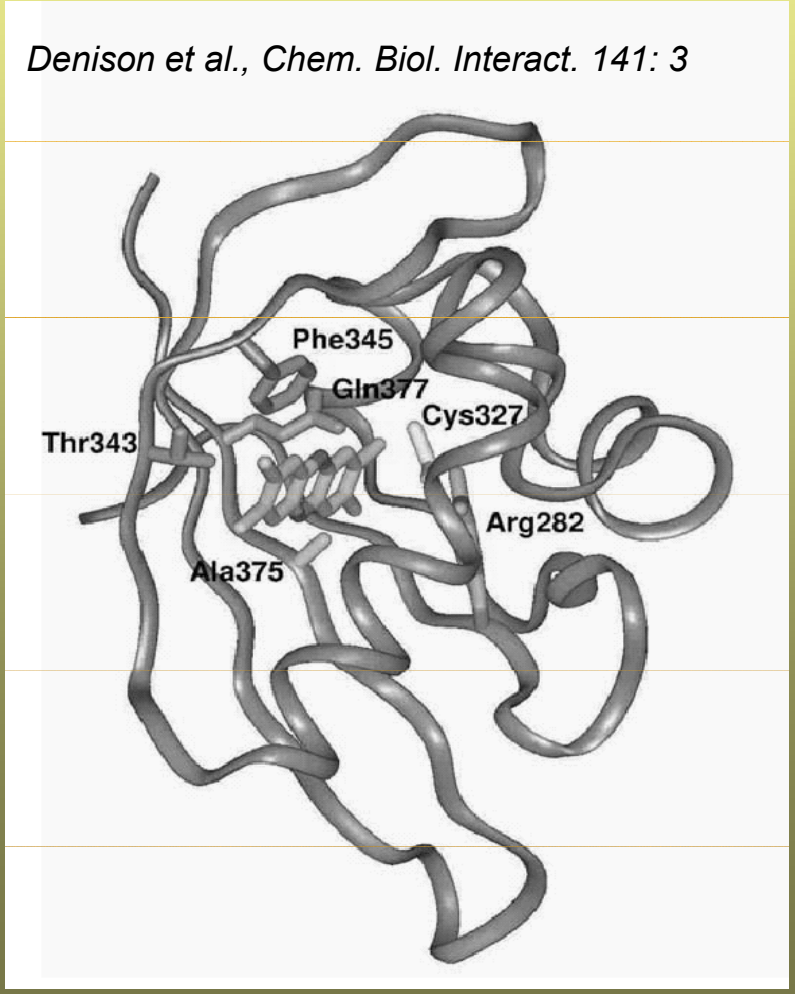
# Aryl hydrocarbon receptor and dioxin-like toxicity
# Dr. Jan Vondráček (IBP.CZ)

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# 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;
- dioxin/like toxicity and TEF/TEQ concept;
- biomarkers of AhR activation and methods of detection of AhR-mediated activity.

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# PAS proteins:

R.J. Kewley et al. / The International Journal of Biochemistry & Cell Biology 36 (2004) 189–204

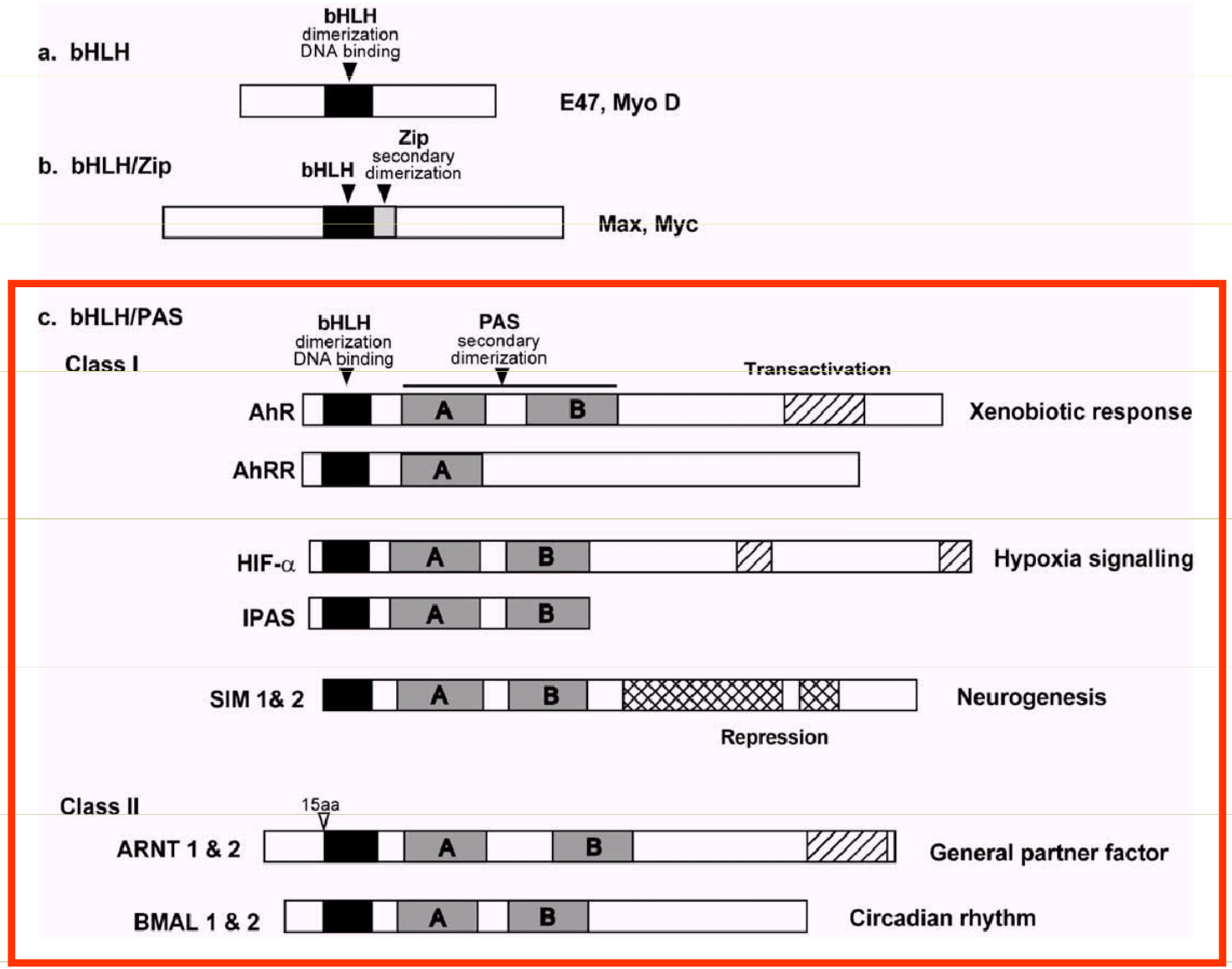


Fig. 1. Schematic representation of the domain structure of some bHLH transcription factor family members.

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# AhR =

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

# AhR domain structure:

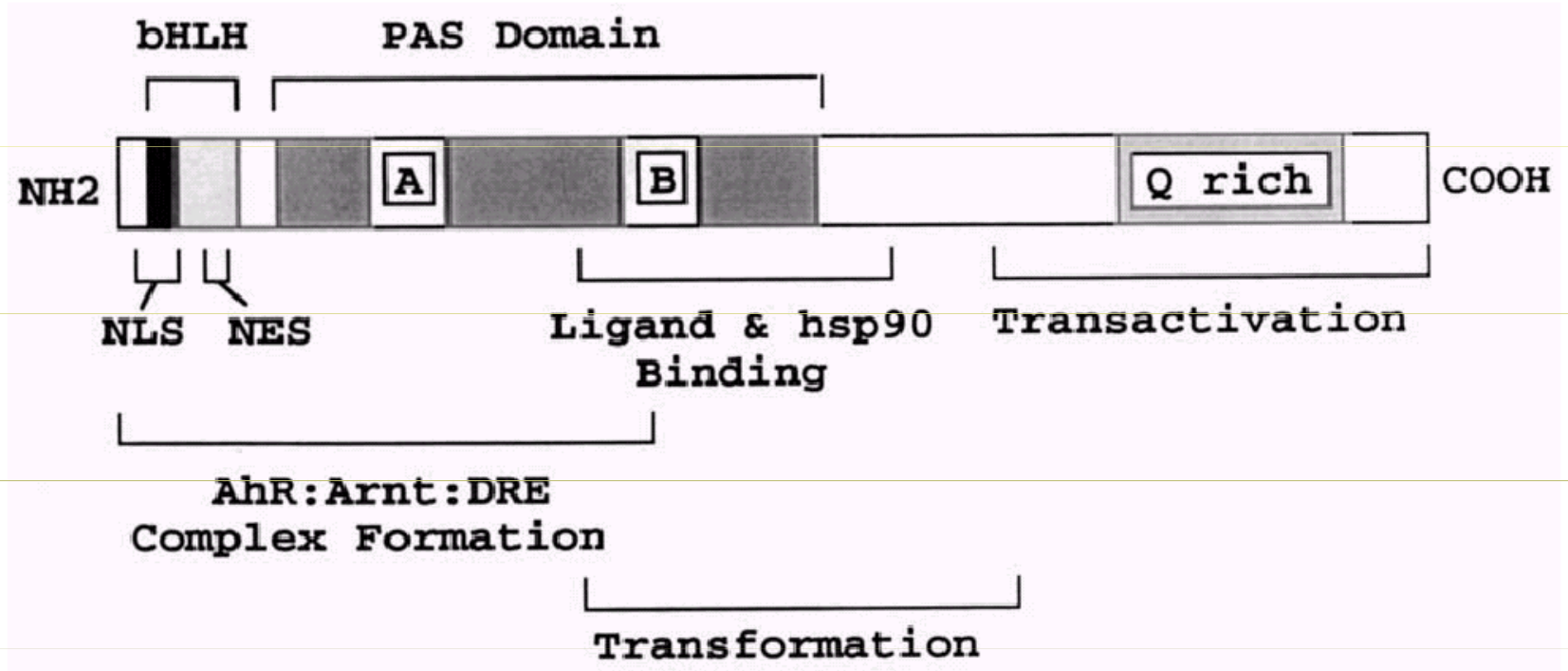


Fig. 2. Domain structure of the AhR.

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

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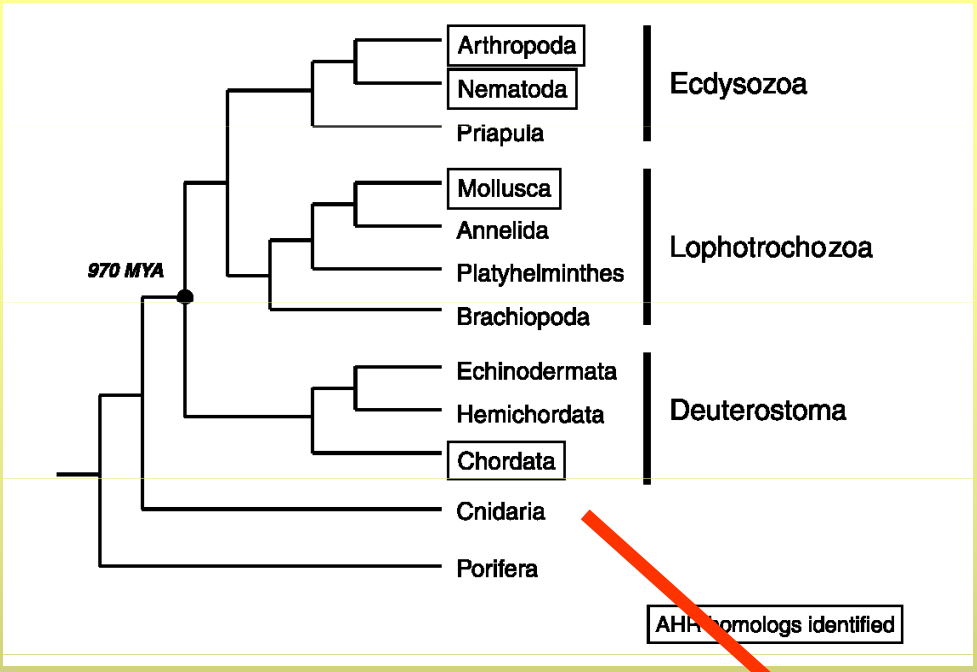
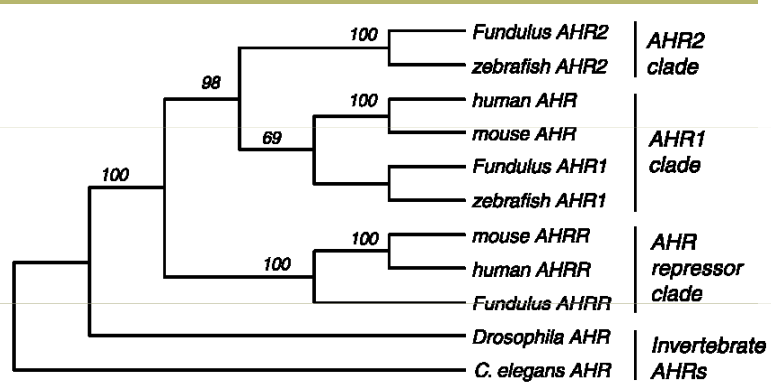
# Evolution of AhR:

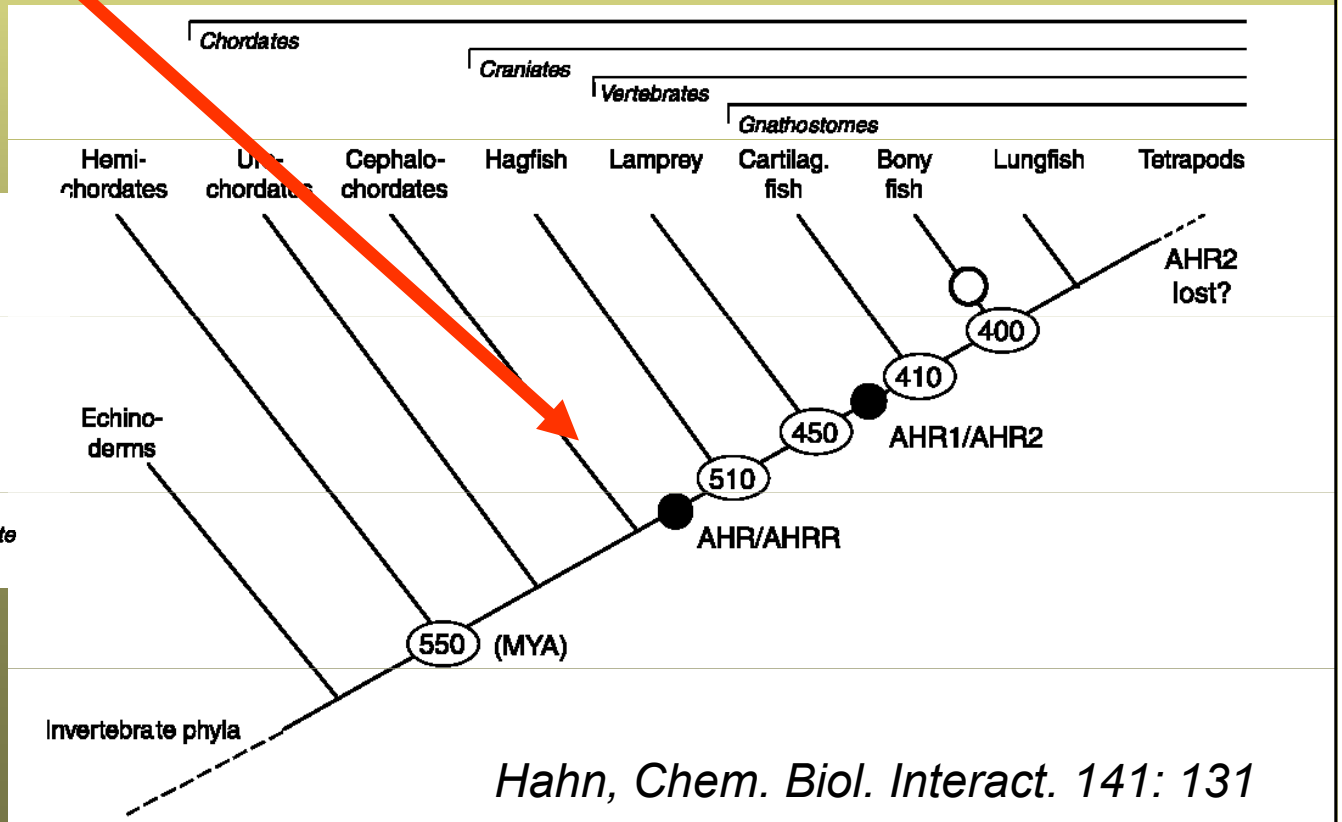
**AHR1**
**AHR2**

**AHRR**

**ARNT**




*Hahn, Chem. Biol. Interact. 141: 131*

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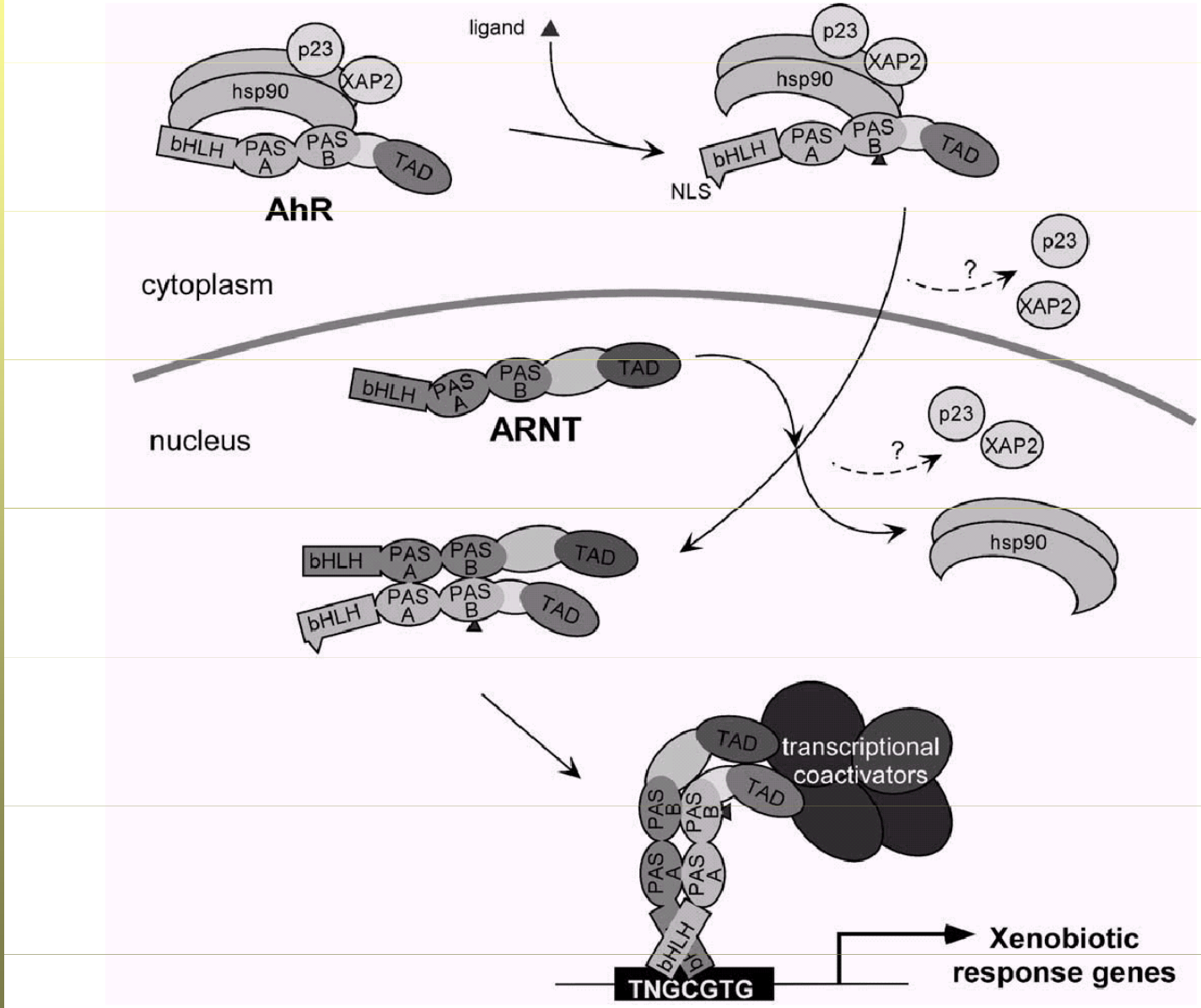
# AhR activation:





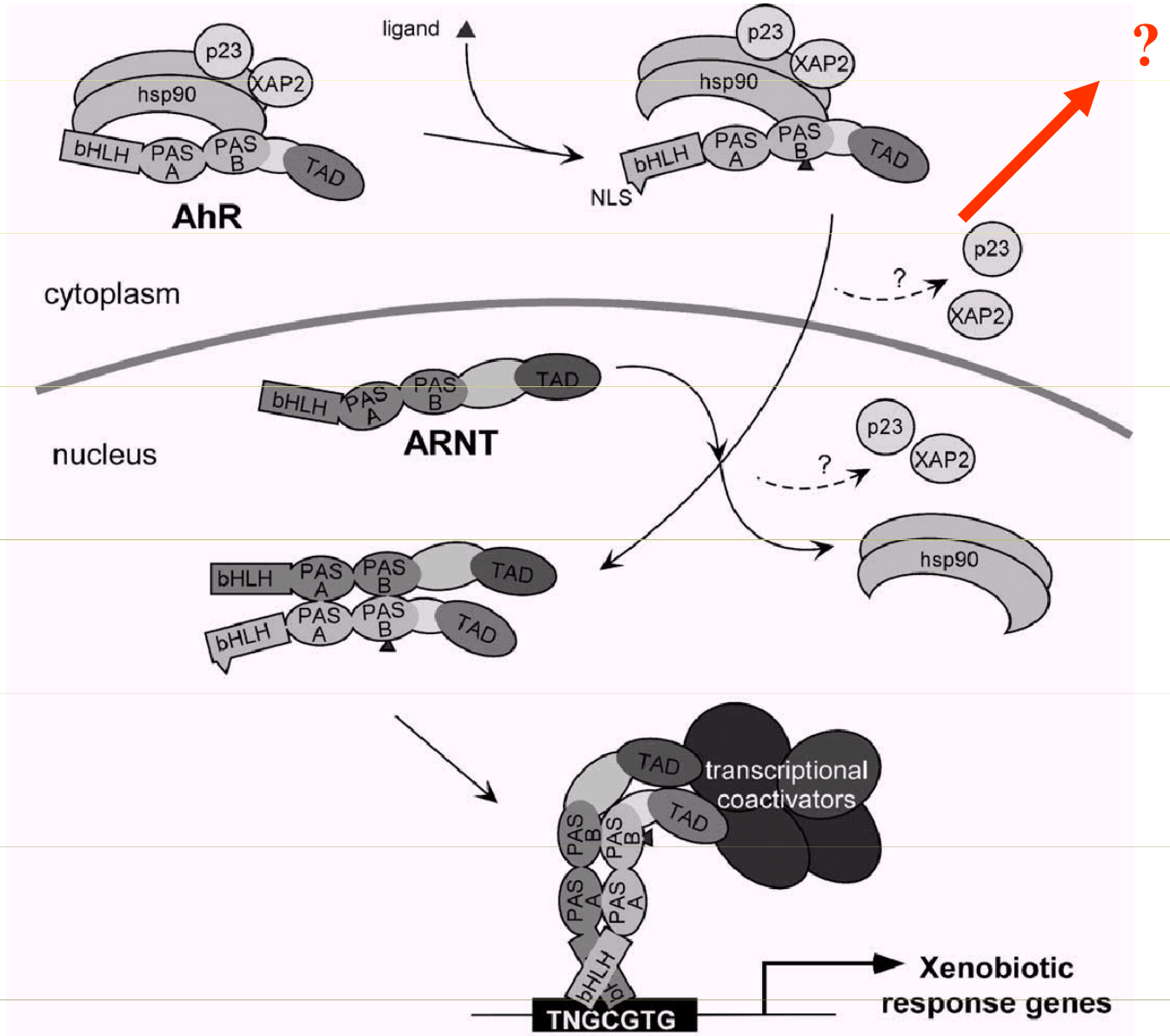
# AhR regulated genes:

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, $p27^{Kip1}$, Jun B, TGF-$\beta$* - regulation of cell cycle and apoptosis;
- *AhRR*.

# AhR activation:



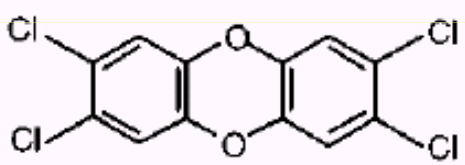


# Physiological role for AhR - AhR-deficient mice:

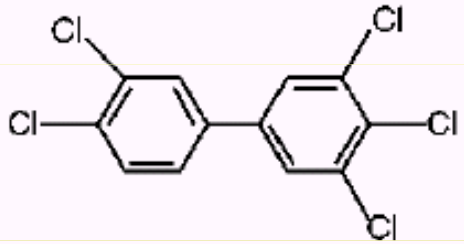
- significant growth retardation;
- devective development of liver and immune system;
- retinoid accummulation in liver;
- abnormal kidney and hepatic vascular structures.

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

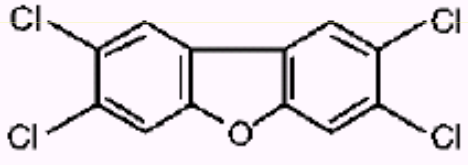
## "Classical" AhR Ligands and CYP1A1 Inducers



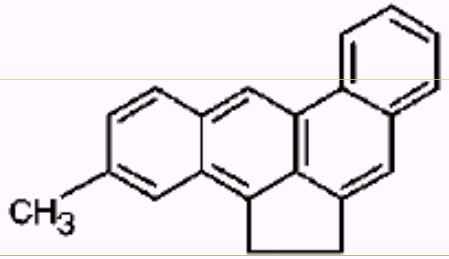

2,3,7,8-Tetrachlorodibenzo-p-dioxin



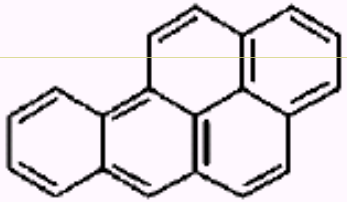

3,4,3',4,'5-Pentachlorobiphenyl



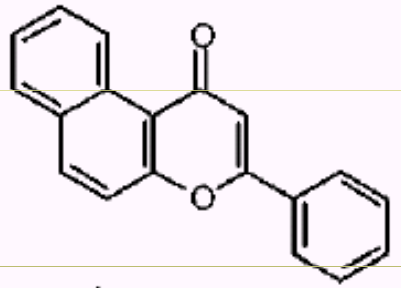

2,3,7,8-Tetrachlorodibenzofuran




3-Methylcholanthrene



Benzo(a)pyrene




β-Naphthoflavone

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

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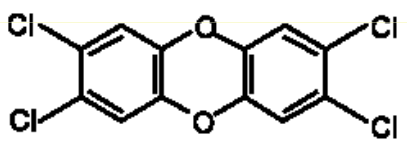
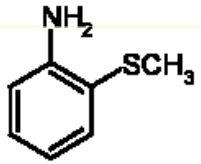
# „Non-classical“ AhR ligands

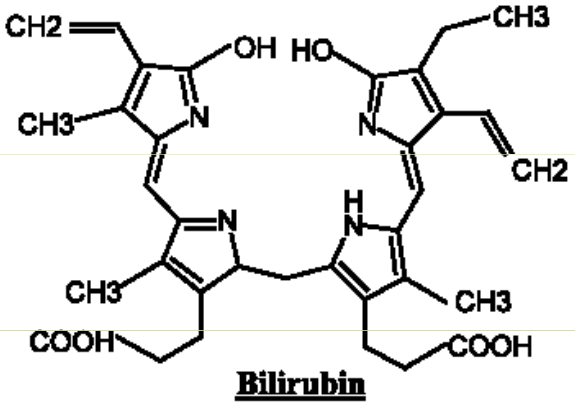
*M.S. Denison et al. / Chemico-Biological Interactions 141 (2002) 3–24*



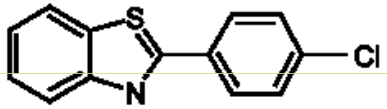

**2,3,7,8-Tetrachlorodibenzo-p-dioxin**



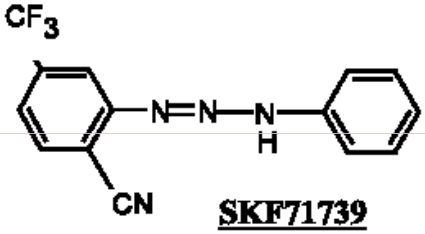

**2-(Methylmercapto)aniline**



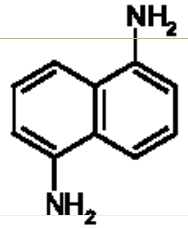

**Bilirubin**



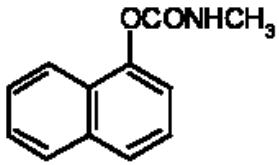

**2-(4'-Chlorophenyl)benzothiazole**



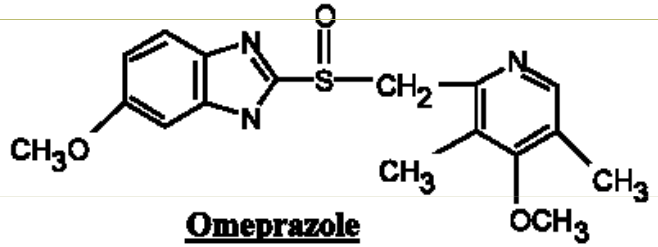

**SKF71739**



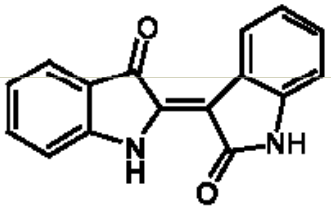

**1,5-Diaminonaphthalene**



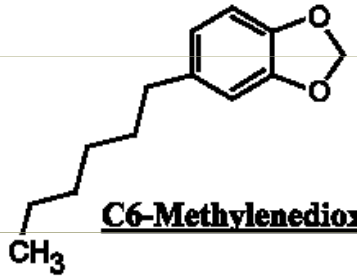

**Carbaryl**



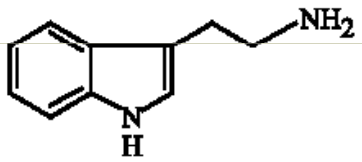

**Omeprazole**




**Indirubin**




**C6-Methylenedioxybenzene**




**Tryptamine**

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

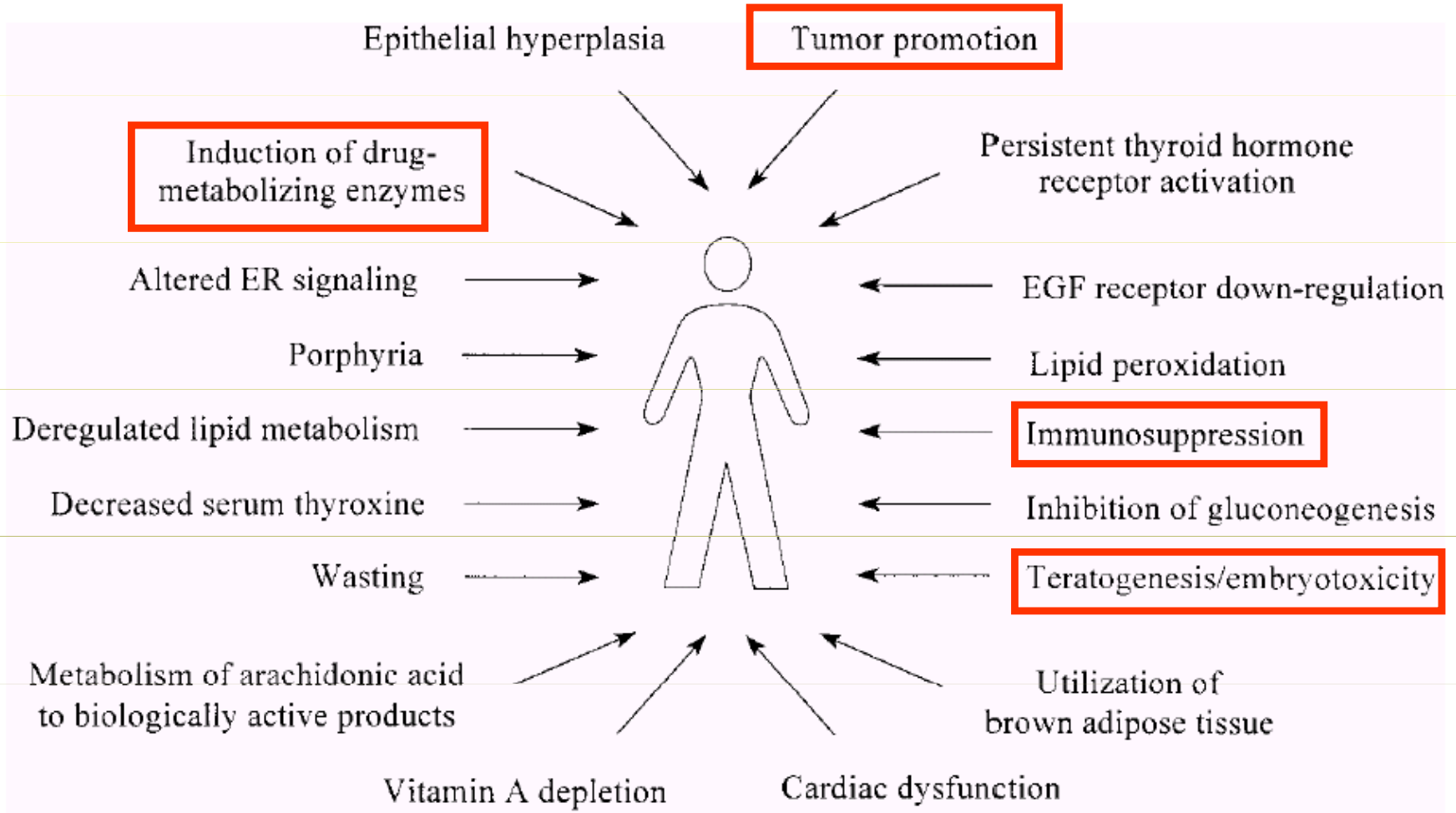


*Figure 1* Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.

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



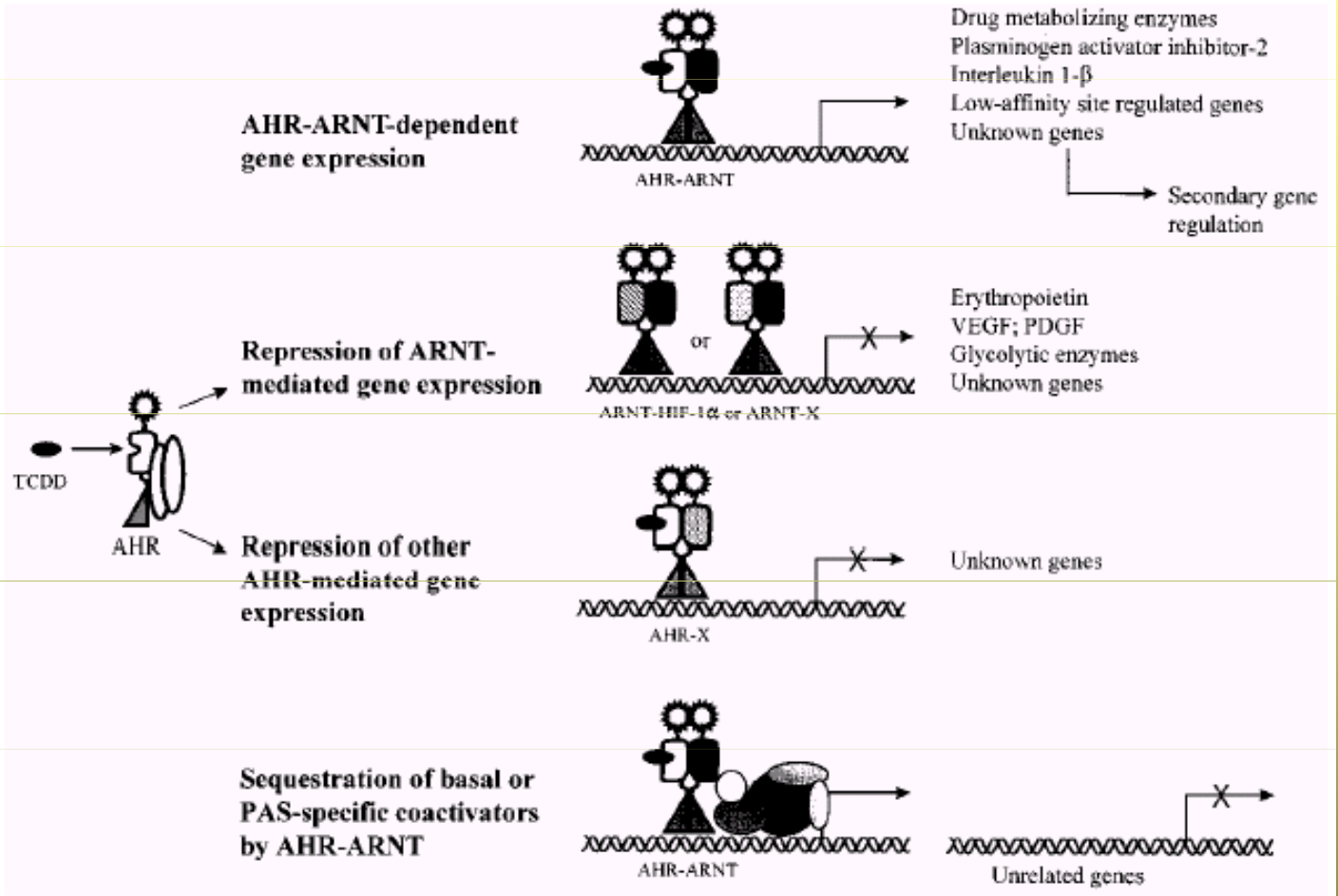


*Figure 6* Possible models for the mechanism of TCDD toxicity, which probably results from alterations in gene expression induced by AHR-ARNT activity. This may be either a direct effect of the activation of AHR-ARNT-regulated genes or an indirect effect resulting from a decrease in the availability of either the AHR or ARNT to participate in different transactivation complexes.

# Toxic equivalency factors (TEF)/TEQ concept:

TEFs provide a simple, single number that is indicative of overall toxicity of a sample containing a mixture of dioxins and dioxin-like compounds. TEFs are consensus values based on REPs across multiple species and/or endpoints. TEFs are based upon a number of endpoints, from chronic in vivo toxicity to in vitro toxicity with the former having the greatest importance in determining overall TEF.

The total potency of a mixture can be expressed in TCDD TEQ concentration:

$$\mathrm{TEQ} = \Sigma\{\mathrm{compound}_1 \times \mathrm{TEF}_1 + \ldots + \mathrm{compound}_n \times \mathrm{TEF}_n\}$$

# Toxic equivalency factors for PCDDs, PCDFs and PCBs:

**Table 4.** Toxic Equivalent Factors established by the WHO (WHO-TEFs) for dioxins and dioxin-like PCBs [4]

| PCDD Congener | WHO-TEF | PCDF Congener | WHO-TEF | PCB Congener | WHO-TEF |
|---|---|---|---|---|---|
| 2,3,7,8-TCDD | 1 | 2,3,7,8-TCDF | 0.1 | *Non-ortho* | |
| 12,3,7,8-PeCDD | 1 | 12,3,7,8-PeCDF | 0.05 | PCB#81 | 0.0005 |
| 123478-HxCDD | 0.1 | 23478-PeCDF | 0.5 | PCB#77 | 0.0005 |
| 123678-HxCDD | 0.1 | 123478-HxCDF | 0.01 | PCB#126 | 0.1 |
| 12,3,7,89-HxCDD | 0.1 | 123678-HxCDF | 0.1 | PCB#169 | 0.01 |
| 1234678-HpCDD | 0.01 | 234678-HxCDF | 0.1 | *Mono-ortho* | |
| OCDD | 0.0001 | 12,3,7,89-HxCDF | 0.1 | PCB#105 | 0.0001 |
| | | 1234678-HpCDF | 0.01 | PCB#114 | 0.0005 |
| | | 1234789-HpCDF | 0.01 | PCB#118 | 0.0001 |
| | | OCDF | 0.0001 | PCB#123 | 0.0001 |
| | | | | PCB#156 | 0.0005 |
| | | | | PCB#157 | 0.0005 |
| | | | | PCB#167 | 0.00001 |
| | | | | PCB#189 | 0.0001 |

*Eljarrat & Barceló, Trends Anal. Chem.22: 655*

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**Table 2.** Comparative lists of POPs selected for environmental and toxicological studies

| POPs selected at the Stockholm Convention (2001) | POPs with an assigned TEF or REP | Emerging POPs |
|---|---|---|
| Aldrin | | |
| Chlordane | | |
| DDT | | |
| Dieldrin | | |
| Endrin | | |
| Heptachlor | | |
| Hexachlorobenzene | | |
| Mirex | | |
| Toxaphene | | |
| PCBs | PCBs | |
| PCDDs/PCDFs | PCDDs/PCDFs | |
| | PCNs | |
| | PBDEs | PBDEs |
| | PBDDs/PBDFs | PBDDs/PBDFs |
| | PBBs | PBBs |
| | PAHs | |

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*Eljarrat & Barceló, Trends Anal. Chem.22: 655*

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# Biomarkers/bioanalytical methods:

- *in vivo* biomarkers: EROD activity, CYP 1A1 and 1B1 expression;
- *in vitro:*

➔ EROD in H4IIE rat hepatoma cells;

➔ CALUX/CAFLUX assays;

➔ GRAB assay (AhR-DNA binding)

➔ yeast bioassay;

➔ immunoassays;

➔ detection of CYP1A mRNA or protein

# Detection of EROD activity:



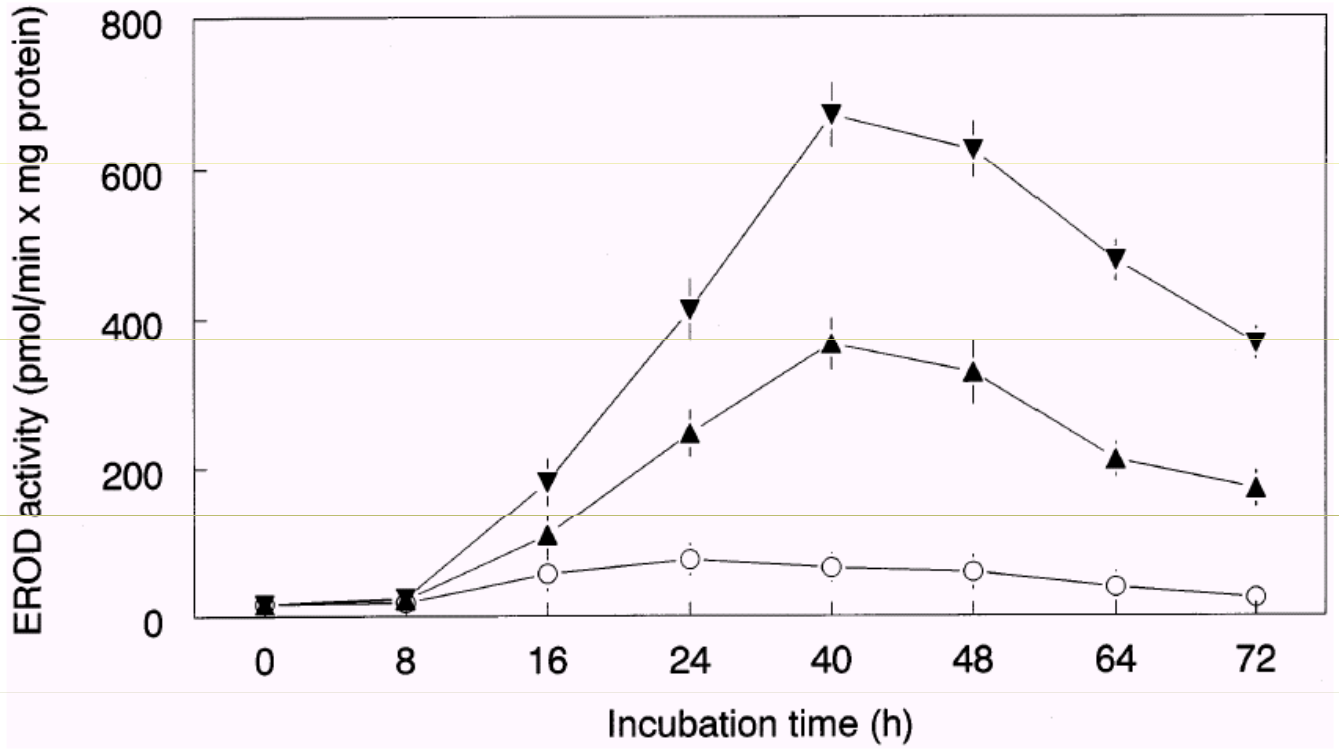
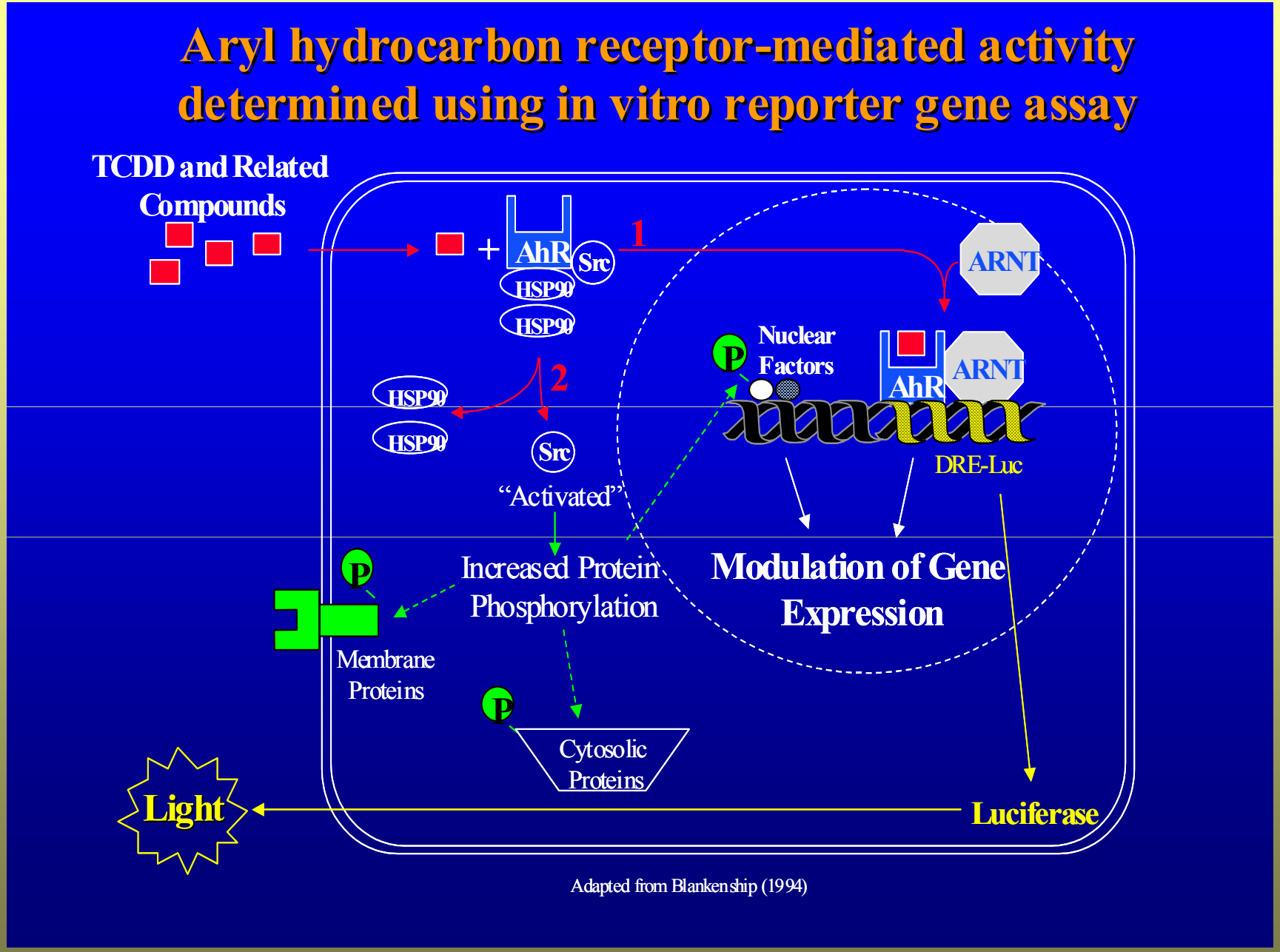



Fig. 2. Time course of induction of CYP1A1-catalyzed 7-ethoxyresorufin *O*-deethylase (EROD) activity in primary cultures of rat hepatocytes, after addition of $1.7 \times 10^{-5}$ M benzo[*a*]pyrene (-▼-), $1.9 \times 10^{-6}$ M benzo[*k*]fluoranthene (-▲-) or $9.4 \times 10^{-5}$ M acenaphthylene (-○-). EROD activity was determined in cell homogenates. The data represent means ± S.D. from four independent experiments.

# CALUX/CAFLUX assays:

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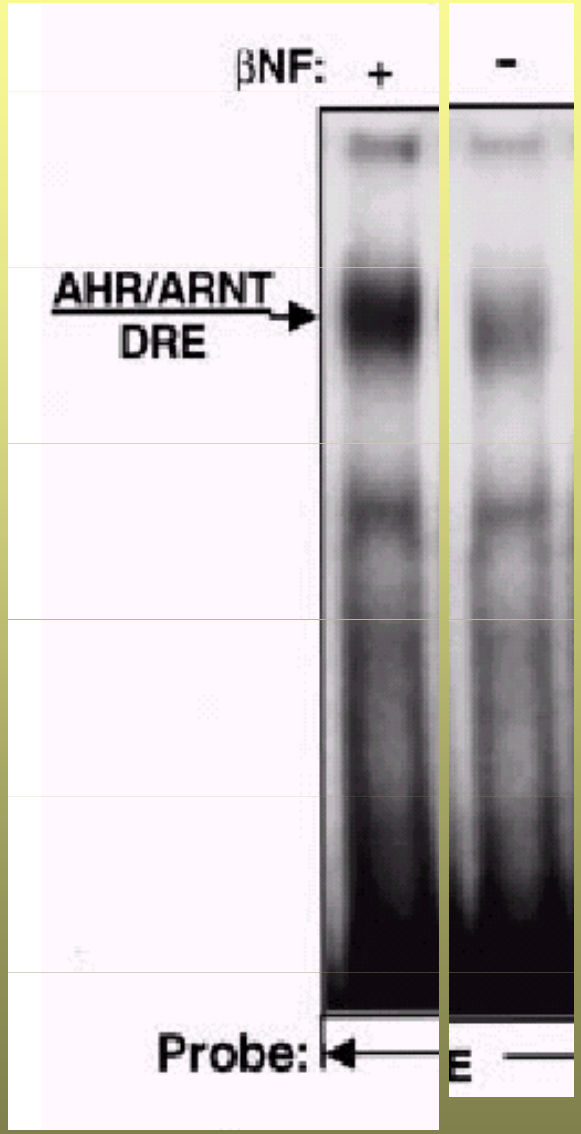
# Gel Retardation of AhR Binding (GRAB) assay:




➔ measures the ability of chemical or chemical mixture to stimulate AhR transformation and DNA binding in vitro