

PAS proteiny jako stresové senzory a vývojové regulátory

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Per-Arnt-Sim – nadrodina proteinů PAS

environmentální senzory, které zprostředkovávají transkripční odpověď na různé typy stimulů:

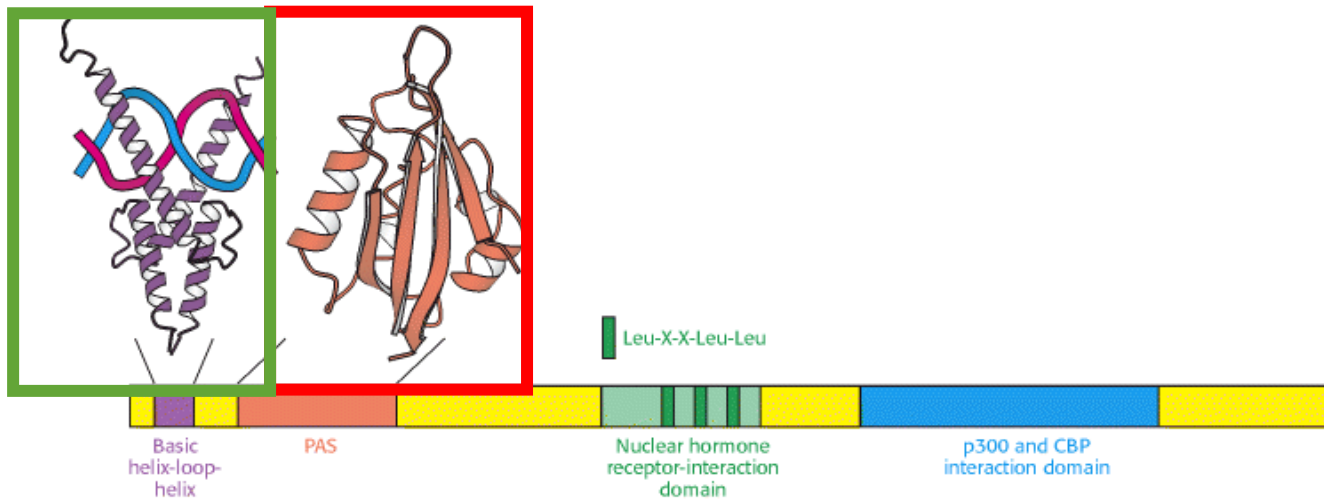
- ✓ cirkadiánní rytmy – vnímání světelných podnětů;
- ✓ vnímání hladiny kyslíku;
- ✓ sensing of toxicants;
- ✓ vývojové regulace – deregulovány v nádorových onemocněních;

Tyto proteiny umožňují adaptaci na rychlé změny v prostředí.

PAS proteiny jsou součástí širší rodiny basic-helix-loop-helix (bHLH) proteinů:

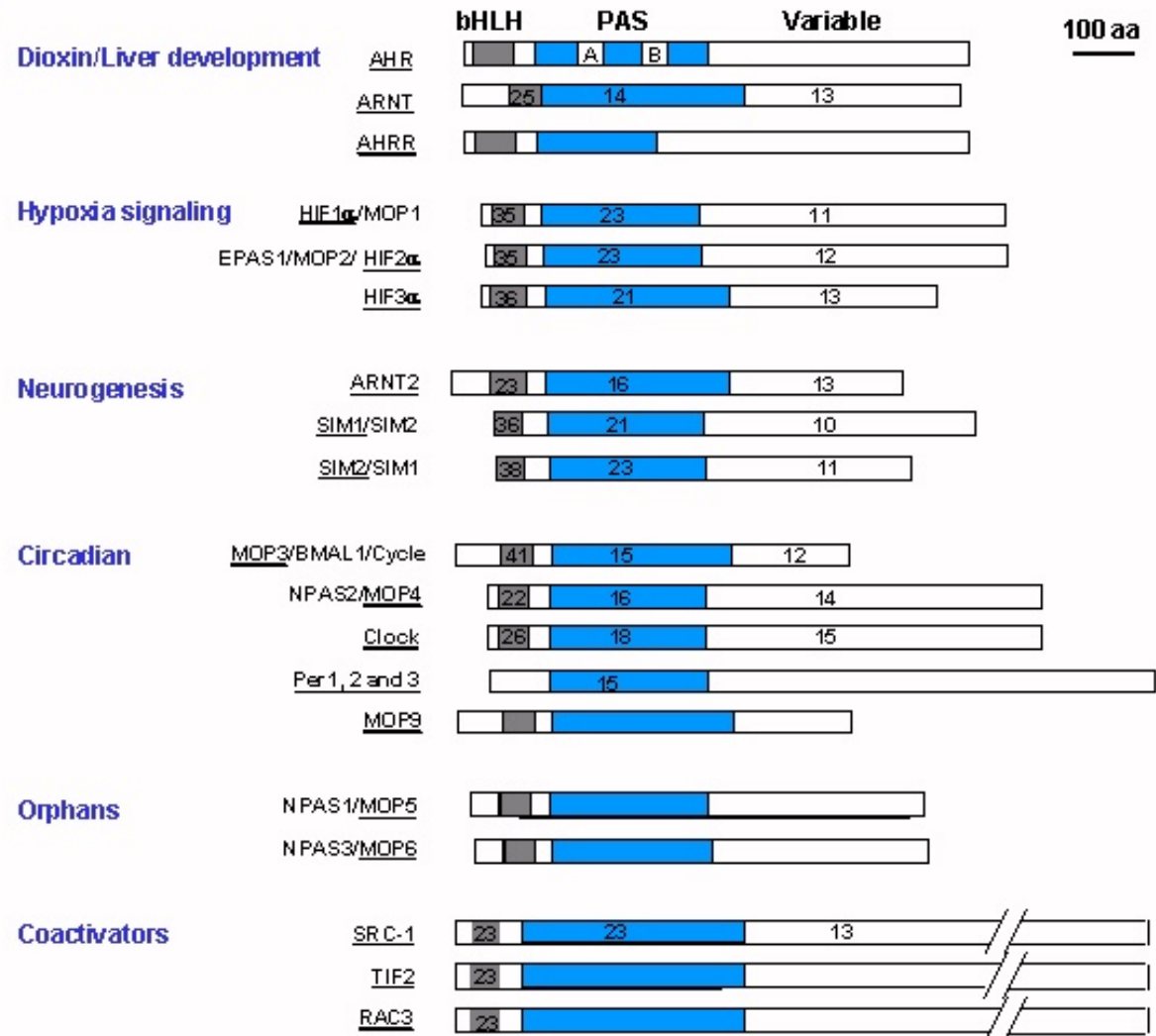
Existují 3 hlavní podskupiny bHLH proteinů:

- (a) pouze s bHLH doménou; nebo ty, kde bHLH doména navazuje na druhou dimerizační doménu:
- (b) doménu leucinových zipů (Zip) nebo
- (c) **PAS doménu.**



PAS proteiny (rodina transkripčních faktorů):

Mammalian PAS Superfamily



PAS doména

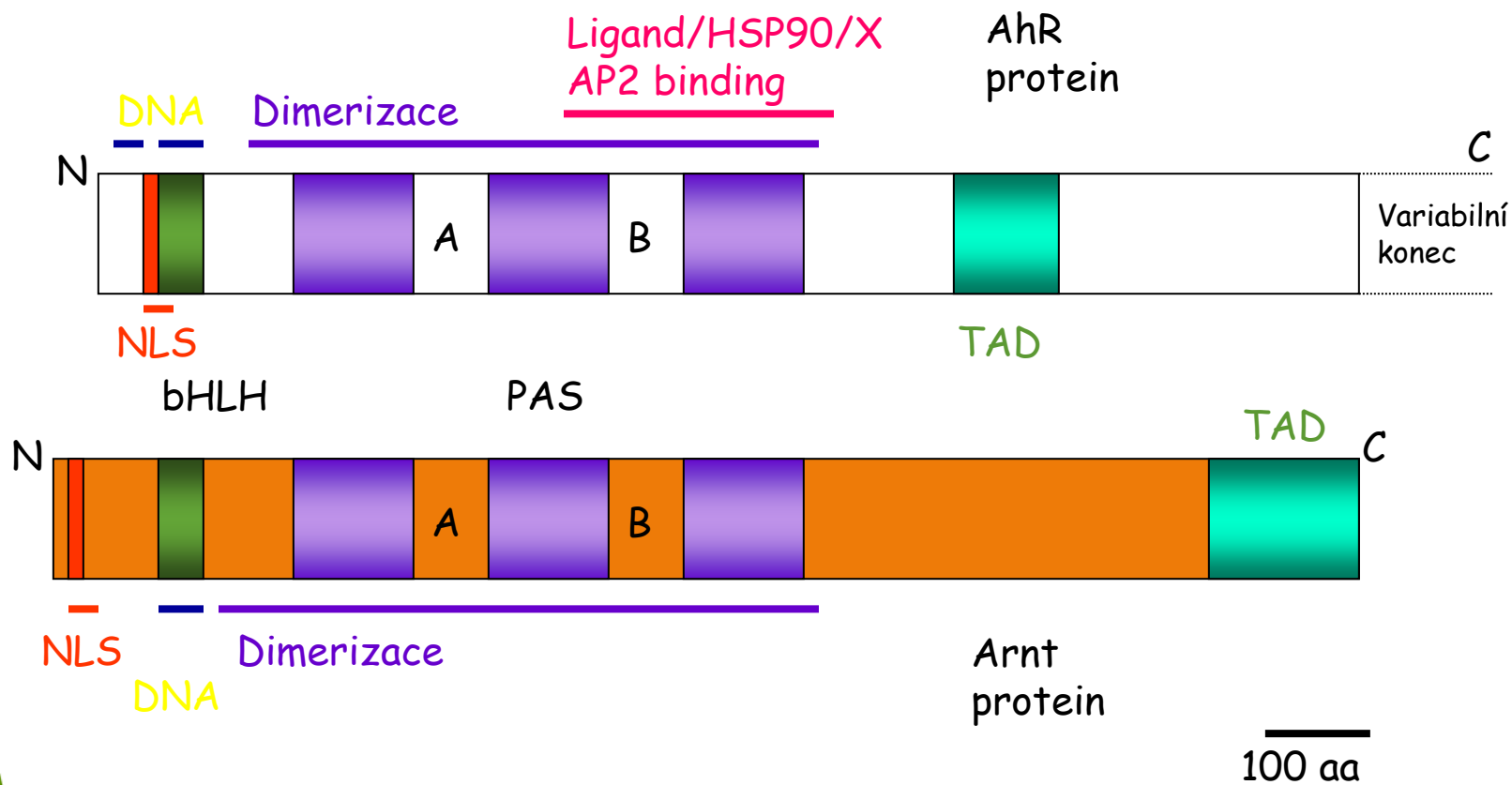
PAS oblast obsahuje 2 sousedící opakování ~130 aminokyselin, PAS A and PAS B.

Tato doména je velmi starý signální prostředek, který zůstává **zachován v průběhu evoluce**. Byla identifikována v proteinech živočichů, bakterií, hub, kvasinek a rostlin:

řada bakterií obsahuje PAS-like proteiny, které detekují světlo a kyslík (Dos, Aer, FixL, PYP);

podobné proteiny vnímají světlo u rostlin (fytochromy PhyA-PhyE, NPH1; faktor interagující s fytochromem - PIF3).

Struktura a funkce domén AhR a ARNT:

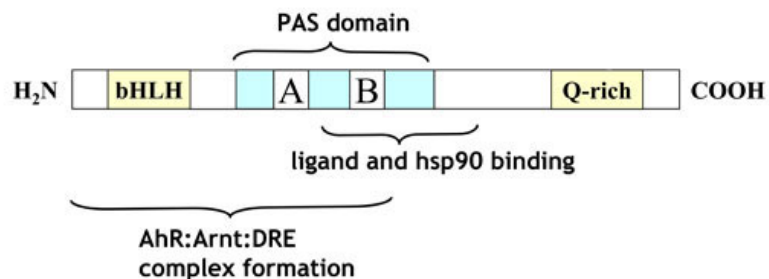
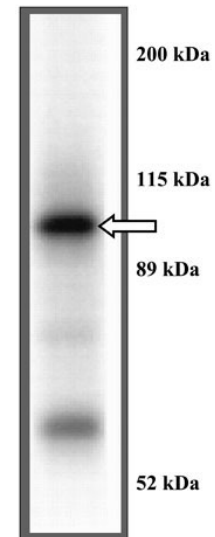
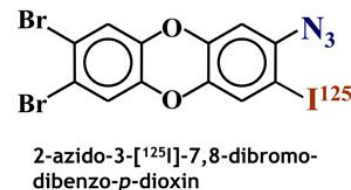
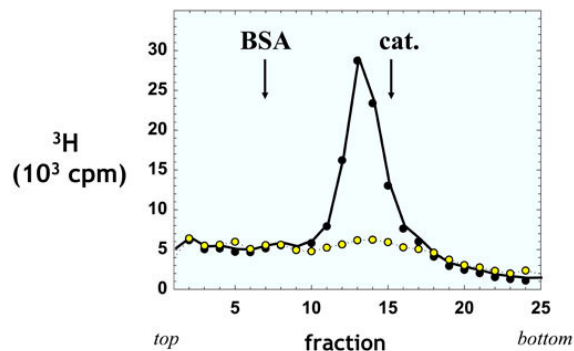


AhR =

- transkripční faktor aktivovaný ligandy;
- zprostředkovává toxicitu řady environmentálních polutantů;
- reguluje metabolizaci xenobiotik a podílí se na bioaktivaci promutagenů.

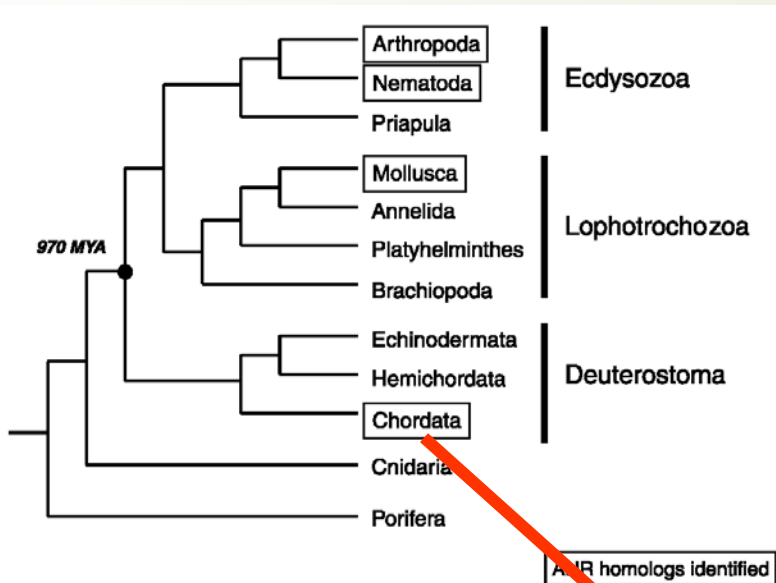
Jak byl objeven AhR?

- různá citlivost inbredních myších kmenů na indukci aktivity enzymů (CYP1A) v jaterních mikrozómech po expozici TCDD a 3-methylcholanthrenu – 60. léta;
- autozomálně dominantní typ dědičnosti;
- izolace unikátního proteinu; klonování genu – 90. léta



Josephy and Mannervik,
Molecular Toxicology,
2nd ed.

Evoluce a AhR:

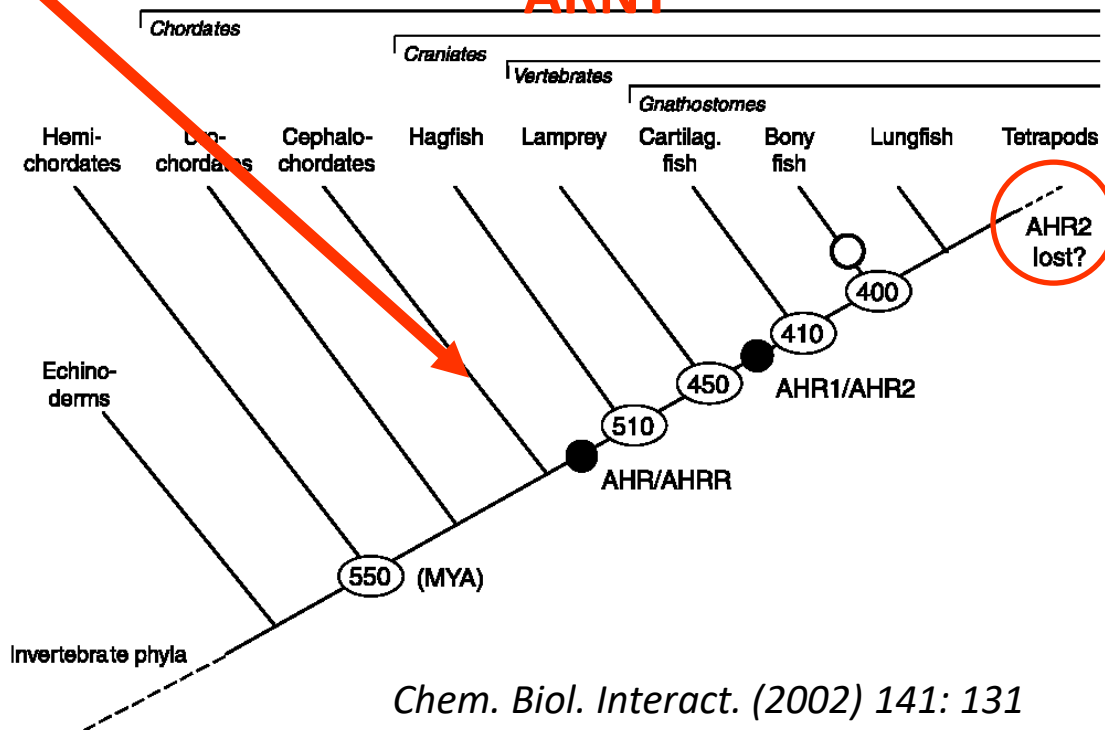
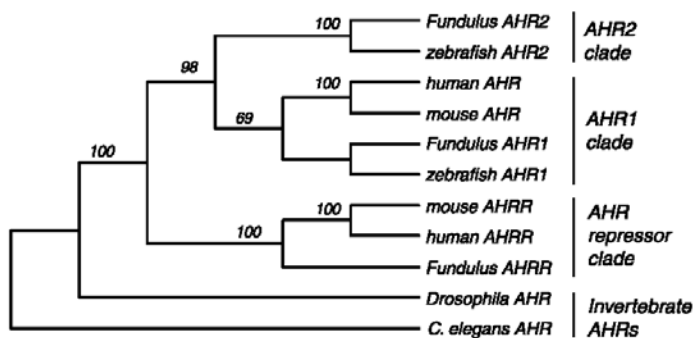


AHR1

AHR2

AHRR

ARNT



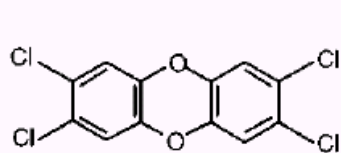
Evoluce a AhR:

Organism:	Name:	Ligand-binding:	Physiological function:
Nematodes: <i>Caenorhabditis elegans</i>	AHR-1	No	Neuronal development; Behavioral effects.
Insects: <i>Drosophila melanogaster</i>	Spineless (Ss)	No	Development; Regulation of homeobox genes and dendrite morphology.
Vertebrates:	AhR (AhR1, AhR2)	Yes	Toxicity mechanisms; Liver and kidney development; Neuronal differentiation? Circadian rhythms?

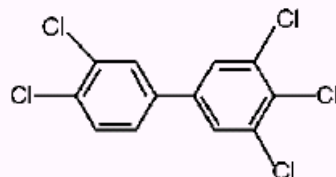
Toxické AhR ligandy:

- polychlorované dibenzo-*p*-dioxiny, dibenzofurany a bifenyly;
- polycyklické aromatické uhlovodíky;

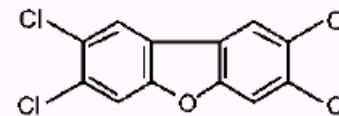
"Classical" AhR Ligands and CYP1A1 Inducers



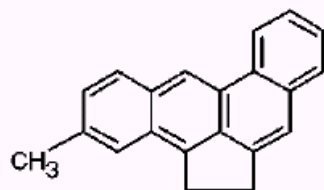
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin



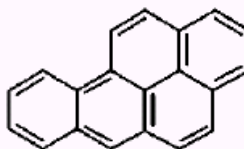
3,4,3',4',5-Pentachlorobiphenyl



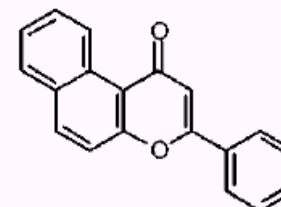
2,3,7,8-Tetrachlorodibenzofuran



3-Methylcholanthrene



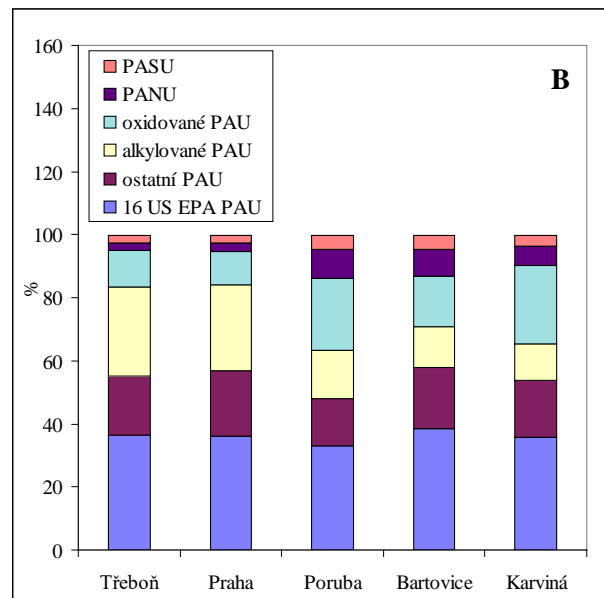
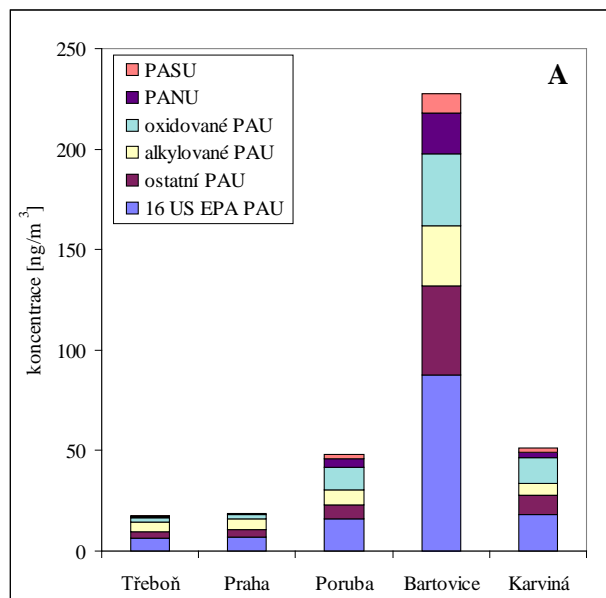
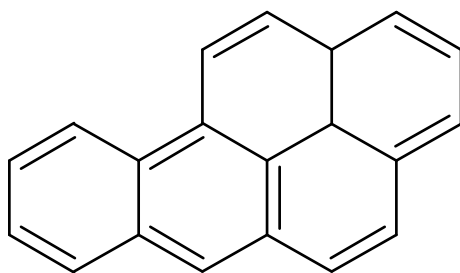
Benzo(a)pyrene



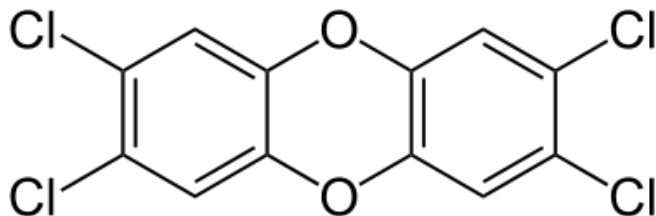
β -Naphthoflavone

Polycyklické aromatické uhlovodíky (PAHs)

- vážný regionální problém v ČR –
- doprava; průmyslová výroba;



2,3,7,8-tetrachlórdibenzo-*p*-dioxin



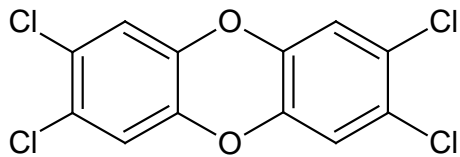
- jedna z nejtoxičtějších látek vznikajících činností člověka;
- modelový zástupce této skupiny, definující jejich toxicitu;

Dioxiny a kontaminace potravín:

Langlebiges Gift

Ein **Dioxin-Skandal** erschütterte Deutschland:

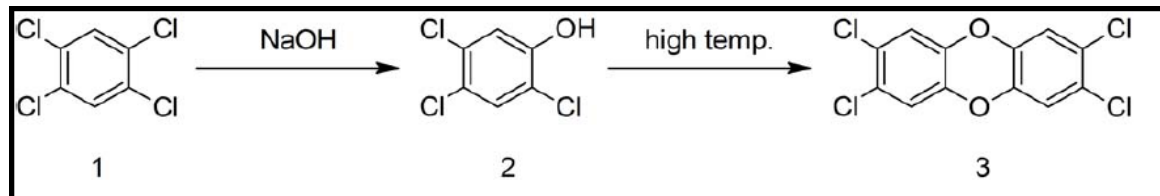
Hühner, Puten und Schweine haben auf deutschen Bauernhöfen vergiftetes Futter gefressen. Ein Futtermittelhersteller hatte technische Fette aus der Diesel-Produktion für Tiernahrung genutzt. Über Eier oder Fleisch nehmen Menschen die Dioxine auf, wo sie sich in Leber und Gewebe einlagern.



Dioxiny a lidské zdraví:

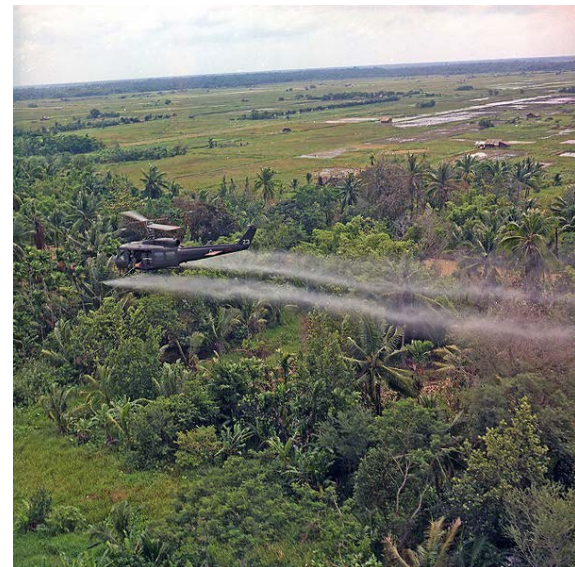
Seveso - 1976

V červenci 1976 došlo k malé chemické továrně zhruba 15 km severně od Milána k explozi a zamoření okolí 2,3,7,8-tetrachlorodibenzo-*p*-dioxinem (TCDD)



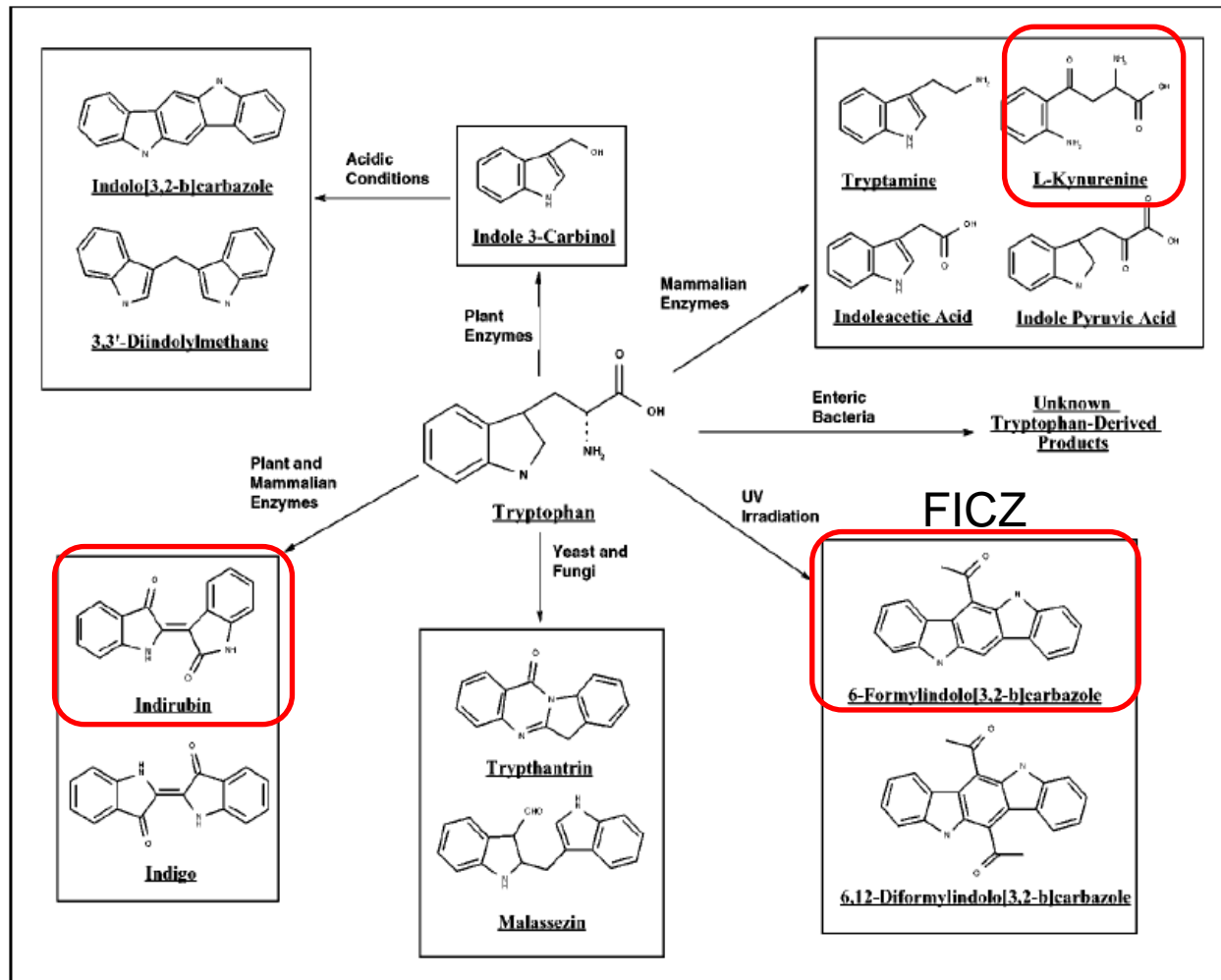
Výroba a používání herbicidů

Vietnam – Agent Orange
Spolana Neratovice – pracovní expozice



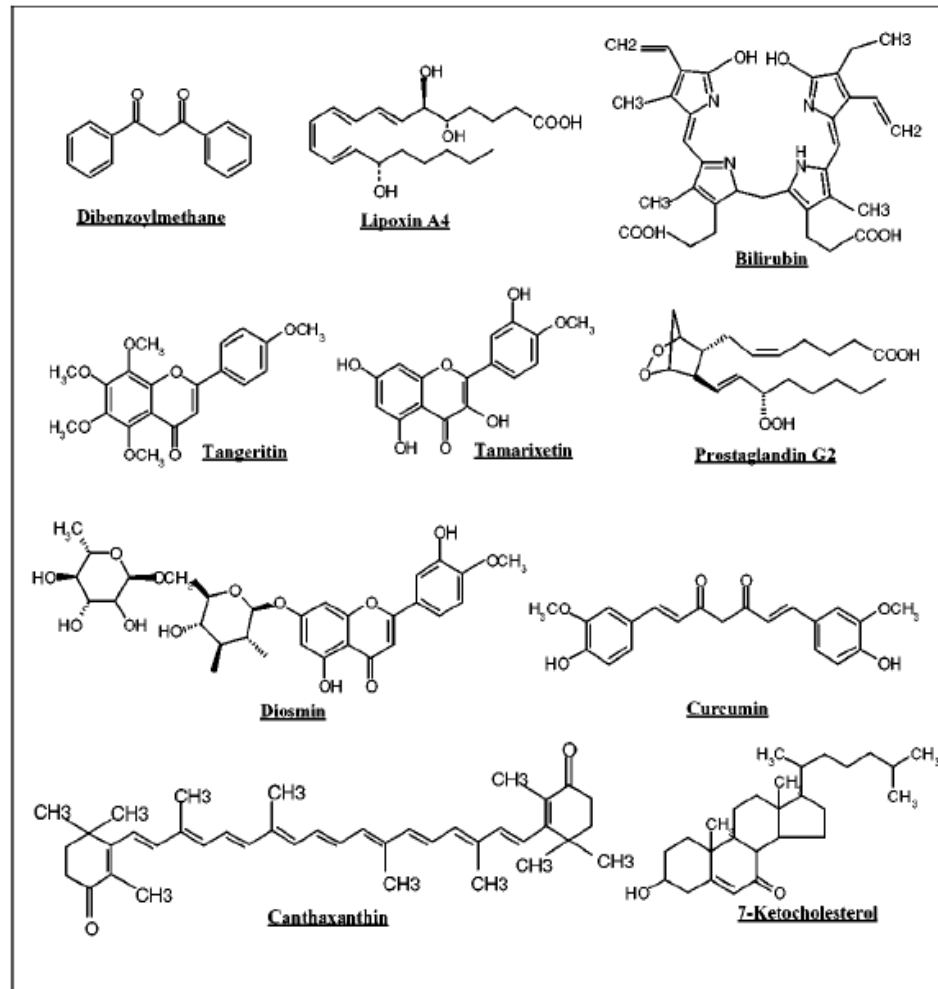
Endogenní a přírodní ligandy AhR???

✓ deriváty tryptofanu;



Jiné přírodní ligandy AhR???

✓ deriváty lipidů a flavonoidy



Toxické účinky dioxinů:

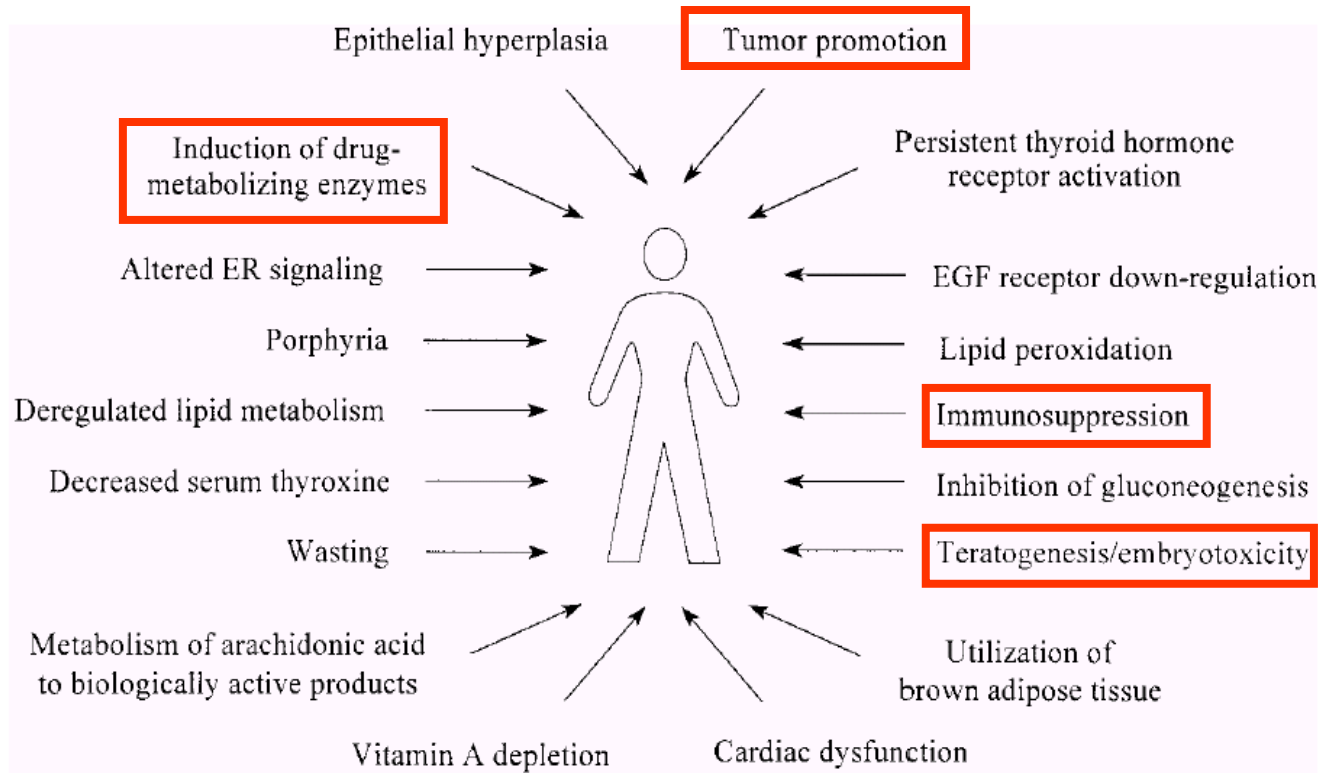
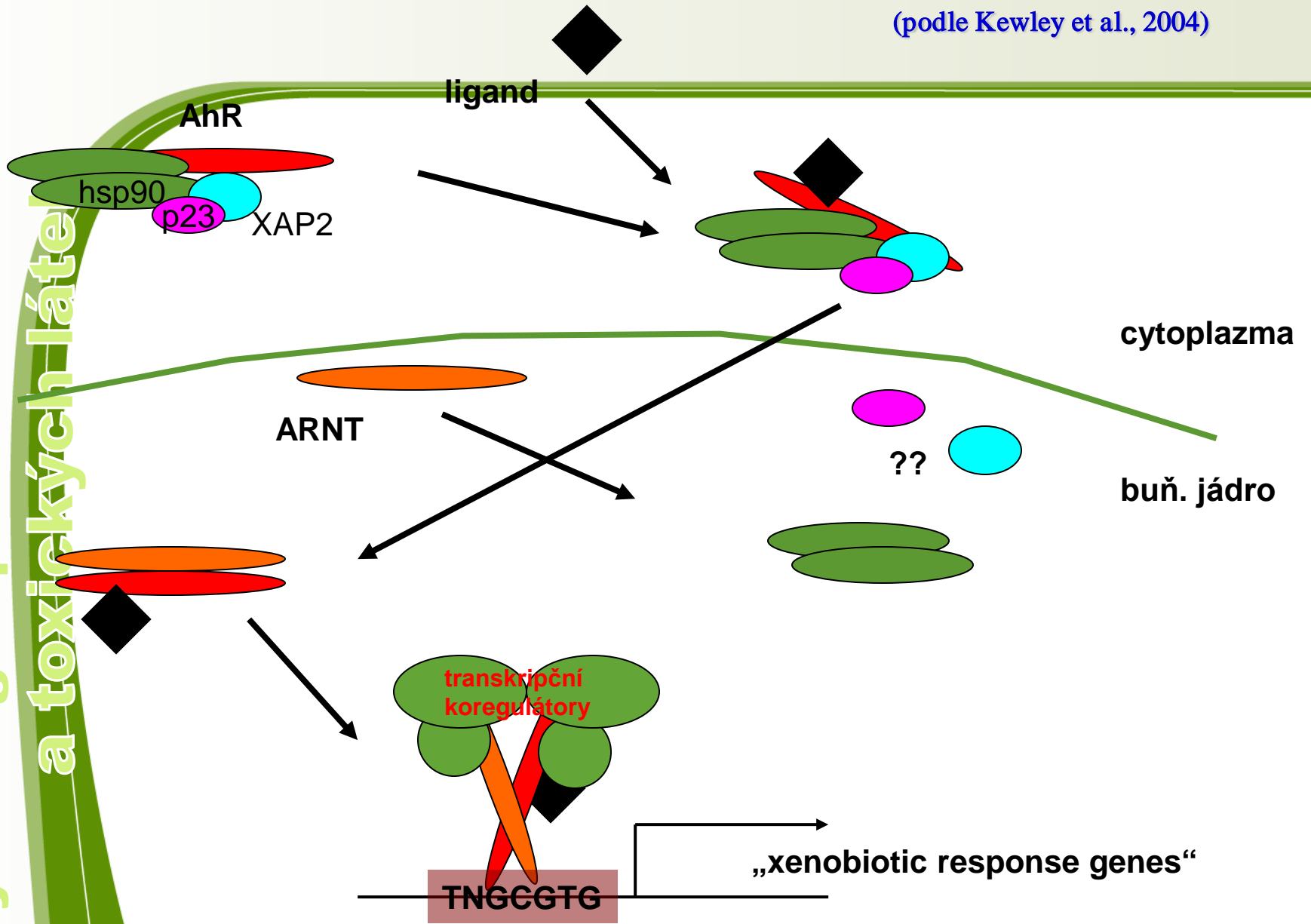


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.

Aktivace AhR: (podle Kewley et al., 2004)



Aktivace AhR:

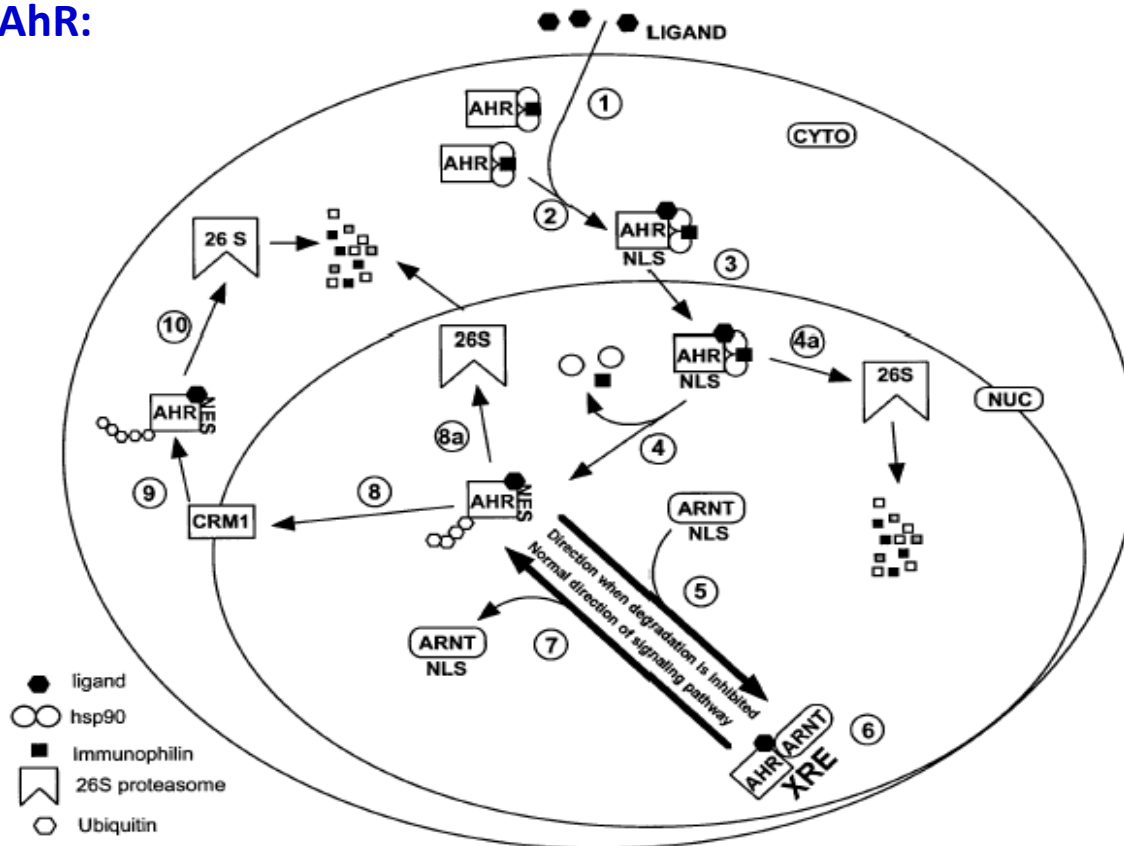
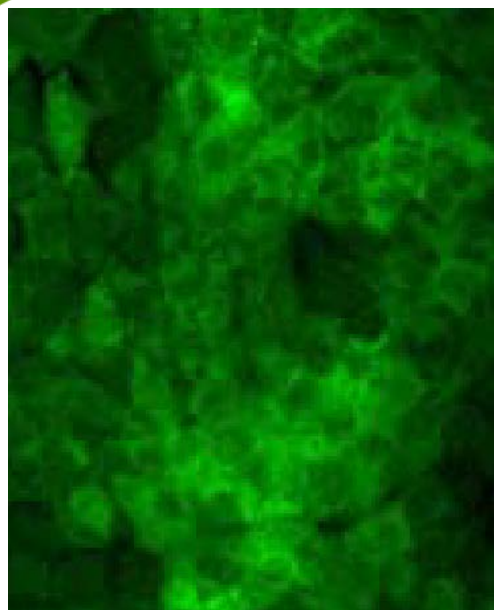
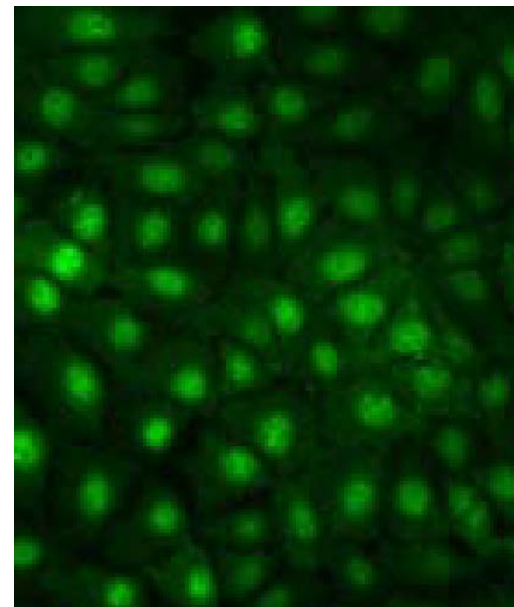


Fig. 1. Model of AHR-mediated signal transduction pathway. (1) Ligand enters cell. (2) Ligand binds to AHR-hsp90-immunophilin complex causing conformational change and exposing the NLS domain. (3) AHR complex is actively imported into the nucleus via NLS and nuclear import receptors. (4a) If receptor complex is in a misfolded conformation, it may be proteolytically degraded. (4) AHR dissociates from hsp90 and immunophilin exposing HLH/PAS domain and NES. (5) AHR dimerizes with ARNT-blocking NES sequence. (6) AHR-ARNT complex binds to XRE regions in DNA. (7) AHR-ARNT complex dissociates from DNA and ARNT exposing NES. (8a) AHR is ubiquitinated in the nucleus and degraded or (8) AHR is exported from nucleus via CRM-1 export receptor. (9) AHR is ubiquitinated in cytoplasm and (10) targeted to 26S proteasome for degradation. Note that the pathway is linear and also note the degradation of the AHR terminal step regardless of whether it occurs within the nucleus or cytoplasm. NLS, nuclear localization signal; CRM-1, chromosome region maintenance protein 1; 26S, 26S proteasome.

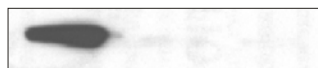
Aktivace AhR:



+ TCDD
→
30 min



Control
TCDD 5 nM
PCB 126 100 nM



← AhR (93 kDa)



← CY1A1 (60 kDa)



← β -actin (40 kDa)

Geny regulované AhR

obsahují v promotorové nebo enhancerové oblasti tzv. xenobiotic response elements (XRE) / dioxin responsive elements (DRE):

- enzymy I. fáze biotransformace - *CYP 1A1, CYP 1A2, CYP 1B1*;
- enzymy II. fáze biotransformace - *UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase*;
- regulátory buněčného cyklu, apoptózy, diferenciace, senescence – *Bax?*, *p27^{Kip1}*, *JunD*, *TGF-β*;
- AhRR.

AhR deficientní myši:

- růstová retardace;
- narušení vývoje jater a imunitního systému;
- hromadění retinoidů v játrech;
- abnormální vaskularizace ledvin a jater;
- řada efektů v imunitním systému a hematopoéze;

- odolné vůči karcinogenním účinkům BaP a teratogenitě TCDD;

Jaterní defekty:

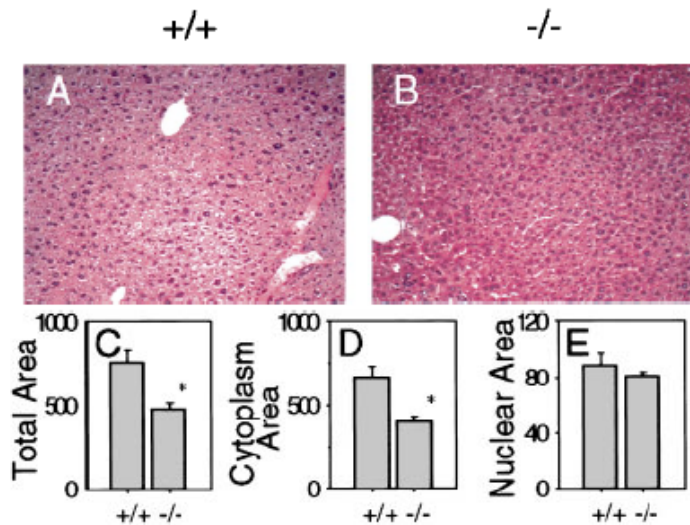
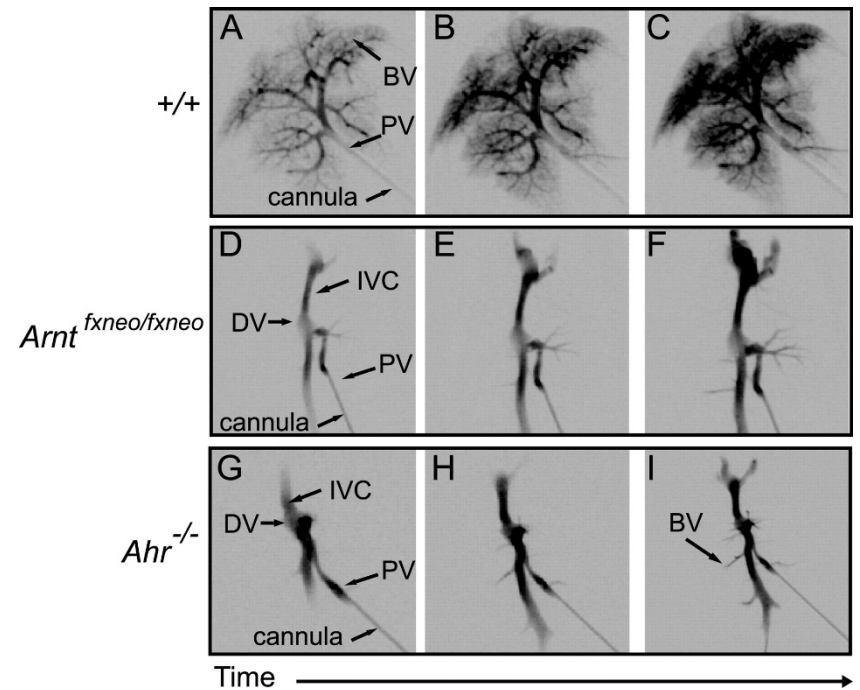


Fig. 1. *Ah* $-/-$ mice have smaller hepatocytes than wild-type mice. Livers of 1-year-old mice were fixed in formalin, and 6- μ m sections were examined after staining with hematoxylin/eosin. (A and B) Thin sections from wild-type (A) and age-matched *Ah* knockout (B) mice are shown, and results of morphometric analyses follow. (C) There is a significant decrease in the total area of the hepatocytes of *Ah* $-/-$ mice. (D and E) Whereas the cytoplasmic area of *Ah* $-/-$ hepatocytes is significantly decreased (D), the nuclear areas of *Ah* $+/+$ and *Ah* $-/-$ hepatocytes are not different (E). Mean and standard errors generated from comparison of six 1-year-old male *Ah* $+/+$ and six age- and sex-matched *Ah* $-/-$ mice are shown; asterisks indicate significance ($P < 0.05$).



BaP není karcinogenní v AhR KO myších:

armak

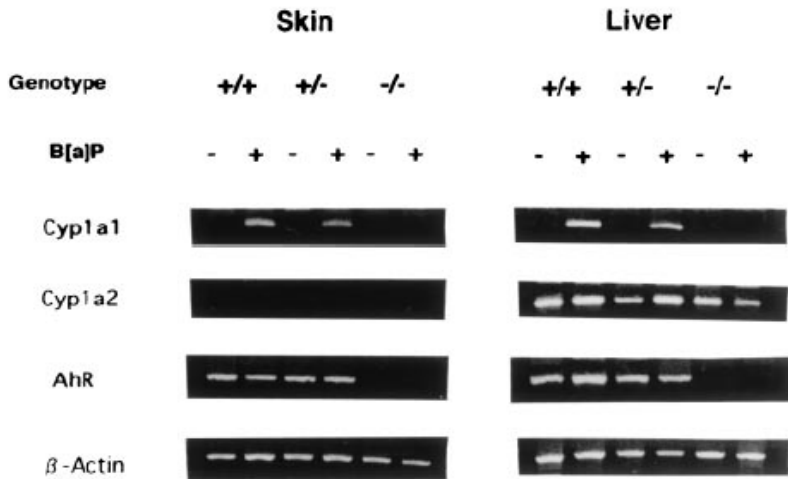


Fig. 1. *Cyp1a1*, *Cyp1a2*, and AhR gene expression in the skin and liver of AhR(+/+), AhR(+/-), and AhR(-/-) mice, with and without B[a]P treatment. One-microgram aliquots of RNA extracted from skin and liver of control and B[a]P-treated mice of the three genotypes were reverse-transcribed and analyzed by PCR using specific primers for the *Cyp1a1*, *Cyp1a2*, and AhR and β-actin genes.

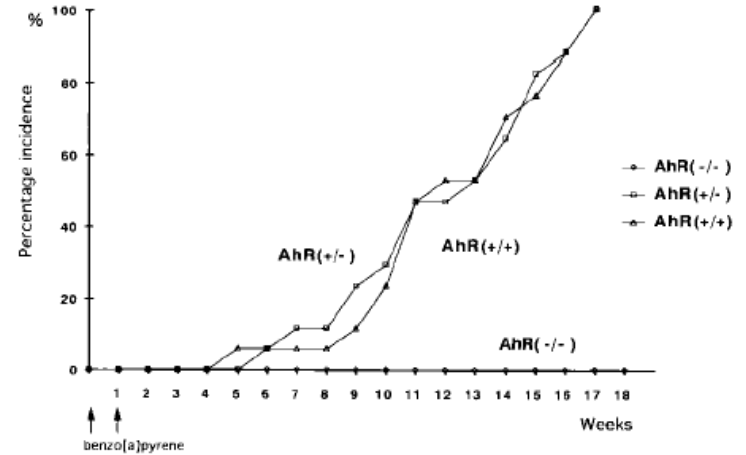


Fig. 2. Subcutaneous tumor induction in wild-type (Δ) and AhR-deficient male mice (+/-, □; -/-, ○) injected with B[a]P.

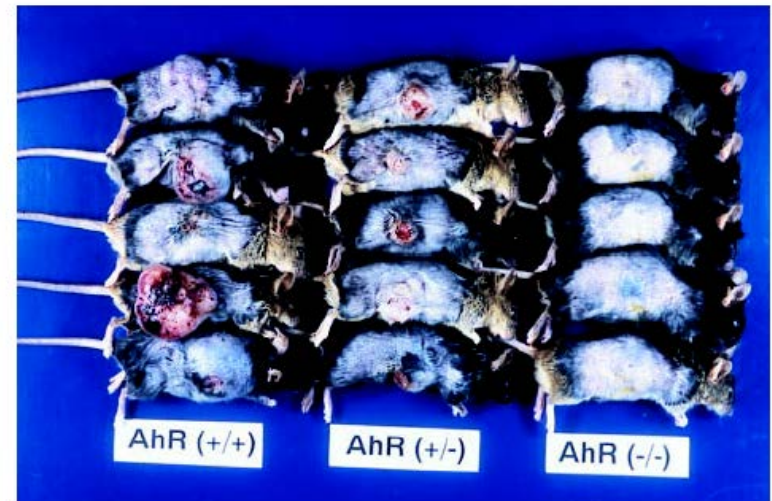
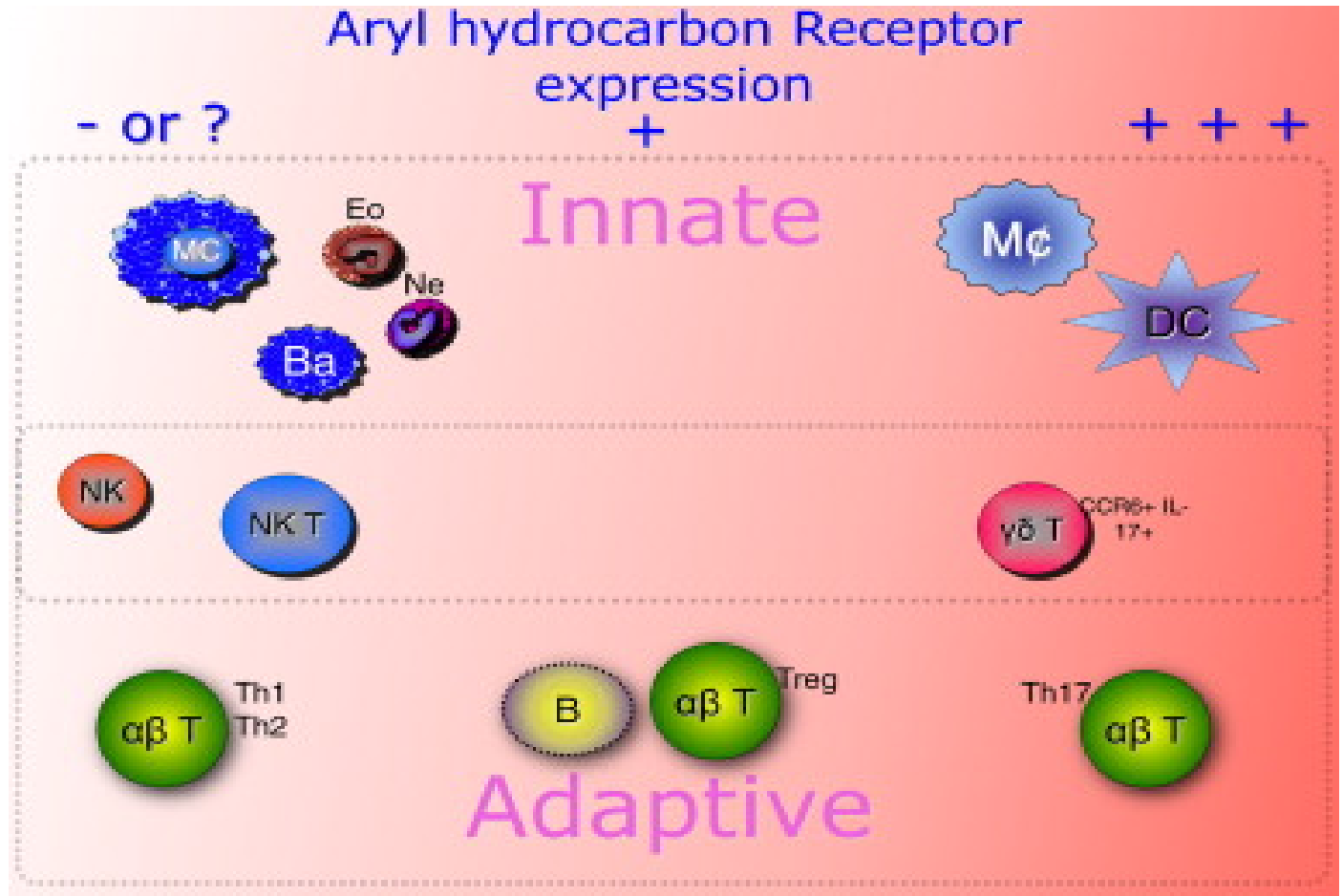


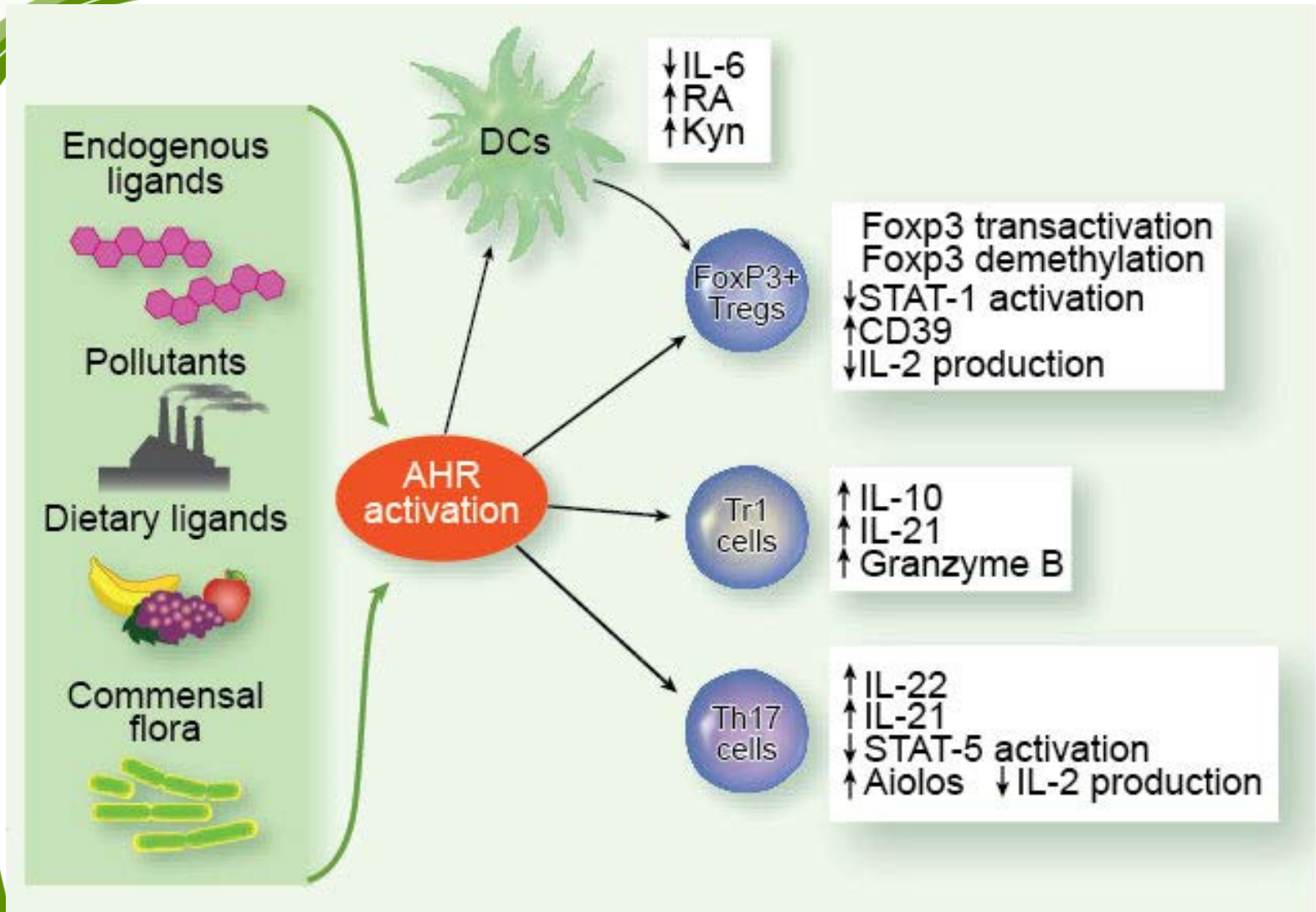
Fig. 3. Gross appearance of flank skins in AhR-wild-type mice (+/+), AhR-heterozygous mice (+/-), and AhR-deficient mice (-/-) injected subcutaneously with B[a]P.

Fyzio

AhR a imunitní systém

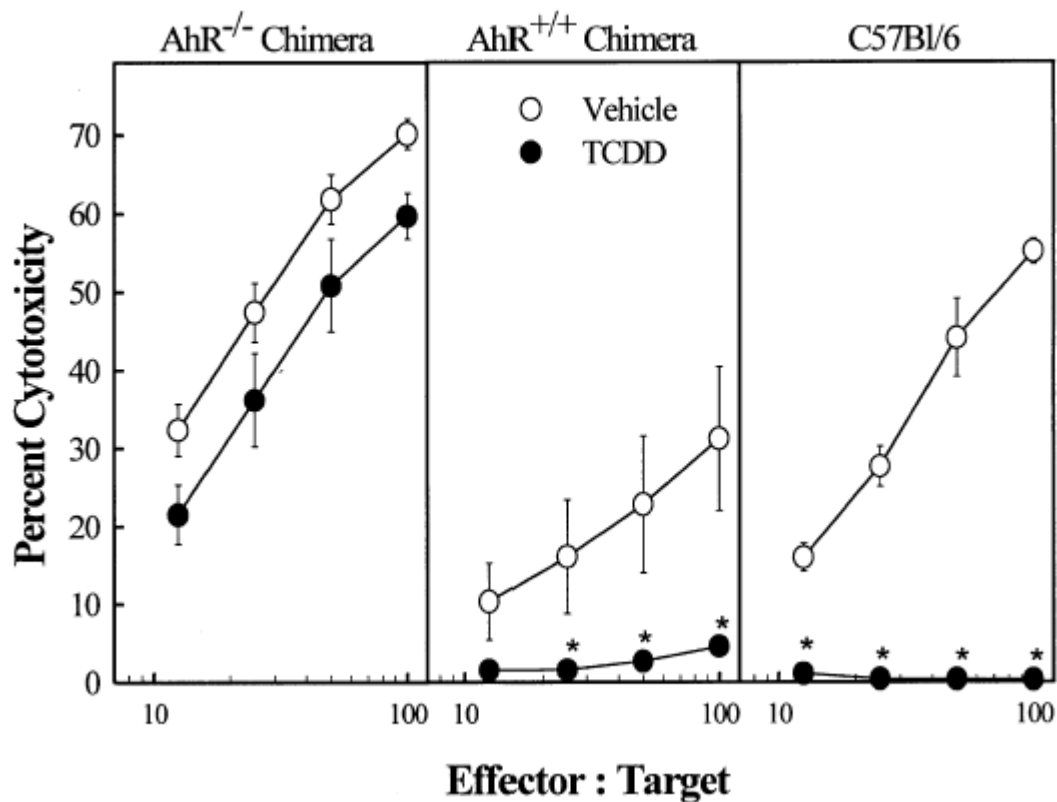


AhR a imunitní systém



AhR je nezbytný pro imunotoxické účinky TCDD:

N.I. Kerkvliet / *International Immunopharmacology* 2 (2002) 277–291



CTL response

Koncepce toxických ekvivalentních faktorů (TEF)

- vzhledem k tomu, že látky dioxinového typu mají společný mechanismus účinku – aktivaci AhR, lze předpokládat, že jejich schopnost aktivovat tento receptor je úměrná jejich toxicitě;
- referenční toxikant – TCDD;
- TEF pro jsou definovány WHO;

$$\text{Toxicita směsi - TEQ} = \sum[C_i] \times \text{TEF}_i$$

Toxické ekvivalentní faktory (TEF)

TABLE 1
Summary of WHO 1998 and WHO 2005 TEF Values

Compound	WHO 1998 TEF	WHO 2005 TEF
Chlorinated dibenzo- <i>p</i> -dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	0.0003
Chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	0.0003
Non- <i>ortho</i> -substituted PCBs		
3,3',4,4'-tetraCB (PCB 77)	0.0001	0.0001
3,4,4',5-tetraCB (PCB 81)	0.0001	0.0003
3,3',4,4',5-pentaCB (PCB 126)	0.1	0.1
3,3',4,4',5,5'-hexaCB (PCB 169)	0.01	0.03
Mono- <i>ortho</i> -substituted PCBs		
2,3,3',4,4'-pentaCB (PCB 105)	0.0001	0.00003
2,3,4,4',5-pentaCB (PCB 114)	0.0005	0.00003
2,3',4,4',5-pentaCB (PCB 118)	0.0001	0.00003
2',3,4,4',5-pentaCB (PCB 123)	0.0001	0.00003
2,3,3',4,4',5-hexaCB (PCB 156)	0.0005	0.00003
2,3,3',4,4',5'-hexaCB (PCB 157)	0.0005	0.00003
2,3',4,4',5,5'-hexaCB (PCB 167)	0.00001	0.00003
2,3,3',4,4',5,5'-heptaCB (PCB 189)	0.0001	0.00003

Bold values indicate a change in TEF value.

AhR interaguje s řadou dalších proteinů:

TABLE 1. Interactions Between Signal Transduction Pathways and AhR^{a, b}

Interactions	References
Direct interactions with AhR	
HSP90	[79]
XAP2	[80–82]
ER, ERR α	[24]
NF κ B (RelA/p65)	[39]
Rb	[44–46]
RIP 140, p300/CBP	[41,51,53]
SRC-1, NCoA-2, pCIP	[41,54]
ERAP 140, SMRT	[49,50]
COUP-TF1	[24]
pp60 ^{src}	[70,71]
tyrosine phosphorylation	[69]
Direct interactions with AhR complex proteins^c	
HIF-1 α , PAS proteins (ARNT)	[32,35]
p300/CBP (ARNT)	[52]
SRC-1, NCoA-2 (ARNT)	[54]
SHP (ARNT)	[78]
AhRR (ARNT)	[20]
ARNT Repressor (ARNT)	[21]
CK2 (XAP2)	[74]
p23 (HSP90)	[76]
XAP2 (HSP90)	[80]
Indirect interactions (cross talk) with AhR	
ER	[8,25,29]
hypoxia	[33,36]
NF κ B	[40–42]
PKC	[59–66]
tyrosine kinases/phosphatases	[69,72,73]
<i>c-myc</i> , AP-1, CK2	[72]
TGF- β	[7]
p27 (Kip 1)	[43]
NF-1	[27]
C2-ceramide	[47]

J.R. Petruitis, G.H. Perdeu / *Chemico-Biological Interactions* 141 (2002) 25–40

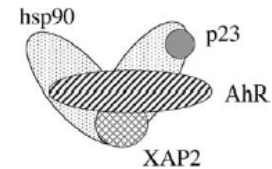


Fig. 4. Model for the arrangement of proteins found in the unliganded AhR complex.

O. Hankinson / *Archives of Biochemistry and Biophysics* 433 (2005) 379–386

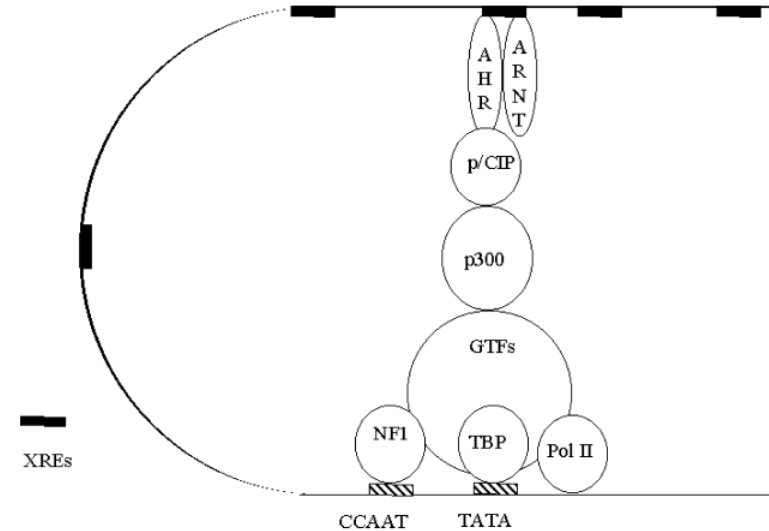


Fig. 3. Hypothetical model of coactivator recruitment at the *Cyp1a1* gene.

AhR-ER α crosstalk

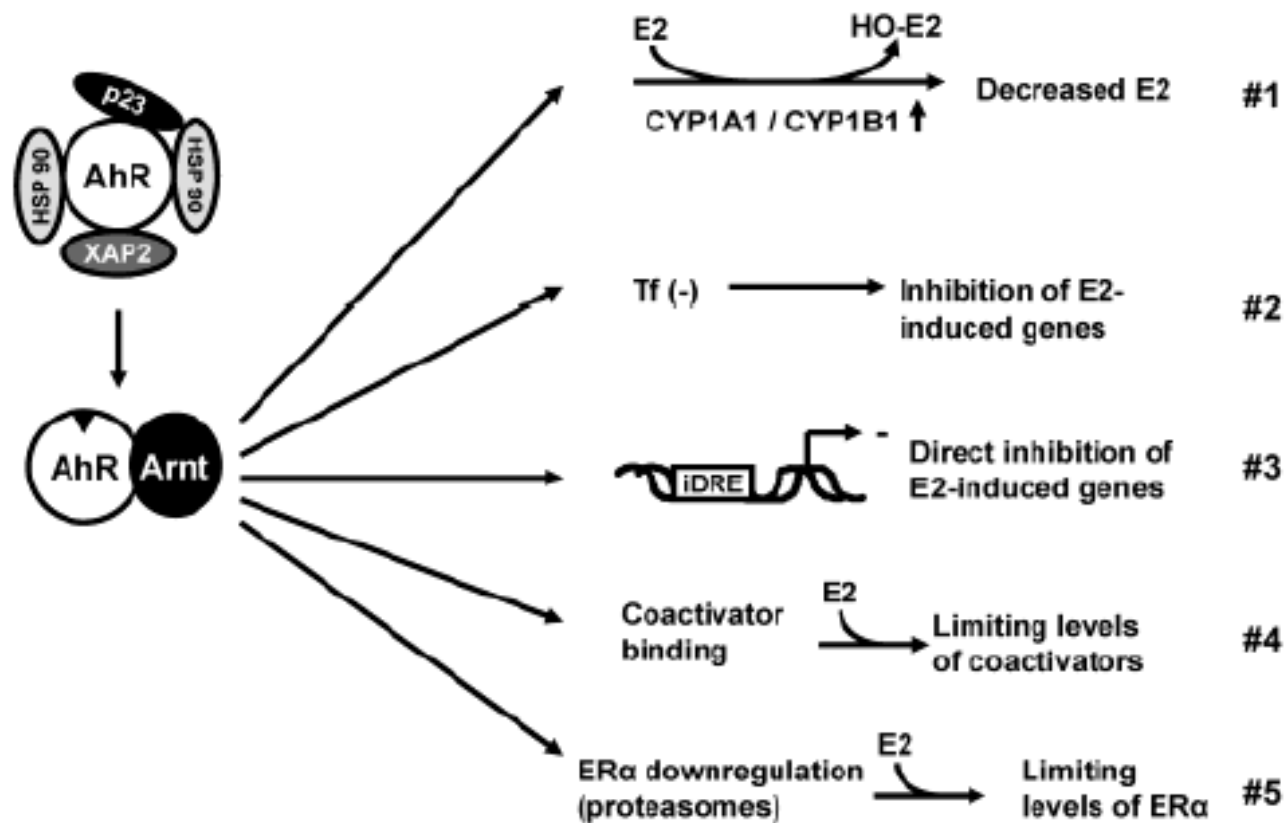


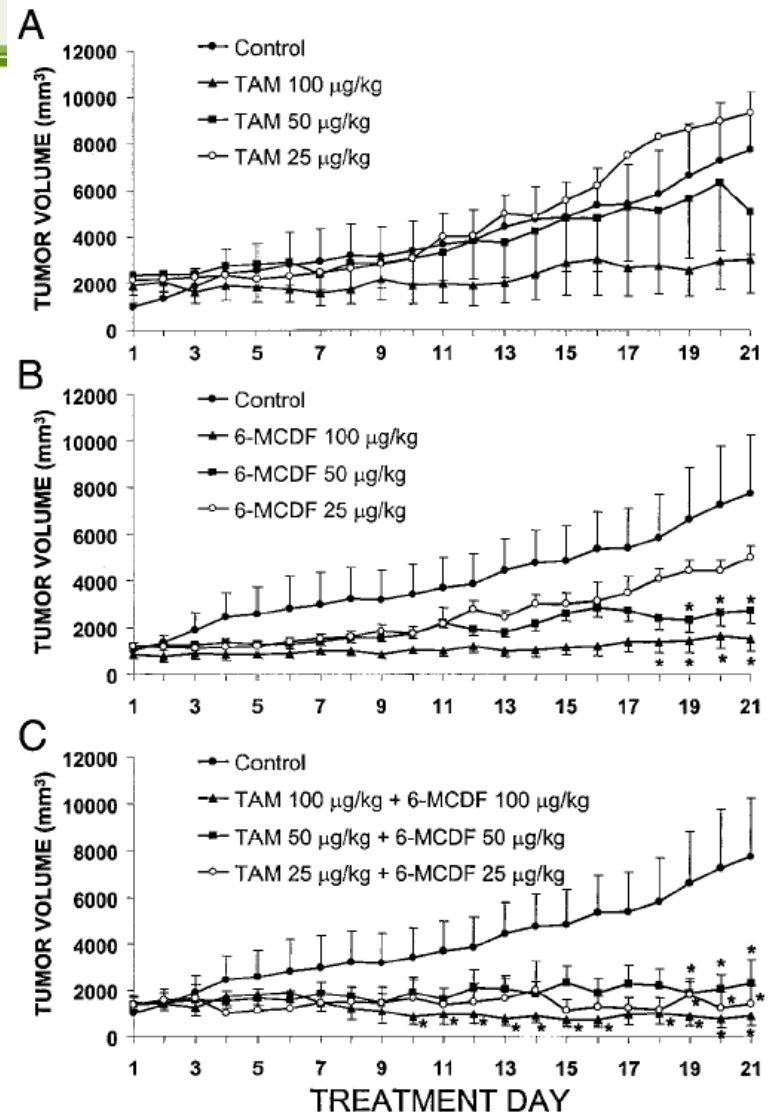
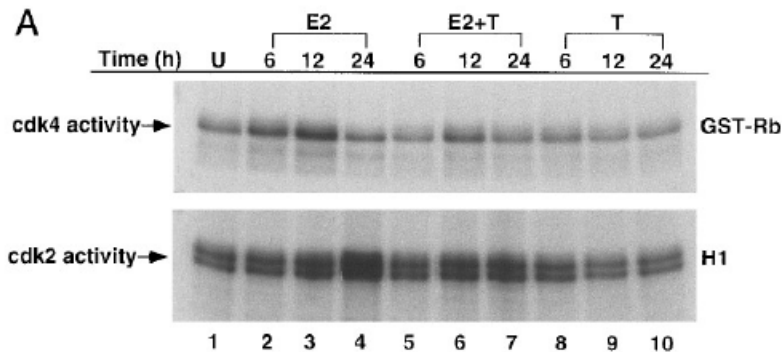
Figure 3. Proposed mechanisms of inhibitory AhR-ER α crosstalk (123-126).

Využití AhR-ER α crosstalk v nádorové terapii?

TABLE I

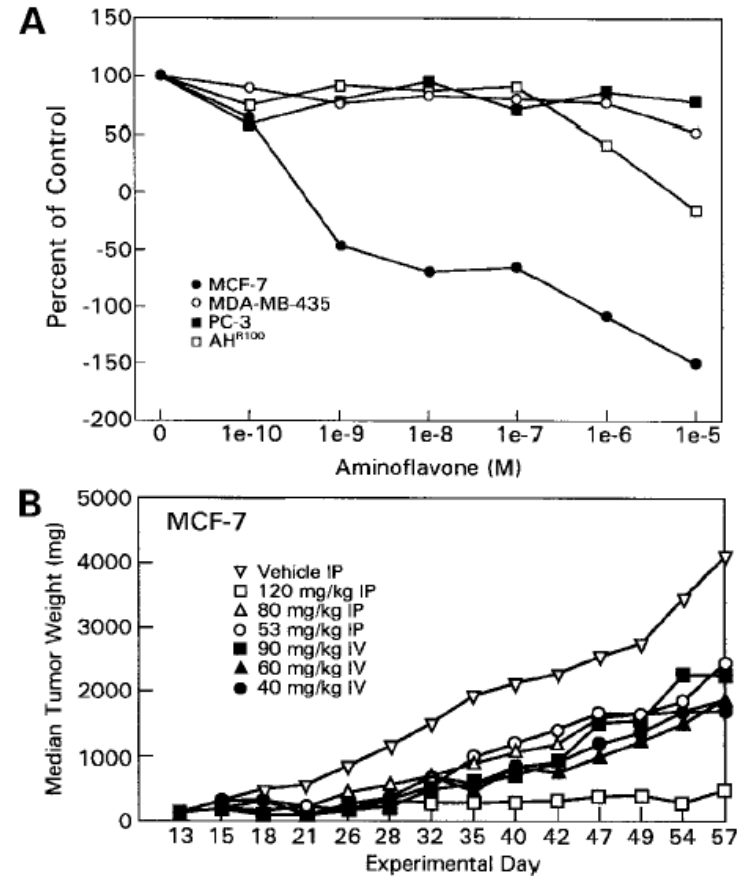
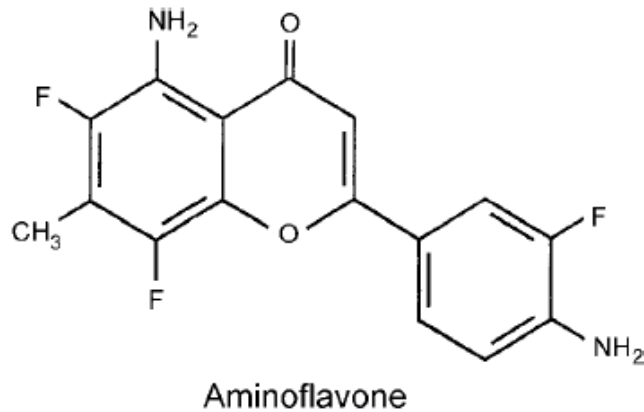
Effects of 17 β -Estradiol and TCDD on Cell Cycle Distribution of MCF-7 Human Breast Cancer Cells^a

Treatment (time, h)	Cell cycle phase (%)		
	G ₀ /G ₁	S	G ₂ /M
Control	89.9 \pm 2.1	4.9 \pm 1.6	5.2 \pm 0.6
E2 (12)	87.7 \pm 2.1	6.0 \pm 1.4	4.4 \pm 0.7
E2 + TCDD (12)	87.2 \pm 0.2	7.9 \pm 0.7	4.9 \pm 0.5
TCDD (12)	89.1 \pm 0.8	6.7 \pm 0.8	4.2 \pm 0.2
E2 (24)	75.1 \pm 0.6 ^b	23.4 \pm 1.7 ^b	1.5 \pm 1.2
E2 + TCDD (24)	81.0 \pm 1.3 ^c	15.8 \pm 1.8 ^d	3.2 \pm 0.7
TCDD (24)	90.8 \pm 0.6	5.2 \pm 0.5	4.0 \pm 0.9

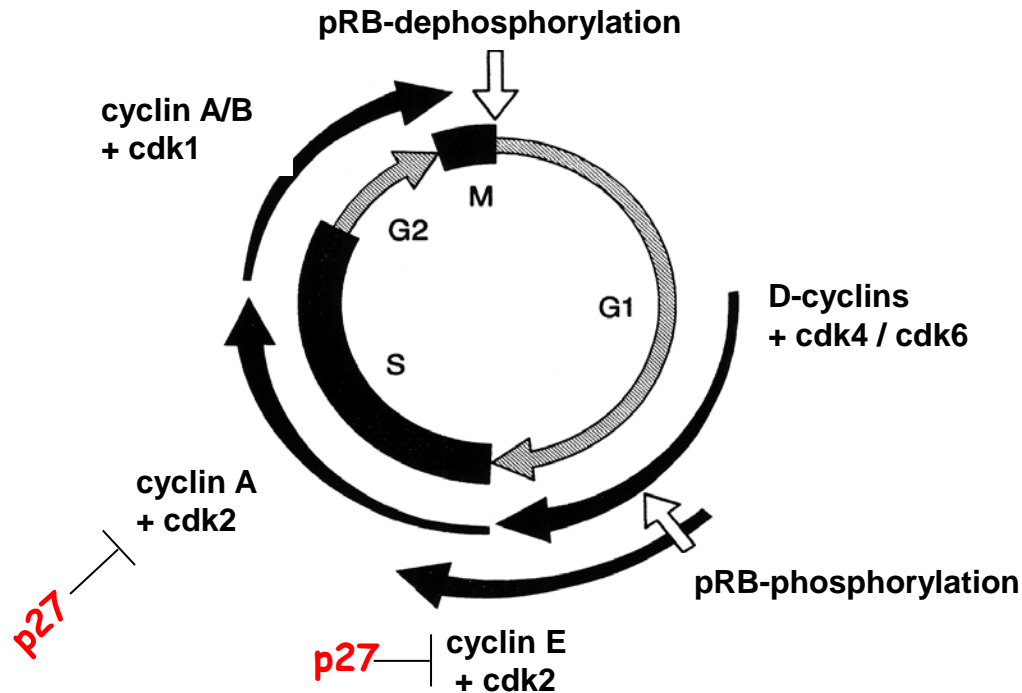


ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 356, 239–248, 1998;
CANCER RESEARCH 61, 3902–3907, 2001

Využití AhR-ER α crosstalk v nádorové terapii?



Úloha AhR v regulaci buněčného cyklu



pRB = retinoblastoma protein
cdk = cyclin-dependent kinase

Úloha AhR v regulaci buněčného cyklu

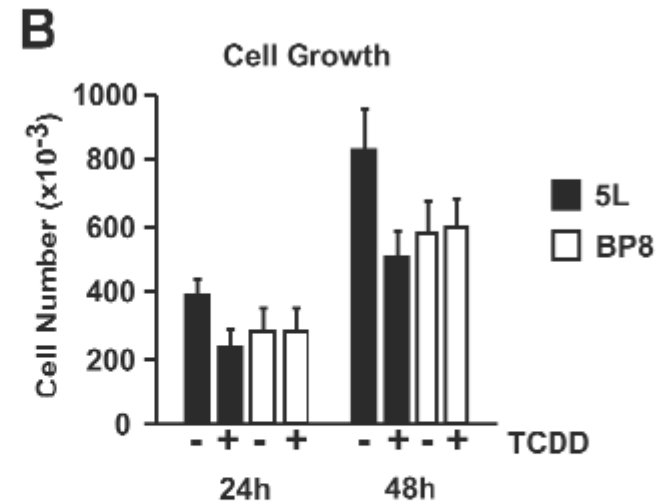
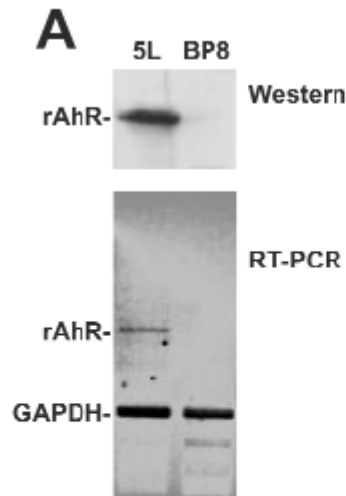
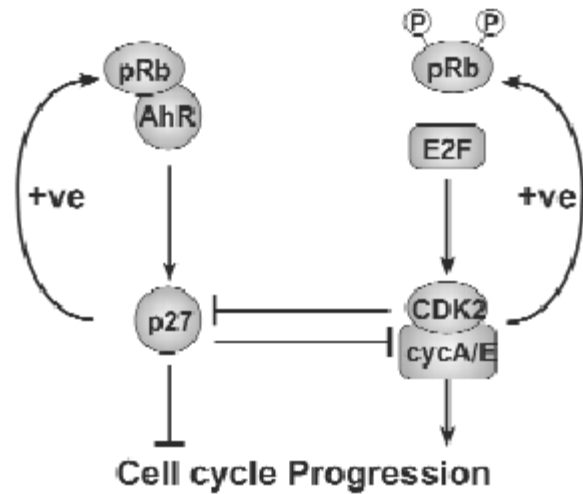
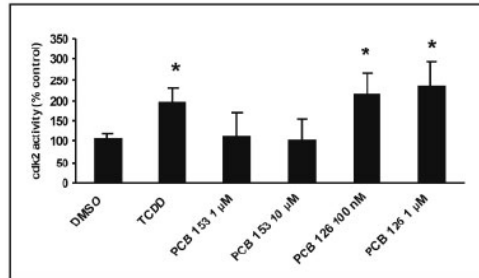
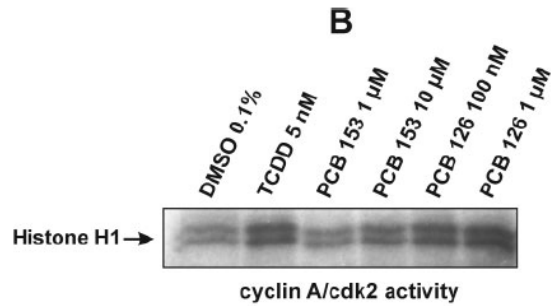
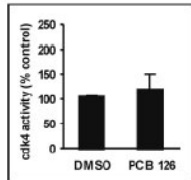
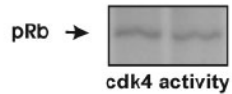
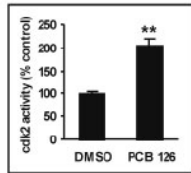
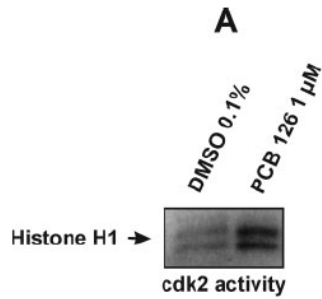


Figure 2. TCDD induces growth inhibition in rat 5L hepatoma cells.

Panel A, total protein from 5L and BP8 cells was fractionated by SDS-PAGE and probed for AhR protein with an anti-AhR antibody (Western). Analysis of AhR expression was also performed by RT-PCR on total RNA from 5L and BP8 cells using primers specific for rat AhR (rAhR) and GAPDH (as a control for RT-PCR).

Panel B, 5L (solid bars) and BP8 (open bars) cells (2×10^5) were grown in the presence of 10 nM TCDD (+) or absence of TCDD (-) for 24h or 48h and counted. The values presented are the mean \pm S.D. of three independent experiments.

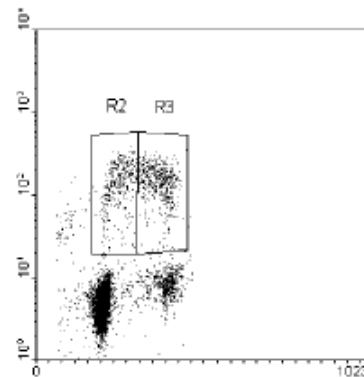


WB-F344

MCF-7

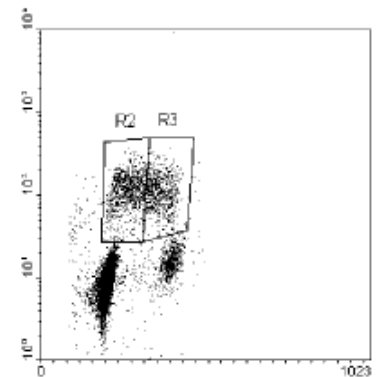
Control

Early S-phase (left): 2.6%
 Late S-phase (right): 3.7%
 Total BrdU positive: 6.6%



BaA

Early S-phase (left): 7.6%
 Late S-phase (right): 7.3%
 Total BrdU positive: 14.9%



Úloha AhR v regulaci buněčného cyklu je pravděpodobně složitější

ARNT – základní dimerizační partner:

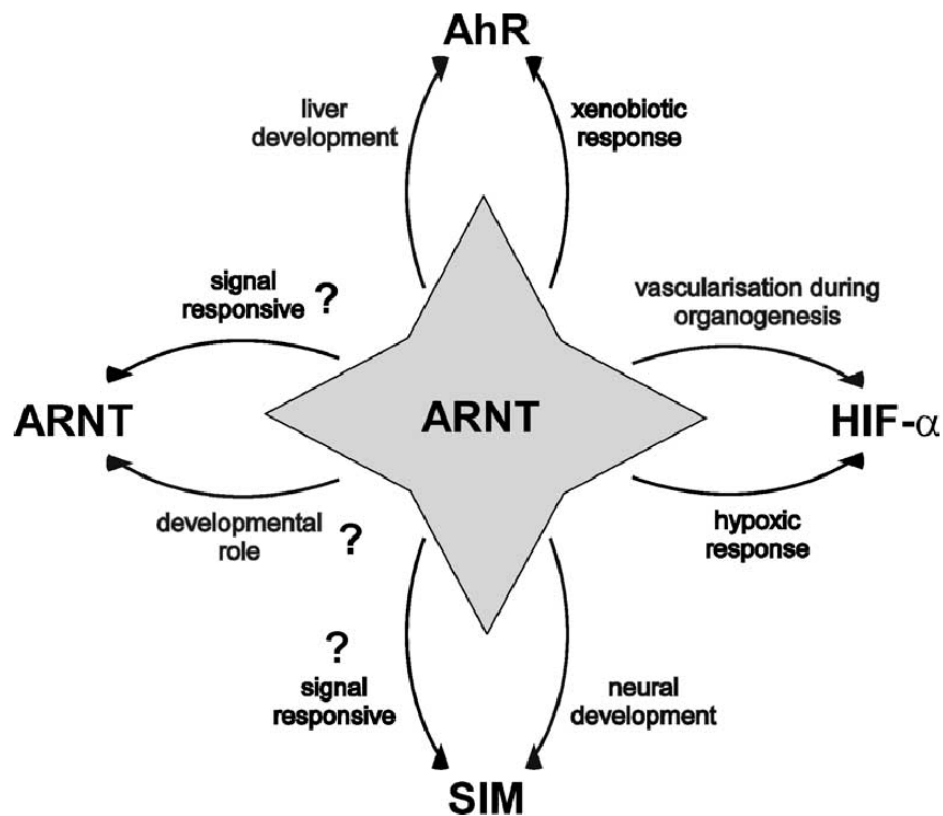
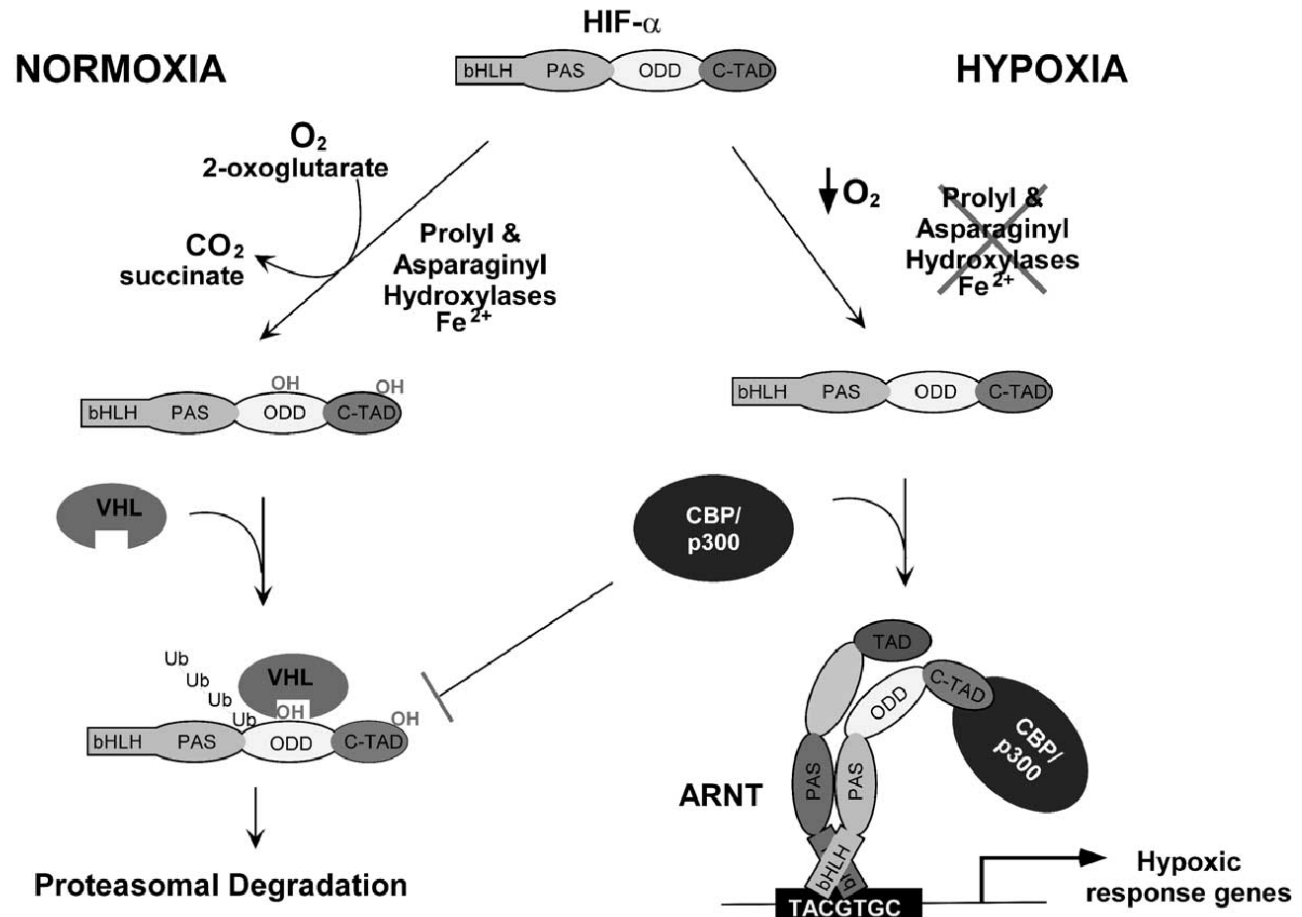
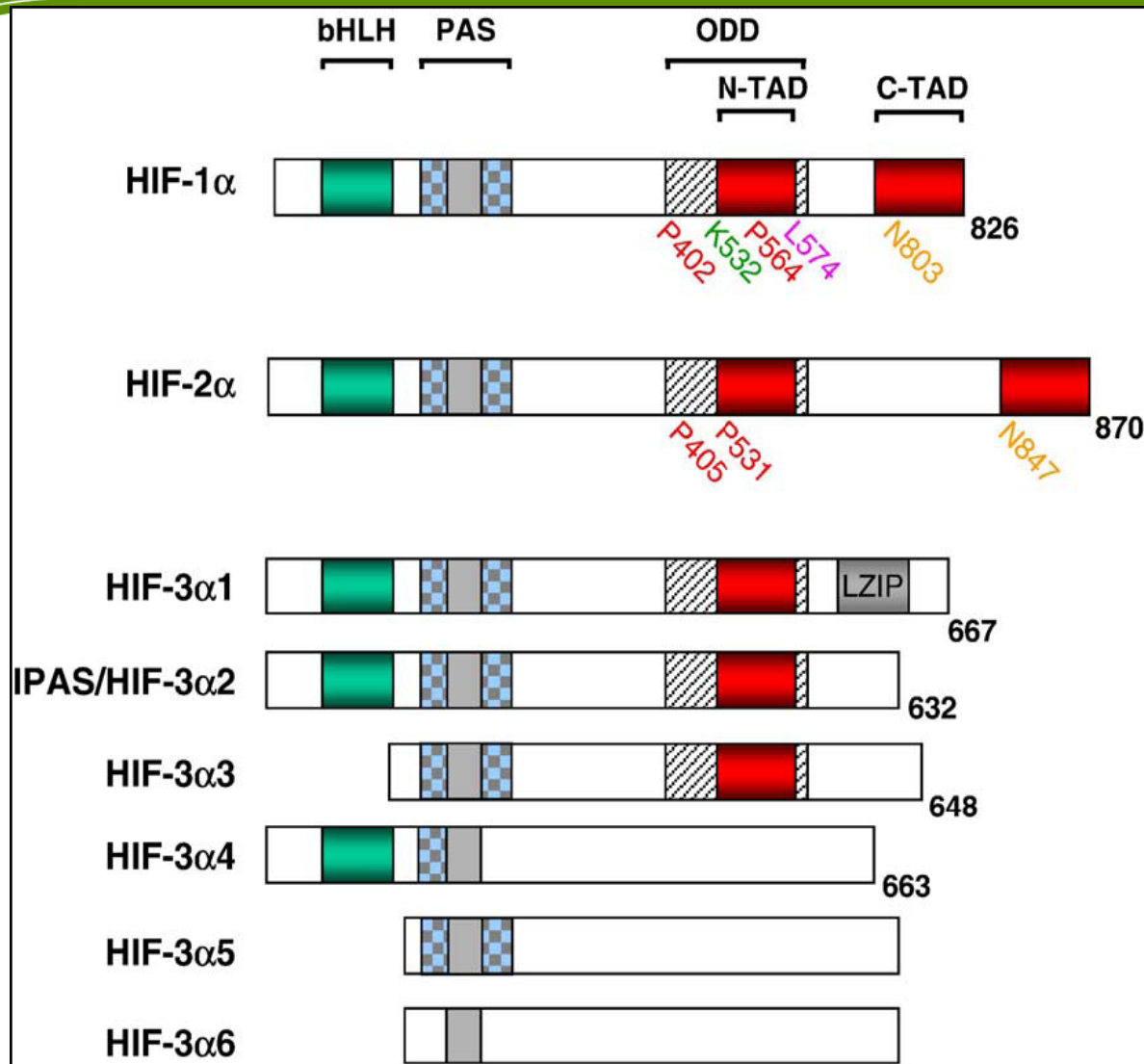


Fig. 4. ARNT is central to transcriptional regulation within the bHLH/PAS family of proteins. ARNT forms both homodimers and heterodimers with the AhR, HIF- α and SIM which play roles both during mammalian development and in response to environmental stimuli in mammals. Symbol '?' indicates where these roles have yet to be characterised.

Faktor indukovaný hypoxií – HIF1



HIF proteiny:



Buněčné prostředí mění hladinu HIF:

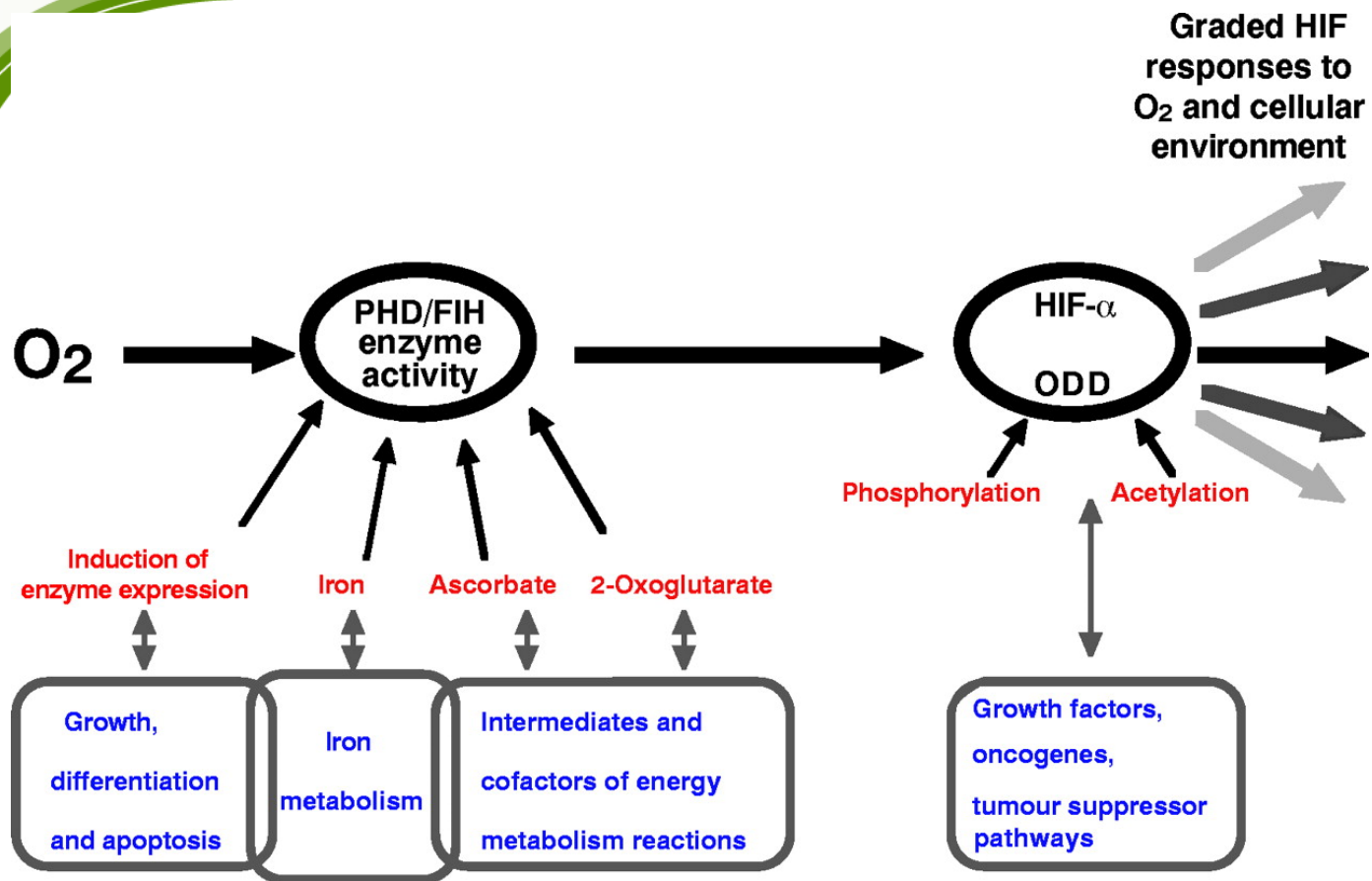
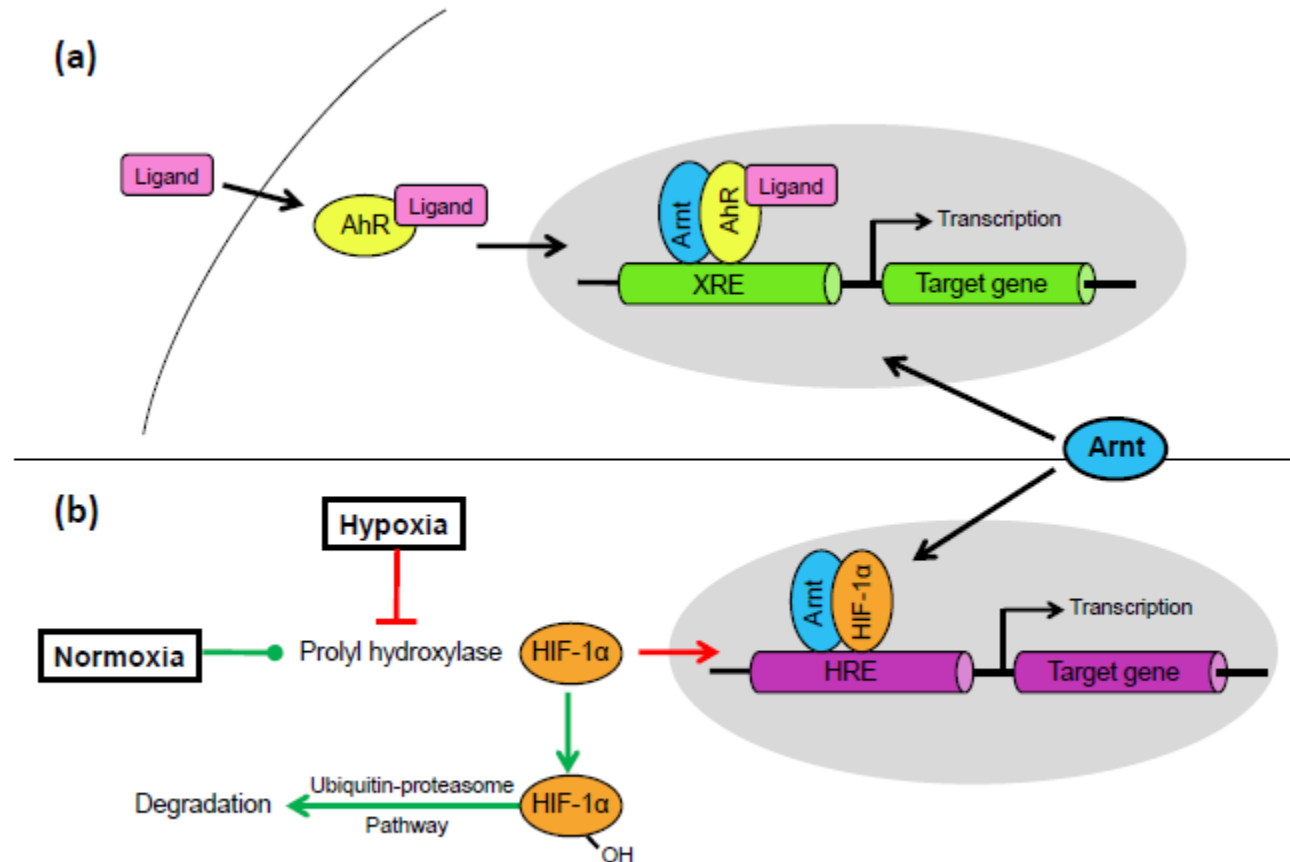


Table 2. HIF-1 target genes.

Function	Gene (abbreviation)	Reference
Erythropoiesis/ iron metabolism	Erythropoietin (EPO)	(Semenza et al., 1991)
	Transferrin (Tf)	(Rolfs et al., 1997)
	Transferrin receptor (Tfr)	(Bianchi et al., 1999)
	Ceruloplasmin	(Lok and Ponka, 1999)
Angiogenesis	Vascular endothelial growth factor (VEGF)	(Levy et al., 1995)
	Endocrine-gland-derived VEGF (EG-VEGF)	(LeCouter et al., 2001)
	Leptin (LEP)	(Grosfeld et al., 2002)
	Transforming growth factor-beta3 (TGF- β 3)	(Scheid et al., 2002)
Vascular tone	Nitric oxide synthase (NOS2)	(Melillo et al., 1995)
	Heme oxygenase 1	(Lee et al., 1997)
	Endothelin 1 (ET1)	(Hu et al., 1998)
	Adrenomedullin (ADM)	(Nguyen and Claycomb, 1999)
Matrix metabolism	α 1B-adrenergic receptor	(Eckhart et al., 1997)
	Matrix metalloproteinases (MMPs)	(Ben-Yosef et al., 2002)
	Plasminogen activator receptors and inhibitors (PAIs)	(Kietzmann et al., 1999)
Glucose metabolism	Collagen prolyl hydroxylase	(Takahashi et al., 2000)
	Adenylate kinase-3	(O'Rourke et al., 1996)
	Aldolase-A,C (ALDA,C)	(Semenza et al., 1996)
	Carbonic anhydrase-9	(Wykoff et al., 2000)
	Enolase-1 (ENO1)	(Semenza et al., 1996)
	Glucose transporter-1,3 (GLU1,3)	(Chen et al., 2001)
	Glyceraldehyde phosphate dehydrogenase (GAPDH)	(Graven et al., 1999)
	Hexokinase 1,2 (HK1,2)	(Mathupala et al., 2001)
	Lactate dehydrogenase-A (LDHA)	(Semenza et al., 1996)
	Pyruvate kinase M (PKM)	(Semenza et al., 1994)
	Phosphofructokinase L (PFKL)	(Semenza et al., 1994)
Phosphoglycerate kinase 1 (PGK1)	(Semenza et al., 1994)	
6-phosphofructo-2-kinase/fructose-2,6-bisphosphate-3 (PFKFB3)	(Minchenko et al., 2002)	
Cell proliferation/ survival	Insulin-like growth factor-2 (IGF2)	(Feldser et al., 1999)
	Transforming growth factor- α (TGF- α)	(Krishnamachary et al., 2003)
	Adrenomedullin (ADM)	(Cormier-Regard et al., 1998)
Apoptosis	Bcl-2/adenovirus E1B 19kD-interacting protein 3 (BNip3)	(Carrero et al., 2000)
	Nip3-like protein X (NIX)	(Bruick, 2000)

ARNT – základní dimerizační partner – vzájemná kompetice??



? AhR-HIF-1 α crosstalk ?

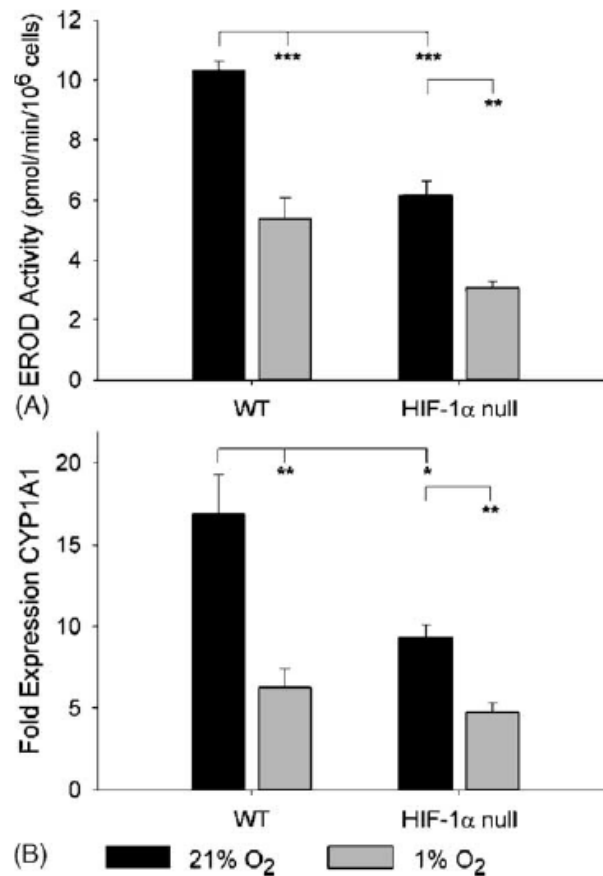
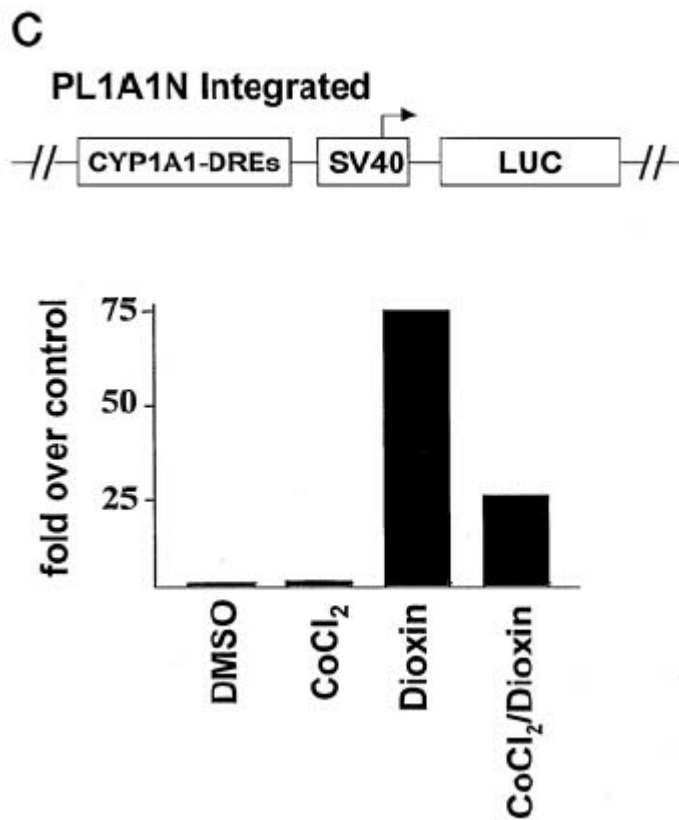
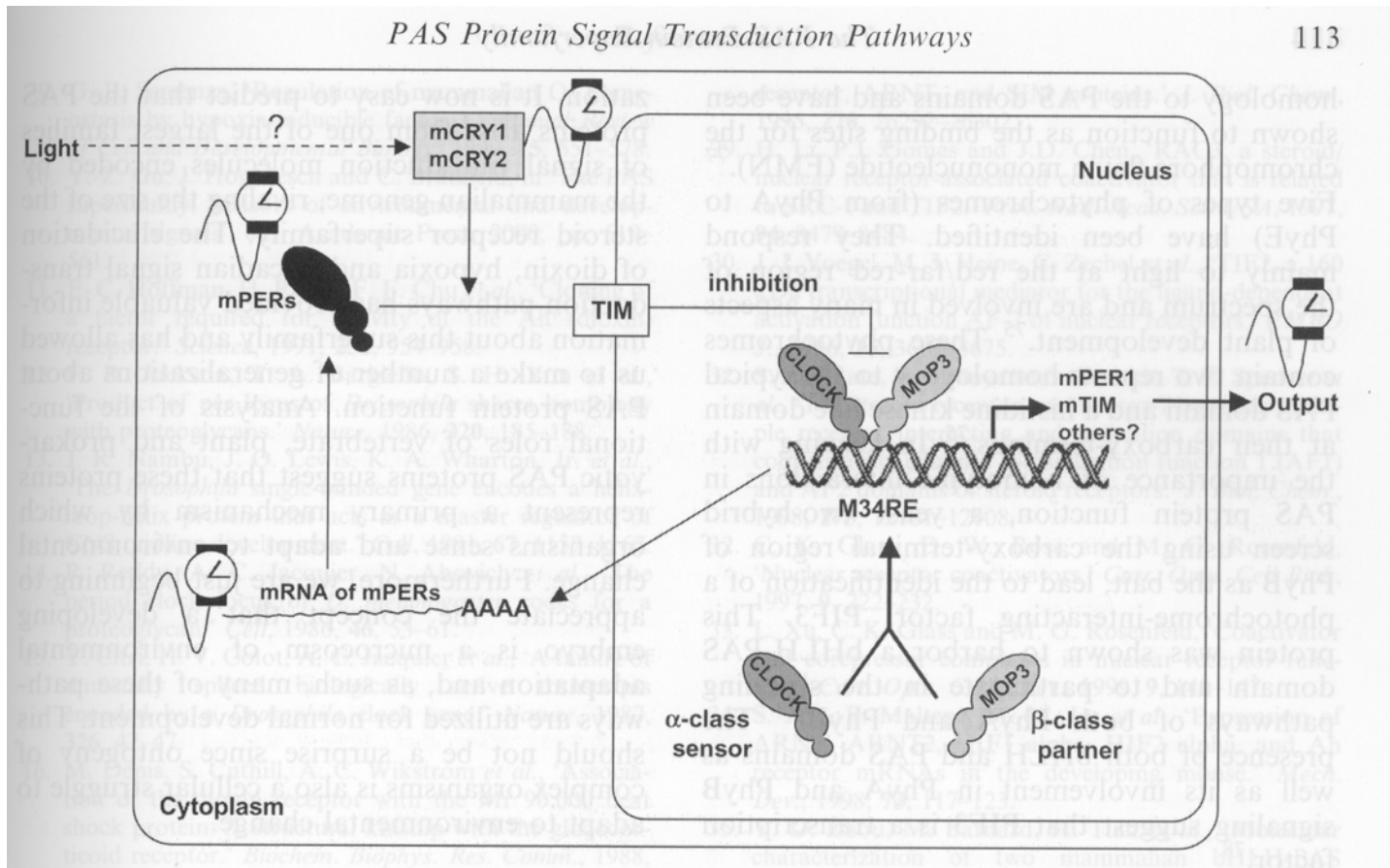


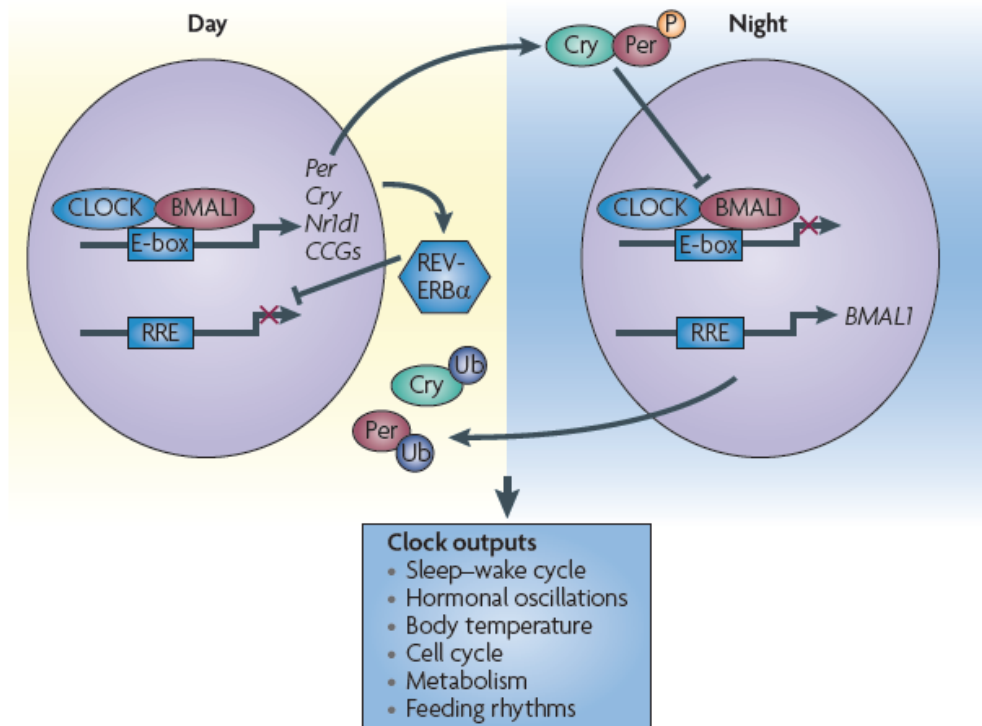
Fig. 3. Enzymatic activity (A) and gene expression (B) of CYP1A1. Rate of conversion of ethoxyresorufin. (A) was assayed in WT and HIF-1 α null cultures under normoxia (21% O₂, black bars) or hypoxia (1% O₂, grey bars) with 5 μ M 3-MC for 24 h. CYP1A1 mRNA levels (B) were measured by real time PCR after 8 h of normoxia (black) or hypoxia (grey) with 5 μ M 3-MC and normalized to untreated, normoxic controls. Values are the mean and standard error or $n = 3$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Regulace cirkadiálních rytmů

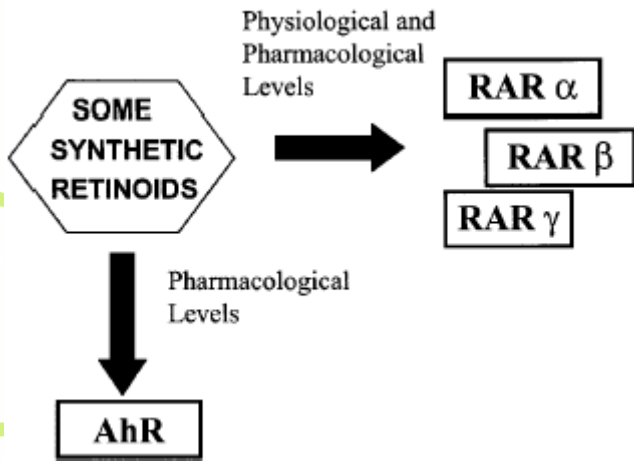


Regulace cirkadiálních rytmů

CLOCK/BMAL1 heterodimer kontroluje expresi genů závislou na cirkadiálních rytmech – PER, CRY – zpětněvazebná regulace vlastní exprese;



AhR-retinoid receptors crosstalk



ATRA

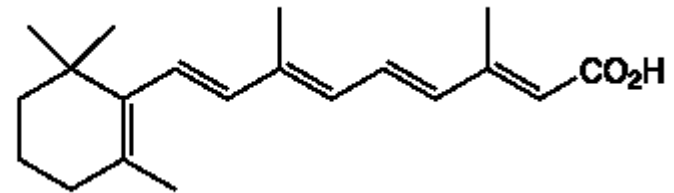


FIGURE 2 Schematic representation of the AhR/Arnt signaling pathway indicating the five steps (see text for descriptions) that have been shown to be modulated by specific retinoids.

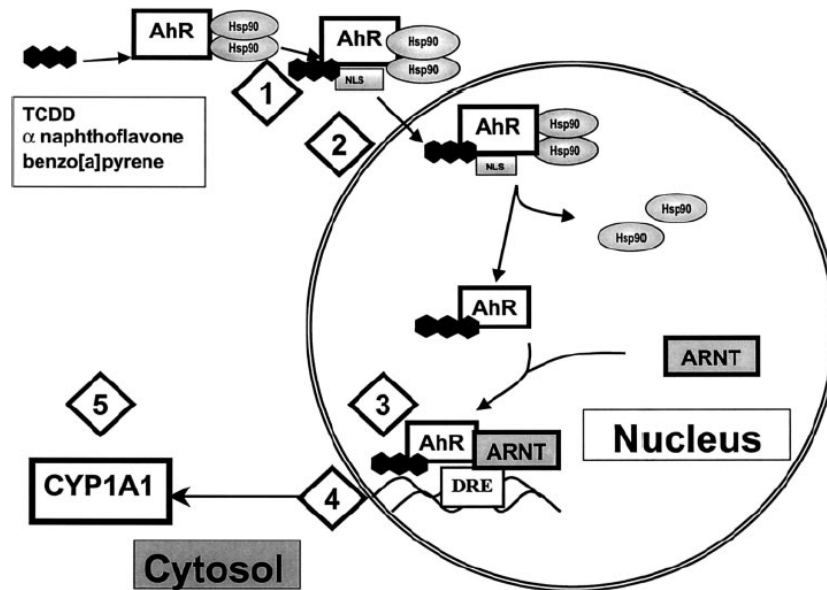


TABLE 2
Effects of Ah Receptor Ligands on Enzyme Activities Involved in Retinoid Metabolism¹

Activity	Effect	Tissue	Reference
Retinoic acid glucuronidation	↑	liver, kidney	Bank et al. 1989
	↑	liver	Sass et al. 1994
Retinoic acid oxidation	↑	liver	Spear et al. 1988
	↑	liver	Fiorella et al. 1995
	±0	liver	Andreola et al. 1997
Retinol esterification	↓	hepatic stellate cells	Nilsson et al. 1996
	↑	kidney	Nilsson et al. 2000
Retinyl ester hydrolysis	±0	liver	Nilsson et al. 2000

¹ TCDD was used in all studies except Sass et al. 1994 (3-methylcholanthrene) and Spear et al. 1998 (3,3',4,4',5,5'-hexabromobiphenyl). All studies were on rats except Andreola et al. 1997 (mice).