptors, all of which appear to function as homodimers. This group includes receptors for estradiol (ER), progesterone (PR), androgens (ARs), glucocorticoids (GRs) and mineralocorticoids (MRs). A second major group contains receptors that form heterodimers with retinoid X receptor (RXR) – the receptor for 9-*cis* retinoic acid. Members of this group include the receptors for all-*trans* retinoic acid (RAR), vitamin D3 (VDR) and thyroid hormone (TR), as well as liver X receptor (LXR), peroxisome proliferator activated receptor (PPAR) and others. A third group consists of receptors that can bind DNA as monomers, such as NGFI-B, RevErb, ROR and SF-1.

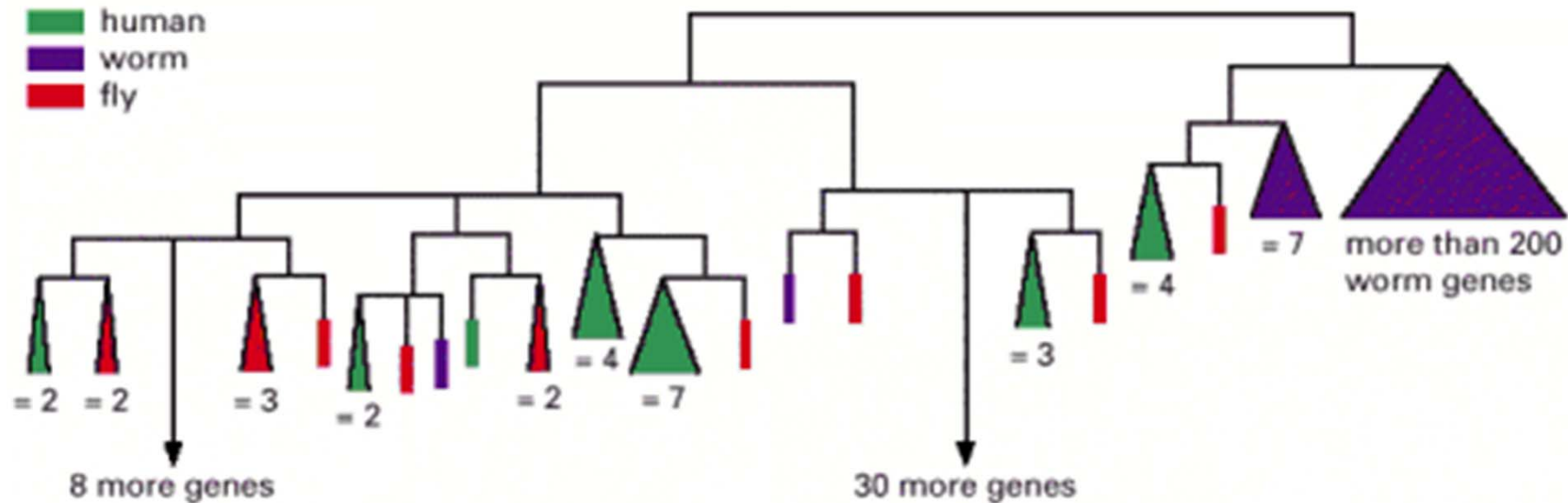


**A:** Unliganded heterodimerizing receptors, exemplified here by VDR, exist as weakly associated heterodimers with RXR, presumably bound nonspecifically to DNA [Haussler et al., 1998]. Binding of the 1,25(OH)<sub>2</sub>D<sub>3</sub> ligand to VDR (1) promotes high-affinity heterodimerization with RXR accompanied by binding of the heterodimer to its direct repeat VDRE (2).



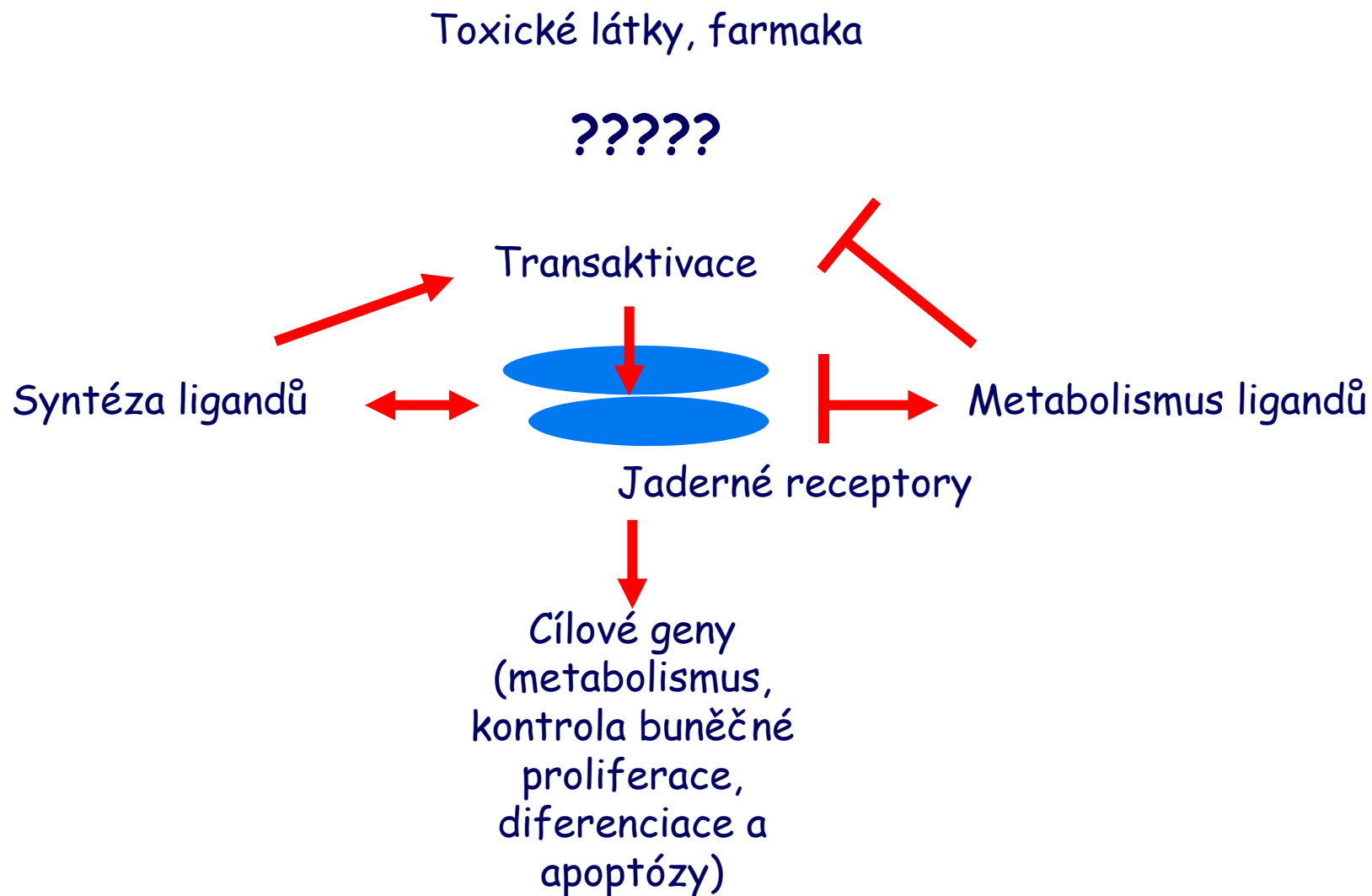
**B:** Unliganded GR, like other receptors in group (d) (see Fig. 2), exists as a complex with heat shock proteins in the cytoplasm. Upon binding its cortisol ligand (1), GR dissociates from the cytoplasmic complex, translocates to the nucleus and forms a homodimer on its palindromic GRE (2). Triggered by a ligand-mediated change in GR conformation, the AF1 and AF2 domains then synergize to promote a series of events (3-6) involving the recruitment of coregulatory complexes similar to those described for the VDR-RXR heterodimer, but with some distinctive features.

## Evolve jaderných receptorů



Many family members have been identified by DNA sequencing only, and their ligand is not yet known; these proteins are therefore referred to as *orphan nuclear receptors*. The importance of such nuclear receptors in some animals is indicated by the fact that 1-2% of the genes in the nematode *C. elegans* code for them, although there are fewer than 50 in humans.

Nízkomolekulární lipofilní sloučeniny jako ligandy = aktivita jaderných receptorů je do značné míry závislá na syntéze a degradaci ligandů a naopak





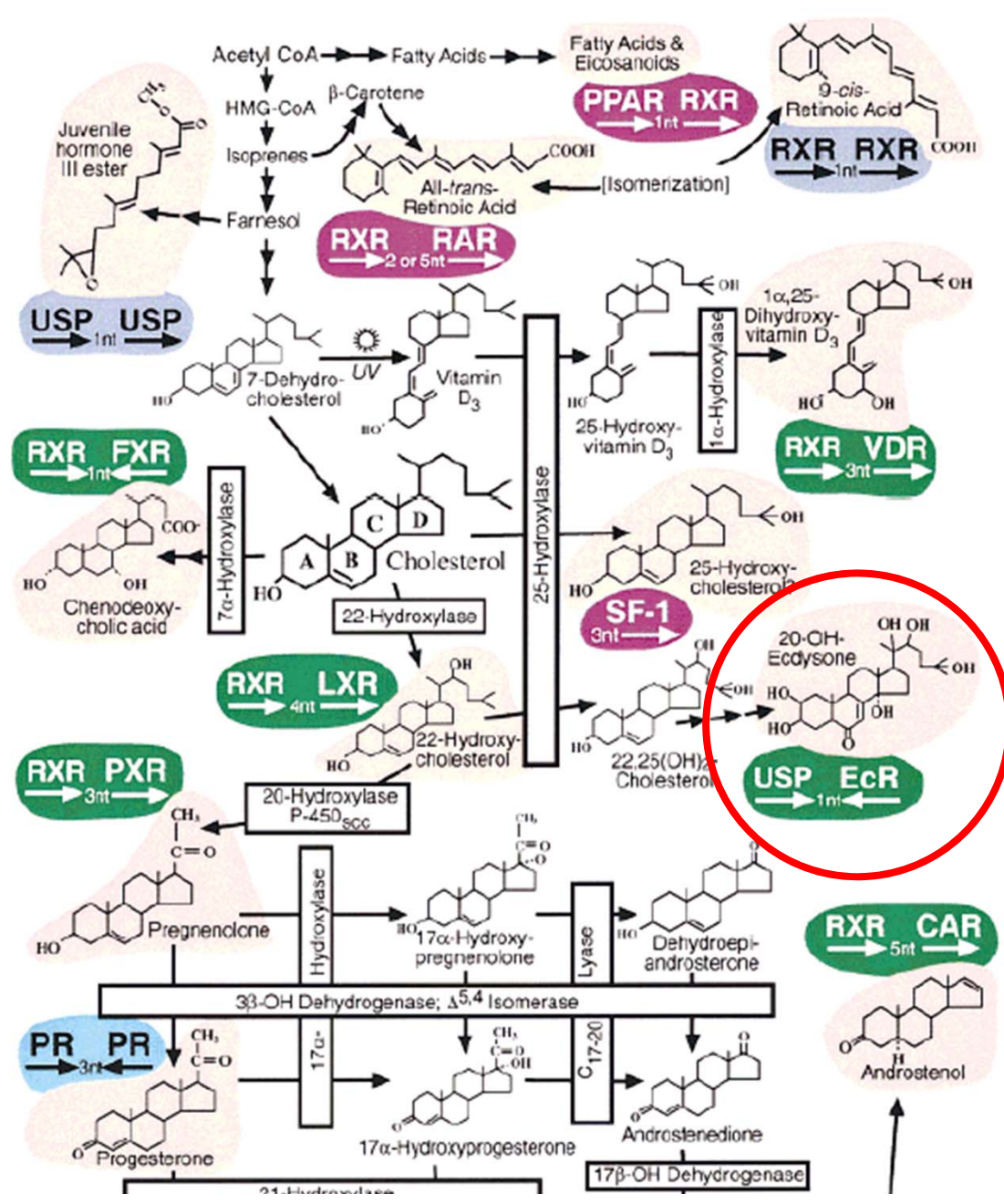
## Úloha jaderných receptorů a enzymů katalyzujících degradaci nebo syntézu jejich ligandů:

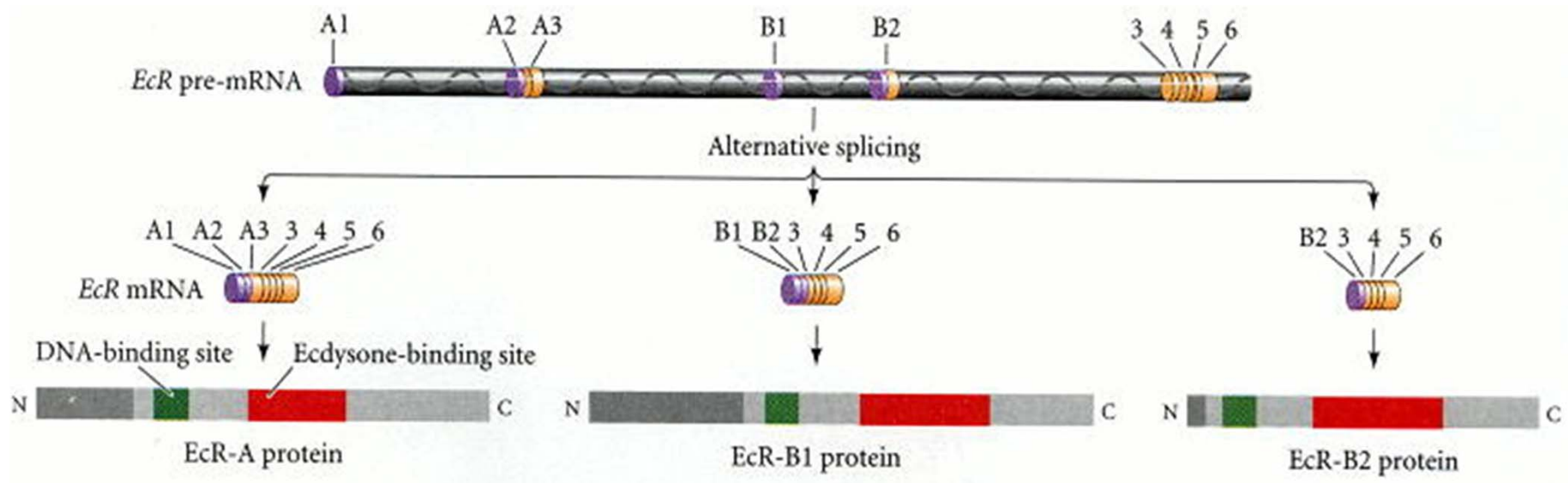
- endokrinní regulace - steroidní hormony, thyroidní hormony;
- regulace signálních drah - eikosanoidy, metabolismus kyseliny retinové, vitamínu D3;
- metabolismus lipidů;
- metabolismus xenobiotik;

**Modelový příklad 1:**

**Metamorfóza hmyzu**







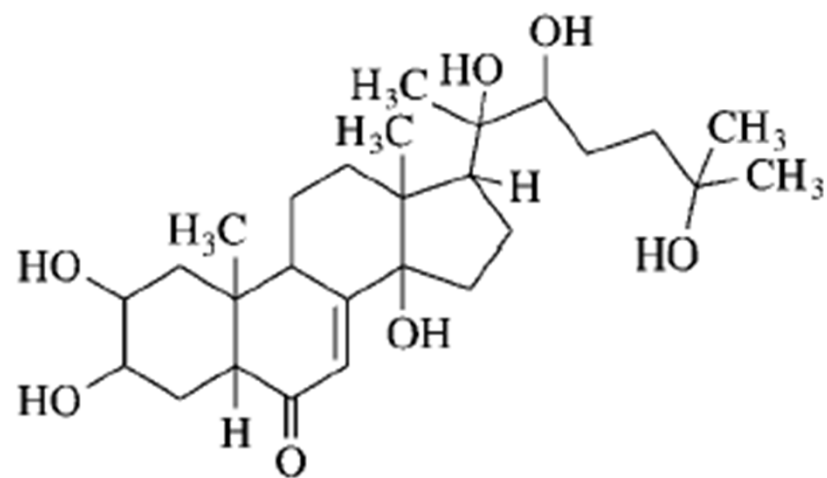
Formation of the ecdysone receptors. Alternative mRNA splicing of the ecdysone receptor (*EcR*) transcript creates three types of *EcR* mRNAs. These generate proteins having the same DNA-binding site (blue) and hydroxyecdysone-binding site (red), but with very different amino termini.

Three isoforms of EcR have been identified in insects, each with a different, stage-specific role in regulation of molting and development. This allows for one steroid hormone to induce a variety of different tissue responses. In general, EcR A is predominant when cells are undergoing a maturation response (from juvenile to adult) and is predominant in imaginal discs, whereas EcR B1 predominates in juvenile cells during proliferation or regression. Little is known about the function of the EcR B2 isoform.

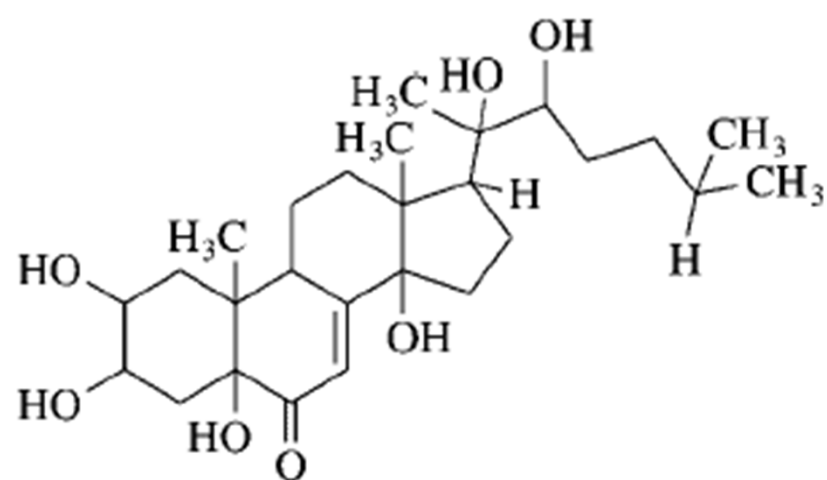
DNA and hormone binding are similar in the three isoforms of EcR. Little is known about the crustacean EcR isoforms and how they change during the molt cycle. However, the EcR that has been cloned from the crab, *Uca pugilator* (U31817, GenBank), shares 85-87% homology with that of *Drosophila* (M74078, GenBank). The differences are primarily in the region of the molecule involved with dimerization. Similar sequence similarities are found between the heterodimeric partner, USP.

There are several ecdysteroids which bind EcR, including 20-hydroxyecdysone, turkesterone, makisterone A, ponasterone A, and muristerone A. Some arthropods may use specific ecdysteroids as their principal molting hormone, but often several ecdysteroids are found within one group. The primary molting hormone for a range of organisms, including some insects and crustacea, is 20-OH ecdysone (20 HE). Among other examples, makisterone A is an important hormone for some crustacea and hemipteran insects.

20-OH Ecdysone  
(20 HE)

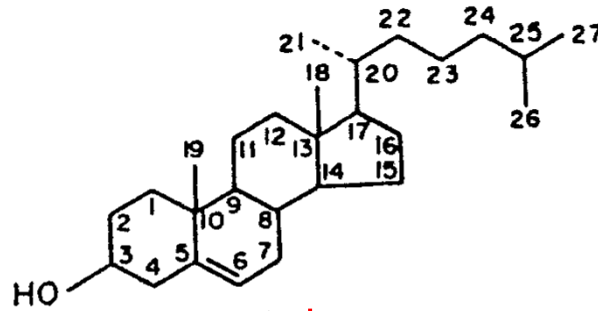


Muristerone A  
(MurA)



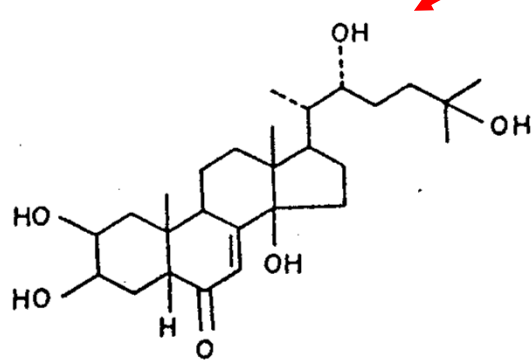
# Synthesis of molting hormones

Cholesterol (from diet- a vitamin for insects)



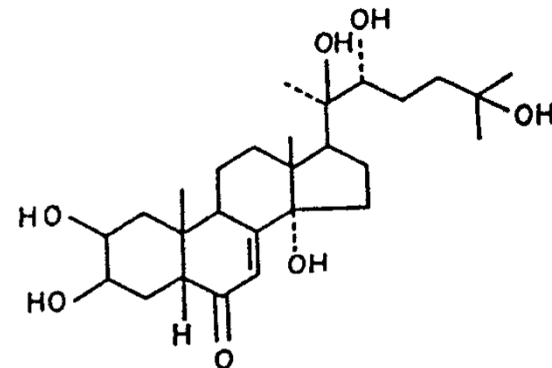
Prothoracic gland

Metabolites (excretion)  
Conjugates (storage)



Ecdysone

mono-oxygenase  
(fat body, epidermis)

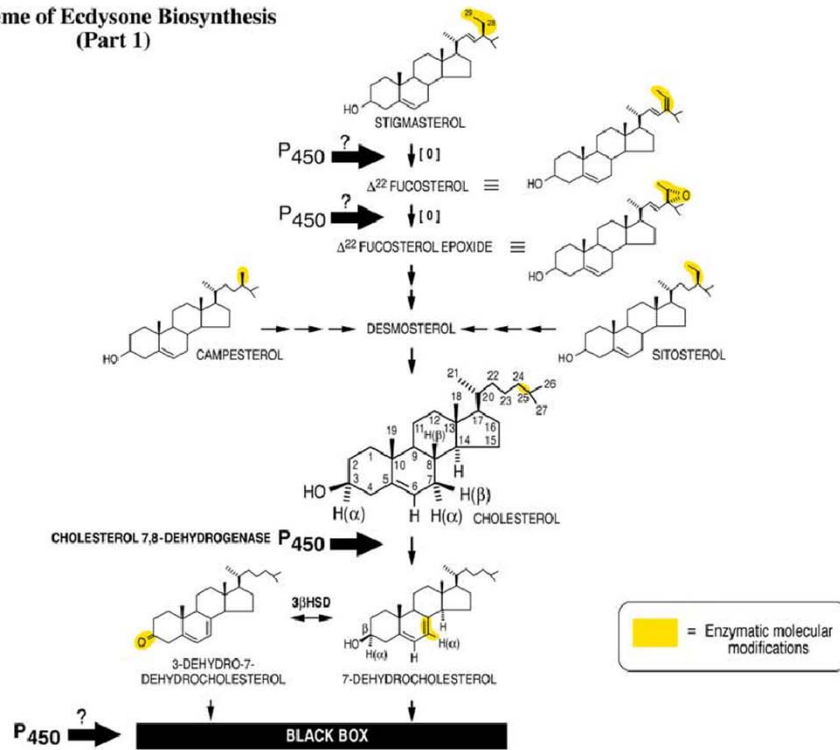


20-Hydroxyecdysone



# Syntéza ecdysteroidů a úloha cytochromů P450:

Scheme of Ecdysone Biosynthesis (Part 1)



Scheme of Ecdysone Biosynthesis (Part 2)

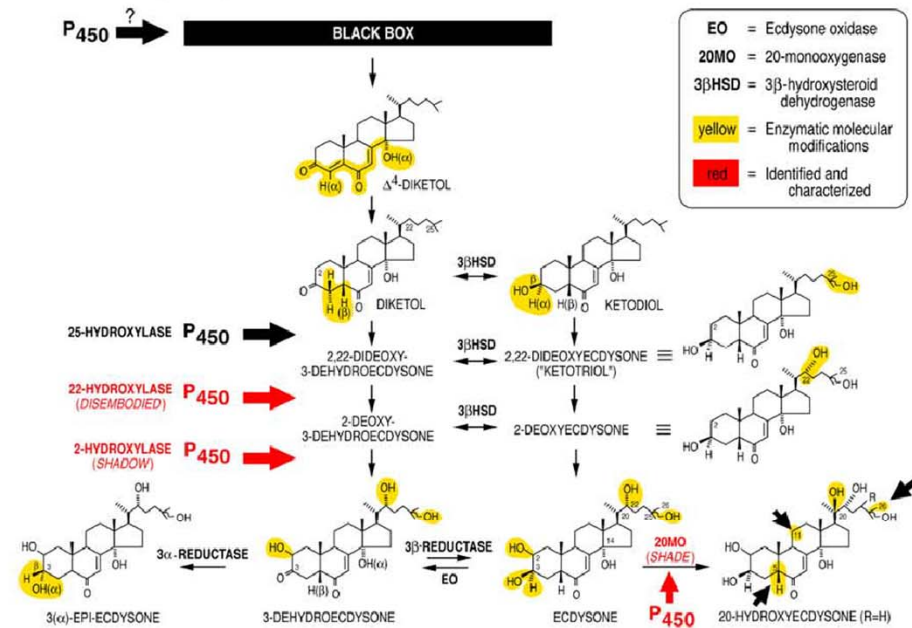


Fig. 5. (Parts 1 and 2). The biosynthesis of 20-hydroxyecdysone from plant sterols. Question marks denote possible involvement of P450 enzymes. Note specifically where the Halloween gene products act (red). 3-Dehydroecdysone is synthesized in the prothoracic glands of many insects (e.g. *Manduca sexta*) and converted to ecdysone in the hemolymph (left column of part 2). For *Drosophila*, ecdysone is synthesized in the prothoracic gland cells of the ring gland (right column of part 2).

**Modelový příklad 2:**  
**Metabolismus xenobiotik**

# Metabolismus a detoxikace polutantů

## 3 fáze metabolismu cizorodých látek:

- **1. fáze biotransformace** – konverze – oxidace, redukce, hydrolýza, hydratace a izomerace;
- **2. fáze biotransformace** – konjugační reakce – glukuronidace, sulfonace, metylace, acetylace, konjugace s glutathionem, konjugace s aminokyselinami;
- **3. fáze biotransformace** – vyloučení konjugovaných metabolitů z buňky – ABC transportéry a další transportní proteiny;

Přehled: L. Skálová a kol., Metabolismus léčiv a jiných xenobiotik, Karolinum, Praha, 2011

# 1. fáze biotransformace



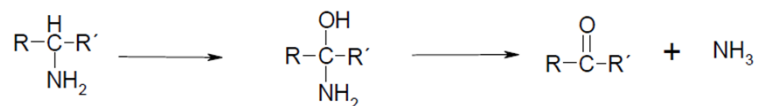
## Oxidace:

- **hydroxylace** – běžná biotransformační reakce (alifatické, aromatické uhlovodíky) – snižuje se lipofilita toxikantu, vzniklé hydroxyderiváty podléhají dalším konverzním nebo konjugačním reakcím;

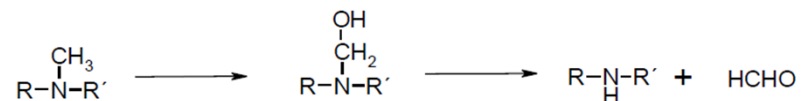
- oxidace alkoholů a aldehydů;



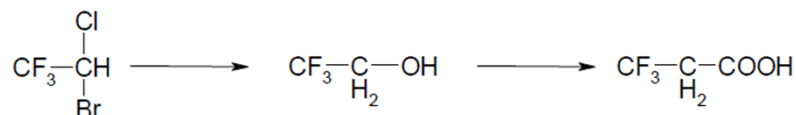
- oxidační deaminace;



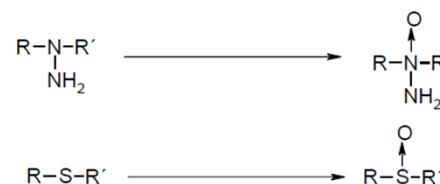
- **dealkylace** - sekundární a terciární aminy, alkoxy-, alkylthiolové skupiny;



- **dehalogenace**;



- *N*-oxidace, *S*-oxidace;



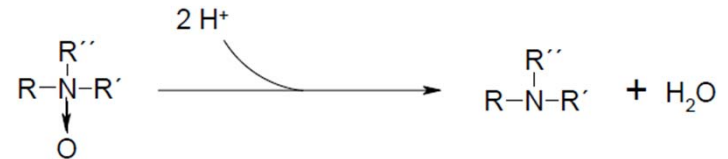
# 1. fáze biotransformace

## Redukce:

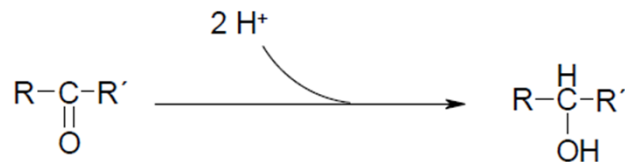
- z hlediska množství metabolizovaných látek méně významné, ale představují hlavní cestu pro detoxikaci některých specifických skupin látek;
- např. redukce nitrosloúčenin a azosloúčenin;



- **redukce N-oxidů a S-oxidů;**



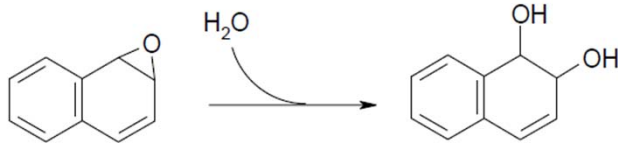
- **redukce karbonylových sloučenin a chinonů;**



# 1. fáze biotransformace

## Hydrolýza:

- estery, epoxidy, amidy, hydrazidy a karbamáty;
- některé hydrolasy (např. epoxidhydrolasa) jsou schopné katalyzovat i hydrataci (tj. adici vody);
- hydrolýze mohou podléhat i některé konjugáty xenobiotik;



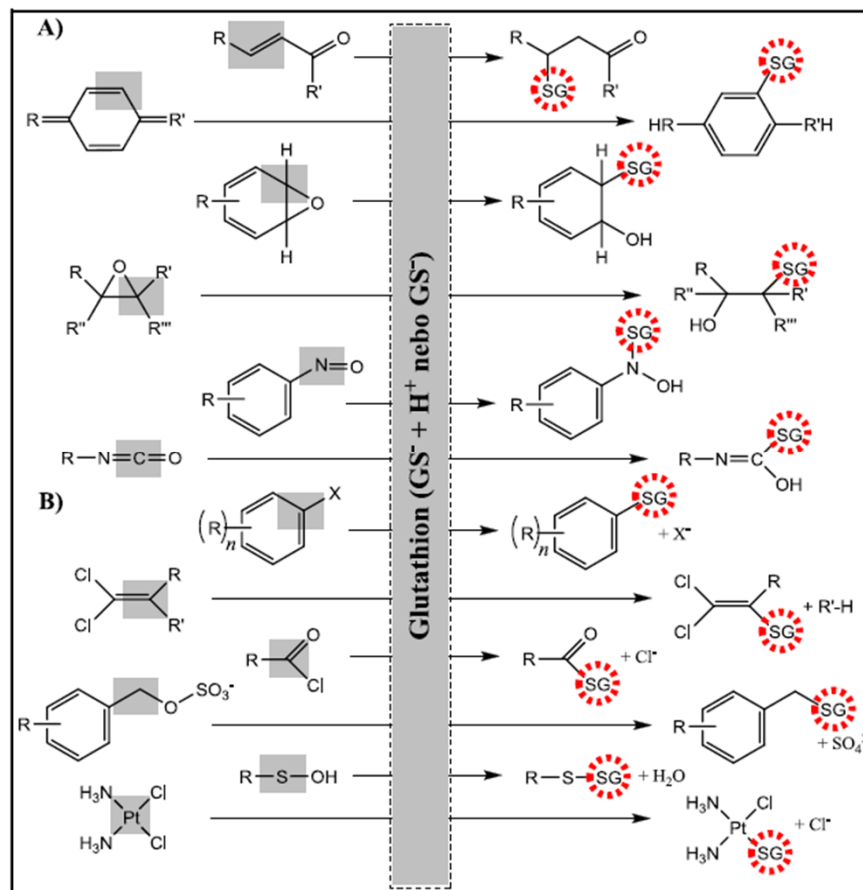
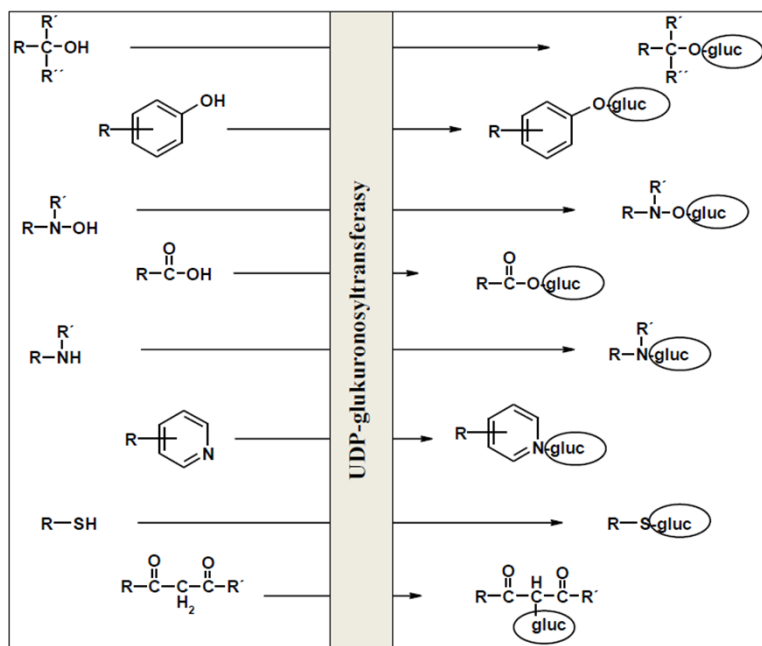
# Enzymy 1. fáze biotransformace

- **cytochromy P450;**
- flavinové monooxygenasy;
- peroxidasy;
- alkoholdehydrogenasy;
- aldehyd dehydrogenasy;
  
- aldo-keto reduktasy;
- dehydrogenasy s krátkým řetězcem (např. karbonylreduktasy);
  
- hydrolasy – zvl. roli epoxidhydrolasa;

## 2. fáze biotransformace

### Konjugační reakce:

- nejvýznamnější pro – konjugace s **UDP-glukuronovou** skupinou, **glutathionem** a sulfonace (s 3'-fosfoadenosin-5'-fosfosulfátem);

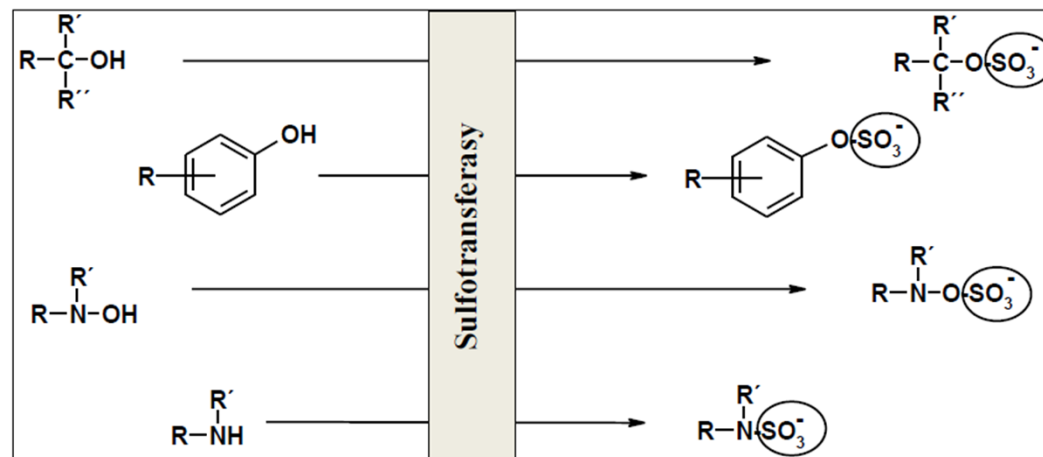
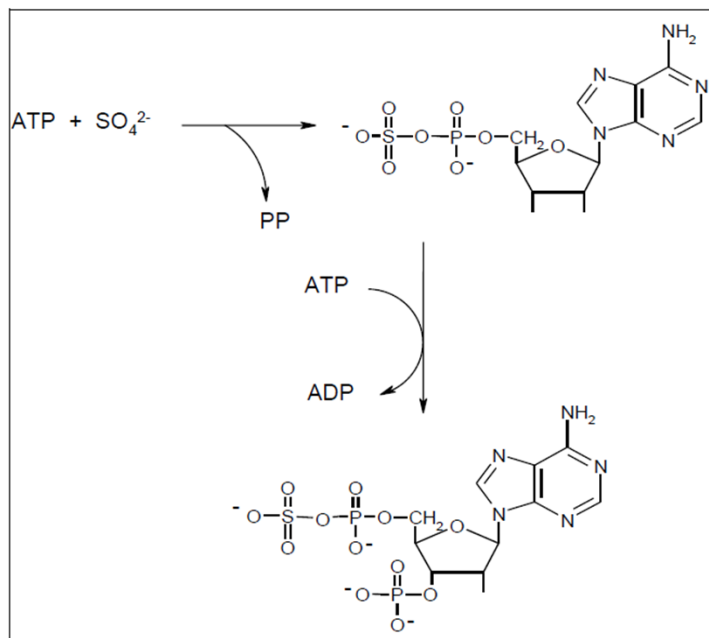




## 2. fáze biotransformace

### Konjugační reakce:

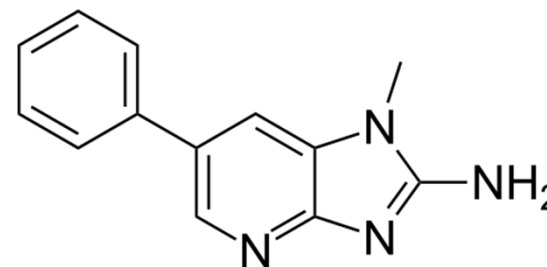
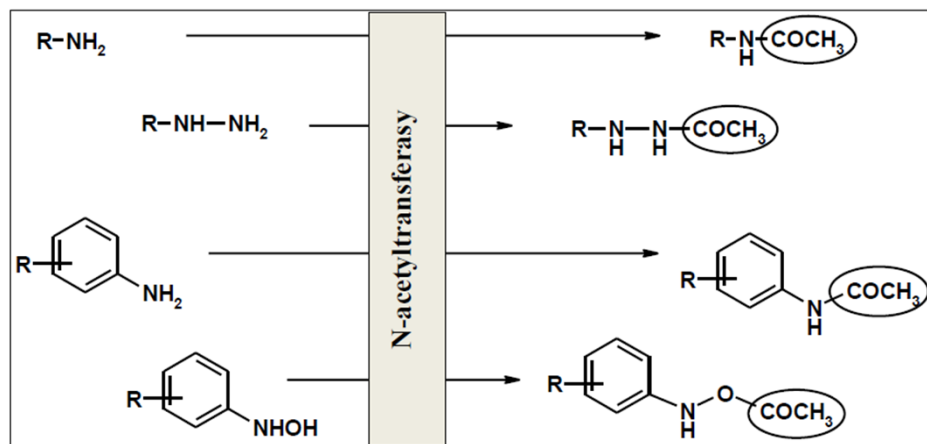
- nejvýznamnější pro – konjugace s UDP-glukuronovou skupinou, glutathionem a **sulfonace** (s **3'-fosfoadenosin-5'-fosfosulfátem**);



## 2. fáze biotransformace

### Konjugační reakce:

- acetylace – cytosolové N-acetyltransferasy;
- bioaktivace heterocyklických aminů – silné genotoxiny;



2-amino-1-methyl-6-fenylimidazo[4,5-b]pyridin  
(PhIP)

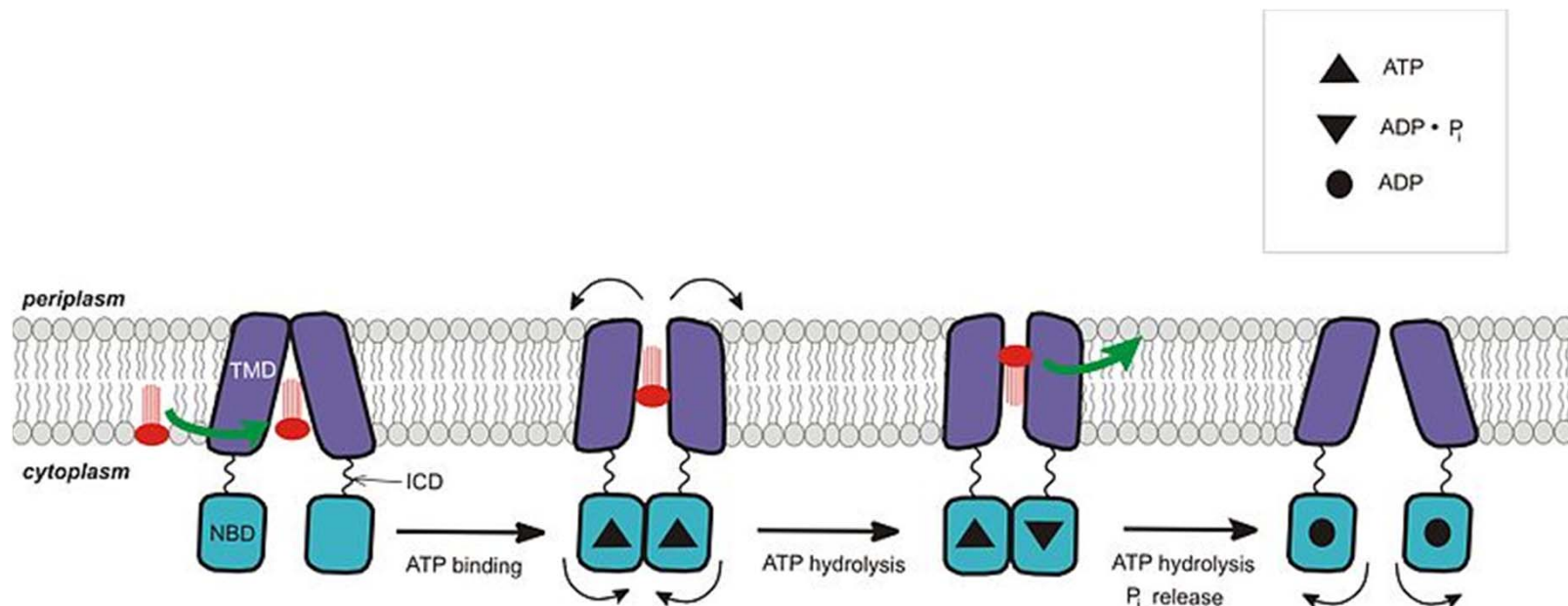
*L. Skálová a kol., Metabolismus léčiv a jiných xenobiotik, Karolinum, Praha, 2011*

## Enzymy 2. fáze biotransformace

- **UDP-glukuronosyltransferasy;**
- **gluthathion-S-transferasy** – cytosolové, mikrosomální;
- **sulfotransferasy;**
- *N*-acetyltransferasy;
- methyltransferasy;

# Transport xenobiotik a jejich metabolitů

- někdy označován jako 3. fáze biotransformace;
- hlavní roli hrají specifické transportní proteiny – přenašeče;
- ABC transportéry (z angl. *ATP-binding cassette*) – BRCP, MDR1/P-gp, MRP proteiny;
- SLC proteiny - přenašeče hydrofilních látek (angl. *solute carrier family*);



Dong et al., 2005, Science 308: 1023-1028.

Table 3 | **XME receptors that regulate XMEs and/or XRTs**

XME receptor	Ligands	XMEs up- or downregulated	XRTs up- or downregulated
AHR	Dioxin, coplanar PAHs, coplanar PHAHs, benzoflavones	CYP1, CYP2A, CYP2C*, CYP2S1*, NQO1, ALDH3A1, GSTA1, UGT1A6/7	?
CAR	Phenobarbital, TCPOBOP, colupulone, androstanes	CYP2B, ALDH, esterase, FMO, methyltransferase, GST, SULT, UGT1A6	ABCC2
FXR	Bile acids	CYP7A1, CYP8B1	ABCB11
HNF-1 $\alpha$	Bile acids	UGT1A7, GSTA2, UGT1A1, UGT2B17	SLC21A6
HNF3 $\alpha,\beta,\gamma$	Epidermal growth factor, fushi tarazu factor-1 $\alpha$ , LPS	CYP2C, CYP3A, CYP7A1	SLC21A10
HNF4 $\alpha$	Long-chain fatty acyl-coA thioesters, chenodeoxycholic acid	CYP2D6	Proteins involved in glucose transport and metabolism
LXR $\alpha,\beta$	Oxysterols	CYP7A1	ABCA1, ABCD2, ABCG1, ABCG4, ABCG5, ABCG8
PPAR $\alpha$	Fibrates, fatty acids	CYP4A1, CYP4A3	ABCC3, ABCC4, proteins involved in glucose transport and metabolism
PPAR $\delta$	Fatty acids, carboprostacyclin	?	?
PPAR $\gamma$	Fatty acids, eicosanoids, thiazolidinediones	CYP4B1	?
PXR (SXR)	Pregnenolone 16 $\alpha$ -carbonitrile, rifampicin, LCA	CYP1, CYP2A, CYP2B, CYP2C, CYP3A, CYP4F, carboxylesterase, MAO, CAT, FMOs, GSTs, UGTs, SULT2A	ABCB1, ABCC2
RAR $\alpha,\beta,\gamma$	Retinoic acids	CYP26A1	?
RXR $\alpha,\beta,\gamma$	9-cis-retinoic acid	?	?
VDR	1 $\alpha$ ,25-dihydroxy-vit D <sub>3</sub>	CYP24A1, CYP27B1	?

Further details of xenobiotic-metabolizing enzymes (XMEs) receptors that regulate XMEs and/or xenobiotic-related transporters (XRTs) can be found in several excellent reports<sup>61,132-135</sup>. \*Only one (or very few) members of the CYP2A and CYP2C subfamilies are upregulated by the aryl hydrocarbon receptor (AHR). All the gene products in this table are given their official names according to the HUGO gene nomenclature homepage. '?' denotes that XMEs and XRTs are expected to be regulated by this XME receptor, but that none have been identified to date. CAR, constitutive androstane receptor; FXR, farnesoid X receptor; HNFs, hepatocyte nuclear factors; LCA, the toxic bile acid lithocholic acid; LPS, lipopolysaccharide; LXRs, liver X receptors; PAHs, polycyclic aromatic hydrocarbons; PHAHs, polyhalogenated aromatic hydrocarbons; PPARs, peroxisome proliferator-activated receptors; PXR, pregnane X receptor; RARs, retinoic acid receptors; RXRs, retinoid X receptors; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; VDR, vitamin D<sub>3</sub> receptor.

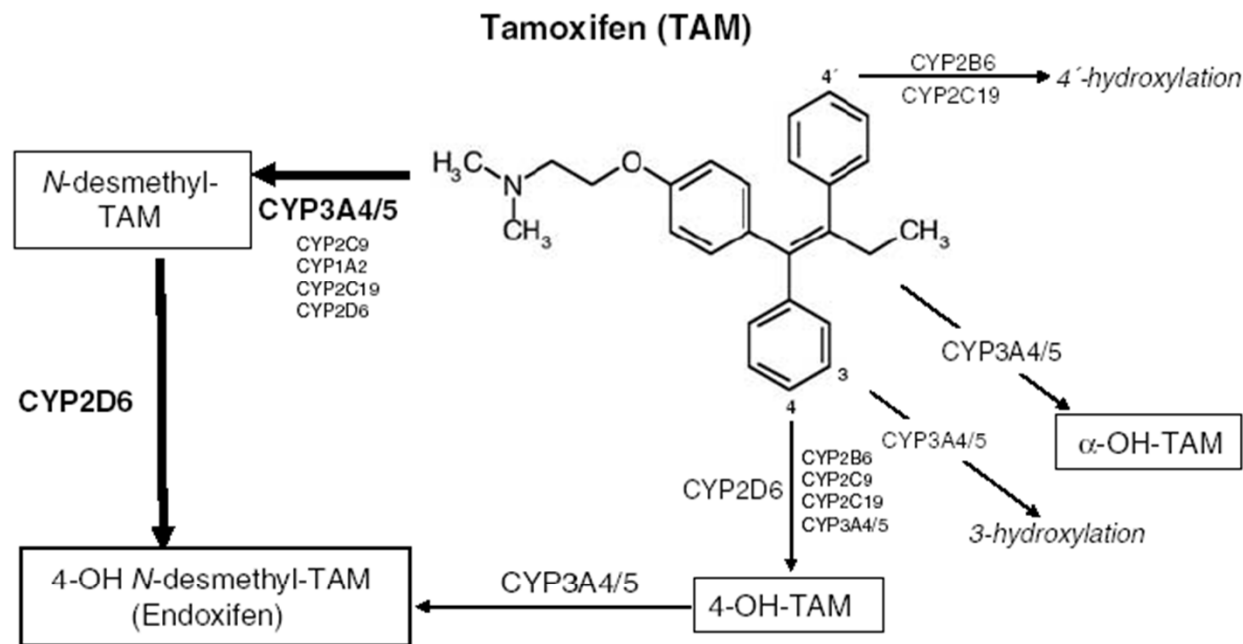
## Aktivace promutagenů:

**Table 2** Precarcinogens metabolized by cytochromes P450

<i>Enzyme</i>	<i>Activation of carcinogens</i>
CYP1A1	Polycyclic aromatic hydrocarbons: benzo( <i>a</i> )pyrene, dimethylbenz[ <i>a</i> ]anthracene, PhIP <sup>a</sup>
CYP1A2	Activation of aryl and heterocyclic amines in industrial settings and food mutagens: <i>N</i> -nitrosodimethylamine, 4-aminobiphenyl, 2-acetyl-amino-fluorene, <i>N</i> -nitrosodiethylamine, PhIP, IQ, aflatoxin B1
CYP1B1	Polycyclic aromatic hydrocarbons: benzo( <i>a</i> )pyrene, dimethylbenz[ <i>a</i> ]anthracene, benz[ <i>a</i> ]anthracene, 3-methylcholanthrene, DMBA, oestradiol
CYP2A6	Activation of tobacco-related <i>N</i> -nitrosamines: NNK, NNAL, NDEA, NNN, NATB, Aflatoxin B1, 1,3-butadiene, 2,6-dichlorobenzonitrile
CYP2B6	Aflatoxin B1 and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
CYP2E1	Low-molecular-weight toxicants and cancer suspect agents: benzene, carbon tetrachloride, chloroform, styrene, vinyl chloride, vinyl bromide, <i>N</i> -nitrosodimethylamine, NNK
CYP3A4/5/7	Diverse carcinogens: aflatoxin B1, aflatoxin G1, benzo( <i>a</i> )pyrene, naphthalene, NNN, 1-nitropyrene, 6-amino-chrysene, oestradiol, senecionine, stergmato-cystine

<sup>a</sup>DMBA, 7,12,-dimethylbenz[*a*]anthracene; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; NATB, *N*-nitrosoanatabine; NDEA, *N*-nitrosodiethylamine; NNAL, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; NNN, *N*9-nitrosornicotine; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine.

# Metabolismus léčiv:



**Figure 2** Chemical structure of tamoxifen and major biotransformation pathways. CYP3A4/5 are the more efficient enzymes responsible for the *N*-demethylation of tamoxifen (TAM), whereas the generations of endoxifen and 4-hydroxytamoxifen (4-OH-TAM) are predominantly catalysed by CYP2D6. Other CYP isoforms, including CYP2C19, CYP2C9, CYP2B6 and CYP1A2, have also been shown to participate in the metabolism of tamoxifen. The most abundant compounds in plasma are *N*-desmethyltamoxifen and endoxifen, and endoxifen has approximately 100 times greater affinity for the oestrogen receptor than tamoxifen and *N*-desmethyltamoxifen. CYP2D6 polymorphisms have been shown to affect the plasma concentrations of endoxifen.

# Jaderné receptory a další proteiny:

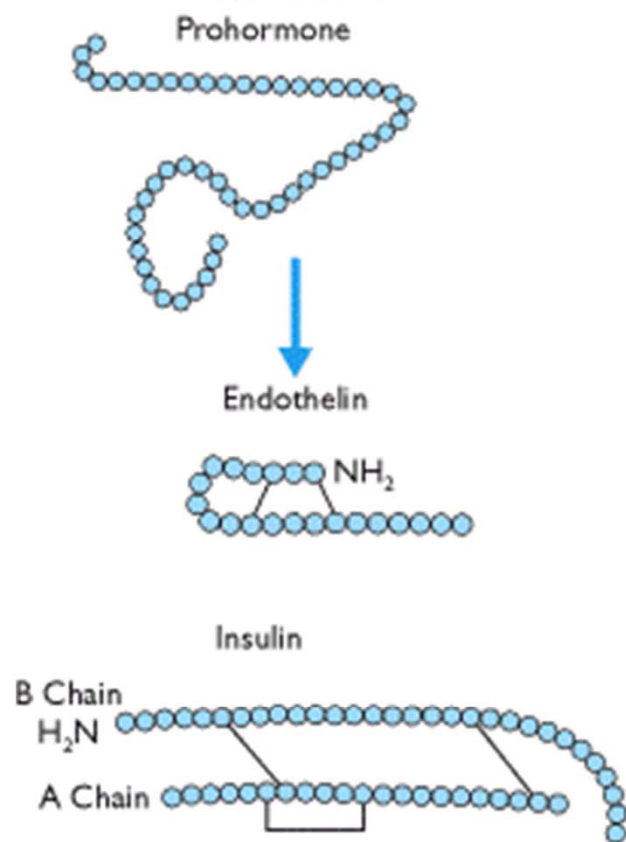
Nuclear receptor	Ligand	CYP enzyme	Cytosolic binding protein	ABC transporter
Retinoid X receptors*	RXR $\alpha,\beta,\gamma$ 9- <i>cis</i> Retinoic acid	-	-	-
Peroxisome proliferator-activated receptors	PPAR $\alpha$	Fatty acids Fibrates ↑ CYP4A1 ↑ CYP4A3	↑ L-FABP	↑ ABCD2, ABCD3 ↑ ABCB4
	PPAR $\delta$	Fatty acids Carboprostacyclin (?)	(?)	(?)
	PPAR $\gamma$	Fatty acids Eicosanoids Thiazolidinediones ↑ CYP4B1	↑ ALBP/aP2 ↑ H-FABP	(?)
Liver X receptors	LXR $\alpha,\beta$ 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	RAR $\alpha,\beta,\gamma$ Retinoic acids	↑ CYP26A1	↑ CRABPII ↑ CRBPI	(?)
Vitamin D receptor	VDR 1,25(OH) $_2$ -vitamin D $_3$	↑ CYP24 ↓ CYP27B1	(?)	(?)



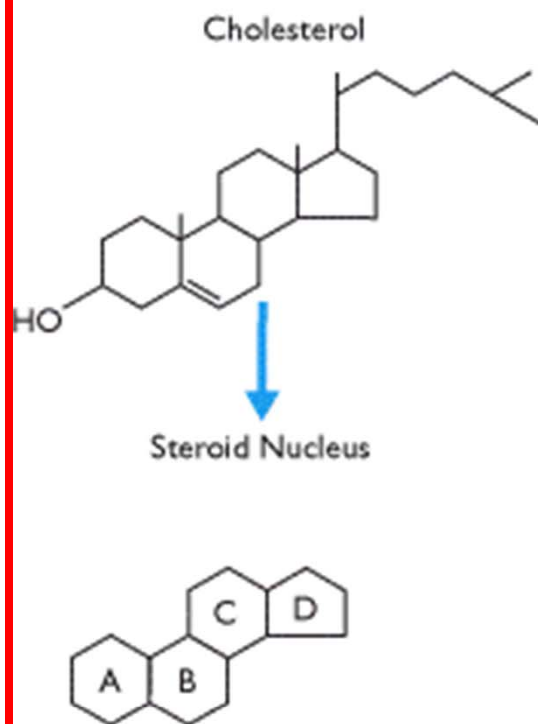
**Modelový příklad 3:**

**Steroidní hormony**

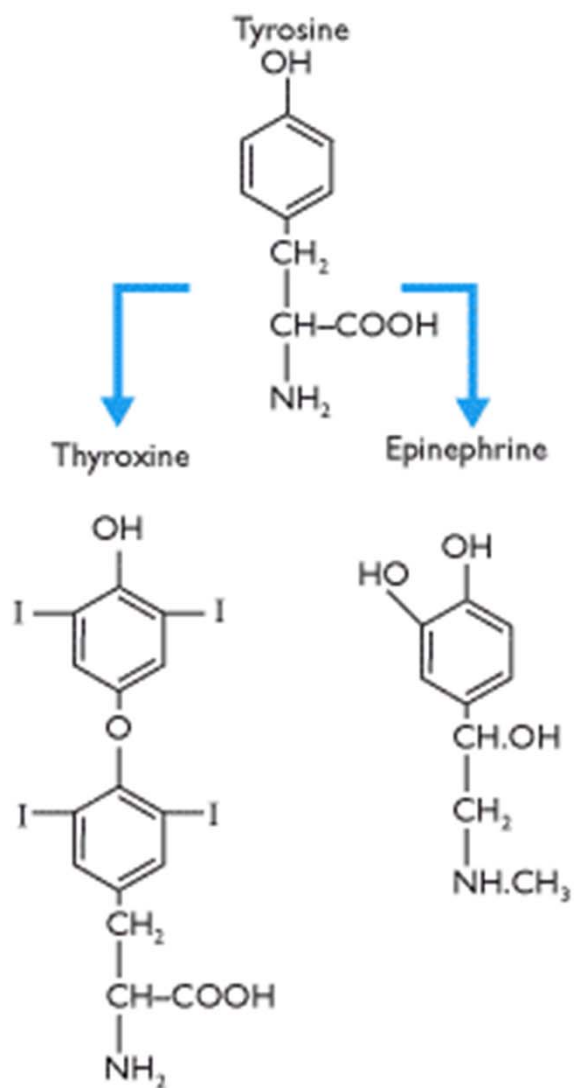
### Protein and Peptide Hormones



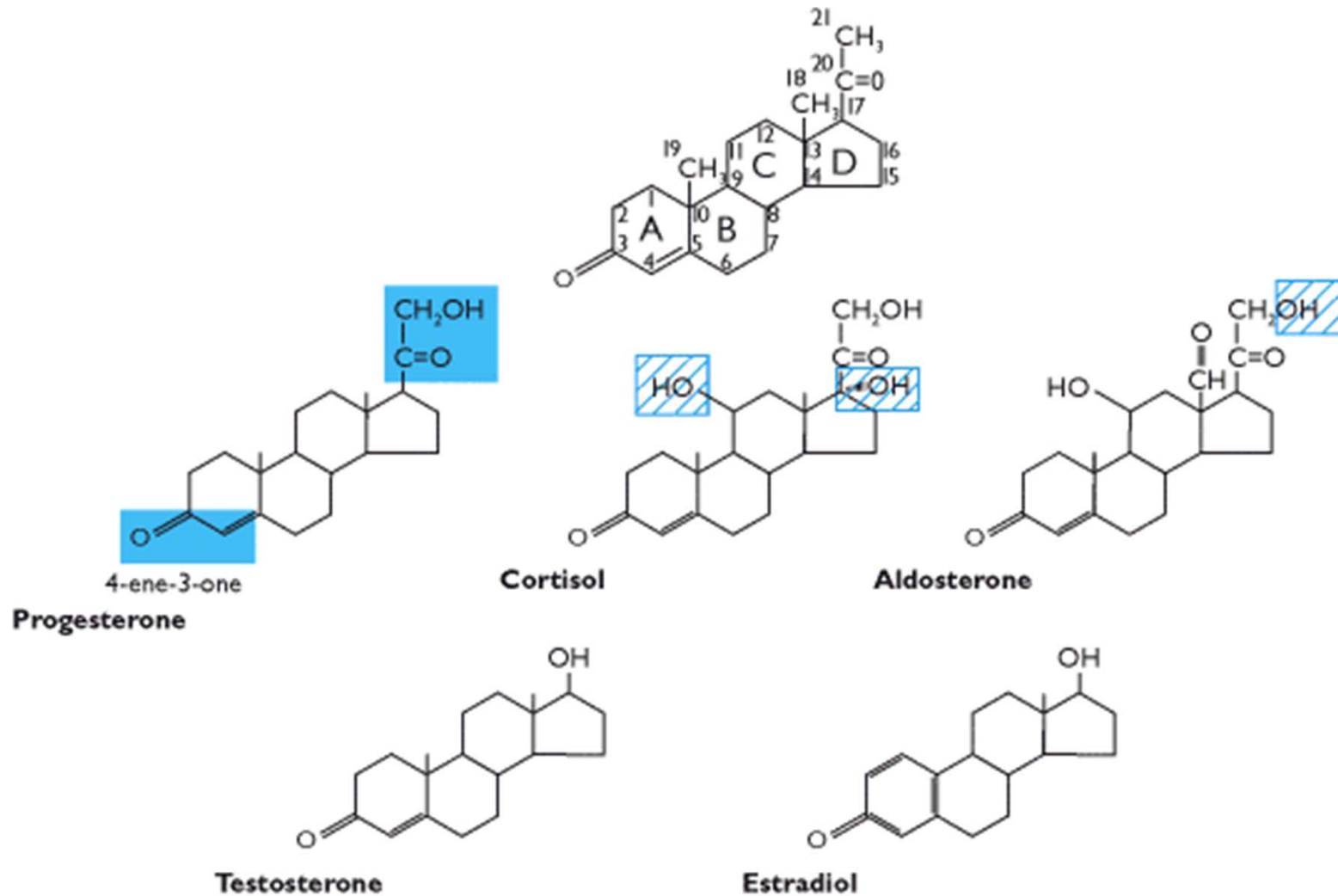
### Steroid Hormones



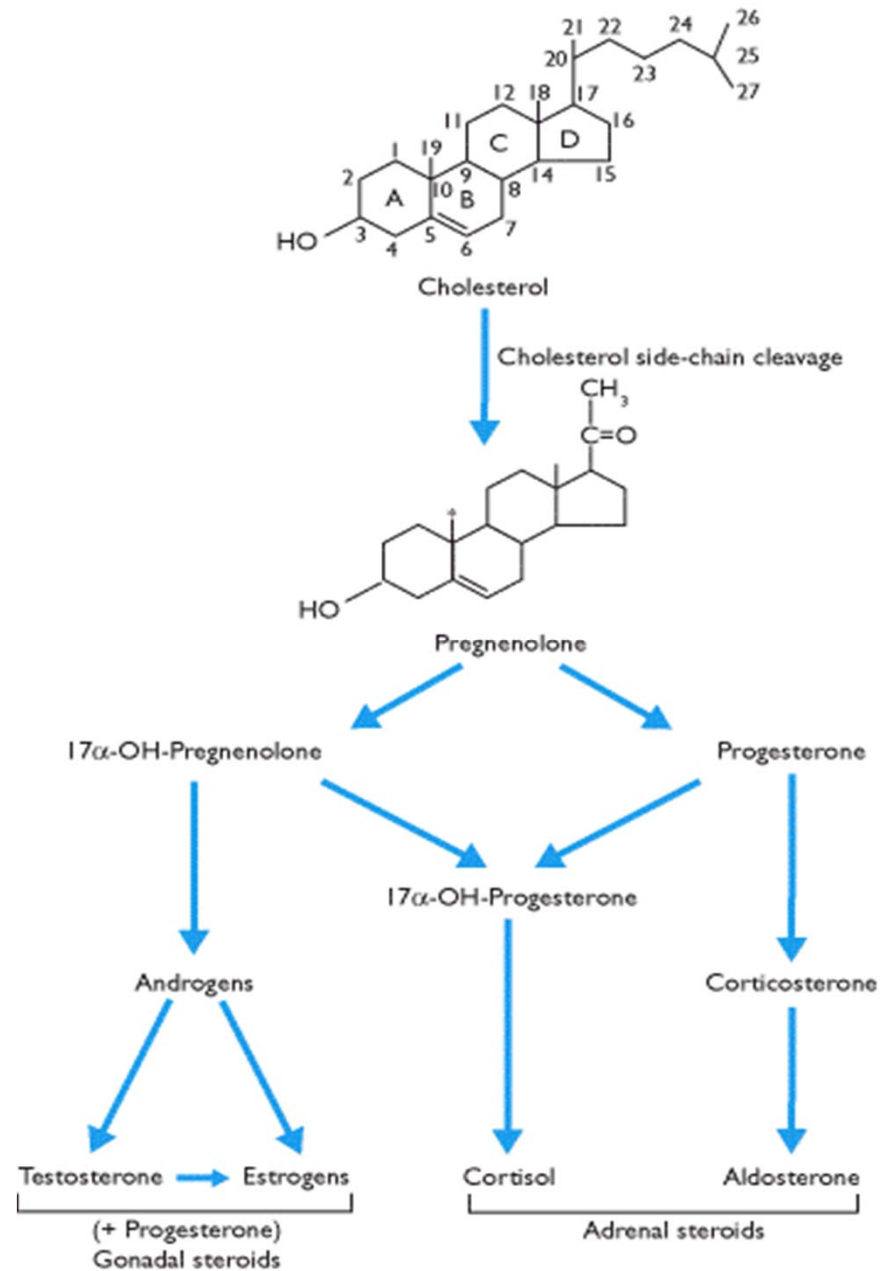
### Tyrosine Derivatives



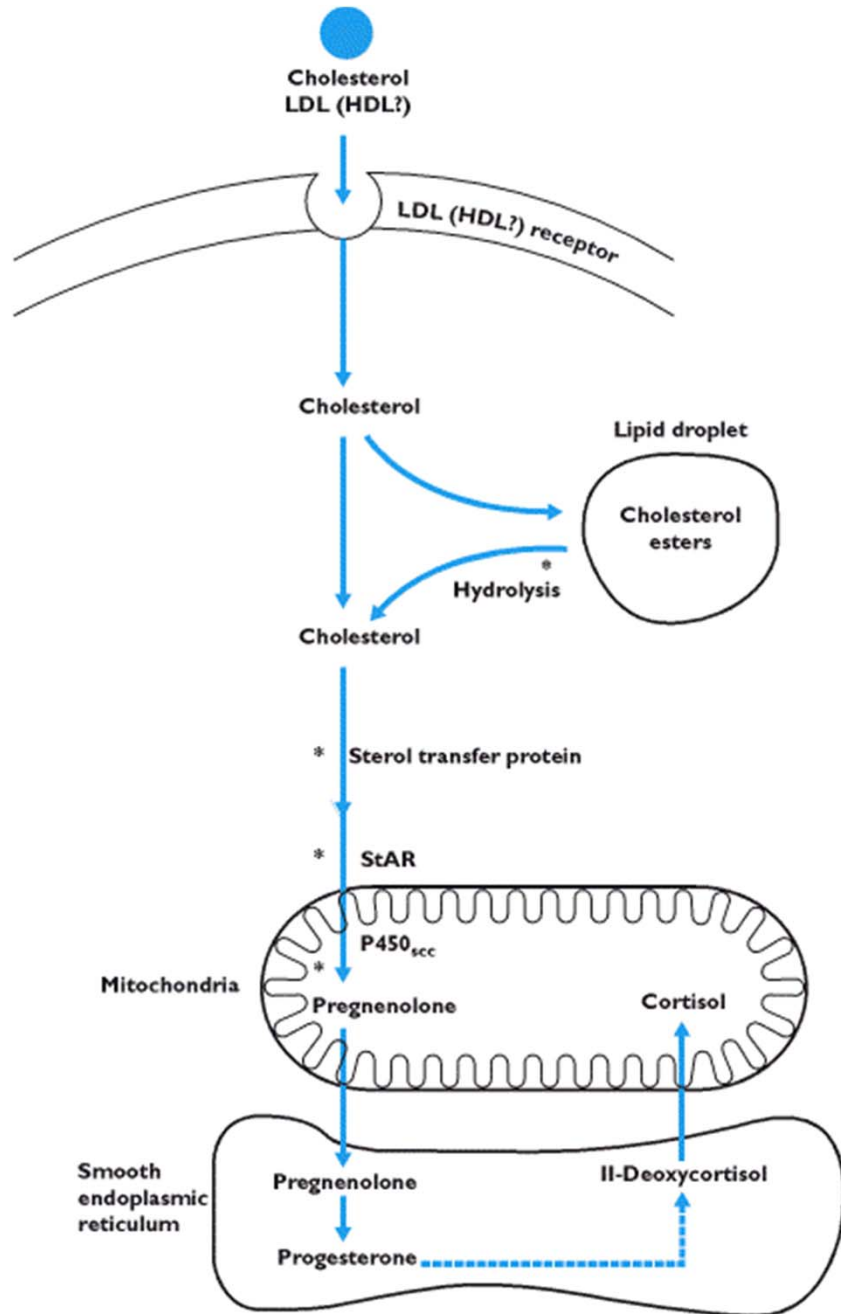
# Pět hlavních skupin steroidních hormonů:



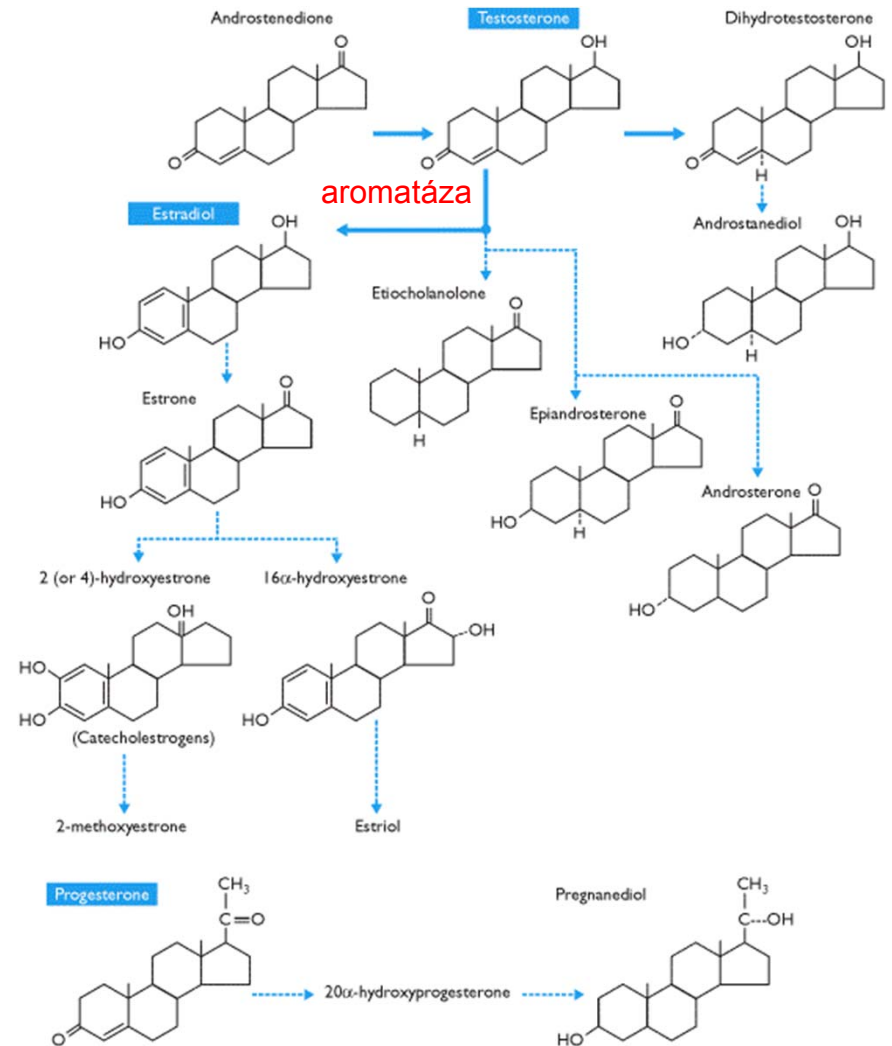
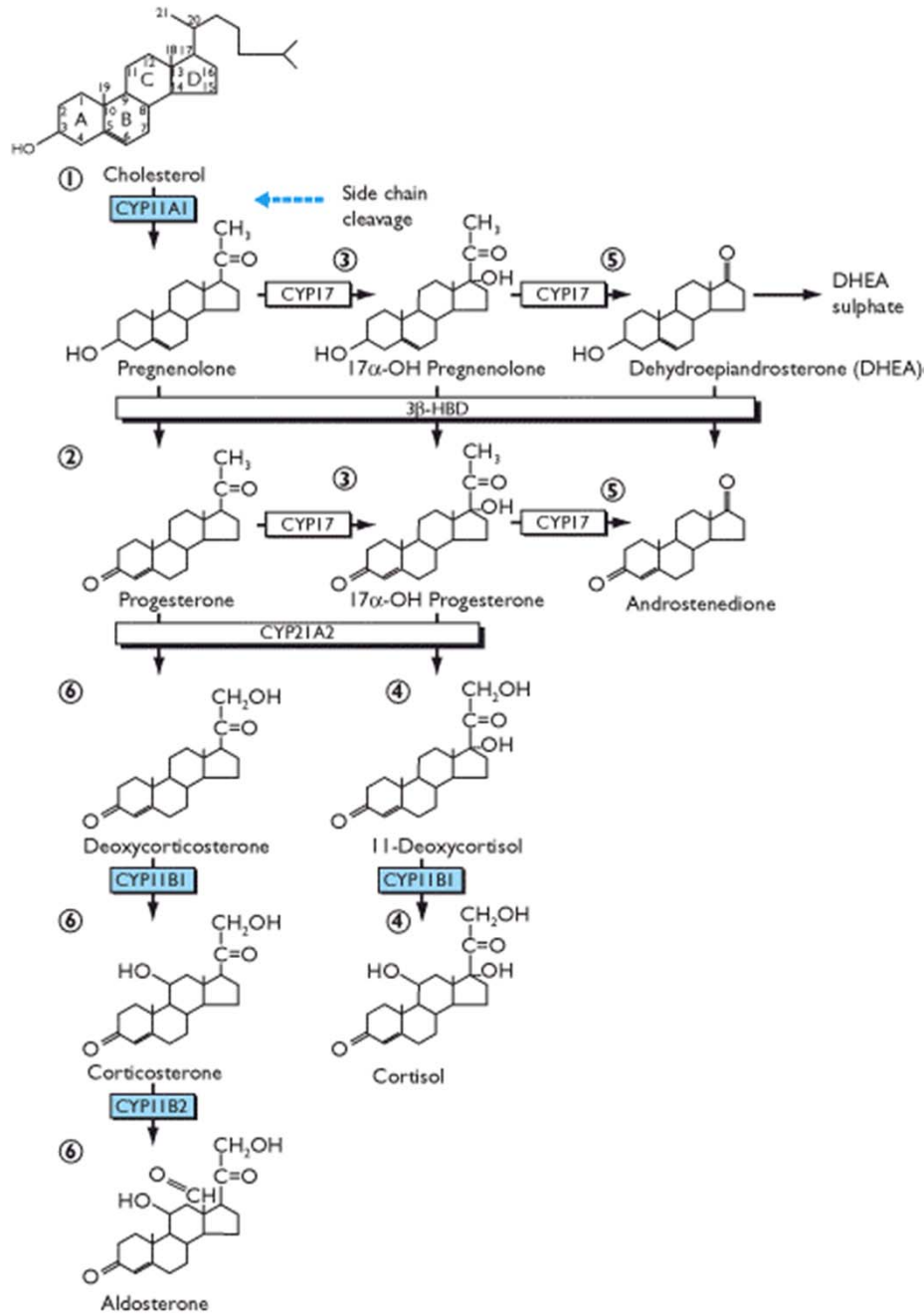
# Biosyntéza steroidních hormonů:

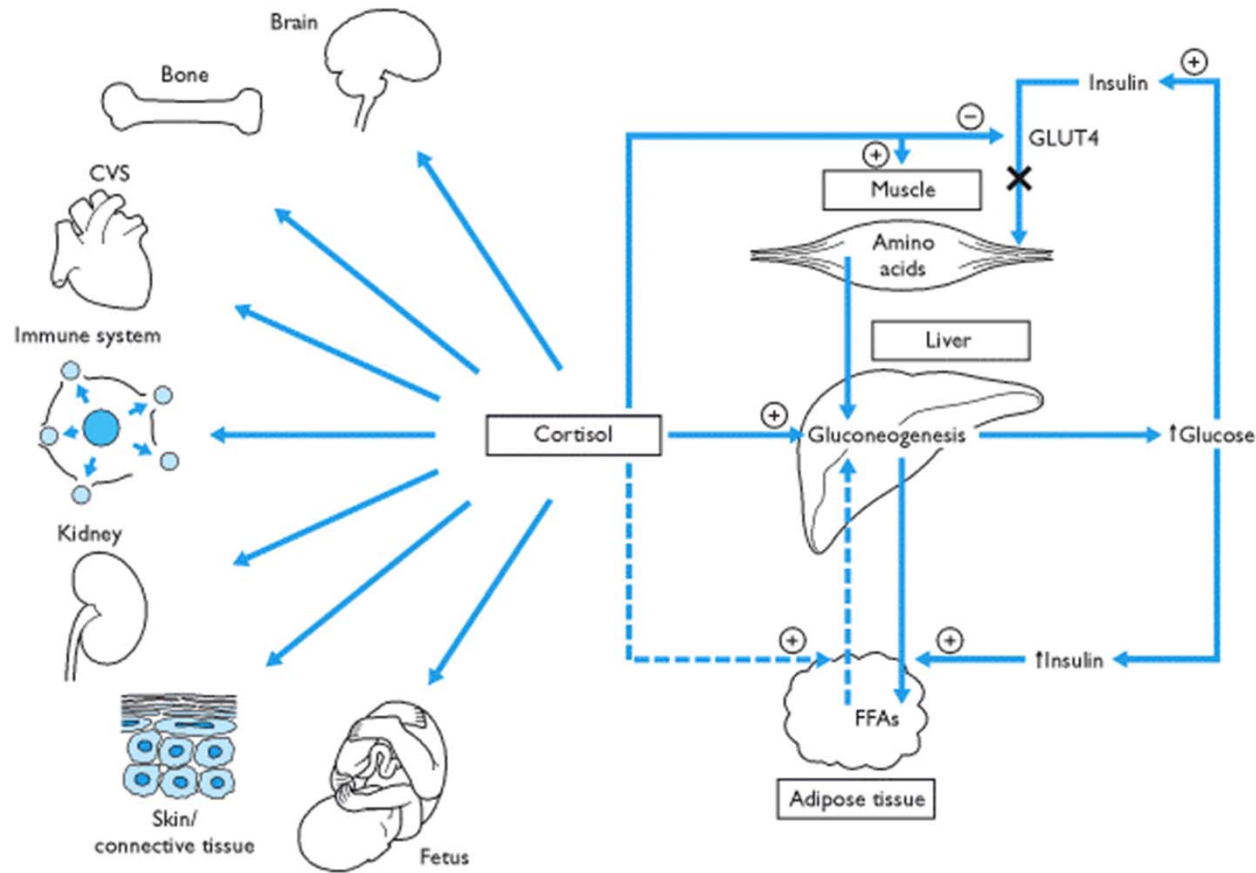


# Diagrammatic outline of the synthesis of cortisol from cholesterol in the adrenal cortex



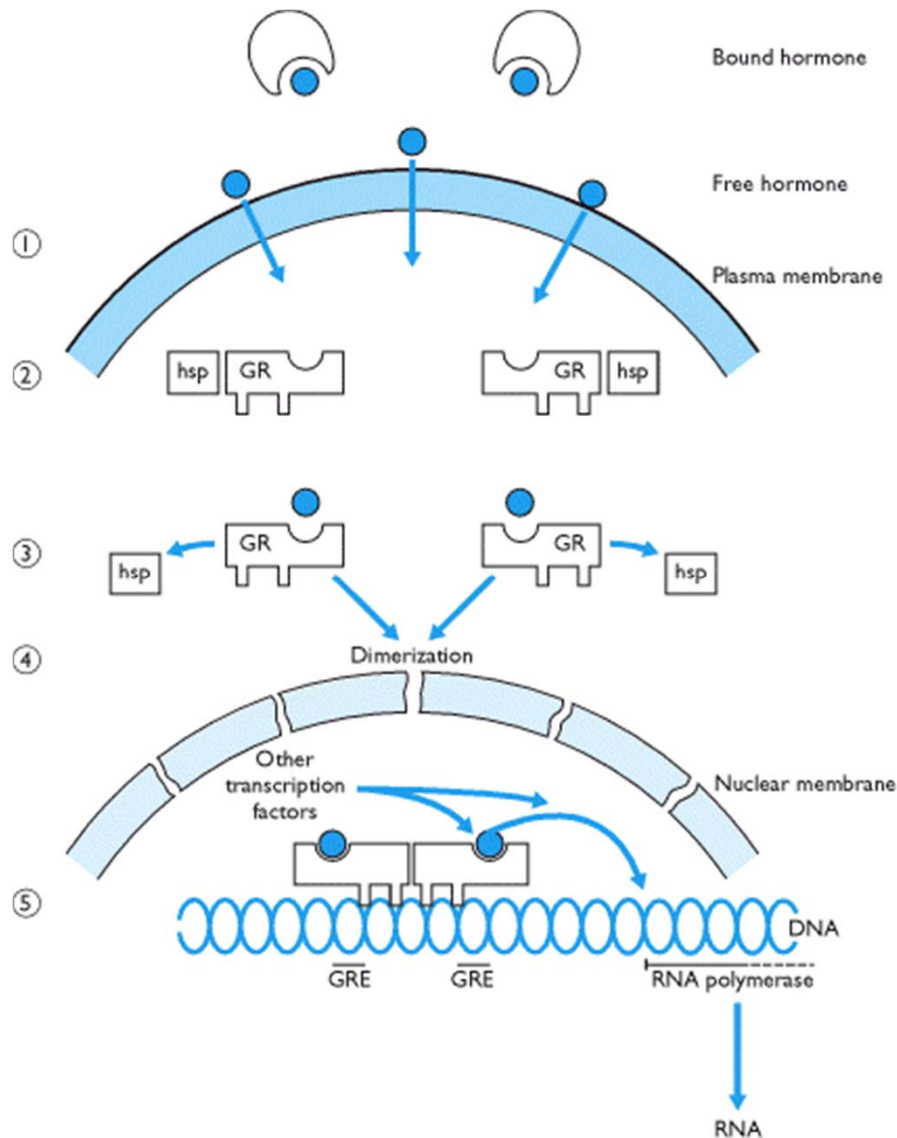
Cholesterol is either obtained from the diet or synthesized from acetate by a CoA reductase enzyme. Approximately 300 mg cholesterol is absorbed from the diet each day and about 600 mg synthesized from acetate. Cholesterol is insoluble in aqueous solutions and its transport from the main site of synthesis, the liver, requires apoproteins to form a lipoprotein complex. In the adrenal cortex, about 80% of cholesterol required for steroid synthesis is captured by receptors which bind low-density lipoproteins (LDL) although recent evidence has shown that high-density lipoprotein (HDL) cholesterol may also be taken up by adrenal cells. The remaining 20% is synthesized from acetate within the adrenal cells by the normal biochemical route.





- Cortisol stimulates the release of amino acids from muscle. These are taken up by the liver and converted to glucose.
- The increased circulating concentration of glucose stimulates insulin release. Cortisol inhibits the insulin-stimulated uptake of glucose in muscle via the GLUT4 transporter.
- Cortisol has mild lipolytic effects. These are overpowered by the lipogenic action of insulin secreted in response to the diabetogenic action of cortisol.
- Cortisol also has varied actions on a wide range of other tissues

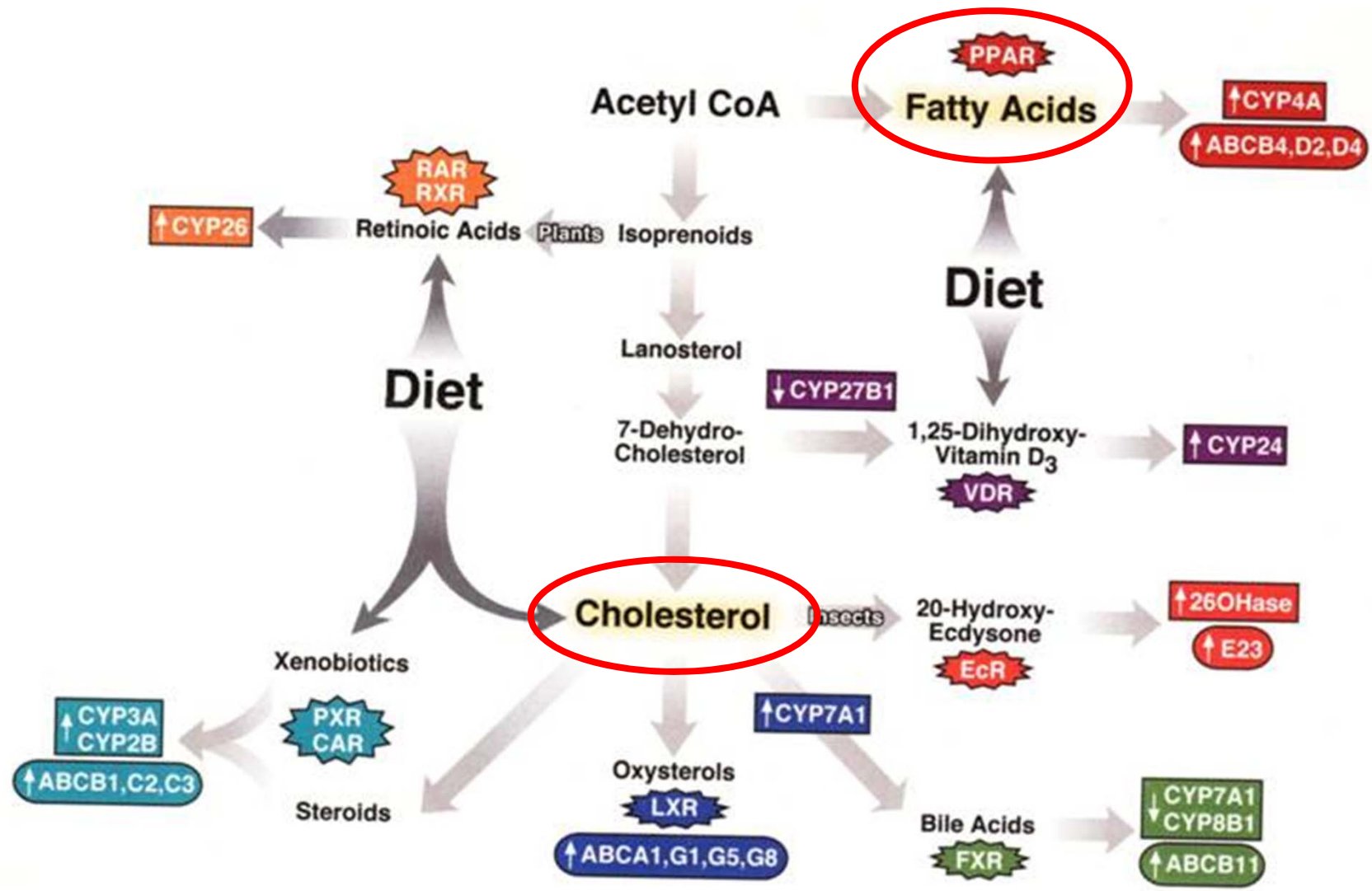
# The glucocorticoid receptor and activation by cortisol



- 1) Unbound, lipophilic cortisol readily crosses cell membranes and in target tissues will combine with the glucocorticoid receptor (GR).
- 2) Like the androgen and progesterone receptors, unliganded GRs are located in the cytoplasm attached to heat shock proteins (hsp-90, hsp-70 and hsp-56).
- 3) When hormones bind to these receptors hsp are released and the hormone receptor complexes translocate to the nucleus.
- 4) These complexes form homo- or heterodimers and the zinc fingers of their DNA-binding domains slot into the glucocorticoid response elements (GREs) in the DNA helix.
- 5) Together with other transcription factors, such as NF- $\kappa$ B or c-jun and c-fos, they initiate RNA synthesis (activation of RNA polymerase) downstream of their binding.



**Modelový příklad 4:  
Metabolismus mastných kyselin**



**Receptory aktivované  
peroxizómovými proliferátory  
(PPAR)**

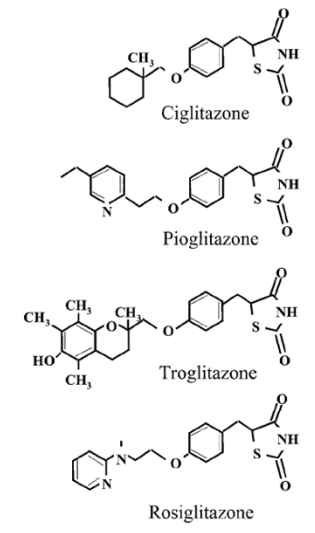
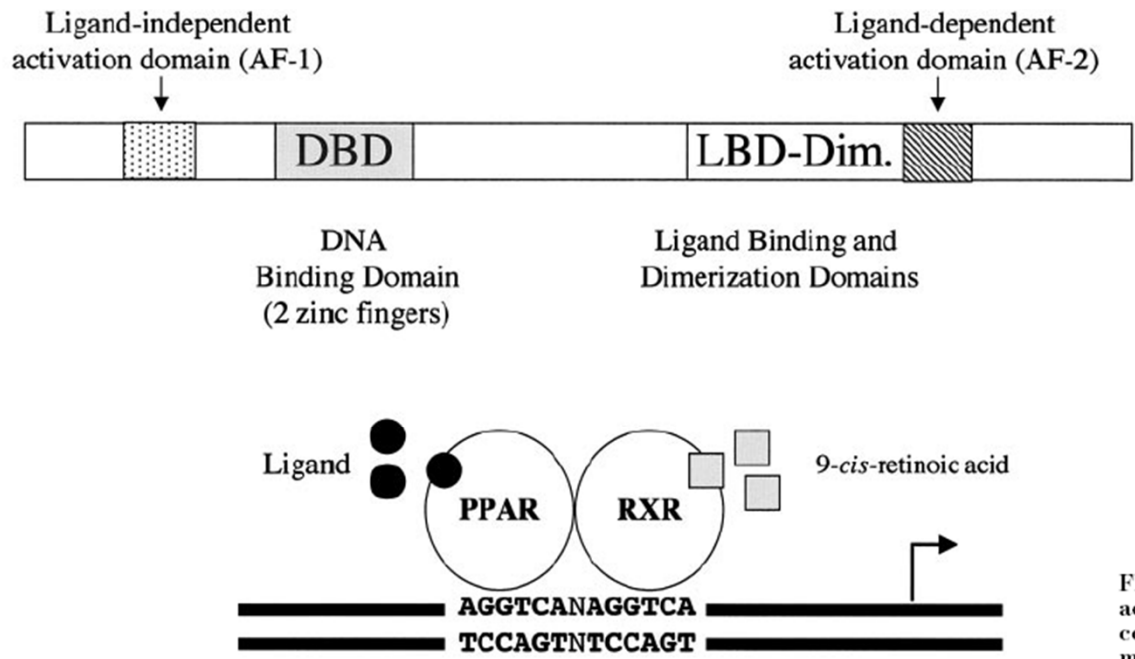
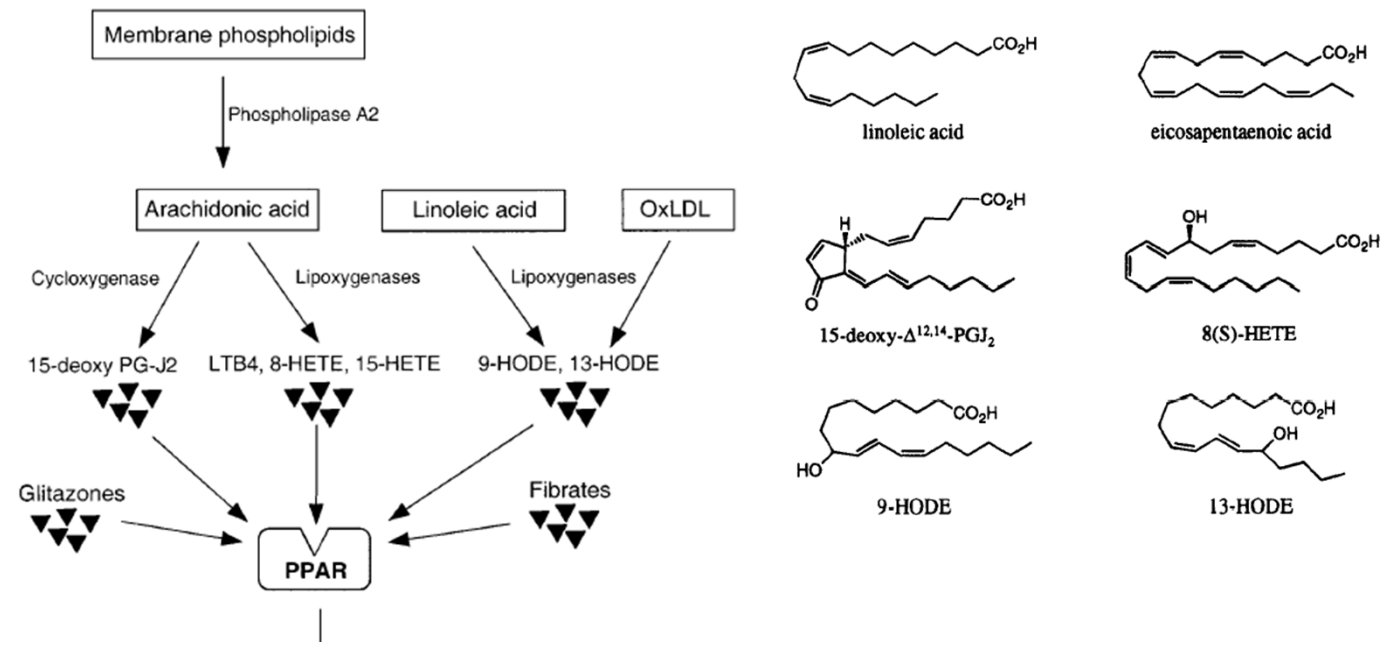


FIG. 1. General structure and mechanism of action of PPARs. PPAR isoforms share a common domain structure and molecular mechanism of action.



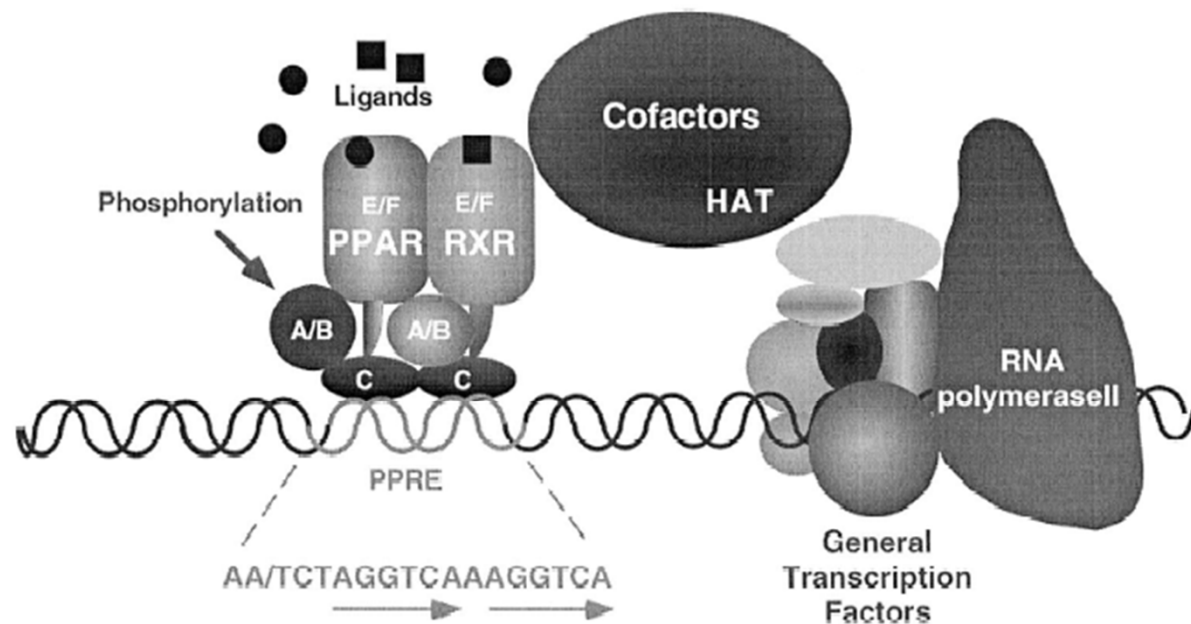


Fig. 3. Mechanisms of transactivation. The PPAR/RXR heterodimer binds to a PPRE (PPAR-response elements) located in the promoter of target genes through the C domain (DNA-binding domain) of PPAR and RXR. Receptor activity is regulated by both phosphorylation of A/B domain and ligand-binding by E/F domain (ligand-binding domain). The activated PPAR/RXR heterodimer associates with cofactors containing histone acetyl-transferase activity (HAT), modifying nucleosome structure and contacting general transcription factors.

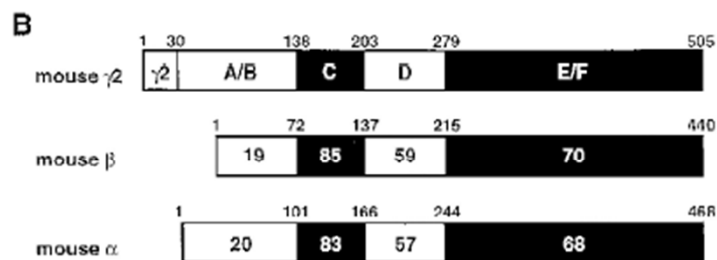
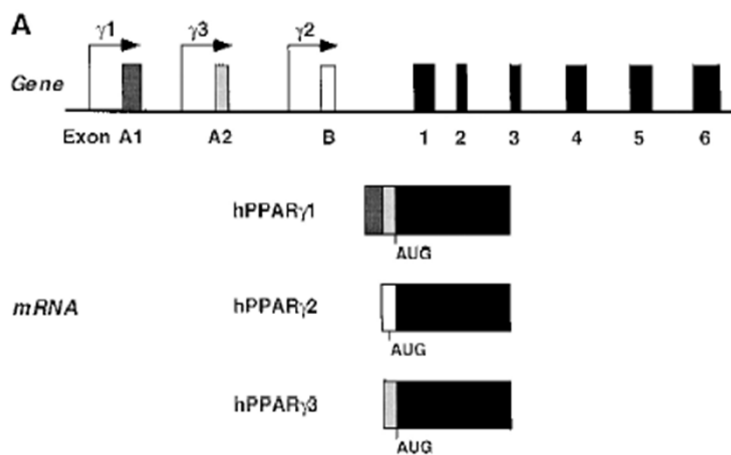


Fig. 1. Structure of PPARs. (A) Genomic organization of the human PPAR $\gamma$  gene (not drawn to scale). Alternative promoter usage and splicing results in three different transcripts. PPAR $\gamma$ 1, PPAR $\gamma$ 2 and PPAR $\gamma$ 3 are transcribed from promoters located upstream of exons A1, B and A2, respectively. PPAR $\gamma$ 1 and PPAR $\gamma$ 3 mRNAs encode the same protein. (B) Structural and functional domains of PPARs.  $\gamma$ 2: PPAR $\gamma$ 2-specific N-terminus of A/B domain. A/B: N-terminal A/B domain containing a ligand-independent activation function 1 (AF-1). (C) DNA-binding domain. (D) Hinge region. (E/F): C-terminal ligand-binding domain containing the ligand-dependent activation function 2 (AF-2). Sequence similarities were determined by the BESTFIT program (GCG package) using reported mouse PPAR $\alpha$  [8], PPAR $\beta$  [14], PPAR $\gamma$ 1 [14] and PPAR $\gamma$ 2 [32] sequences.

Gene	Localization of PPRE	PPRE	function of gene product
ACO	(-570/-558)	$\overrightarrow{\text{TGACCT}}\overrightarrow{\text{TGTCTT}}$	First step in fatty acid $\beta$ -oxidation
	(-214/-202)	$\overrightarrow{\text{TGACCT}}\overrightarrow{\text{CTACCT}}$	
HD	(-2939/-2927)	$\overleftarrow{\text{TGACCT}}\overrightarrow{\text{aTGAACCTaTTACCT}}$	Second and third step in fatty acid $\beta$ -oxidation
C-ACS	(-175/-154)	$\overrightarrow{\text{TGACTG}}\overrightarrow{\text{aTGCCCTgaaAGACCT}}$	Conversion of fatty acids into acyl-CoA derivatives
CYP4A6	(-650/-662)	$\overrightarrow{\text{TCACCTT}}\overrightarrow{\text{aTGCCCTAGTTCA}}$	Formation of dicarboxylic acids by $\omega$ -oxidation
	(-728/-740)	$\overleftarrow{\text{GGACCT}}\overrightarrow{\text{aTGCCCTT}}\overrightarrow{\text{TGTCTT}}$	
	(-27/-1)	$\overrightarrow{\text{TGACCT}}\overrightarrow{\text{aTGCCCA}}$	
HMG-CoAS	(-104/-92)	$\overrightarrow{\text{AGACCT}}\overrightarrow{\text{aTGCCCC}}$	Liver ketogenesis
MCAD	(-301/-336)	$\overleftarrow{\text{TGGTCA}}\overrightarrow{\text{gcctTCACCT-TTACC}}\overrightarrow{\text{ggagagaa}}$ $\overleftarrow{\text{AGGTCA}}$	First step in $\beta$ -oxidation of medium-chain fatty acids
L-FABP	(-68/-56)	$\overrightarrow{\text{TGACCT}}\overrightarrow{\text{aTGCCCT}}$	Liver fatty acid binding protein
aP2	(-5222/-5209)	$\overleftarrow{\text{GGATCA}}\overrightarrow{\text{aAGTTCA}}$	Adipose tissue fatty acid binding protein
ME	(-328/-340)	$\overrightarrow{\text{TCAACT}}\overrightarrow{\text{aTGACCC}}$	Malate decarboxylation, providing NADPH for fatty acid synthesis
PEPCK	(-999/-987)	$\overrightarrow{\text{AGACCT}}\overrightarrow{\text{-TATCCC}}$	Gluconeogenesis and glycero-neogenesis
LPL	(-169/-157)	$\overrightarrow{\text{TGCCCT}}\overrightarrow{\text{aTCCCC}}$	Hydrolysis of triglyceride-rich particles
apo A-I	(-212/-197)	$\overleftarrow{\text{TGAACC}}\overrightarrow{\text{cTGACCC}}\overrightarrow{\text{cTGCCCT}}$	Protein component HDL, co-factor LCAT
apo A-II	(-734/-716)	$\overrightarrow{\text{CAACCT}}\overrightarrow{\text{aTACCCT}}$	Protein component HDL
<b>Consensus</b>		$\overrightarrow{\text{TGACCT}}\overrightarrow{\text{aTGACCT}}$	

Fig. 2. Functional PPRES. The DR-1s are indicated by a solid arrow which is indicated above the sequence when the coding strand is depicted; a dotted arrow indicates eventual additional half sites located adjacent to the DR-1 element. Abbreviations used in this figure include: ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; aP2, adipocyte fatty acid binding protein P2; apo, apolipoprotein; L-FABP, liver fatty acid binding protein; HD, enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase; HMG-CoAS, HMG-CoA synthase; LPL, lipoprotein lipase; MCAD, medium-chain acyl-CoA dehydrogenase; ME, malic enzyme.

The **peroxisome proliferator-activated receptors (PPAR  $\alpha$ ,  $\gamma$ ,  $\delta$ )** are activated by polyunsaturated fatty acids, eicosanoids, and various synthetic ligands. Consistent with their distinct expression patterns, gene-knockout experiments have revealed that each PPAR subtype performs a specific function in fatty acid homeostasis.

**PPAR $\alpha$**  is a global regulator of fatty acid catabolism. PPAR $\alpha$  activation up-regulates the transcription of liver fatty acid-binding protein, which buffers intracellular fatty acids and delivers PPAR $\alpha$  ligands to the nucleus. In addition, expression of two members of the adrenoleukodystrophy subfamily of ABC transporters, ABCD2 and ABCD3, is similarly up-regulated to promote transport of fatty acids into peroxisomes where catabolic enzymes promote  $\beta$ -oxidation. The hepatocyte CYP4A enzymes complete the metabolic cascade by catalyzing  $\omega$ -oxidation, the final catabolic step in the clearance of PPAR $\alpha$  ligands.

**PPAR $\gamma$**  was identified initially as a key regulator of adipogenesis, but it also plays an important role in cellular differentiation, insulin sensitization, atherosclerosis, and cancer. Ligands for PPAR $\gamma$  include fatty acids and other arachidonic acid metabolites, antidiabetic drugs (e.g., thiazolidinediones), and triterpenoids. In contrast to PPAR $\alpha$ , PPAR $\gamma$  promotes fat storage by increasing adipocyte differentiation and transcription of a number of important lipogenic proteins.

Ligands for **PPAR $\delta$**  include long-chain fatty acids and carboprostacyclin. PPAR $\delta$  may affect lipid metabolism in peripheral tissues; it can be antagonized by other small lipophilic agents, including 22(*S*)-hydroxycholesterol, certain unsaturated fatty acids, and geranylgeranyl pyrophosphate.

Nízkomolekulární lipofilní sloučeniny jako ligandy = aktivita jaderných receptorů je do značné míry závislá na syntéze a degradaci ligandů a naopak

