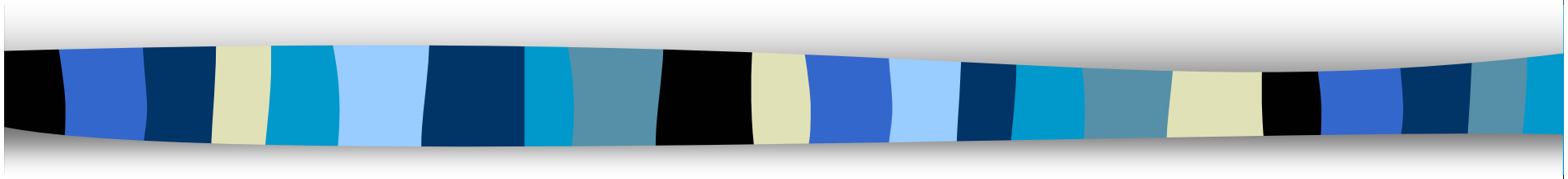
Fyziologie působení farmak a toxických látek



Přednáška č. 1

PAS proteiny jako stresové senzory a vývojové regulátory

Náplň předmětu:

Fyziologie komunikace

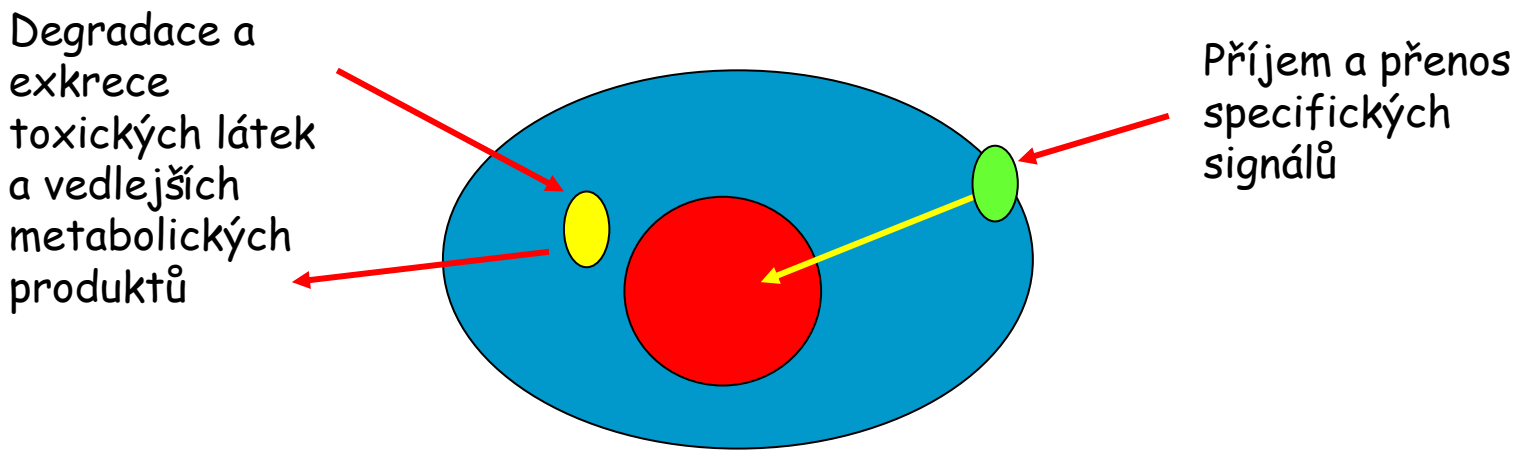
Nízkomolekulární látky přírodního a antropogenního původu:

- Signalizace
- Toxicita
- Fyziologické podmínky vs. lidské zásahy

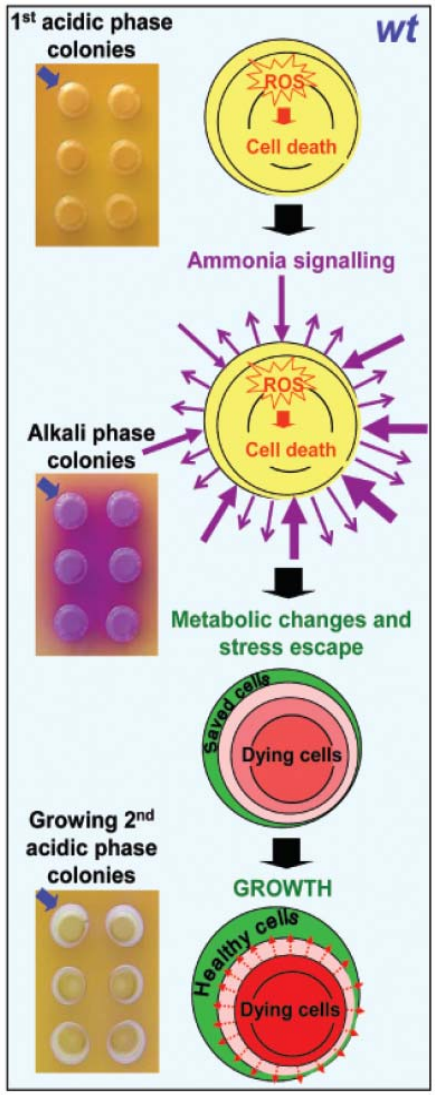
Mechanismy jejich působení na buněčné úrovni

Již na úrovni jednobuněčných organismů je nezbytná schopnost:

1. **Přijímat a identifikovat signály z vnějšího prostředí – např. za účelem výměny genetické informace;**
2. **Eliminovat toxické látky přijímané z vnějšího prostředí/vznikající jak vedlejší produkty metabolismu;**



Modelová forma komunikace – NH_4 jako signální molekula mezi koloniemi kvasinek:



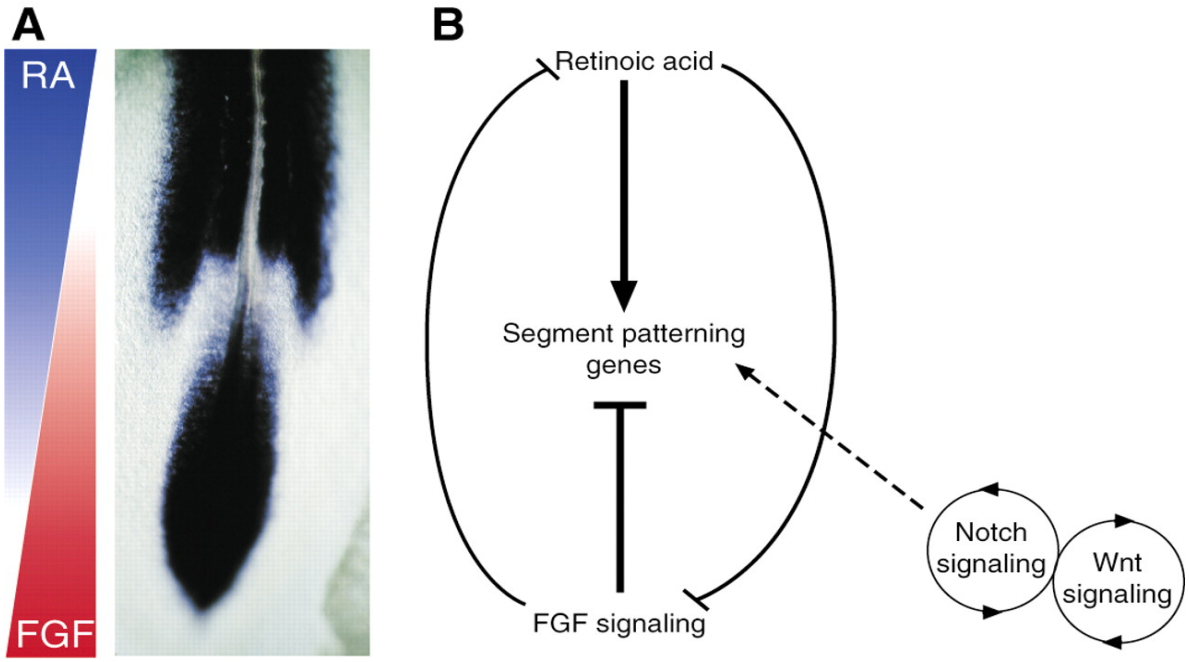
Model of ammonia-triggered differentiation in Saccharomyces cerevisiae colonies. In first-acidic phase colonies, ROS and other harmful products are produced by cells throughout the whole colonies and induce the regulated yeast cell death (YCD). To escape damage, yeast cells start to emit (outgoing violet arrows) and accept (incoming arrows) ammonia signal, which triggers metabolic changes that consequently allow cells to lower their ROS production. Healthy cells located mainly at the colony border (where the concentration of ROS is low) can thus escape cell death. Consequently, at the colony border, there are mainly slowly growing and dividing healthy cells (green) in later developmental phases (second acidic phase), while in the colony centre, dying cells (red) predominate.Compounds released (red arrows) from these cells in late stages of YCD sustain border cell growth and reproduction. The blue arrow indicates the position of the colony considered in the model.

Palková a Váchová, FEMS Microbiol Rev 30 (2006) 806–824

U mnohobuněčných organismů (živočichů) se vyvinuly stovky signálních drah a dalších mechanismů:

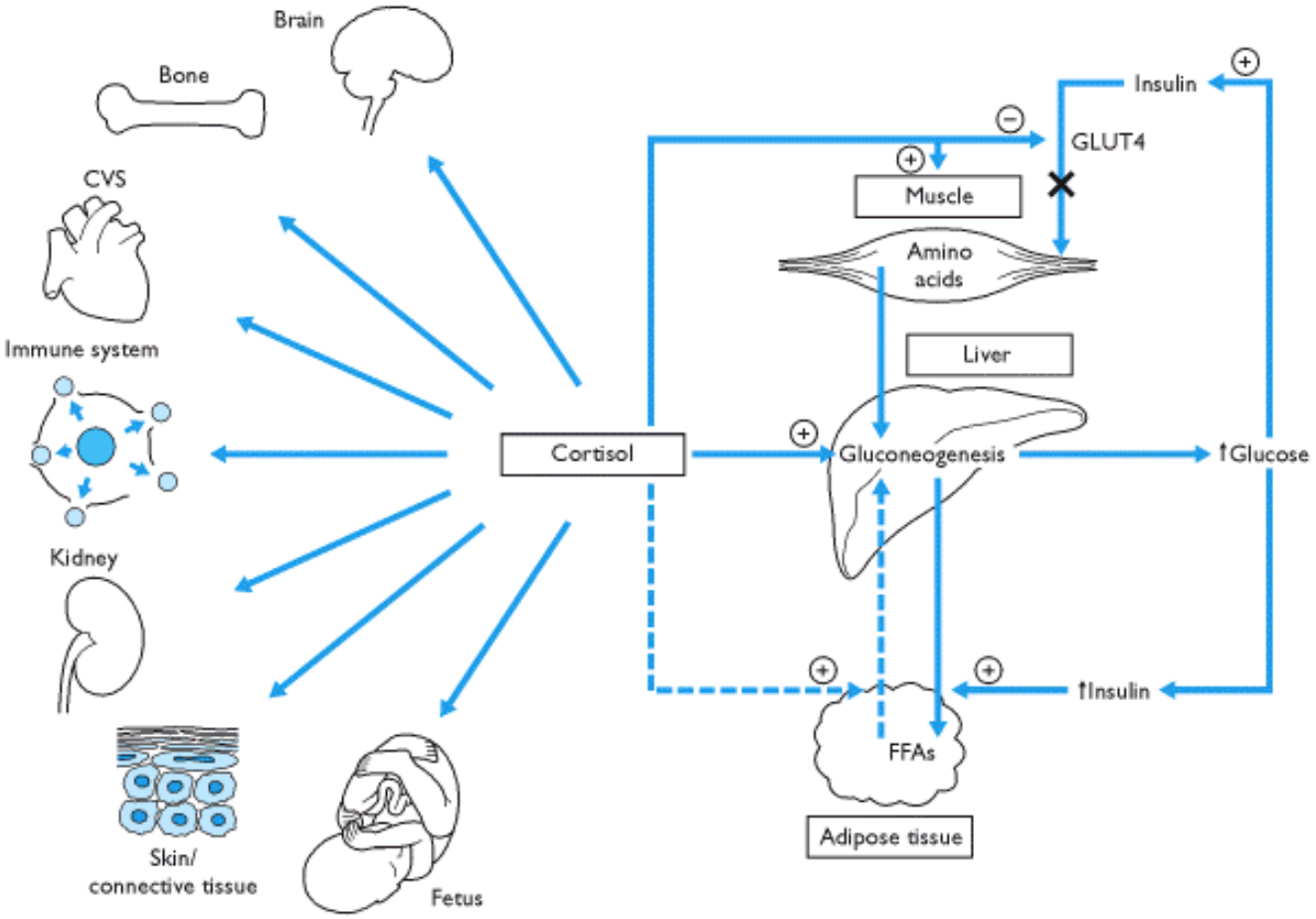
1. Embryonální a postnatální vývoj;
2. Regulace metabolismu a obecně, homeostázy;
3. Pohlavní rozmnožování;
4. Tvorba a degradace signálních molekul i toxických sloučenin; přenos signálu

http://www.luc.edu/faculty/wwasser/dev/zebra.mov



Dubrulle, J. et al. Development 2004;131:5783-5793

A model for somitogenesis. (A) Double in situ hybridization of a 2-day-old chicken embryo with Raldh2 (retinaldehyde dehydrogenase 2) and Fgf8 (fibroblast growth factor 8) probes. Anterior is towards the top. These genes participate in the establishment of mutually inhibitory, antagonistic gradients of retinoic acid (RA) and fibroblast growth factor (FGF) signaling. (B) Molecular mechanisms leading to a segmental pattern. Segment patterning genes are periodically activated by the segmentation clock, whose main regulators are the Notch and Wnt signaling pathways. The spatial activation of the segment patterning genes is defined by the RA and FGF antagonistic gradients: RA positively regulates their transcription, whereas FGF signaling represses RA activity and inhibits presomitic mesoderm maturation.

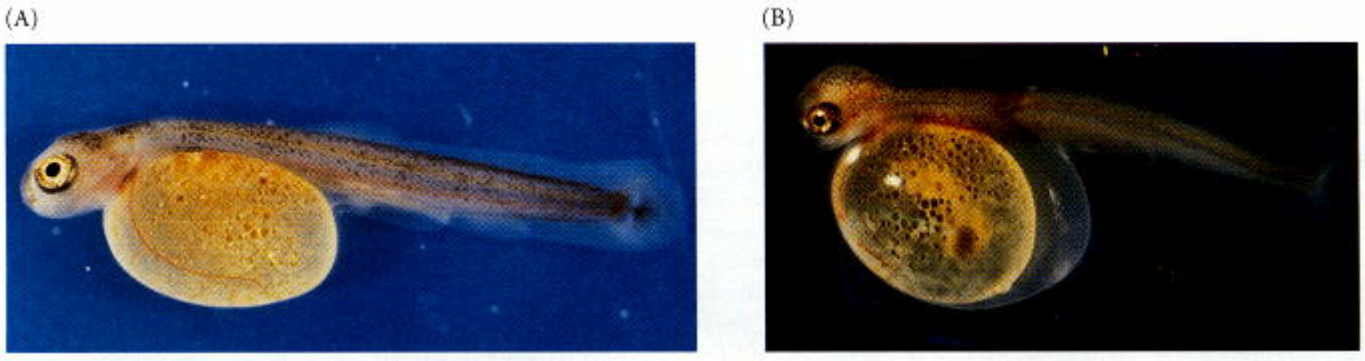


Cortisol stimulates the release of amino acids from muscle. These are taken up by the liver and converted to glucose. The increased circulating concentration of glucose stimulates insulin release. Cortisol inhibits the insulin-stimulated uptake of glucose in muscle via the GLUT4 transporter. Cortisol 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

Endocrinology: An Integrated Approach. Nussey and Whitehead (2001) London: Taylor&Francis

Zásahy z vnějšího prostředí:

1. Produkty sekundárního metabolismu rostlin a hub;
2. Zásahy člověka – cílené – aplikace chemických látek jako jsou pesticidy, syntetické feromony; terapie;
3. Zásahy člověka – nezamýšlené – toxické sloučeniny; odpad.



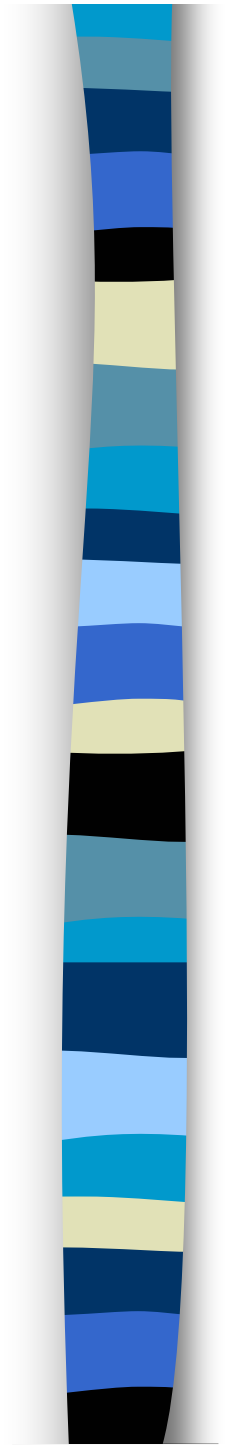
Lake trout 4 weeks after hatching. (A) Normal larva with its golden yellow yolk sac. (B) Dioxin-exposed larva exhibiting a blue yolk sac. The yolk sac has swelled with water and has numerous sites of hemorrhage. Such fish often have reduced growth, as well as heart and facial anomalies. (Photograph courtesy of R. E. Peterson.)

Developmental Biology. 6th ed. Gilbert, Scott F. Sunderland (MA): Sinauer Associates, Inc.; 2000.

Co všechno se dá najít v odpadních vodách:

(Kolpin et al., ENVIRONMENTAL SCIENCE & TECHNOLOGY / VOL. 36, NO. 6, 2002)

chemical (method)	CASRN	*N*	RL (μg/L)	freq (%)	max (μg/L)	med (μg/L)	use	MCL or *HAL* (*23*) (μg/L)	lowest LC_{50} for the most sensitive indicator species (μg/L)/no. of aquatic studies identified (*24*)
Veterinary and Human Antibiotics									
carbodox (1)	6804-07-5	104	0.10	0	ND	ND	antibiotic	–	–/1
chlortetracycline (1)	57-62-5	115	0.05	0	ND	ND	antibiotic	–	88000[a]/3
chlortetracycline (2)	57-62-5	84	0.10	2.4	0.69	0.42	antibiotic	–	88000[a]/3
ciprofloxacin (1)	85721-33-1	115	0.02	2.6	0.03	0.02	antibiotic	–	–/0
doxycycline (1)	564-25-0	115	0.1	0	ND	ND	antibiotic	–	–/0
enrofloxacin (1)	93106-60-6	115	0.02	0	ND	ND	antibiotic	–	40[b]/29
erythromycin-H_2O (1)	114-07-8	104	0.05	21.5	1.7	0.1	erythromycin metabolite	–	665000[b]/35
lincomycin (1)	154-21-2	104	0.05	19.2	0.73	0.06	antibiotic	–	–/0
norfloxacin (1)	70458-96-7	115	0.02	0.9	0.12	0.12	antibiotic	–	–/6
oxytetracycline (1)	79-57-2	115	0.1	0	ND	ND	antibiotic	–	102000[a]/46
oxytetracycline (2)	79-57-2	84	0.10	1.2	0.34	0.34	antibiotic	–	102000[a]/46
roxithromycin (1)	80214-83-1	104	0.03	4.8	0.18	0.05	antibiotic	–	–/0
sarafloxacin (1)	98105-99-8	115	0.02	0	ND	ND	antibiotic	–	–/0
sulfachloropyridazine (2)	80-32-0	84	0.05	0	ND	ND	antibiotic	–	–/0
sulfadimethoxine (1)	122-11-2	104	0.05	0	ND	ND	antibiotic	–	–/5
sulfadimethoxine (2)	122-11-2	84	0.05	1.2	0.06	0.06	antibiotic	–	–/5
sulfamerazine (1)	127-79-7	104	0.05	0	ND	ND	antibiotic	–	100000[c]/17
sulfamerazine (2)	127-79-7	84	0.05	0	ND	ND	antibiotic	–	100000[c]/17
sulfamethazine (1)	57-68-1	104	0.05	4.8	0.12	0.02	antibiotic	–	100000[c] 17
sulfamethazine (2)	57-68-1	84	0.05	1.2	0.22	0.22	antibiotic	–	100000[c]/17
sulfamethizole (1)	144-82-1	104	0.05	1.0	0.13	0.13	antibiotic	–	–/0
sulfamethoxazole (1)	723-46-6	104	0.05	12.5	1.9	0.15	antibiotic	–	–/0
sulfamethoxazole (3)	723-46-6	84	0.023	19.0	0.52	0.066	antibiotic	–	–/0
sulfathiazole (1)	72-14-0	104	0.10	0	ND	ND	antibiotic	–	–/0
sulfathiazole (2)	72-14-0	84	0.05	0	ND	ND	antibiotic	–	–/0
tetracycline (1)	60-54-8	115	0.05	0	ND	ND	antibiotic	–	550000[b]/3
tetracycline (2)	60-54-8	84	0.10	1.2	0.11	0.11	antibiotic	–	550000[b]/3
trimethoprim (1)	738-70-5	104	0.03	12.5	0.71	0.15	antibiotic	–	3000[c]/4
trimethoprim (3)	738-70-5	84	0.014	27.4	0.30	0.013	antibiotic	–	3000[c]/4
tylosin (1)	1401-69-0	104	0.05	13.5	0.28	0.04	antibiotic	–	–/0
virginiamycin (1)	21411-53-0	104	0.10	0	ND	ND	antibiotic	–	–/0



Prescription Drugs									
albuterol (salbutamol) (3)	18559-94-9	84	0.029	0	ND	ND	antiasthmatic	–	–/0
cimetidine (3)	51481-61-9	84	0.007	9.5	0.58[d]	0.074[d]	antacid	–	–/0
codeine (3)	76-57-3	46	0.24	6.5	0.019	0.012	analgesic	–	–/0
codeine (4)	76-57-3	85	0.1	10.6	1.0[d]	0.2[d]	analgesic	–	–/0
dehydronifedipine (3)	67035-22-7	84	0.01	14.3	0.03	0.012	antianginal	–	–/0
digoxin (3)	20830-75-5	46	0.26	0	ND[d]	ND[d]	cardiac stimulant	–	10000000[a]/24
digoxigenin (3)	1672-46-4	84	0.008	0	ND	ND	digoxin metabolite	–	–/0
diltiazem (3)	42399-41-7	84	0.012	13.1	0.049	0.021	antihypertensive	–	–/0
enalaprilat (3)	76420-72-9	84	0.15	1.2	0.046[d]	0.046[d]	enalapril maleate (antihypertensive) metabolite	–	–/0
fluoxetine (3)	54910-89-3	84	0.018	1.2	0.012[d]	0.012[d]	antidepressant	–	–/0
gemfibrozil (3)	25812-30-0	84	0.015	3.6	0.79	0.048	antihyperlipidemic	–	–/0
metformin (3)	657-24-9	84	0.003	4.8	0.15[d]	0.11[d]	antidiabetic	–	–/0
paroxetine metabolite (3)	–	84	0.26	0	ND[d]	ND[d]	paroxetine (antidepressant) metabolite	–	–/0
ranitidine (3)	66357-35-5	84	0.01	1.2	0.01[d]	0.01[d]	antacid	–	–/0
warfarin (3)	81-81-2	84	0.001	0	ND	ND	anticoagulant	–	16000[c]/ 33
Nonprescription Drugs									
acetaminophen (3)	103-90-2	84	0.009	23.8	10	0.11	antipyretic	–	6000[a]/ 14
caffeine (3)	58-08-2	84	0.014	61.9	6.0	0.081	stimulant	–	40000[e]/ 77
caffeine (4)	58-08-2	85	0.08	70.6	5.7	0.1	stimulant	–	40000[e]/ 77
cotinine (3)	486-56-6	84	0.023	38.1	0.90	0.024	nicotine metabolite	–	–/0
cotinine (4)	486-56-6	54	0.04	31.5	0.57	0.05	nicotine metabolite	–	–/0
1,7-dimethylxanthine (3)	611-59-6	84	0.018	28.6	3.1[d]	0.11[d]	caffeine metabolite	–	–/0
ibuprofen (3)	15687-27-1	84	0.018	9.5	1.0	0.20	antiinflammatory	–	–/0

Other Wastewater-Related Compounds

1,4-dichlorobenzene (4)	106-46-7	85	0.03	25.9	4.3	0.09	deodorizer	*75*	1100[c]/190
2,6-di-*tert*-butylphenol (4)	128-39-2	85	0.08	3.5	0.11[d]	0.06[d]	antioxidant	–	–/2
2,6-di-*tert*-butyl-1,4-benzoquinone (4)	719-22-2	85	0.10	9.4	0.46	0.13	antioxidant	–	–/0
5-methyl-1H-benzotriazole (4)	136-85-6	54	0.10	31.5	2.4	0.39	antiocorrosive	–	–/0
acetophenone (4)	98-86-2	85	0.15	9.4	0.41	0.15	fragrance	–	155000[e]/21
anthracene (4)	120-12-7	85	0.05	4.7	0.11	0.07	PAH	–	5.4[e]/188
benzo[*a*]pyrene (4)	50-32-8	85	0.05	9.4	0.24	0.04	PAH	0.2	1.5[a]/428
3-*tert*-butyl-4-hydroxy anisole (4)	25013-16-5	85	0.12	2.4	0.2[d]	0.1[d]	antioxidant	–	870[c]/14
butylated hydroxy toluene (4)	128-37-0	85	0.08	2.4	0.1[d]	0.1[d]	antioxidant	–	1440[a]/15
bis(2-ethylhexyl) adipate (4)	103-23-1	85	2.0	3.5	10[f]	3[f]	plasticizer	400	480[a]/9
bis(2-ethylhexyl) phthalate (4)	117-81-7	85	2.5	10.6	20[f]	7[f]	plasticizer	6	7500[a]/309
bisphenol A (4)	80-05-7	85	0.09	41.2	12	0.14	plasticizer	–	3600[e]/26
carbaryl (4)	63-25-2	85	0.06	16.5	0.1[d]	0.04[d]	insecticide	*700*	0.4[a]/1541
***cis*-chlordane** (4)	5103-71-9	85	0.04	4.7	0.1	0.02	insecticide	2	7.4[b]/28
chlorpyrifos (4)	2921-88-2	85	0.02	15.3	0.31	0.06	insecticide	*20*	0.1[a]/1794
diazinon (4)	333-41-5	85	0.03	25.9	0.35	0.07	insecticide	*0.6*	0.56[a]/1040
dieldrin (4)	60-57-1	85	0.08	4.7	0.21	0.18	insecticide	0.2	2.6[c]/1540
diethylphthalate (4)	84-66-2	54	0.25	11.1	0.42	0.2	plasticizer	–	12000[c]/129
ethanol,2-butoxy-phosphate (4)	78-51-3	85	0.2	45.9	6.7	0.51	plasticizer	–	10400[e]/7
fluoranthene (4)	206-44-0	85	0.03	29.4	1.2	0.04	PAH	–	74[e]/216
lindane (4)	58-89-9	85	0.05	5.9	0.11	0.02	insecticide	0.2	30[c]/1979
methyl parathion (4)	298-00-0	85	0.06	1.2	0.01	0.01	insecticide	*2*	12[a]/888
4-methyl phenol (4)	106-44-5	85	0.04	24.7	0.54	0.05	disinfectant	–	1400[a]/74
naphthalene (4)	91-20-3	85	0.02	16.5	0.08	0.02	PAH	*20*	910[c]/519
N,*N*-diethyltoluamide (4)	134-62-3	54	0.04	74.1	1.1	0.06	insect repellant	–	71250[c]/9
4-nonylphenol (4)	251-545-23	85	0.50	50.6	40[g]	0.8[g]	nonionic detergent metabolite	–	130[e]/135
4-nonylphenol monoethoxylate (4)	–	85	1.0	45.9	20[g]	1[g]	nonionic detergent metabolite		14450[a]/4
4-nonylphenol diethoxylate (4)	–	85	1.1	36.5	9[g]	1[g]	nonionic detergent metabolite	–	5500[a]/6
4-octylphenol monoethoxylate (4)	–	85	0.1	43.5	2[g]	0.2[g]	nonionic detergent metabolite	–	–/0
4-octylphenol diethoxylate (4)	–	85	0.2	23.5	1[g]	0.1[g]	nonionic detergent metabolite	–	–/0
phenanthrene (4)	85-01-8	85	0.06	11.8	0.53	0.04	PAH	–	590[a]/192
phenol (4)	108-95-2	85	0.25	8.2	1.3[f]	0.7[f]	disinfectant	*400*	4000[c]/2085
phthalic anhydride (4)	85-44-9	85	0.25	17.6	1[f]	0.7[f]	plastic manufacturing	–	40400[c]/5
pyrene (4)	129-00-0	85	0.03	28.2	0.84	0.05	PAH	–	90.9[a]/112
tetrachloroethylene (4)	127-18-4	85	0.03	23.5	0.70[d]	0.07[d]	solvent, degreaser	5	4680[c]/147
triclosan (4)	3380-34-5	85	0.05	57.6	2.3	0.14	antimicrobial disinfectant	–	180[e]/3
tri(2-chloroethyl) phosphate (4)	115-96-8	85	0.04	57.6	0.54	0.1	fire retardant	–	66000[b]/8
tri(dichlorisopropyl) phosphate (4)	13674-87-8	85	0.1	12.9	0.16	0.1	fire retardant	–	3600[b]/9
triphenyl phosphate (4)	115-86-6	85	0.1	14.1	0.22	0.04	plasticizer	–	280[c]/66

Steroids and Hormones									
***cis*-androsterone** (5)	53-41-8	70	0.005	14.3	0.214	0.017	urinary steroid	–	–/0
cholesterol (4)	57-88-5	85	1.5	55.3	10[d]	1[d]	plant/animal steroid	–	–/0
cholesterol (5)	57-88-5	70	0.005	84.3	60[h]	0.83	plant/animal steroid	–	–/0
coprostanol (4)	360-68-9	85	0.6	35.3	9.8[d]	0.70[d]	fecal steroid	–	–/0
coprostanol (5)	360-68-9	70	0.005	85.7	150[h]	0.088	fecal steroid	–	–/0
equilenin (5)	517-09-9	70	0.005	2.8	0.278	0.14	estrogen replacement	–	–/0
equilin (5)	474-86-2	70	0.005	1.4	0.147	0.147	estrogen replacement	–	–/0
17α-ethynyl estradiol (5)	57-63-6	70	0.005	15.7	0.831	0.073	ovulation inhibitor	–	–/22
17α-estradiol (5)	57-91-0	70	0.005	5.7	0.074	0.03	reproductive hormone	–	–/0
17β-estradiol (4)	50-28-2	85	0.5	10.6	0.2[d]	0.16[d]	reproductive hormone	–	–/0
17β-estradiol (5)	50-28-2	70	0.005	10.0	0.093	0.009	reproductive hormone	–	–/0
estriol (5)	50-27-1	70	0.005	21.4	0.051	0.019	reproductive hormone	–	–/0
estrone (5)	53-16-7	70	0.005	7.1	0.112	0.027	reproductive hormone	–	–/11
mestranol (5)	72-33-3	70	0.005	10.0	0.407	0.074	ovulation inhibitor	–	–/0
19-norethisterone (5)	68-22-4	70	0.005	12.8	0.872	0.048	ovulation inhibitor	–	–/0
progesterone (5)	57-83-0	70	0.005	4.3	0.199	0.11	reproductive hormone	–	–/0
stigmastanol (4)	19466-47-8	54	2.0	5.6	4[d]	2[d]	plant steroid	–	–/0
testosterone (5)	58-22-0	70	0.005	2.8	0.214	0.116	reproductive hormone	–	–/4

Per-Arnt-Sim – PAS superfamily of proteins

environmental sensors, which mediate transcriptional responses to various types stimuli:

- ✔ circadian rhythms;
- ✔ oxygen sensing;
- ✔ sensing of toxicants;

- ✔ developmental role/cancer;

These proteins enable adaptation to rapid changes in the environment.

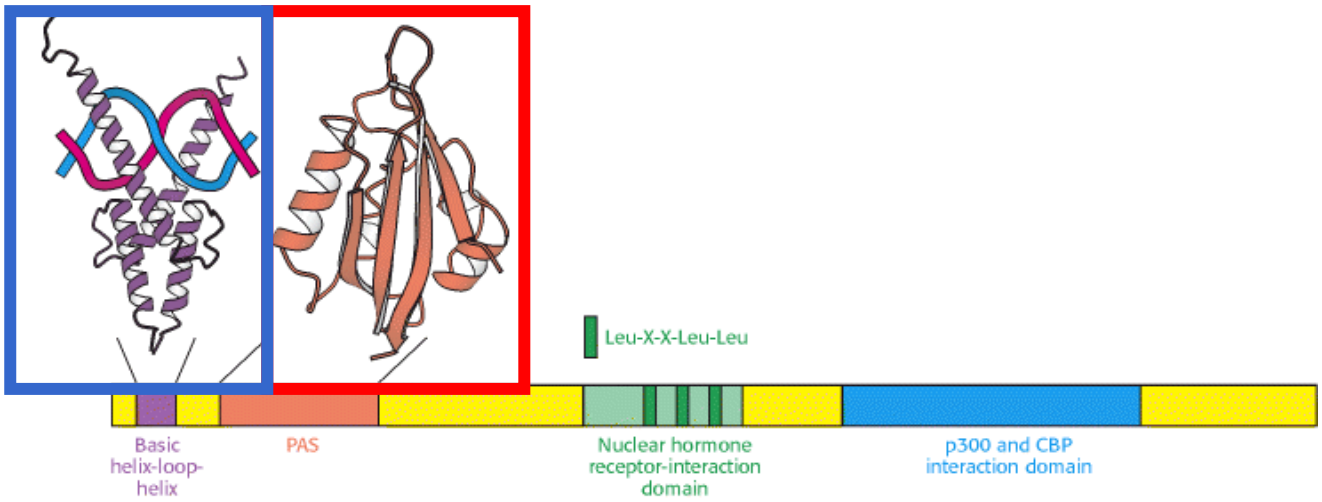
PAS proteiny jsou součástí širší rodiny bHLH proteinů:

There are three main sub-families of bHLH proteins:

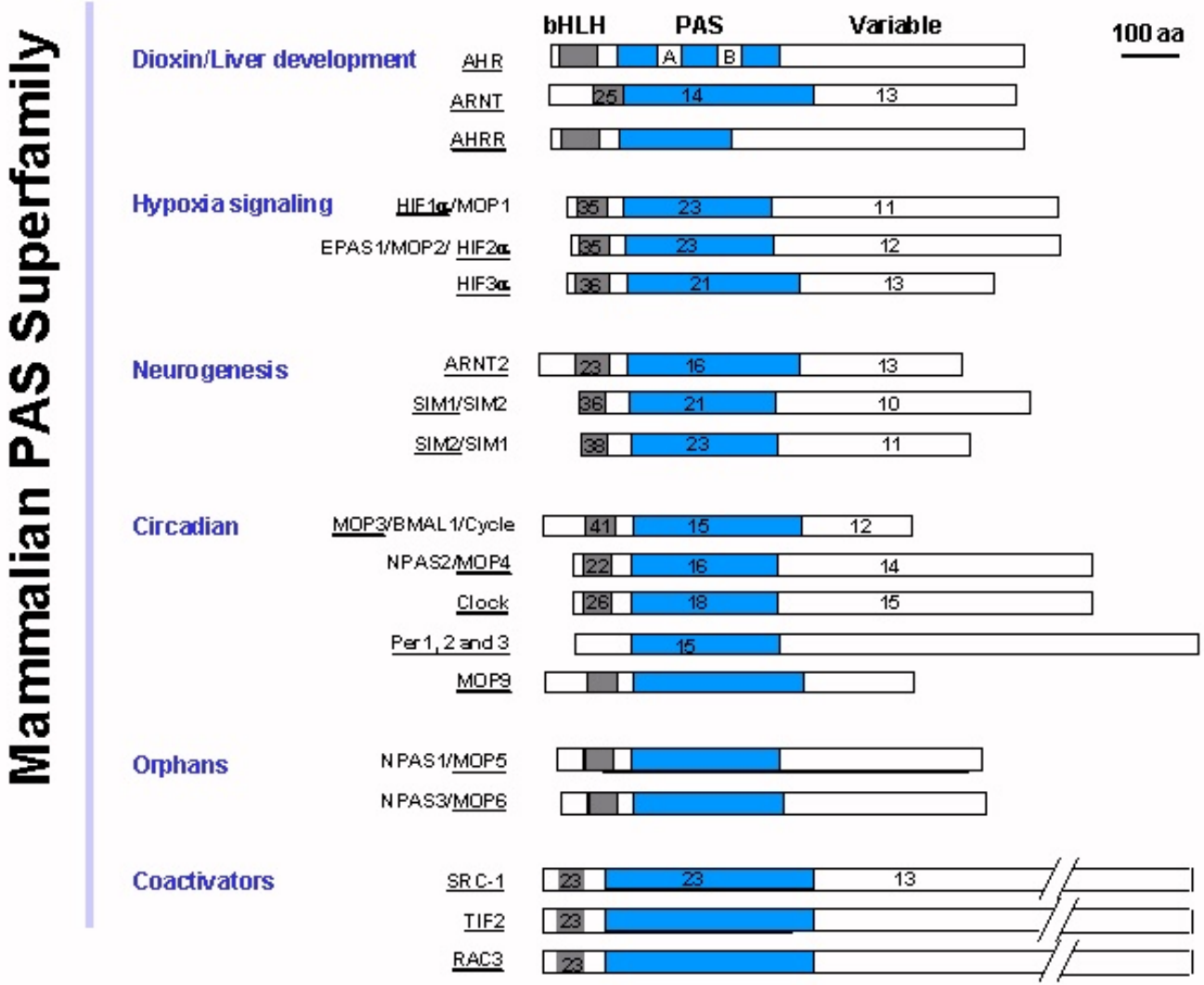
(a) those containing only the bHLH domain; and those where the bHLH domain is contiguous with a second dimerisation domain, either

(b) the leucine zipper (Zip) or

(c) the PER/aryl hydrocarbon receptor nuclear translocator (ARNT)/single
minded (SIM) (PAS) homology domain.



PAS proteiny (rodina transkripčních faktorů):



http://mcardle.oncology.wisc.edu/bradfield/

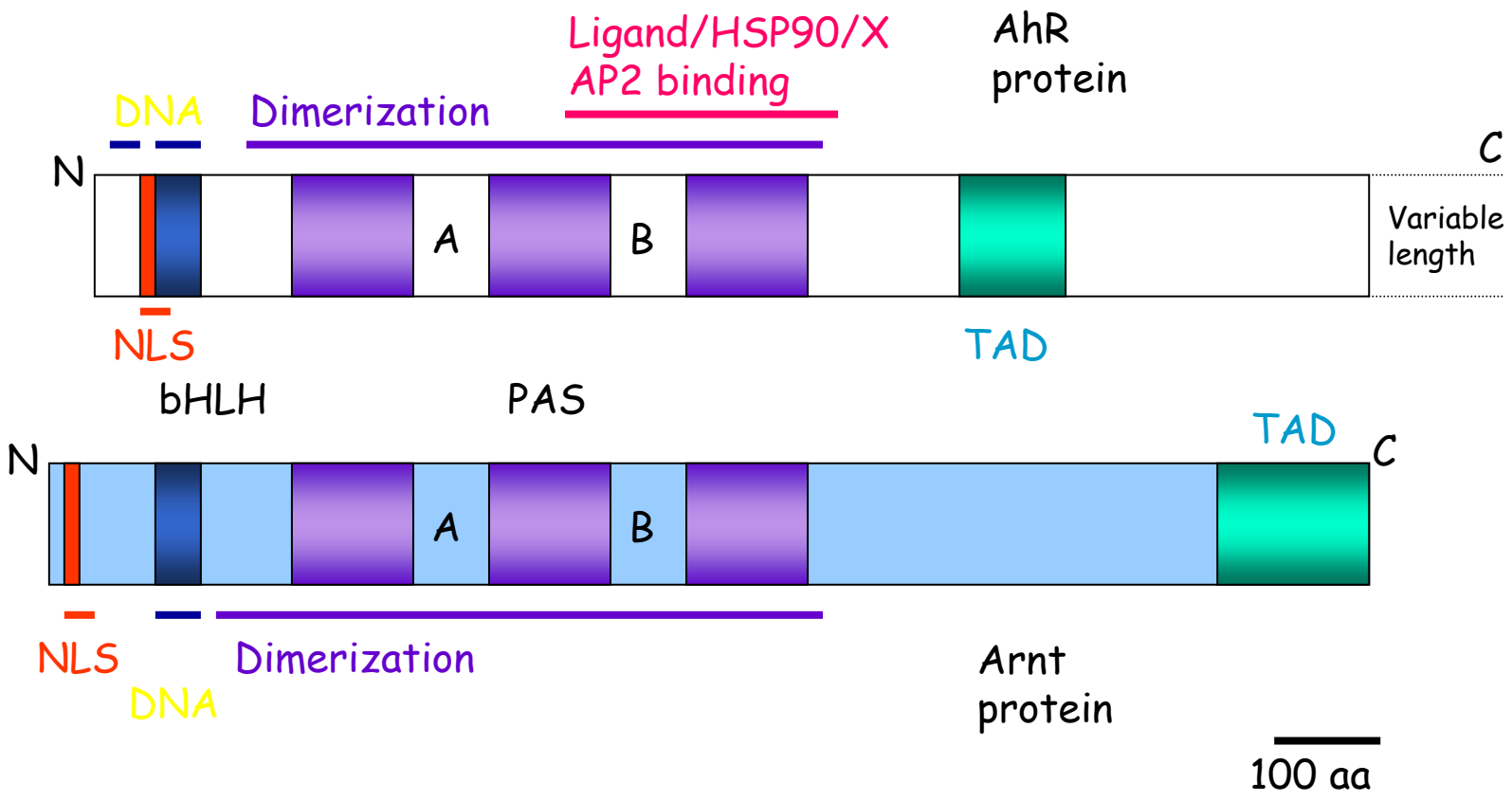
PAS domain

The PAS region consists of two adjacent degenerate repeats of ~130 amino acids, PAS A and PAS B. The domain is an **ancient signalling device conserved through evolution**, having been identified in proteins throughout the animal kingdom, in bacteria, fungi and yeast in addition to mammals and flies, where the most commonly studied bHLH/PAS proteins originate.

Many bacteria contain PAS-like proteins that detect light and oxygen (Dos, Aer, FixL, PYP).

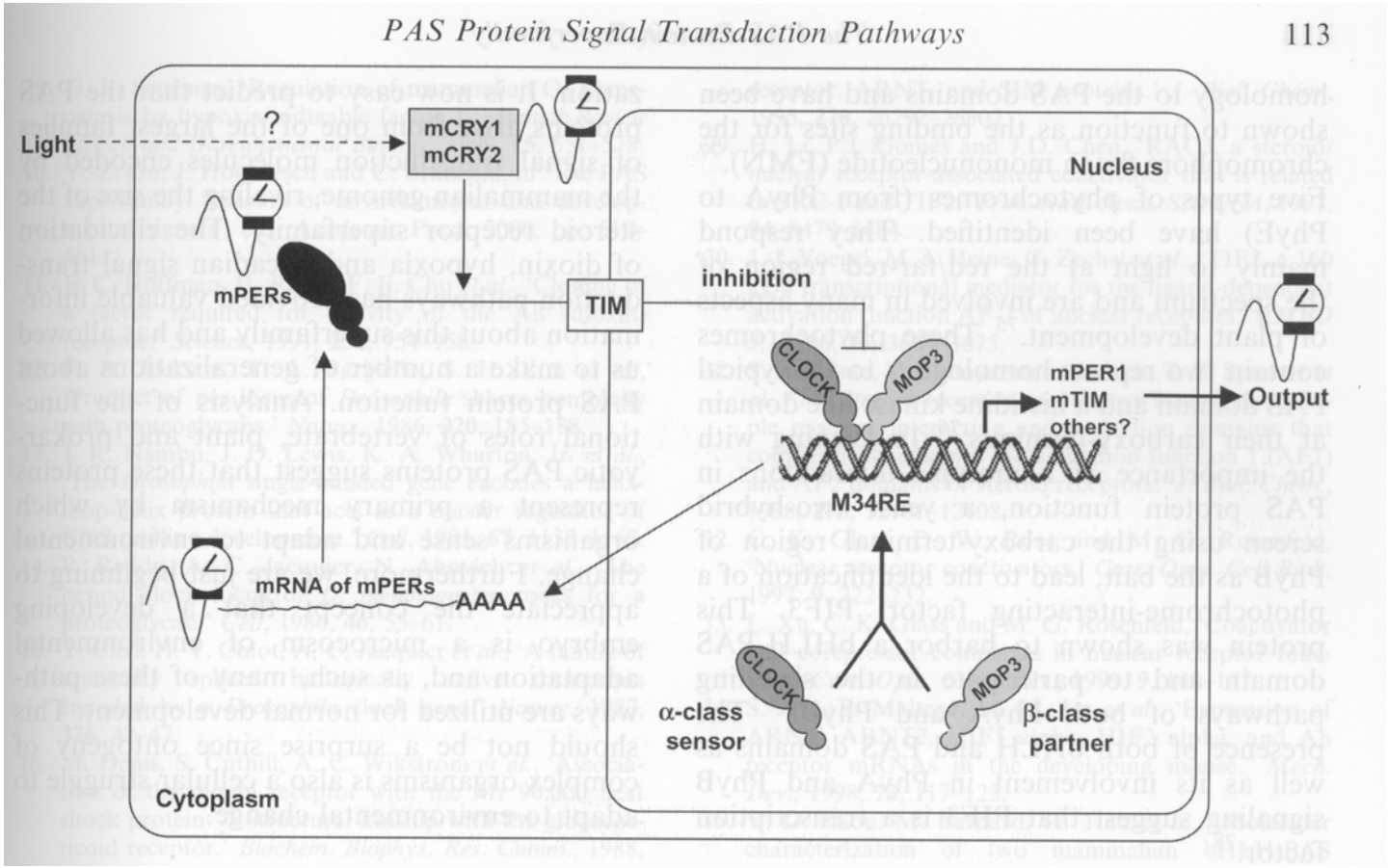
Similar proteins sense light in plants (phytochromes PhyA-PhyE, NPH1; phytochrome interacting factor PIF3).

Domain structure and function of PAS proteins:



(Gu et al., Annu Rev Pharmacol Toxicol. 2000;40:519-61.)

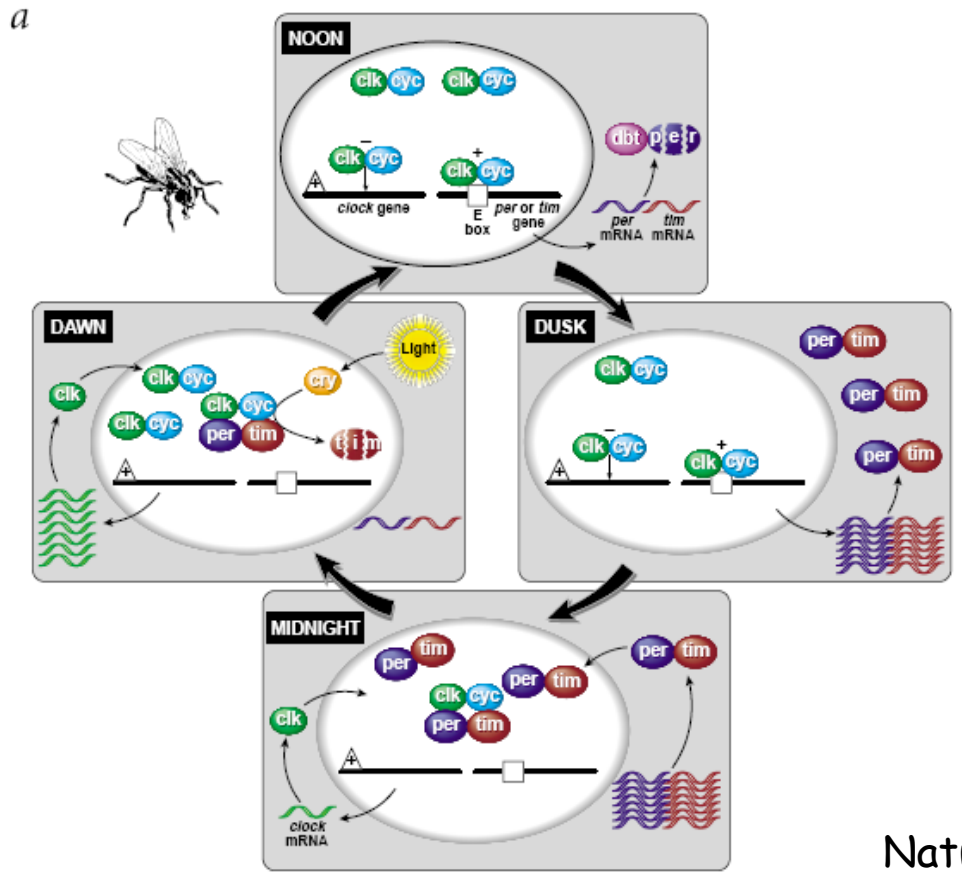
The circadian response pathway

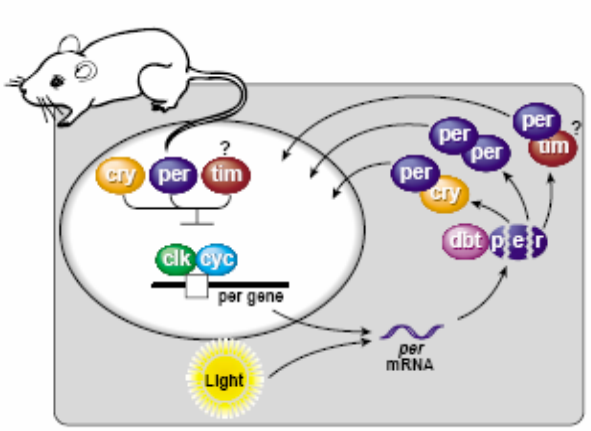


(Comprehensive Toxicology, vol. 14)

Daily changes in light/dark require physiological and behavioral adaptation:

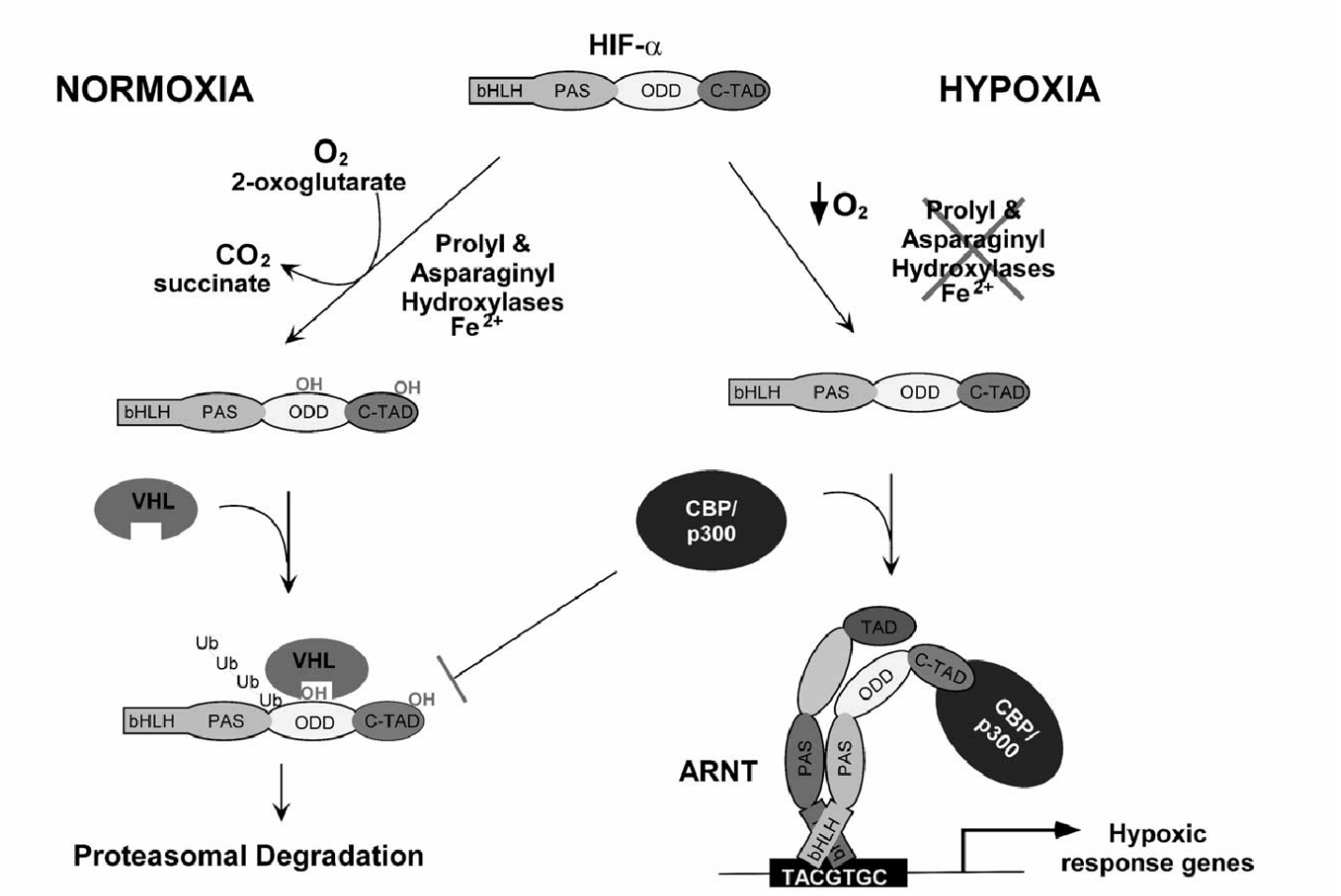
✔ CLOCK/MOP3 heterodimer controls expression of circadian responsive gene products – PER, TIM;





Nature Genetics 26, 23 - 27 (2000)

The hypoxia response pathway



The International Journal of Biochemistry & Cell Biology 36 (2004) 189–204

The ability to maintain O2 homeostasis is essential for survival of mammals. The hyperoxic state, or high O2 tension, can result in the generation of reactive oxygen intermediates and potentially lethal damage to membranes and DNA. The hypoxic state, or low O2 tension, can result in levels of ATP insufficient to maintain essential cellular functions. The hypoxic state occurs in a number of medical conditions, such as cancer and ischemias, inspiring research into understanding the cellular mechanisms for detecting and responding to low levels of oxygen. Responses to hypoxia are mediated by three bHLH/PAS proteins, HIF-1a, HIF-2a (also known as Endothelial PAS domain protein 1, HIF-like factor and member of PAS family 2), and HIF-3a.

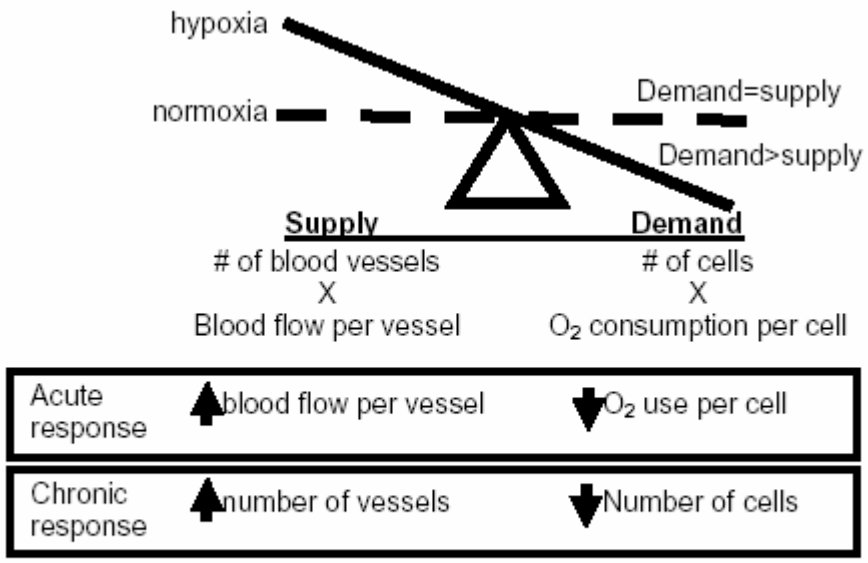
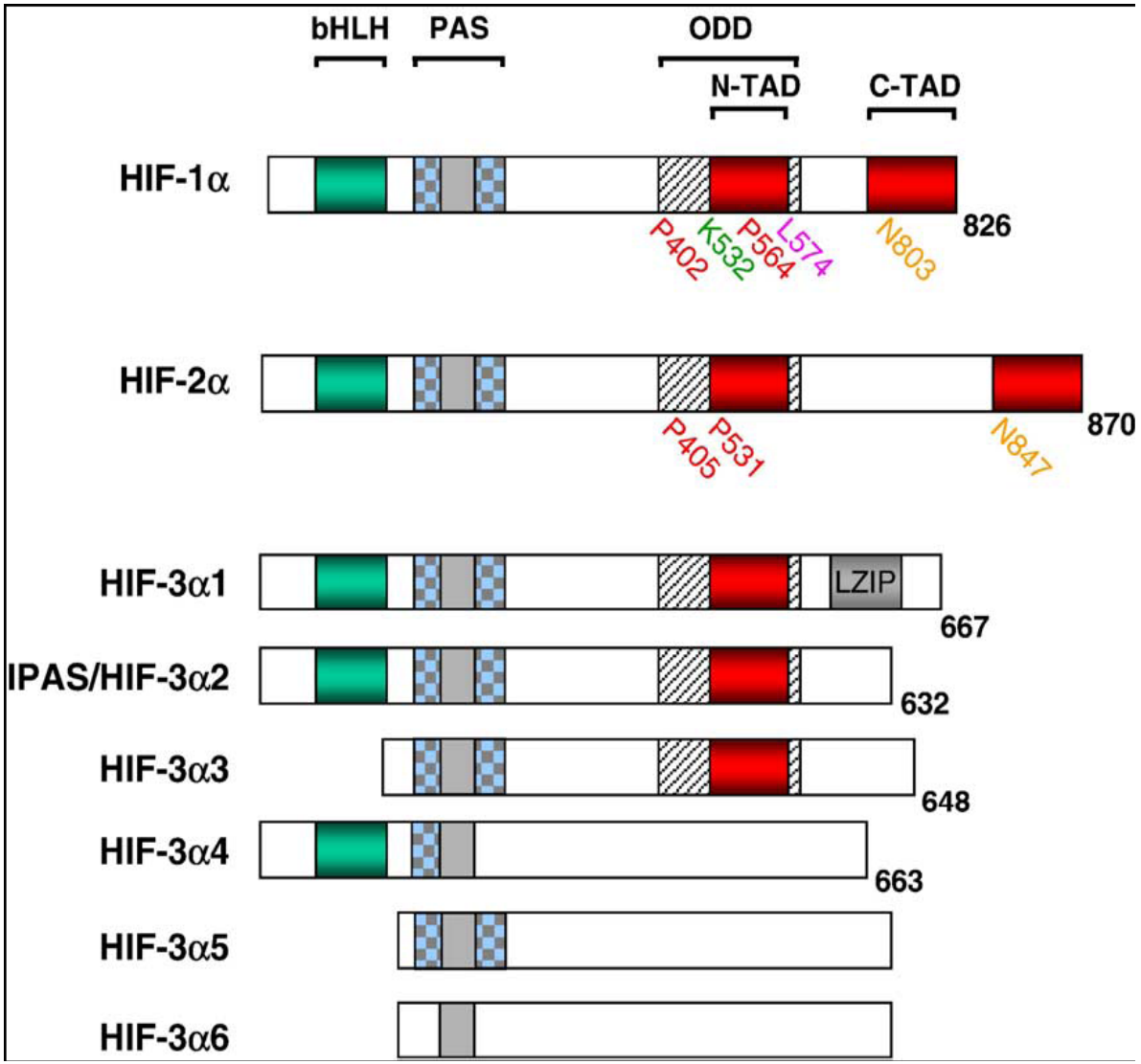


Fig. 1. Supply and demand governs oxygen availability.

Mutation Research 569 (2005) 87–100

HIF subfamily



Biochimica et Biophysica Acta 1755 (2005) 107 – 120

Hypoxia-inducible factor (HIF-1α):

Hypoxia-inducible factor-1 (HIF-1), composed of HIF-α and HIF-β (ARNT) subunits, is a heterodimeric transcriptional activator. In response to hypoxia, stimulation of growth factors, and activation of oncogenes as well as carcinogens, HIF-1a is overexpressed and/or activated and targets those genes which are required for angiogenesis, metabolic adaptation to low oxygen and survival of cells. HIF-1 is critical for both physiological and pathological processes.

Several dozens of putative direct HIF-1 target genes have been identified on the basis of one or more cis-acting hypoxia-response elements that contain an HIF-1 binding site. A variety of regulators including growth factors, genetic alterations, stress activators, and some carcinogens have been documented for regulation of HIF-1 in which several signaling pathways are involved depending on the stimuli and cell types. Activation of HIF-1 in combination with activated signaling pathways and regulators is implicated in tumour progression and prognosis.

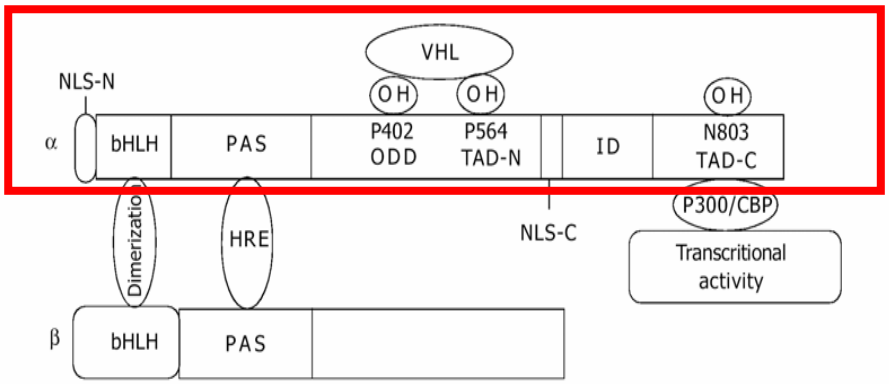
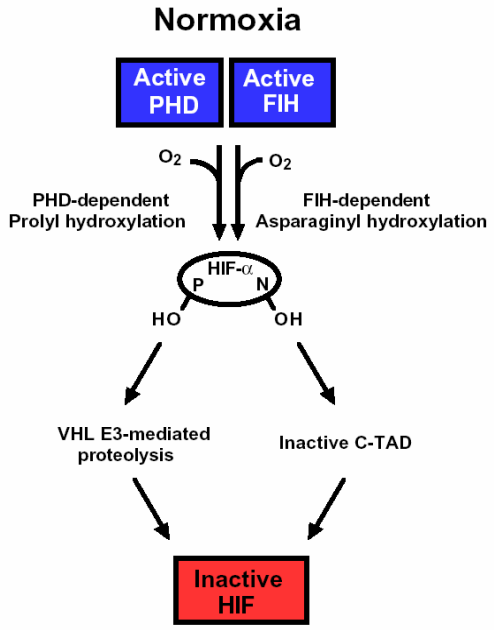


Figure 1 Molecular structure of HIF-1α and HIF-1β. bHLH domain mediates dimerization of the two subunits. PAS domain is responsible for DNA binding. Proline residues of 402 and 564 at ODD domain are hydroxylized by proline hydroxylase and recognized by VHL and then targeted to the ubiquitin proteasome pathway. Asn803 at the C-terminal transactivation domain (TAD-C) is hydroxylized by FIH-1 (factor inhibiting HIF-1) with a result of inhibition of HIF-1α interaction with co-activator p300 and consequently inhibits transcriptional activity. The nuclear location signal at C-terminal functions in HIF-1α translocation into nuclei.

World J Gastroenterol 2004;10(8):1082-1087



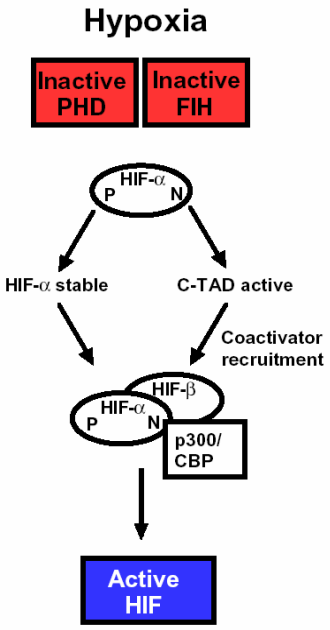
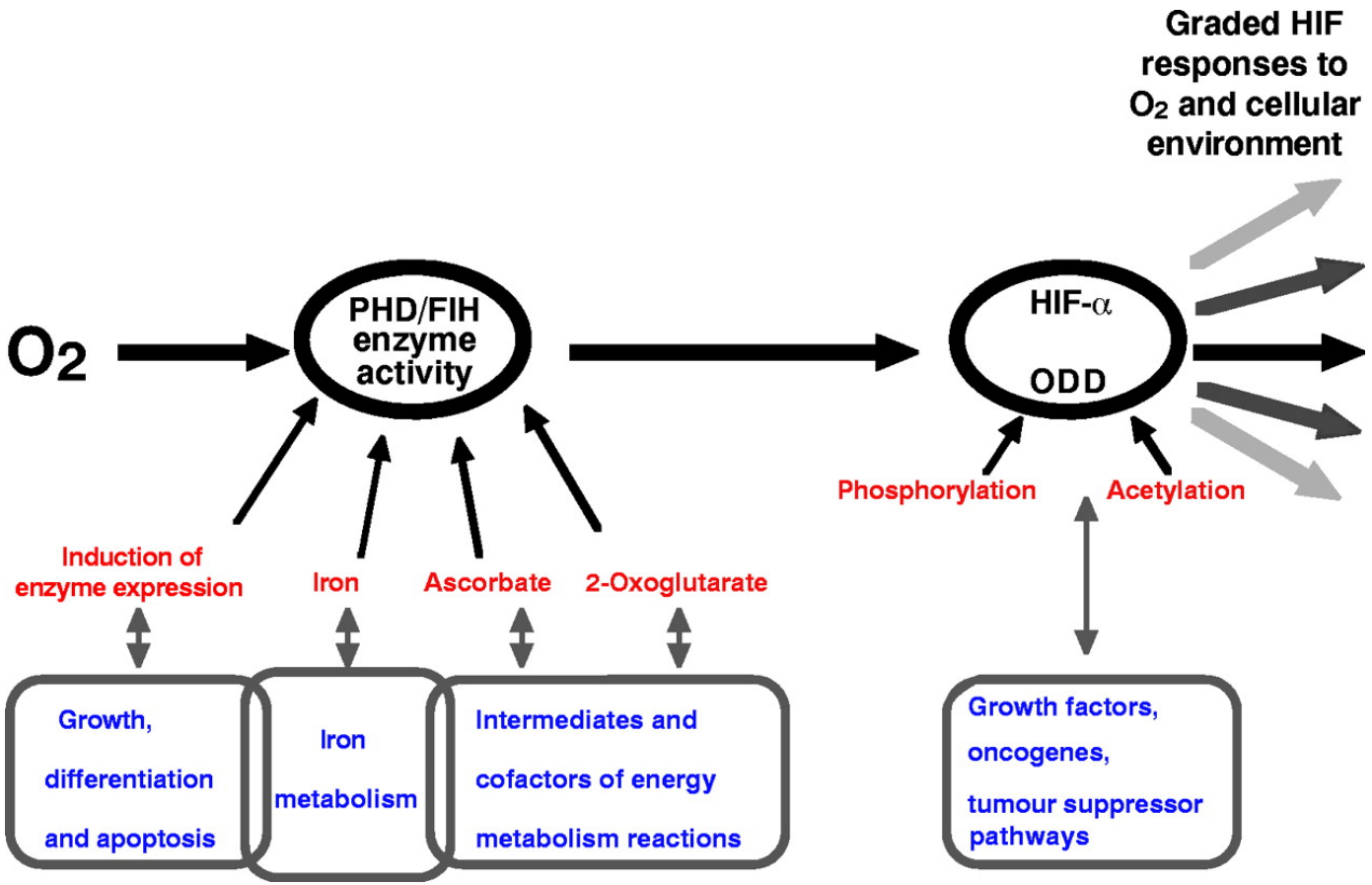


Fig. 1. Two independent hydroxylation pathways regulate HIF activity in response to cellular oxygen level. In normoxia, oxygen availability enables PHD-dependent prolyl hydroxylation of the HIF-α ODD. This prolyl hydroxylation allows binding of the VHL E3 ligase leading to ubiquitylation and degradation of HIF-α subunits. Oxygen availability also enables FIH-dependent asparaginyl hydroxylation of the C-TAD, blocking interaction with the p300/CBP co-activator. In hypoxia, the PHD and FIH enzymes are inactive and the lack of hydroxylation results in stable HIF-α able to form a DNA-binding heterodimer with HIF-β and recruit p300/CBP at the C-TAD.

Masson, N. et al. J Cell Sci 2003;116:3041-3049

HIF-dependent responses to O2 may be modulated by the cellular environment:



Masson, N. et al. J Cell Sci 2003;116:3041-3049

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- β3)	(Scheid et al., 2002)
Vascular tone	Nitric oxide synthase (NOS2)	(Melillo et al., 1995)
	Heme oxygenease 1	(Lee et al., 1997)
	Endothelin 1 (ET1)	(Hu et al., 1998)
	Adrenomedulin (ADM)	(Nguyen and Claycomb, 1999)
	α1B-adrenergic receptor	(Eckhart et al., 1997)
Matrix metabolism	Matrix metalloproteinases (MMPs)	(Ben-Yosef et al., 2002)
	Plasminogen activator receptors and inhibitors (PAIs)	(Kietzmann et al., 1999)
	Collagen prolyl hydroxylase	(Takahashi et al., 2000)
Glucose metabolism	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/gructose-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 EIB 19kD-interacting protein 3 (BNip3)	(Carrero et al., 2000)
	Nip3-like protein X (NIX)	(Bruick, 2000)

Molecular Pharmacology Fast Forward. Published on August 3, 2006 as doi:10.1124/mol.106.027029

ARNT – základní dimerizační partner:

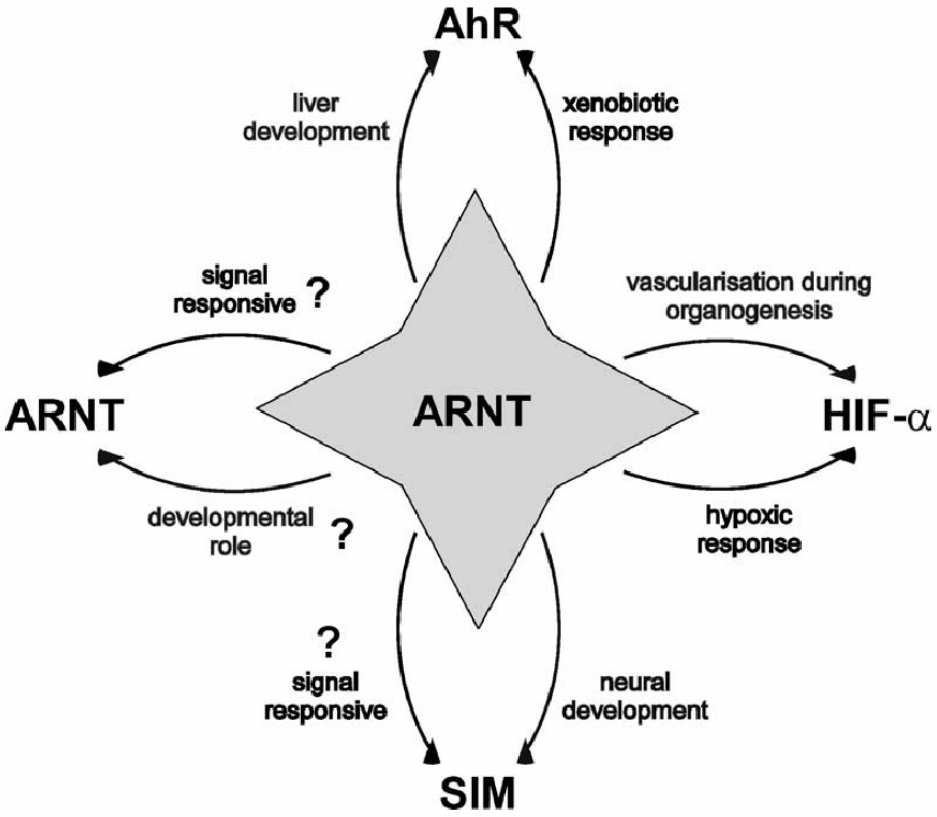


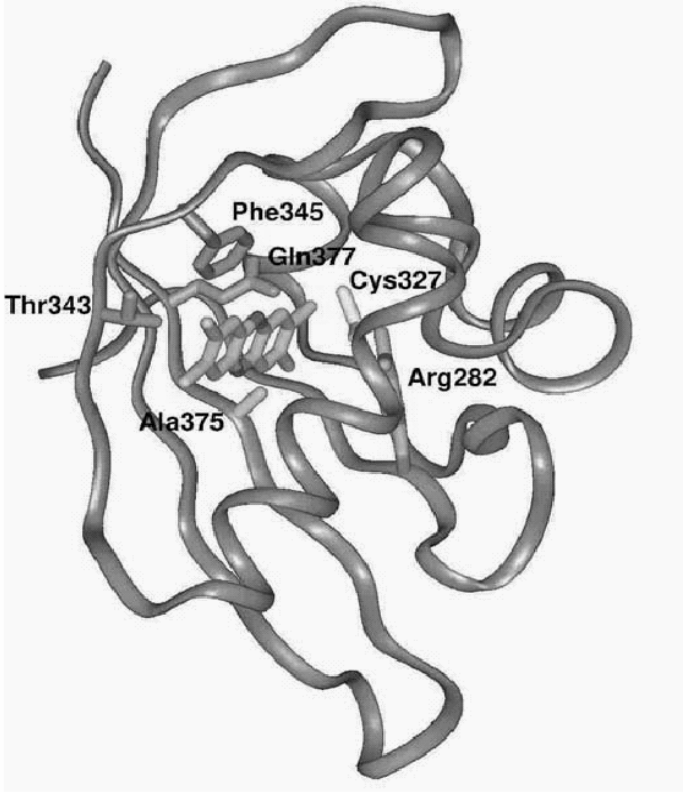
Fig. 4. ARNT is central to transcriptional regulation within the bHLH/PAS family of proteins. ARNT forms both homodimers and heterodimers with the AhR, HIF-α and SIM which play roles both during mammalian development and in response to environmental stimuli in mammals. Symbol '?' indicates where these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

The International Journal of Biochemistry & Cell Biology 36 (2004) 189–204

Jak HIF-1α, tak ARNT představují proteiny nezbytné pro přežití – KO myši odumírají již v průběhu embryonálního vývoje.

The Ah receptor pathway

Denison et al., Chem. Biol. Interact. 141: 3

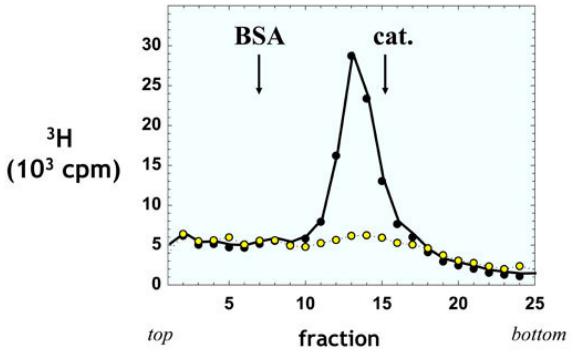


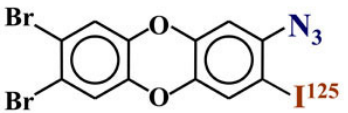
AhR =

- ligand-activated transcription factor;
- important mediator of toxicity of POPs;
- regulator of xenobiotic metabolism and activation of promutagens.

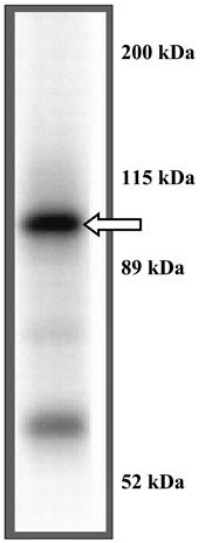
AhR discovery

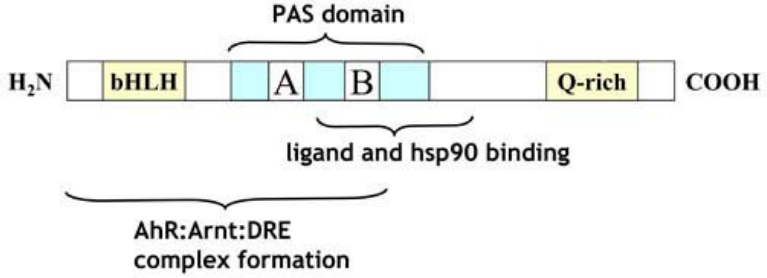
- different sensitivity of inbred mouse strains to TCDD and 3-MC – inducers of CYP1A activity in liver microsomes;
- autosomal dominant Mendelian trait;
- isolation of protein; cloning





2-azido-3-[^{125}I]-7,8-dibromo-dibenzo-*p*-dioxin





Molecular Toxicology, 2nd ed.

Overview of aryl hydrocarbon receptor and dioxin-like toxicity:

- what is AhR;
- evolution perspective;
- activation of AhR; AhR-dependent genes
- toxic effects associated with AhR activation;
- AhR interactions
- the role of AhR in cell cycle regulation

AhR domain structure:

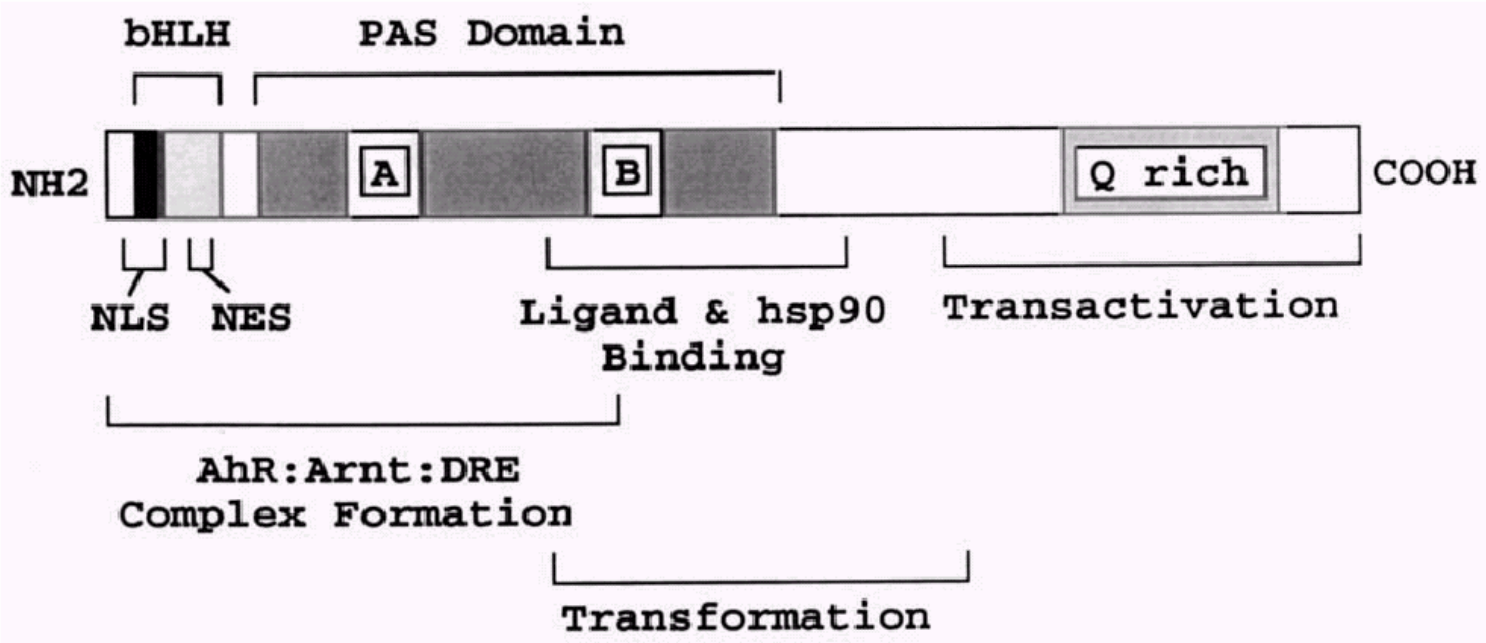


Fig. 2. Domain structure of the AhR.

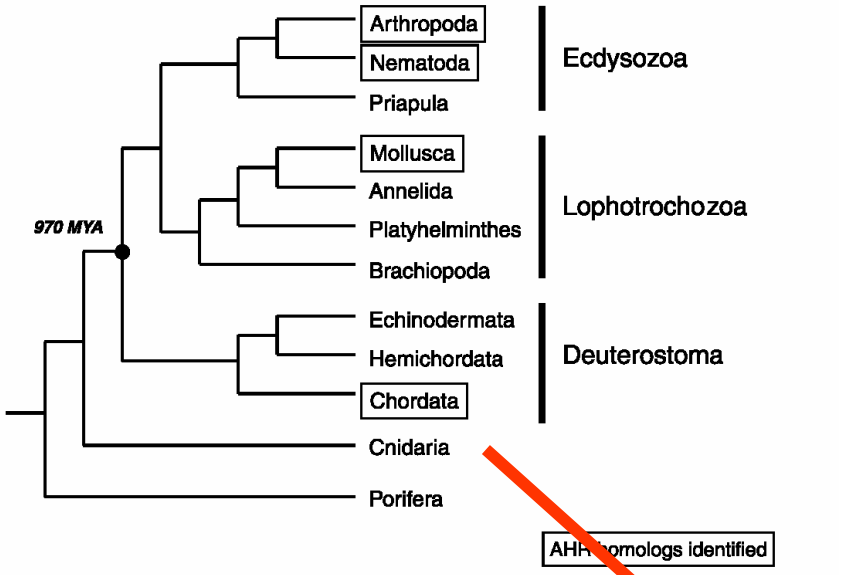
Denison et al., Chem. Biol. Interact. 141: 3

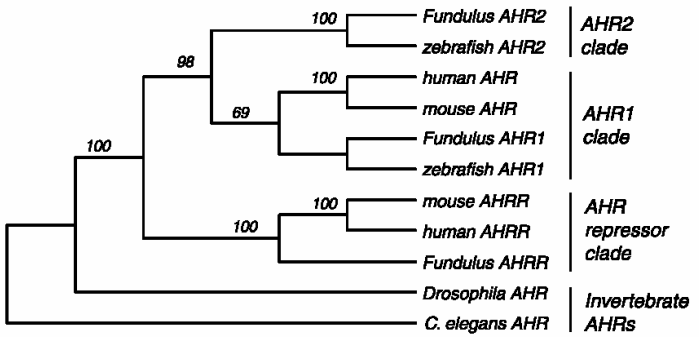
Evolution of AhR:

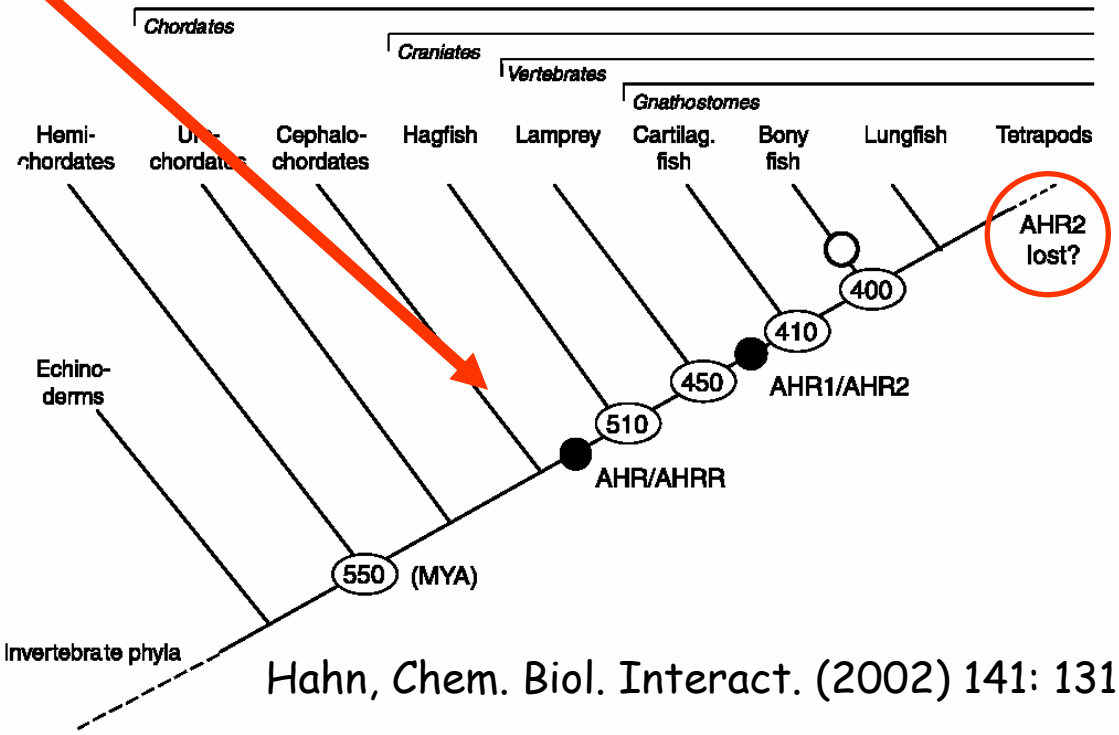
AHR1
AHR2

AHRR

ARNT







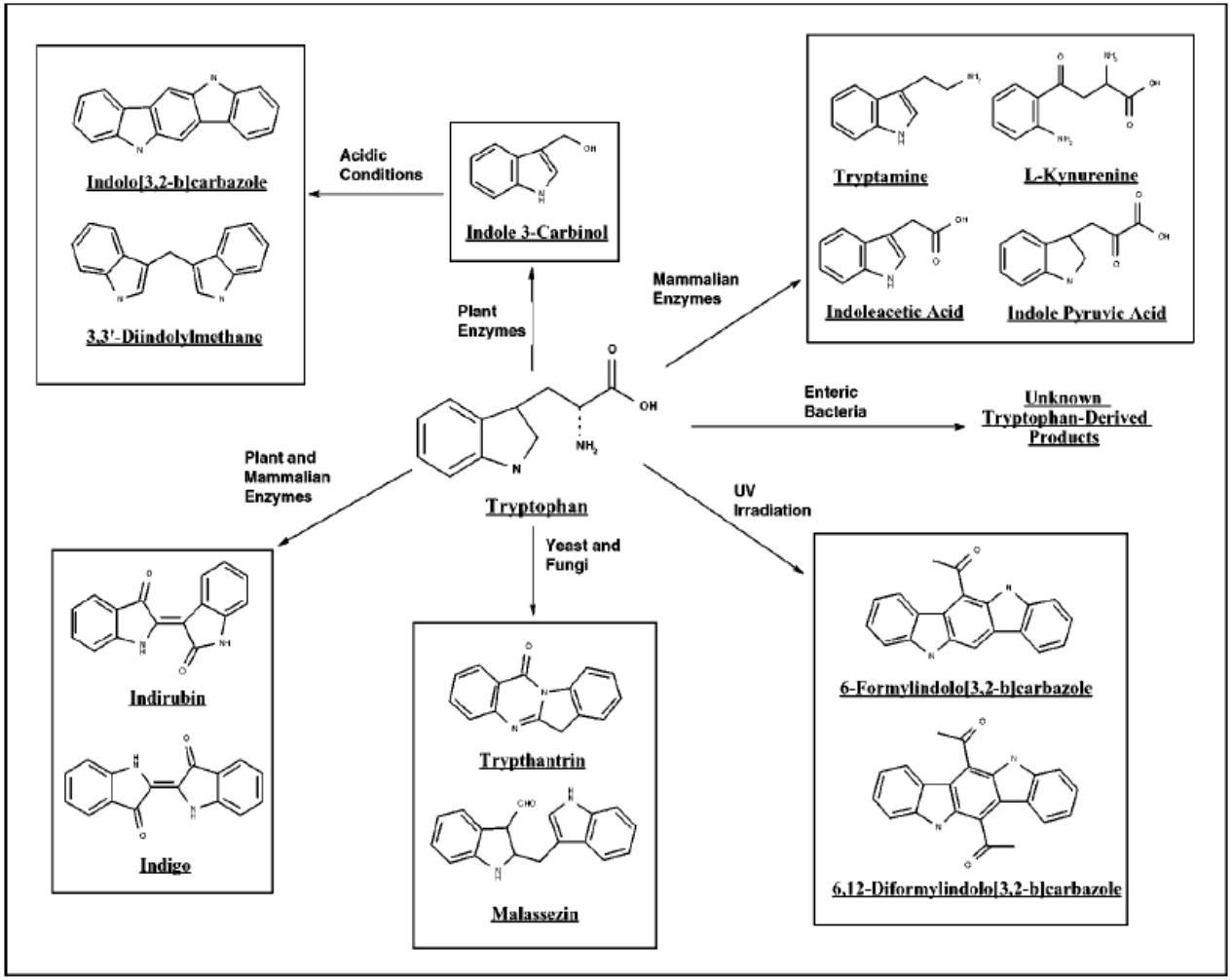
Hahn, Chem. Biol. Interact. (2002) 141: 131

Evolution of AhR:

Organism:	**Name:**	**Ligand-binding:**	**Physiological function:**
Nematodes: *Caenorhabditis elegans*	AHR-1	No	Neuronal development; Behavioral effects.
Insects: *Drosophila melanogaster*	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 rhytms?

Natural ligands of AhR???????????

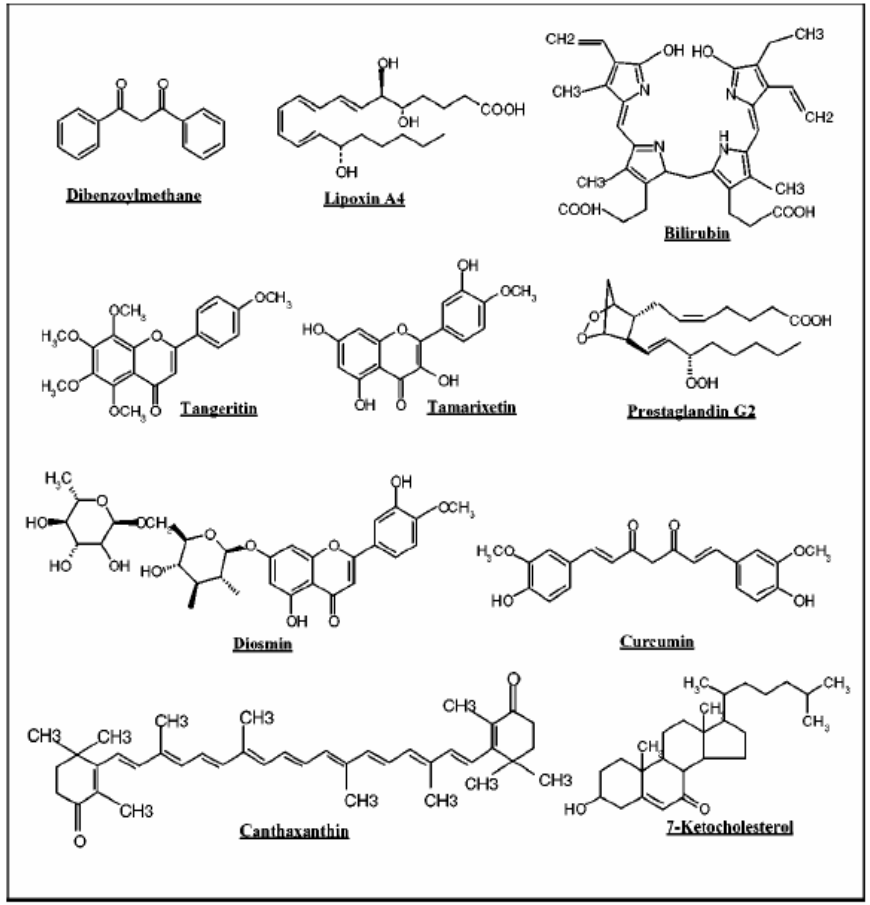
- light hypothesis – tryptophane derivatives



Denison & Nagy, Annu. Rev. Pharmacol. Toxicol. 43:309

Natural ligands of AhR???????????

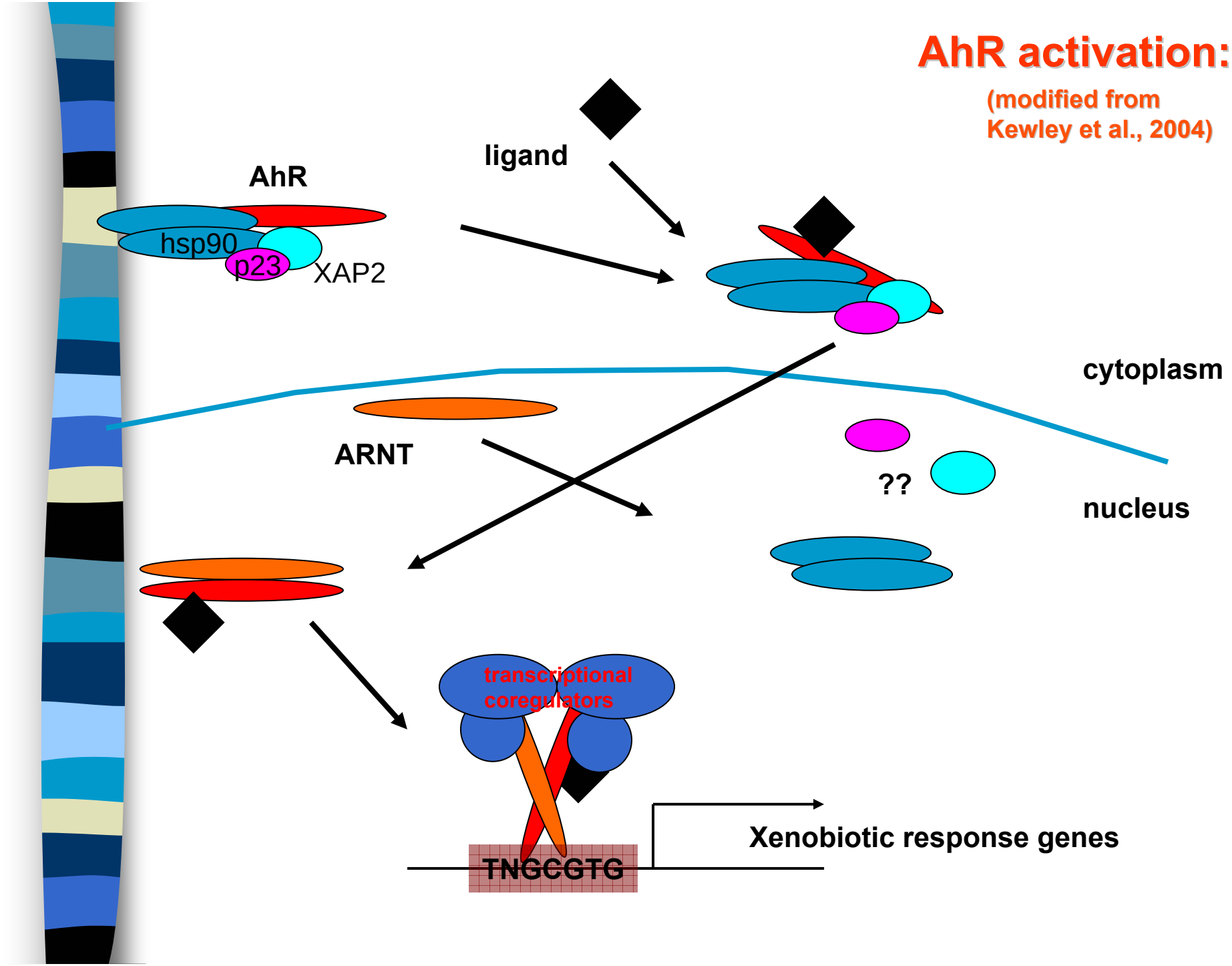
- lipid compounds and flavonoids



Denison & Nagy, Annu. Rev. Pharmacol. Toxicol. 43:309

AhR activation:

(modified from Kewley et al., 2004)



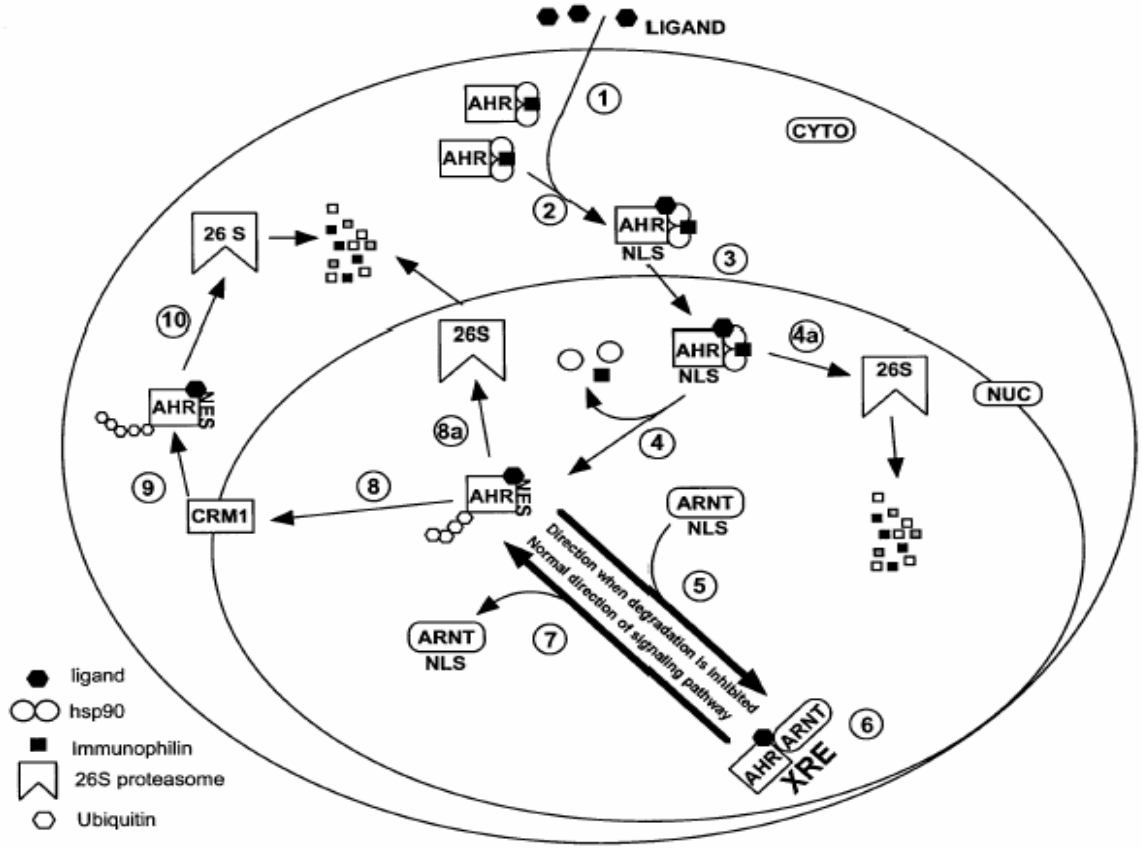
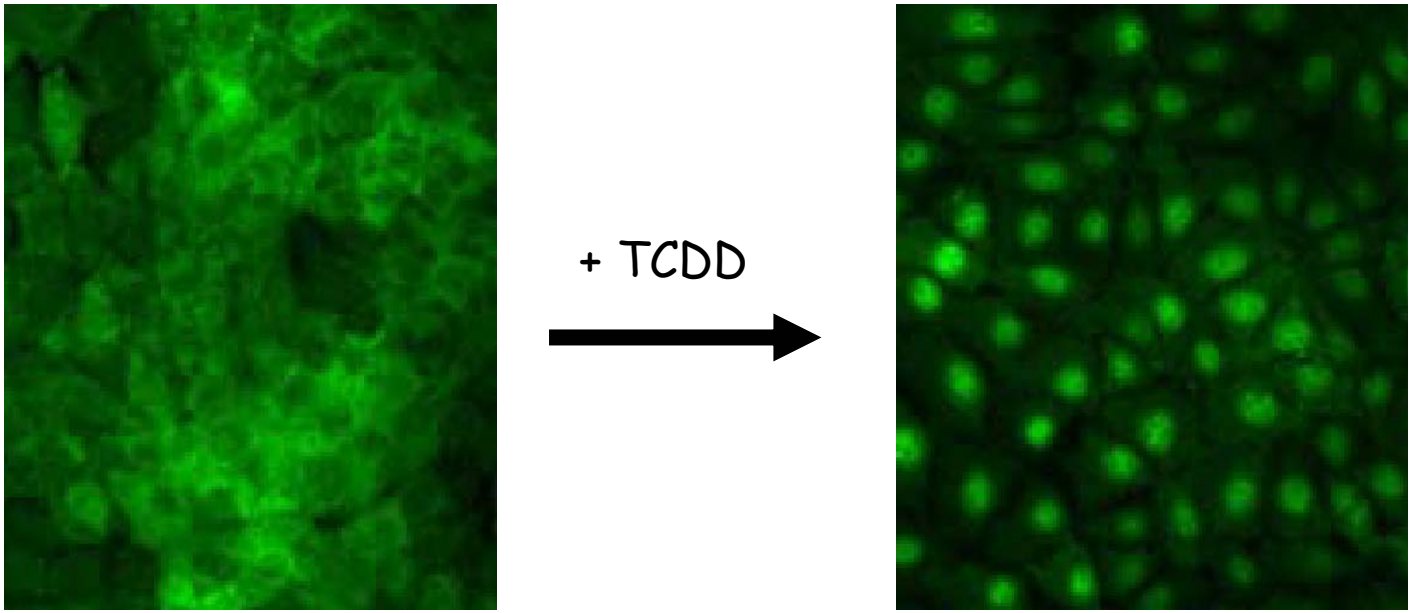
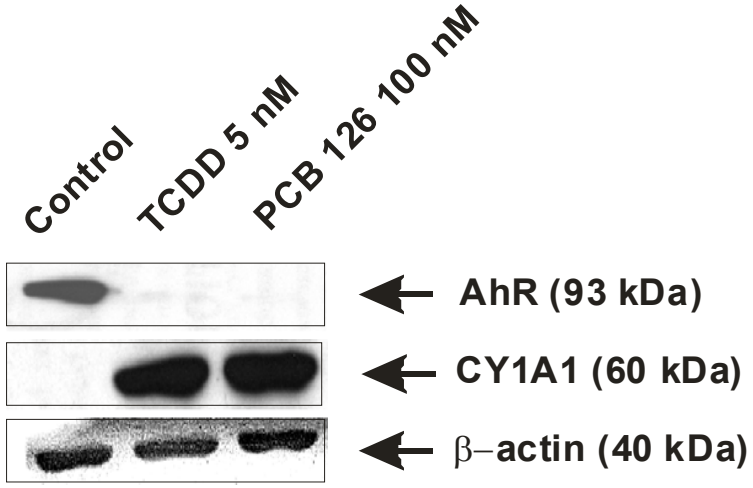


Fig. 1. Model of AHR-mediated signal transduction pathway. (1) Ligand enters cell. (2) Ligand binds to AHR·hsp90·immunophilin complex causing conformational change and exposing the NLS domain. (3) AHR complex is actively imported into the nucleus via NLS and nuclear import receptors. (4a) If receptor 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 ubiquinated in the nucleus and degraded or (8) AHR is exported from nucleus via CRM-1 export receptor. (9) AHR is ubiquinated 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.

Chemico-Biological Interactions 141 (2002) 41–61


+ TCDD

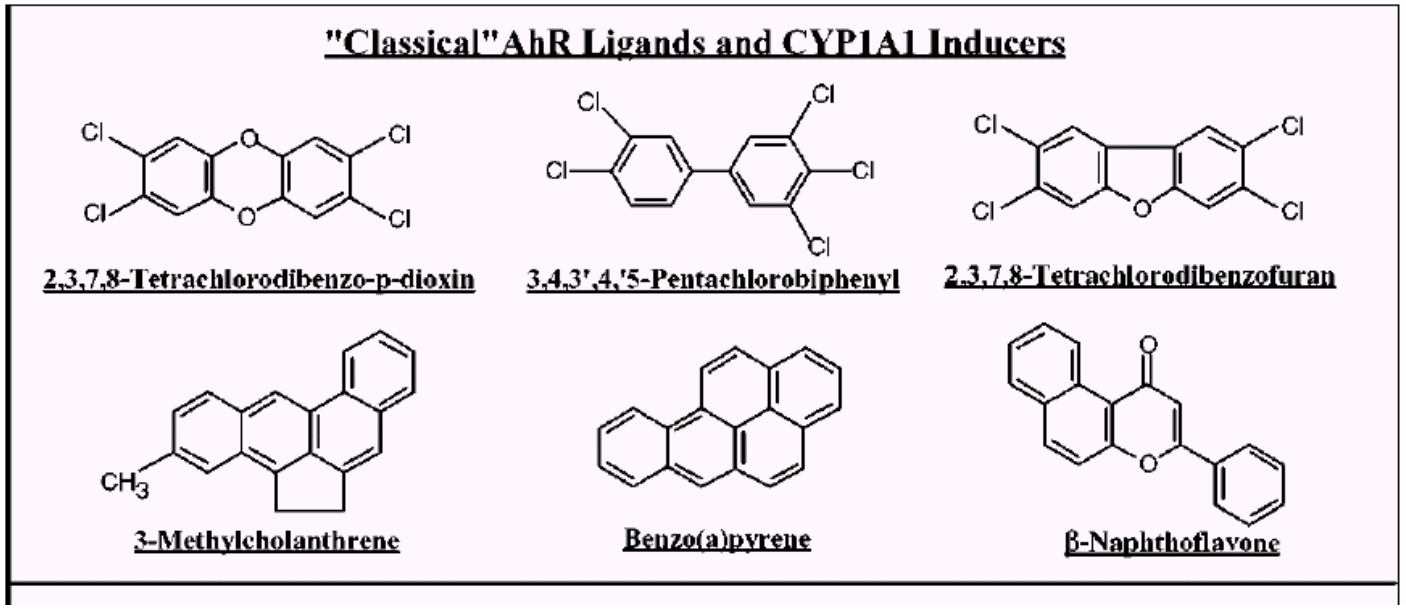

Control
TCDD 5 nM
PCB 126 100 nM
AhR (93 kDa)
CY1A1 (60 kDa)
β–actin (40 kDa)

AhR regulated genes:

contain xenobiotic response elements (XRE) or dioxin responsive elements (DRE) in their promoter region:

- phase I enzymes - *CYP 1A1, CYP 1A2, CYP 1B1;*
- phase II enzymes - *UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase;*
- other genes - *Bax?, $p27^{Kip1}$, JunD, TGF-β* - regulation of cell cycle and apoptosis;
- *AhRR*.

AhR toxicants:



Denison & Nagy, Annu. Rev. Pharmacol. Toxicol. 43:309

Toxic effects of dioxins:

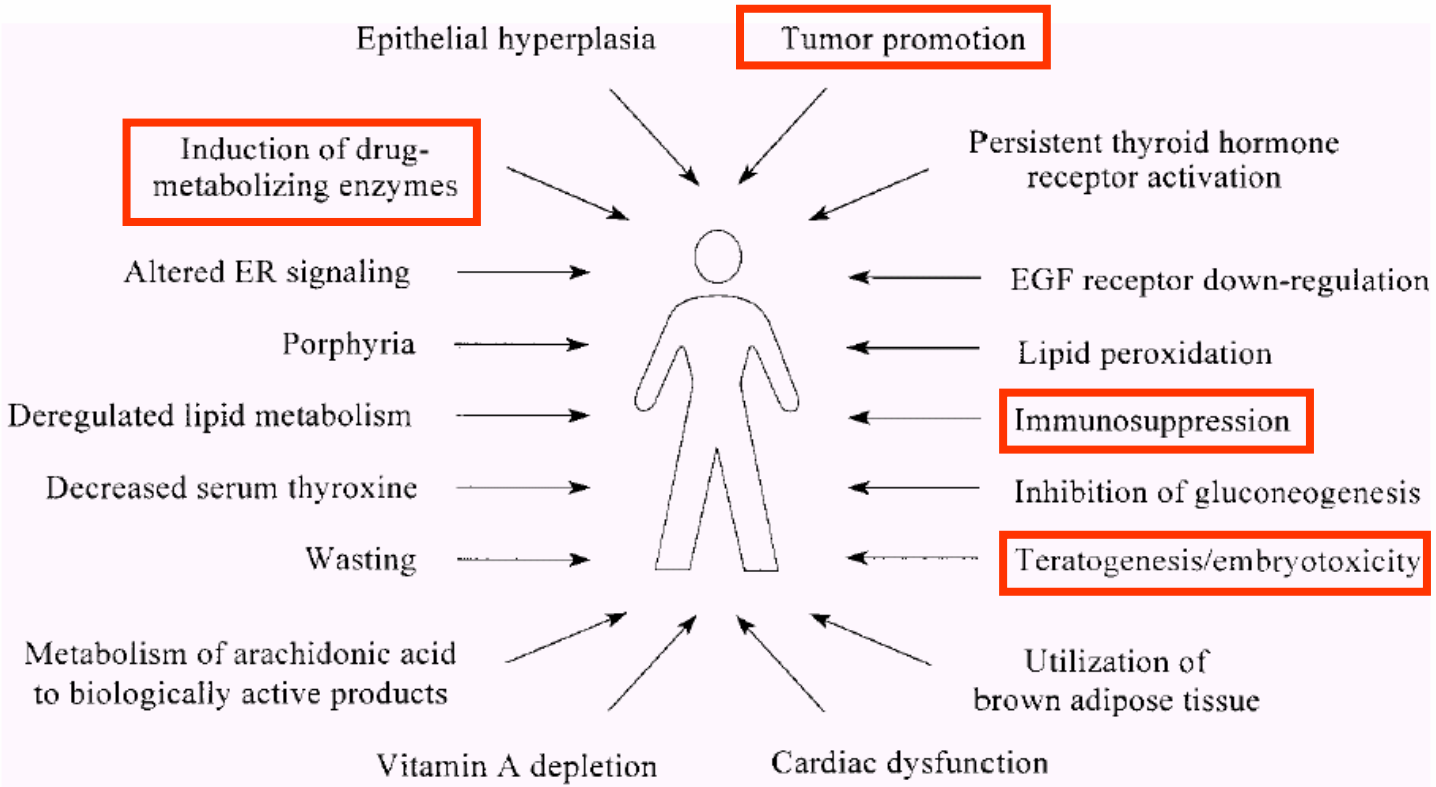
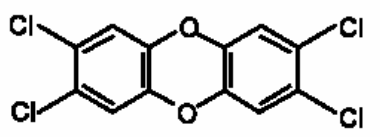


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.

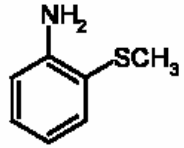
Schmidt & Bradfield, Annu. Rev. Cell Dev. Biol. 12:55

„Non-classical" AhR ligands

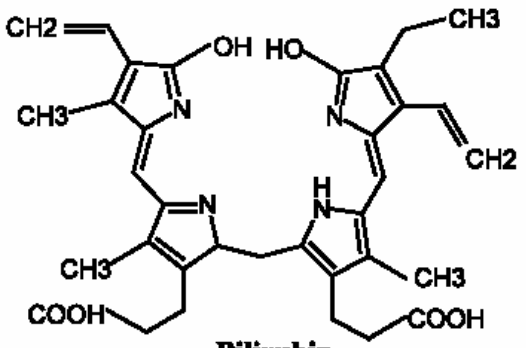
M.S. Denison et al. / Chemico-Biological Interactions 141 (2002) 3–24



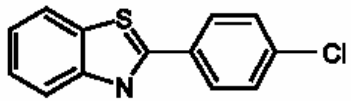
2,3,7,8-Tetrachlorodibenzo-p-dioxin



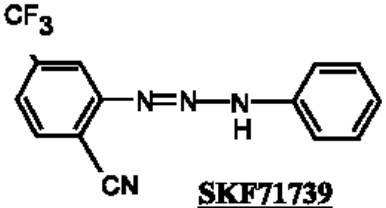
2-(Methylmercapto)aniline



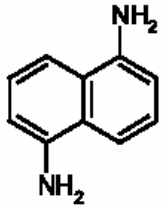
Bilirubin



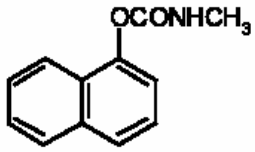
2-(4'-Chlorophenyl)benzothiazole



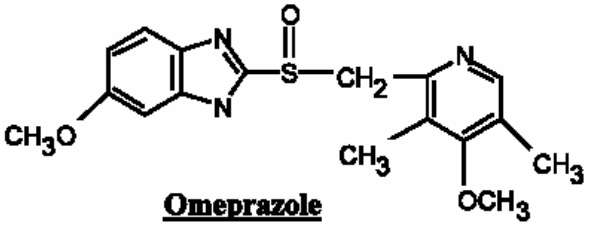
SKF71739



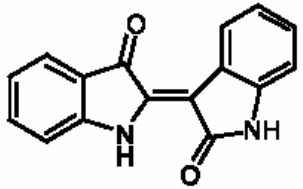
1,5-Diaminonaphthalene



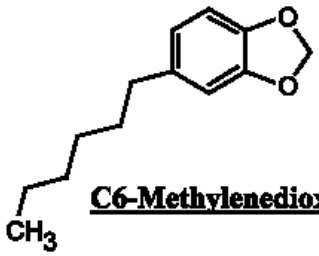
Carbaryl



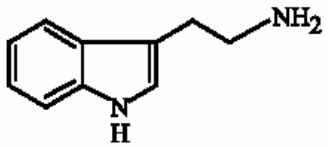
Omeprazole



Indirubin



C6-Methylenedioxybenzene



Tryptamine

Physiological role for AhR - AhR-deficient mice:

- ✓ significant growth retardation;
- ✓ devective development of liver and immune system;
- ✓ retinoid accummulation in liver;
- ✓ abnormal kidney and hepatic vascular structures.

- ✓ resistant to BaP-induced carcinogenesis and TCDD-induced teratogenesis;
- ✓ no inducible expression of CYP 1A1 and 2.

Liver defects:

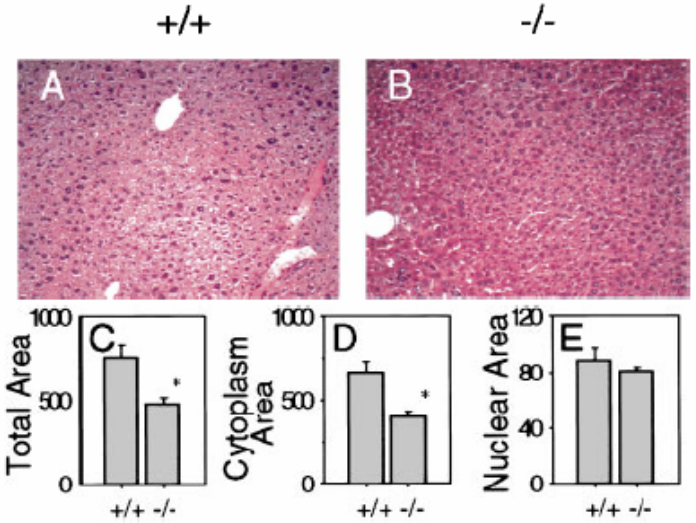
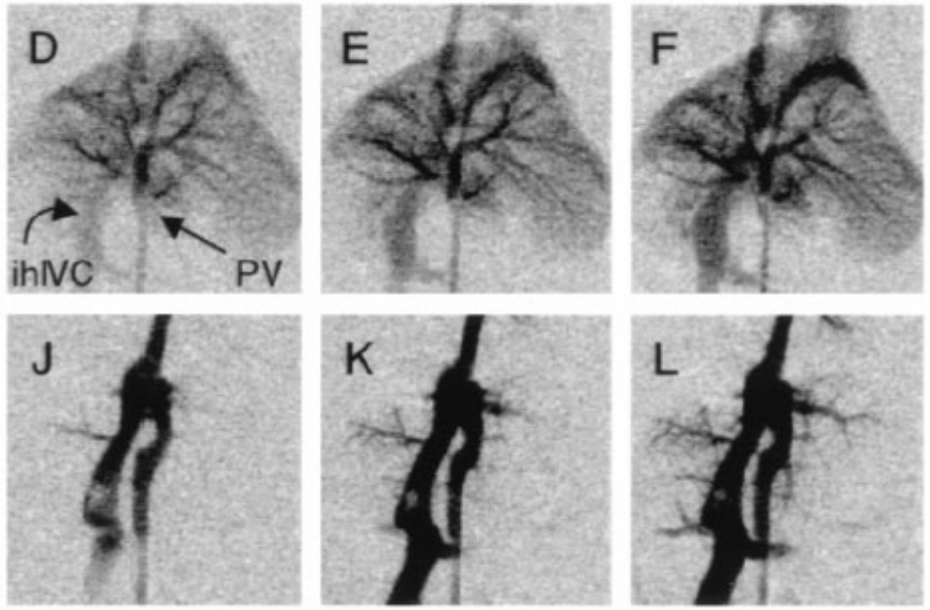


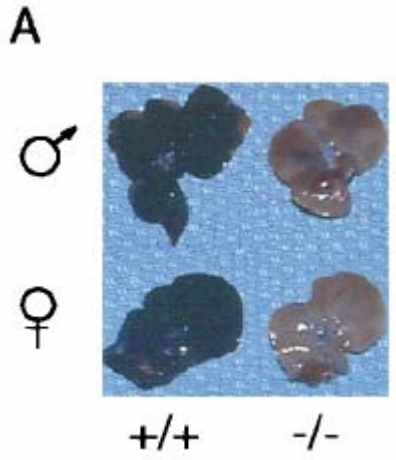
Fig. 1. *Ah* −/− mice have smaller hepatocytes than wild-type mice. Livers of 1-year-old mice were fixed in formalin, and 6-μm sections were examined after staining with hematoxylin/eosin. (*A* and *B*) Thin sections from wild-type (*A*) and age-matched *Ah* knockout (*B*) mice are shown, and results 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 ($P < 0.05$).

PNAS 2000 vol. 97:10447

+/+



-/-



BaP není karcinogenní v AhR KO myších:

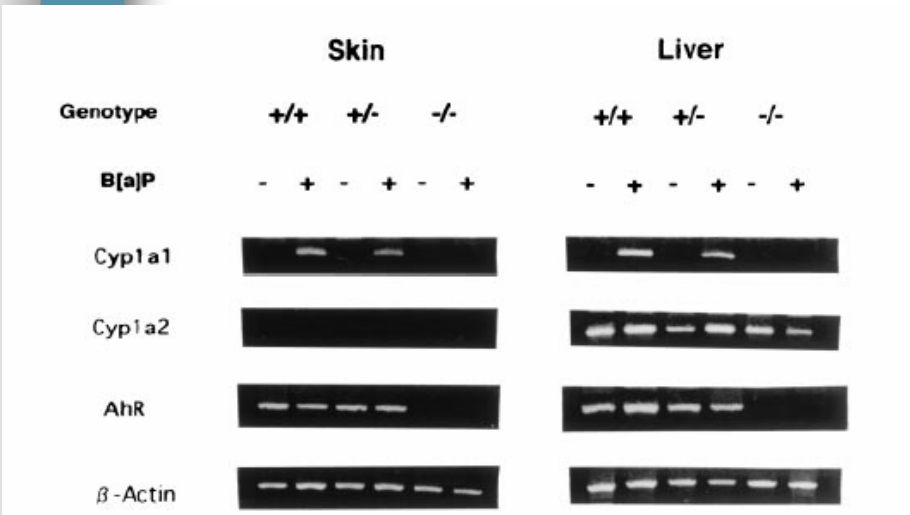


Fig. 1. *Cyp1a1*, *Cyp1a2*, and AhR gene expression in the skin and liver of AhR(+/+), AhR(+/−), and AhR(−/−) mice, with and without B[*a*]P treatment. One-microgram aliquots of RNA extracted from skin and liver of control and B[*a*]P-treated mice of the three genotypes were reverse-transcribed and analyzed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

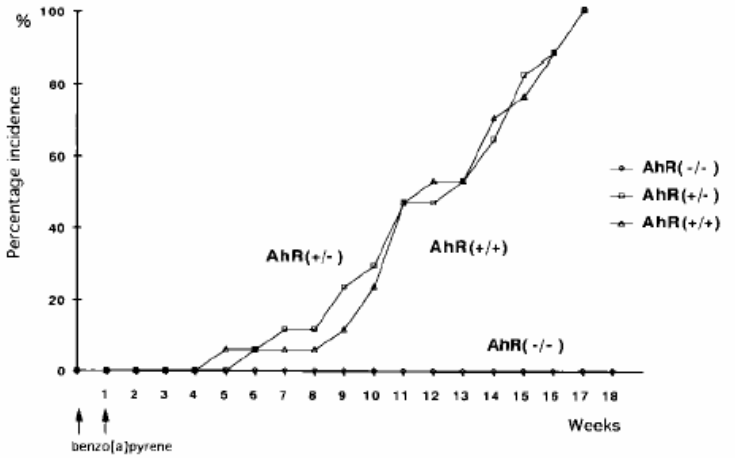


Fig. 2. Subcutaneous tumor induction in wild-type (△) and AhR-deficient male mice (+/−, □; −/−, ○) injected with B[*a*]P.

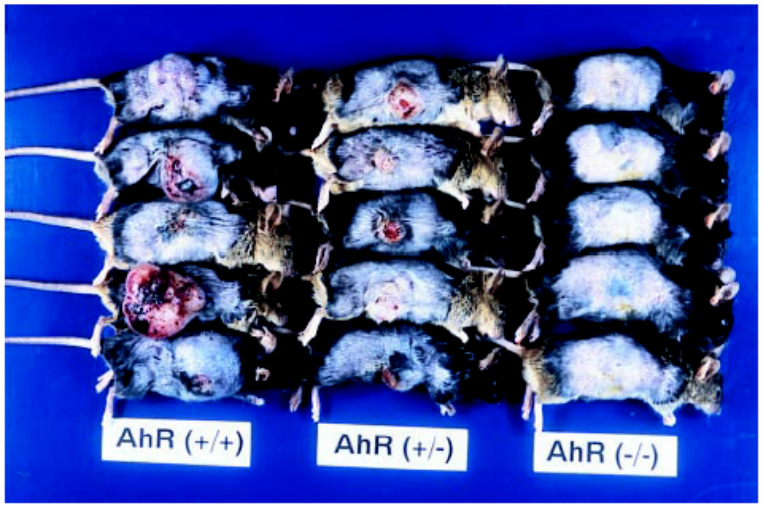
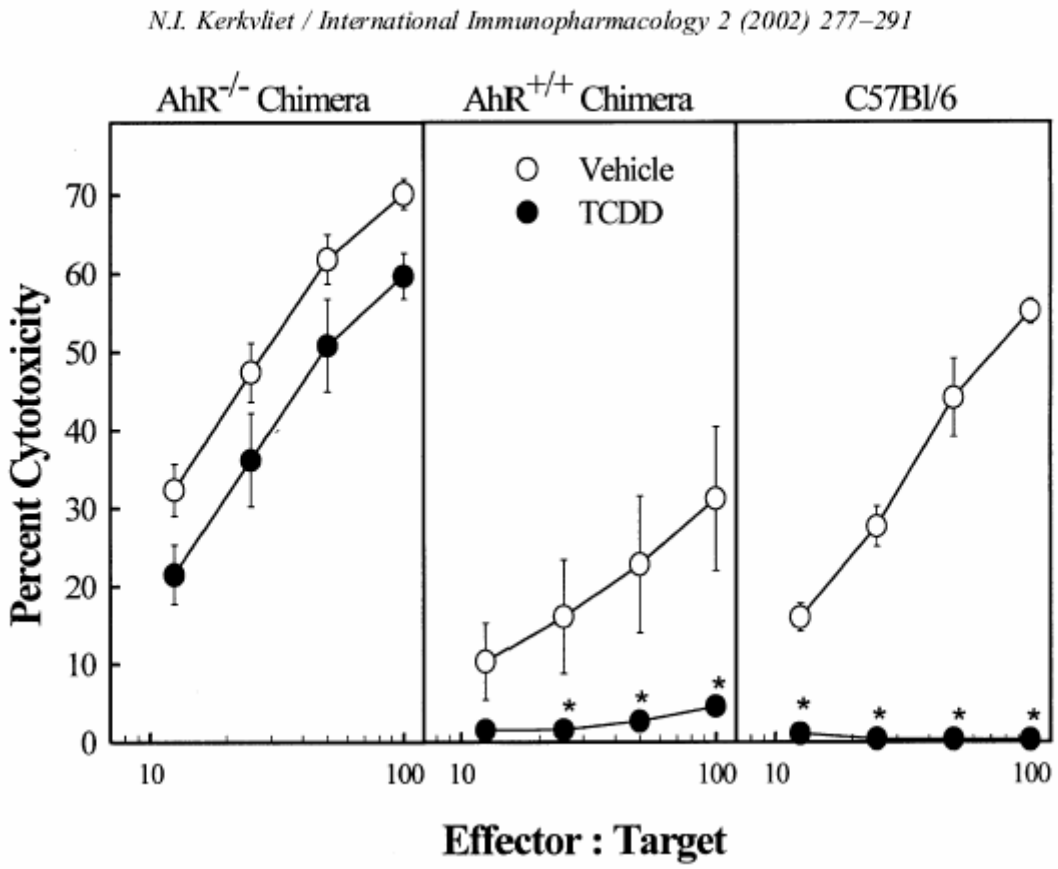


Fig. 3. Gross appearance of flank skins in AhR-wild-type mice (+/+), AhR-heterozygous mice (+/−), and AhR-deficient mice (−/−) injected subcutaneously with B[*a*]P.

PNAS (2000) 97: 779-782

AhR je nezbytný pro imunotoxické účinky TCDD:



CTL response

Interactions of AhR with other proteins

TABLE 1. Interactions Between Signal Transduction Pathways and AhR[a,b]

Interactions	*References*
Direct interactions with AhR	
HSP90	[79]
XAP2	[80–82]
ER, ERRα	[24]
NFκB (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-1α, 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]
NFκB	[40–42]
PKC	[59–66]
tyrosine kinases/phosphatases	[69,72,73]
c-myc, AP-1, CK2	[72]
TGF-β	[7]
p27 (Kip 1)	[43]
NF-1	[27]
C2-ceramide	[47]

J.R. Petrulis, G.H. Perdew / Chemico-Biological Interactions 141 (2002) 25–40

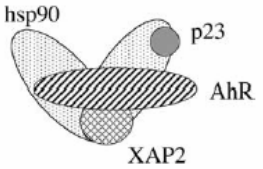


Fig. 4. Model for the arrangement of proteins found in the unliganded AhR complex.

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

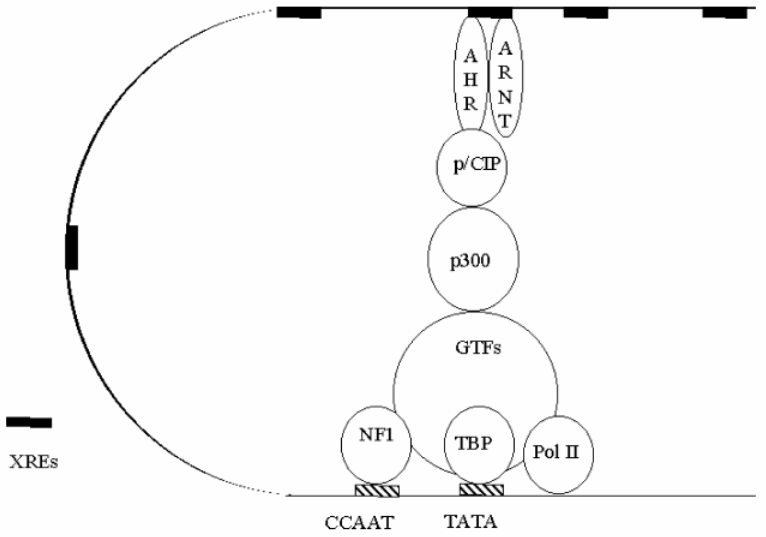


Fig. 3. Hypothetical model of coactivator recruitment at the *Cyp1a1* gene.

J Biochem Mol Toxicol 16:317–325, 2002;

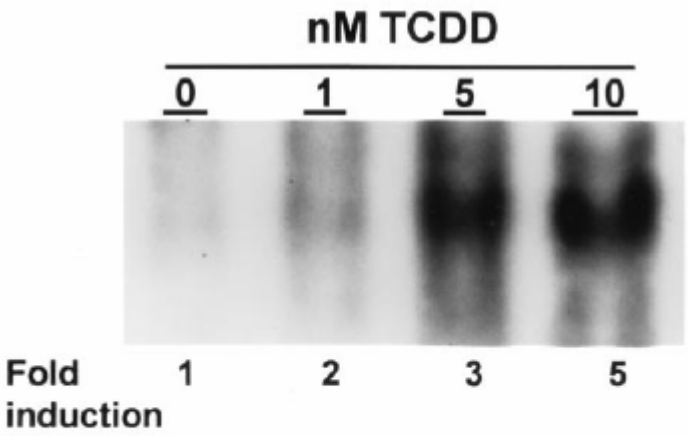
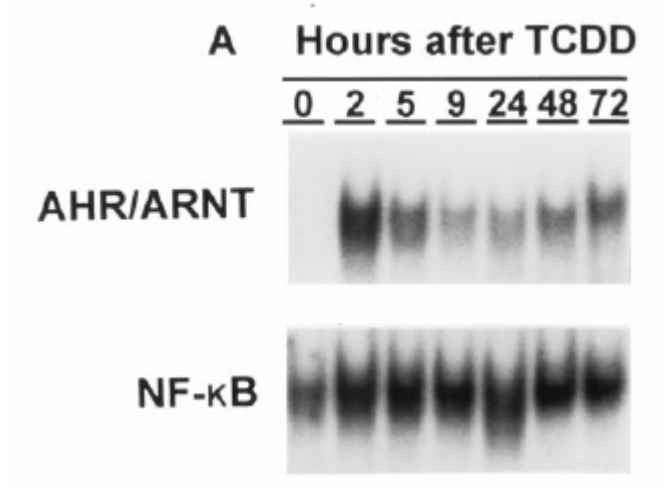
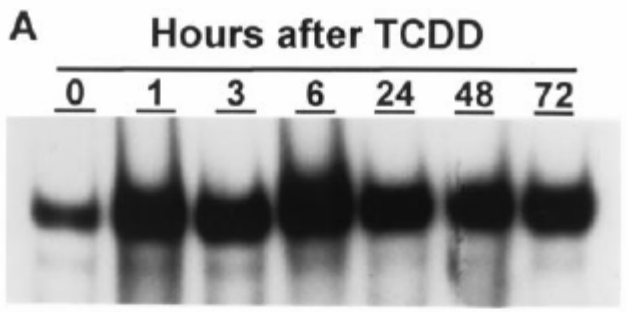
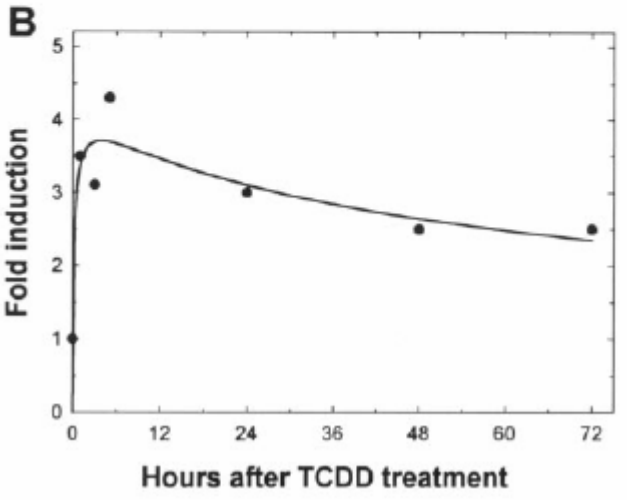


FIG. 2. Induction of c-Jun mRNA by TCDD. Hepa-1 cells were treated for 24 hr with TCDD in 0.05% DMSO at the indicated concentrations. Total RNA was extracted from these cells, fractionated in agarose–formaldehyde gels, and transferred and hybridized to a mouse c-jun probe as described in the Methods Section. Fold induction, determined by densitometry, is indicated below each lane.



AP-1





Biochemical Pharmacology, Vol. 59, pp. 997–1005, 2000.

AhR-ERα crosstalk

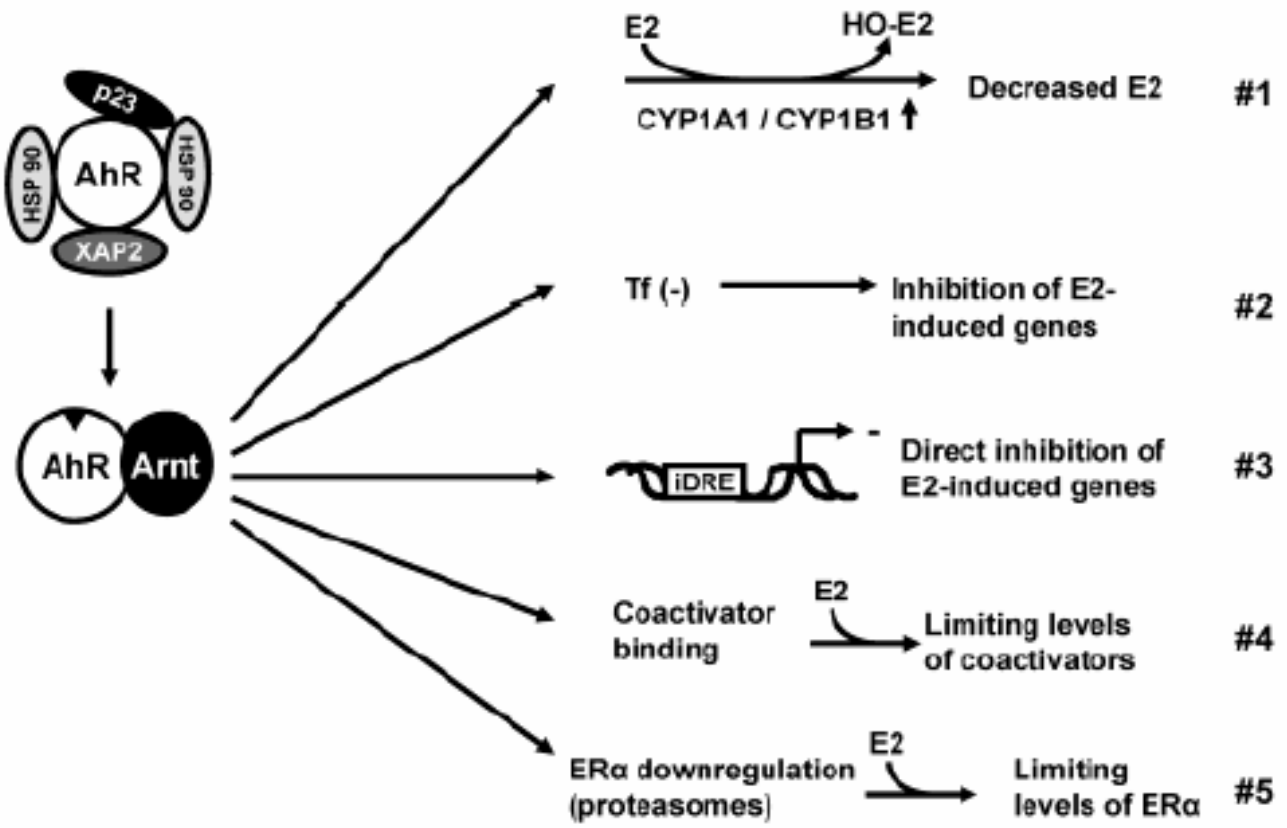


Figure 3. Proposed mechanisms of inhibitory AhR−ERα cross-talk (*123*–*126*).

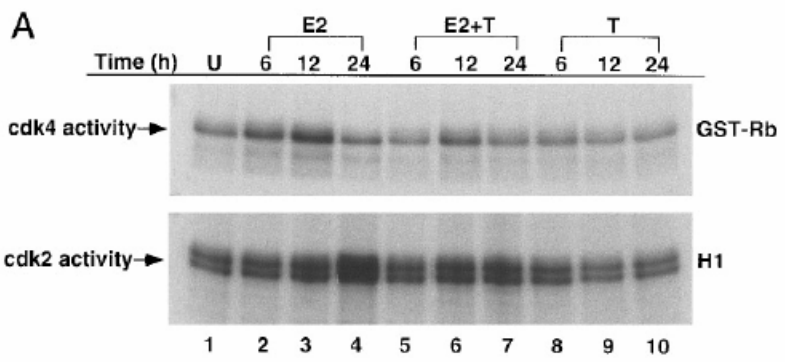
Chem. Res. Toxicol., Vol. 16, No. 7, 2003

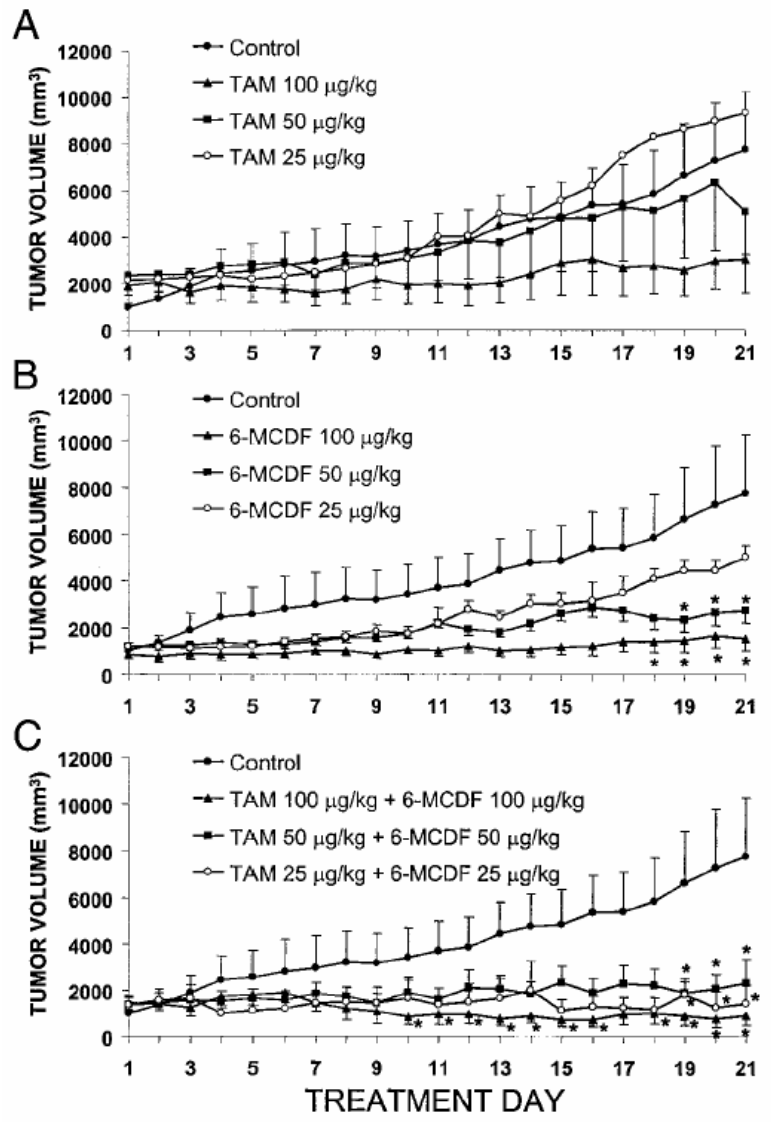
Využitií AhR-ERa crosstalk v nádorové terapii?

TABLE I

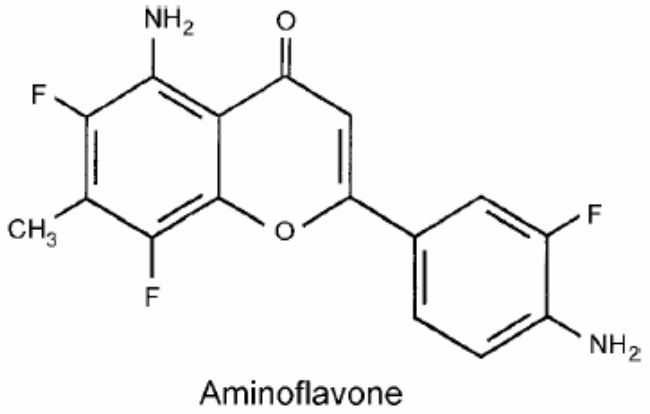
Effects of 17β-Estradiol and TCDD on Cell Cycle Distribution of MCF-7 Human Breast Cancer Cells[a]

Treatment (time, h)	Cell cycle phase (%)		
	G_0/G_1	S	G_2/M
Control	89.9 ± 2.1	4.9 ± 1.6	5.2 ± 0.6
E2 (12)	87.7 ± 2.1	6.0 ± 1.4	4.4 ± 0.7
E2 + TCDD (12)	87.2 ± 0.2	7.9 ± 0.7	4.9 ± 0.5
TCDD (12)	89.1 ± 0.8	6.7 ± 0.8	4.2 ± 0.2
E2 (24)	75.1 ± 0.6[b]	23.4 ± 1.7[b]	1.5 ± 1.2
E2 + TCDD (24)	81.0 ± 1.3[c]	15.8 ± 1.8[d]	3.2 ± 0.7
TCDD (24)	90.8 ± 0.6	5.2 ± 0.5	4.0 ± 0.9

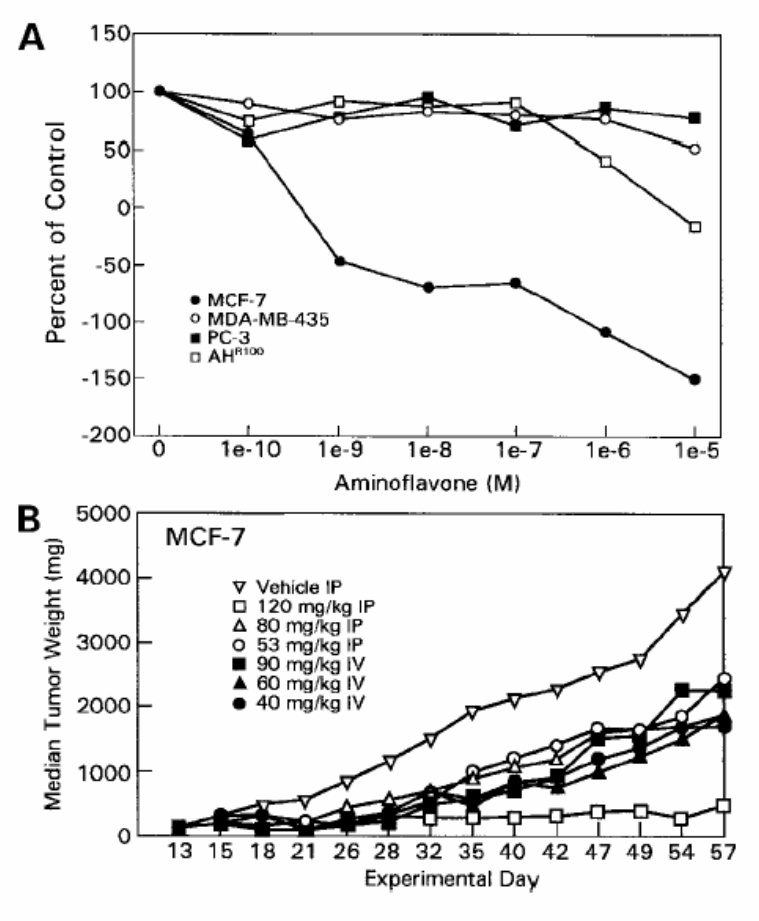




ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 356, 239–248, 1998;
CANCER RESEARCH 61, 3902–3907, 2001

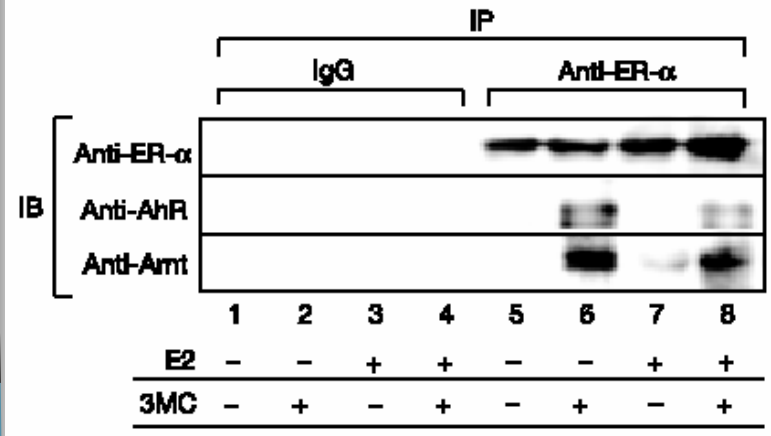


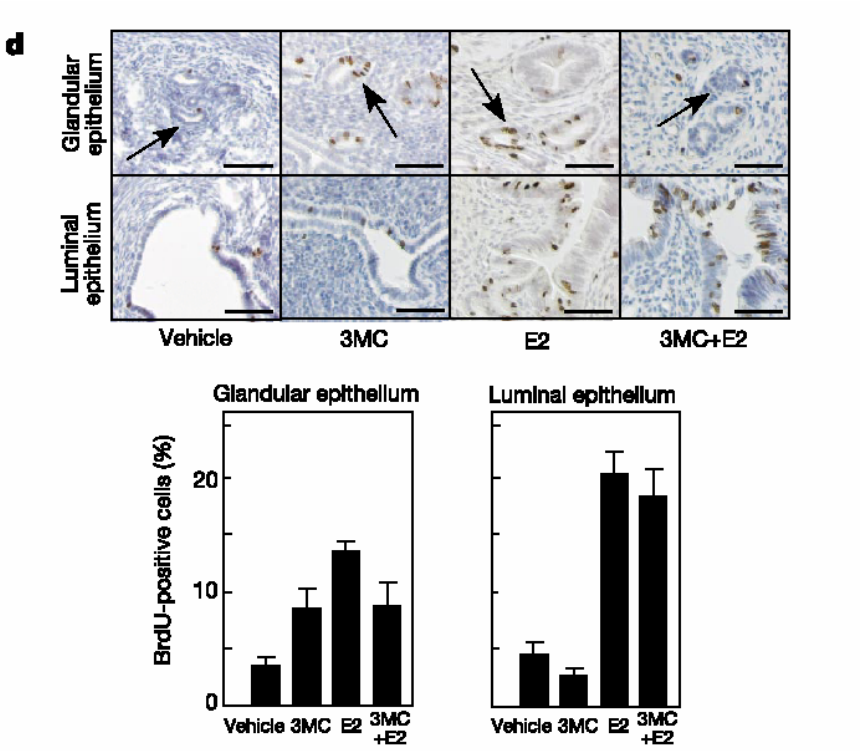
Aminoflavone



Mol Cancer Ther 2004;3(6):715–25

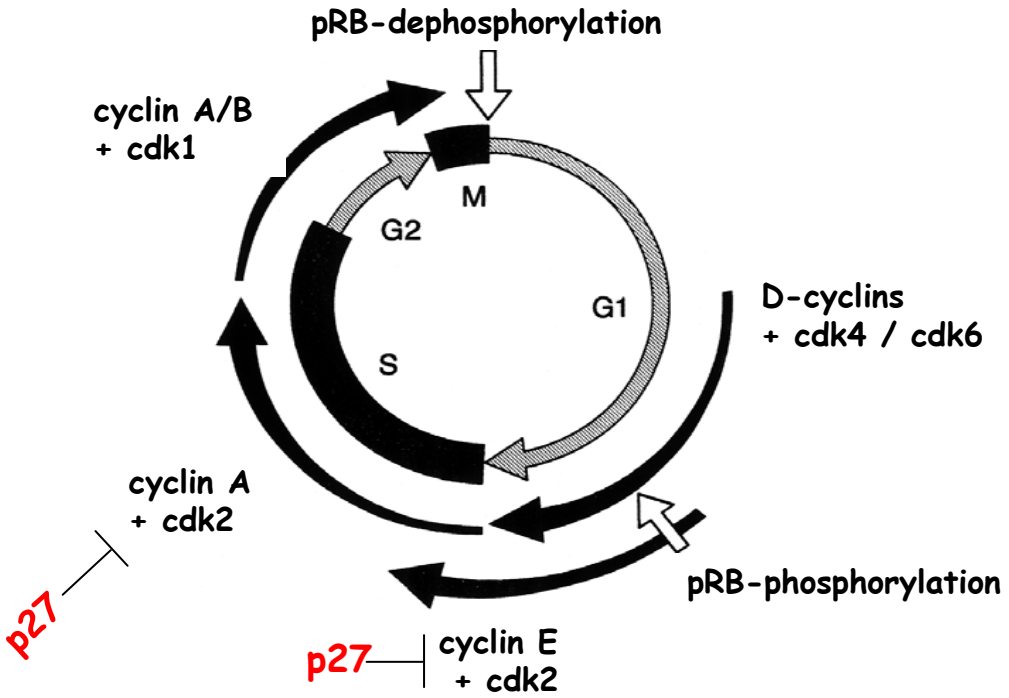
Direct AhR-ER interaction?



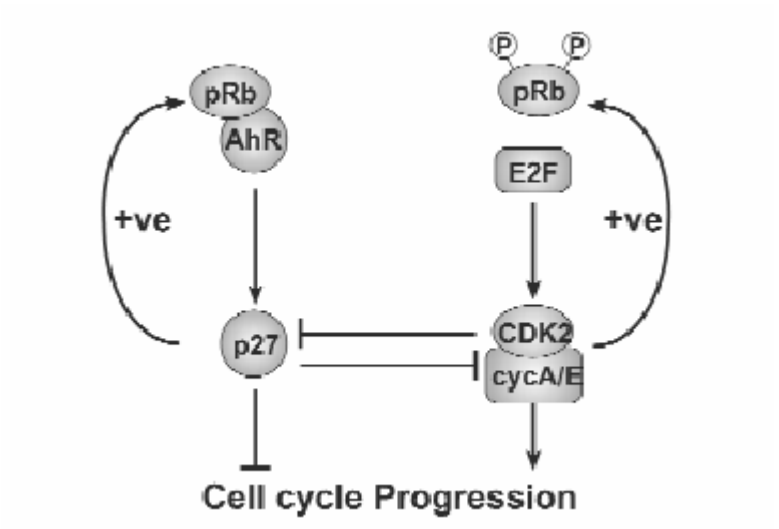


Ohtake et al., 2003, NATURE 423 www.nature.com/nature

Regulation of the eukaryotic cell cycle



pRB = retinoblastoma protein
cdk = cyclin-dependent kinase



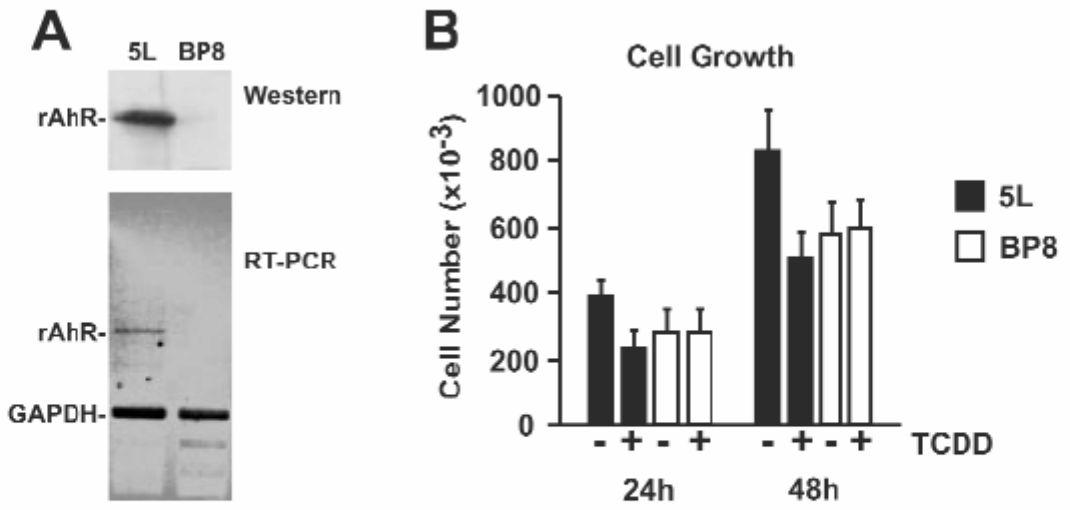
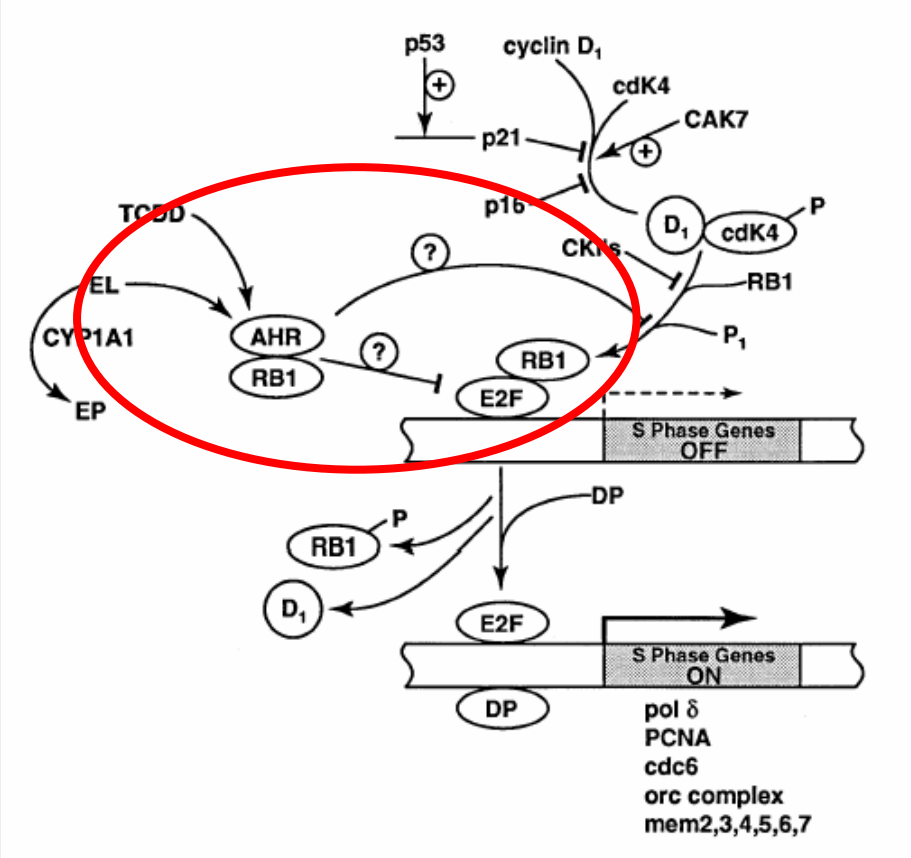


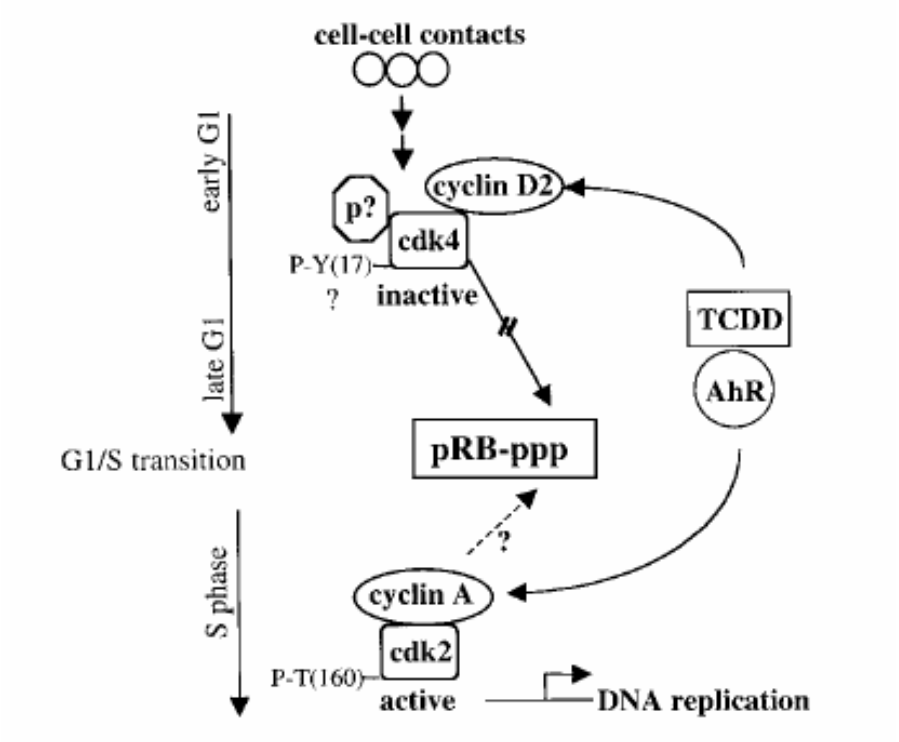
Figure 2.. TCDD induces growth inhibition in rat 5L hepatoma cells.
Panel A, total protein from 5L and BP8 cells was fractionated by SDS-PAGE and probed for AhR protein with an anti-AhR antibody (Western). Analysis of AhR expression was also performed by RT-PCR on total RNA from 5L and BP8 cells using primers specific for rat AhR (rAhR) and GAPDH (as a control for RT-PCR).
Panel B, 5L (solid bars) and BP8 (open bars) cells ($2x10^5$) 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 ± S.D. of three independent experiments.

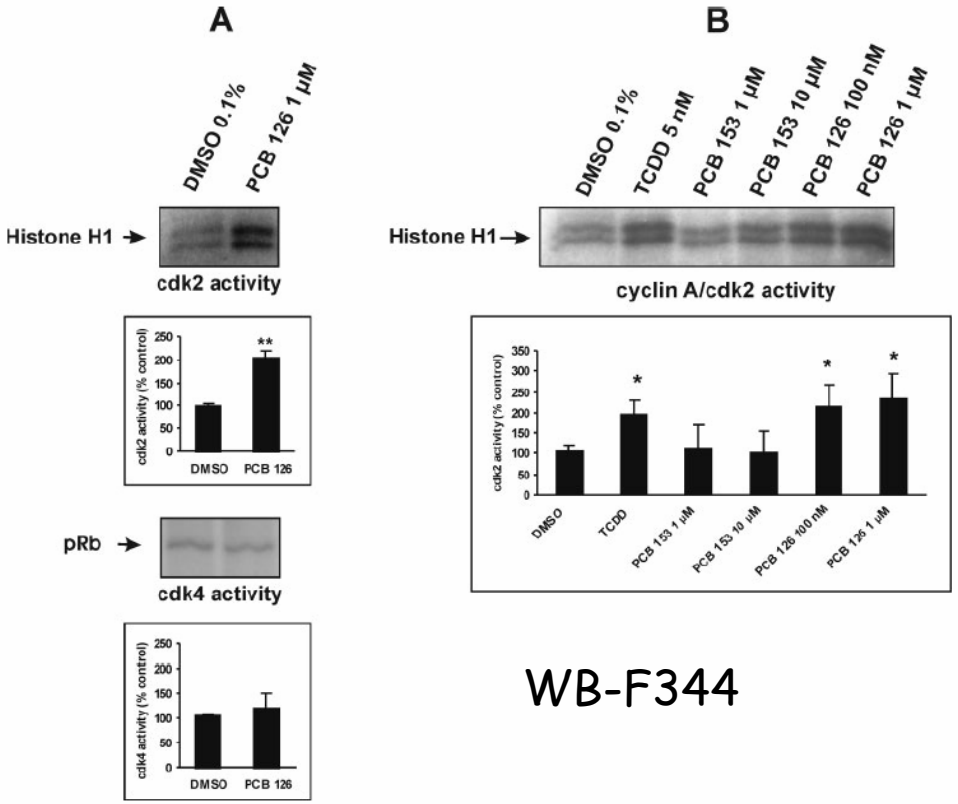
Progress in Cell Cycle Research, Vol. 5, 261-267, (2003)

Puga, Elferink



Dietrich

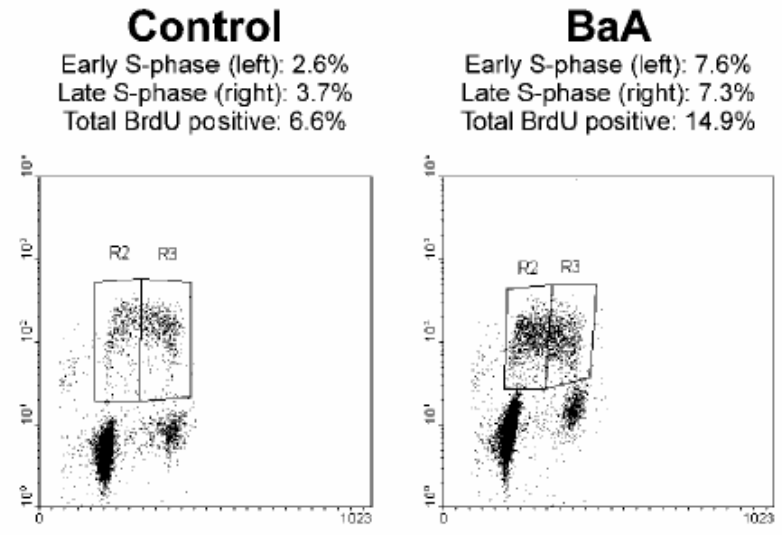




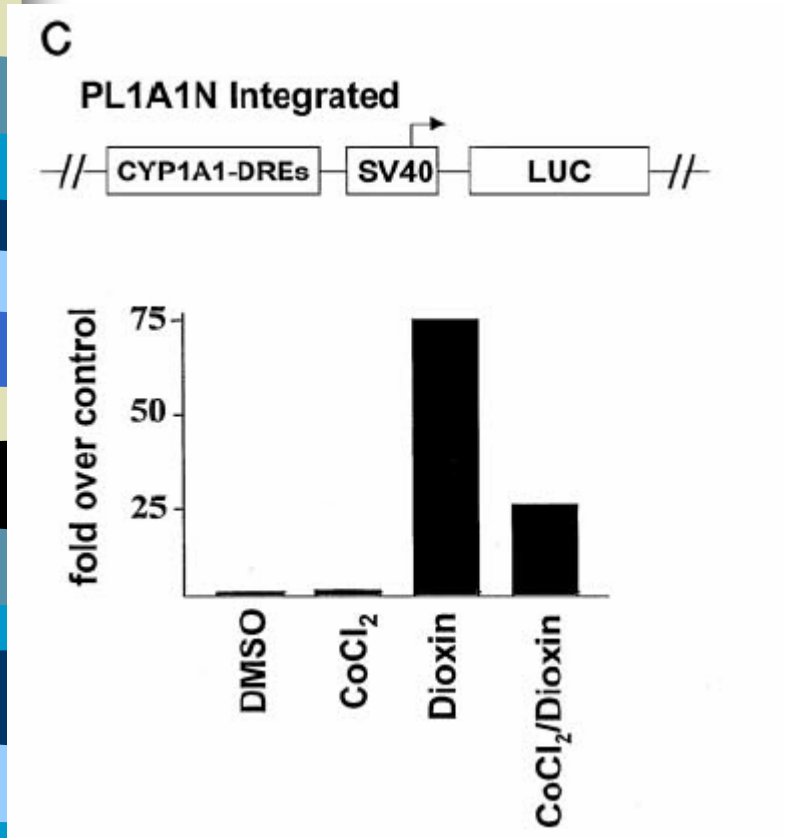
WB-F344

Úloha AhR v regulaci buněčného cyklu je pravděpodobně složitější

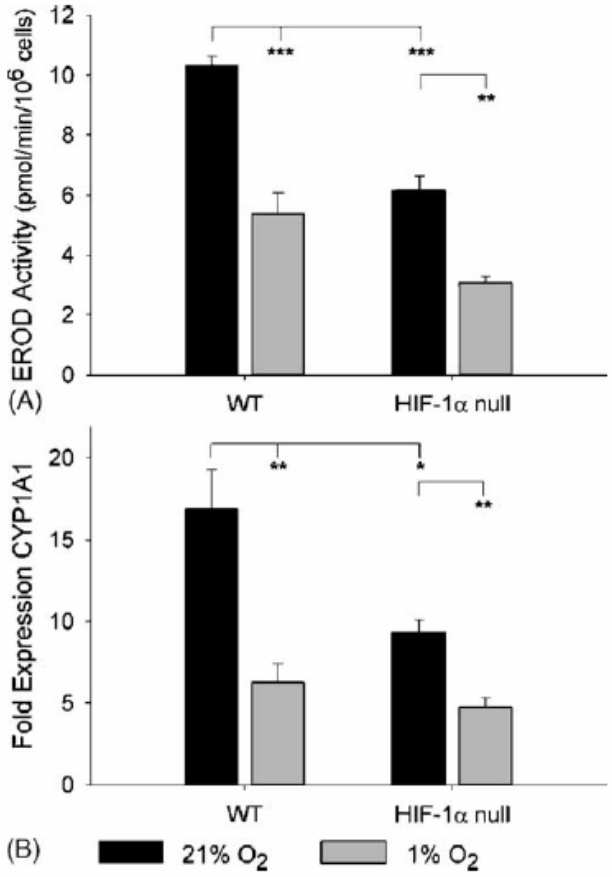
MCF-7



? AhR-HIF-1α crosstalk ?



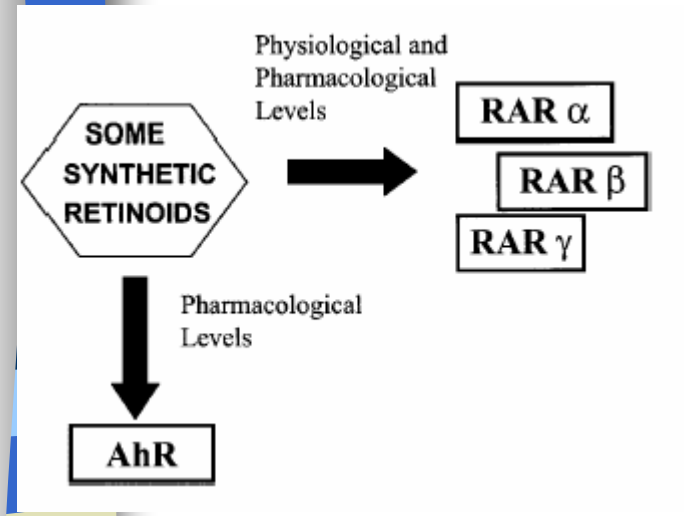
JBC 274, 12115–12123, 1999

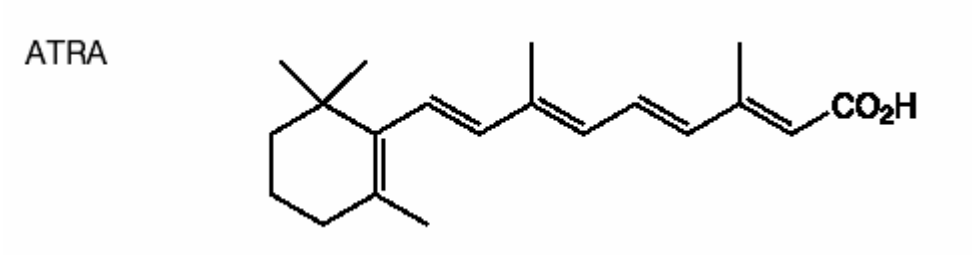


ig. 3. Enzymatic activity (A) and gene expression (B) of CYP1A1. Rate of conversion of ethoxyresorufin. (A) was assayed in WT and HIF-1α null cultures under normoxia (21% O_2, black bars) or hypoxia (1% O_2, grey bars) with 5 μM 3-MC for 24 h. CYP1A1 mRNA levels (B) were measured by real time PCR after 8 h of normoxia black) or hypoxia (grey) with 5 μM 3-MC and normalized to untreated, normoxic controls. Values are the mean and standard error for $n=3$: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Toxicology Letters 155 (2005) 151–159

AhR-retinoid receptors crosstalk





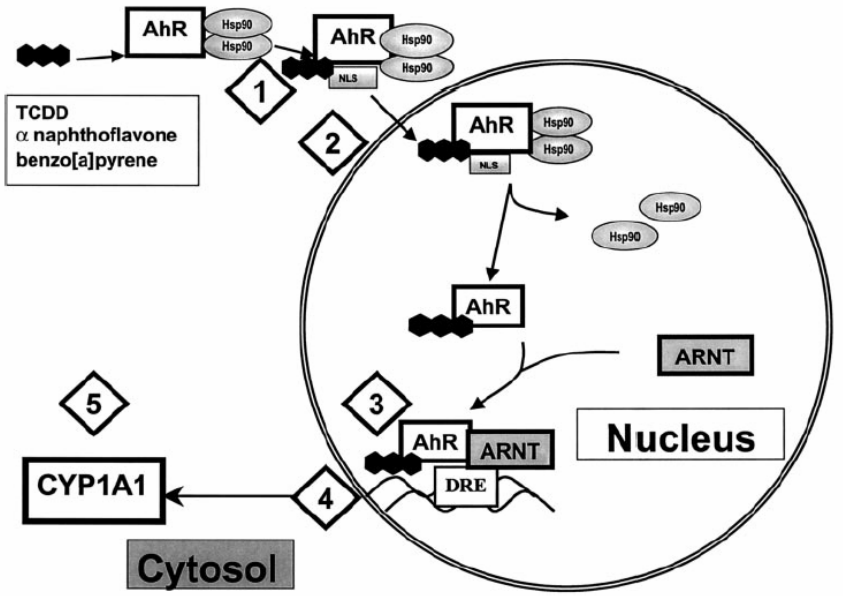


FIGURE 2 Schematic representation of the AhR/Arnt signaling pathway indicating the five steps (see text for descriptions) that have been shown to be modulated by specific retinoids.

J. Nutr. 133: 277S–281S, 2003.

TABLE 2
Effects of Ah Receptor Ligands on Enzyme Activities Involved in Retinoid Metabolism[1]

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).

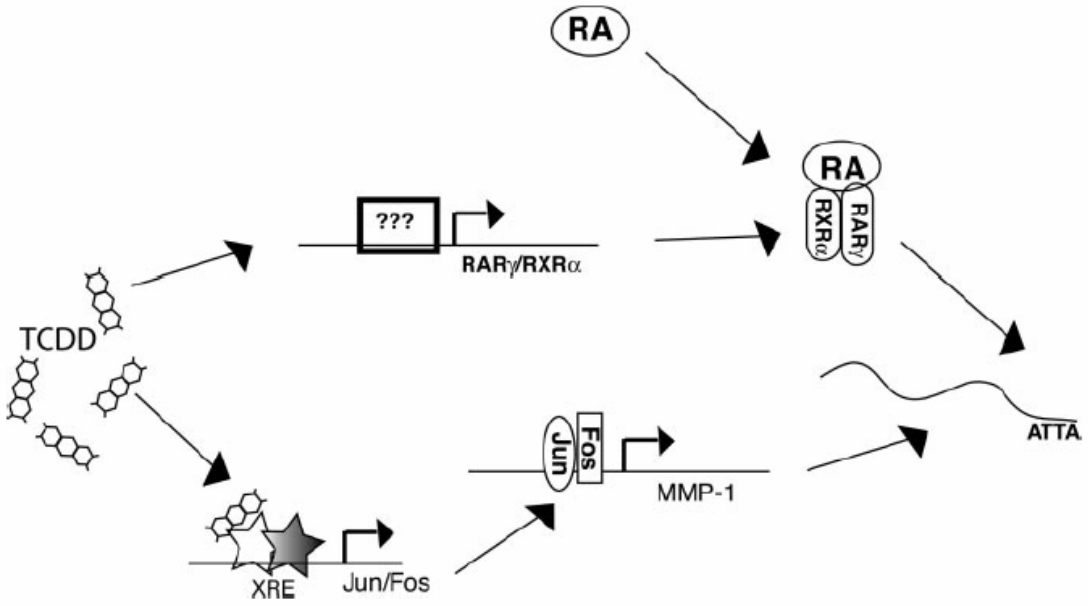


FIG. 9. **Schematic depiction of the activation of MMP-1 mRNA levels by TCDD and atRA in NHKs.** The data presented in this report suggest that TCDD is having an impact on MMP-1 expression in NHKs through at least two mechanisms: 1) by inducing the binding of Fos and Jun proteins to the AP-1 elements in its promoter and thereby activating transcription; and 2) by altering the expression of RARγ and RXRα expression, which leads to an enhancement of MMP-1 mRNA stability following exposure to atRA.

JBC (2004) 279(24):25284-93.