다이옥신의 수용체작용을 통한 내분비계 장애작용 검색기법

박 현 성

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다이옥신의 독성은 체중의 감소, 간 대사효소의 증가, 면역억제, 정자 수의 감소, 발암 촉진 등 매우 다양한 양상을 나타내고 있다. 그러나 아직도 다이옥신이 어떻게 다양한 독성을 나타내는지에 대한 설명은 미비한 상태이다. 다만 다이옥신에 의해 증가되는 싸이토크롬 P450 1A1 (CYP1A1) 효소 유전자의 전사활성화 기전연구를 통하여 다이옥신의 작용은 생체 내에 존재하는 다이옥신 수용체에 의하여 매개된다는 사실을 알게 되었다. 전사 활성화 인자로서의 다이옥신 수용체는 복합 이성질체로 구성되어 있다. 즉 다이옥신과 특이적으로 결합하는 AhR과 핵 내에 항상 존재하는 Arnt라는 두개의 단백질로 구성되어 있다. AhR과 Arnt는 모두 bHLH-PAS motif를 갖는 단백질로 그 그룹에 속한 다른 단백질들과 유사한 작용기전을 가지고 있다. AhR은 세포질 내에 HSP90등의 단백질과 함께 존재하다가 세포질 막을 통과하여 확산에 의하여 도달한 지용성 라이겐드 즉 다이옥신과 높은 친화도 (Kd = ~ 10 9M)로 결합하고, AhR은 HSP90와 분리되어 핵 내로 이동하게 된다. AhR은 핵 내에 존재하는 Arnt와 결합한 후에 특이적 염기서열 (5-T/G n GCGTG A/C G/C A-3)에 결합하여 해당유전자의 전사를 촉진한다. 두 단백질은 모두 아미노 말단에 위치하는 bHLH 및 PAS domain 을 통하여 서로 결합하며 두 단백질의 결합은 DNA 결합에 선행 조건임을 알 수 있었다. 또한 전사활성화 부위 즉 전사에 요구되는 coactivator 등과 결합하는 부위는 AhR의 카복시 말단에 위치하며, 이 부위는 CYP1A1 유전자의 전사개시 부분의 클로모좀 구조를 보다 느슨하게 변형시키는데 요구됨을 알 수 있었다. Arnt는 핵 수용체의 RXR과 같이 AhR외에도 다른 짝 단백질과 결합하여 다른 유전자 발현에 공동으로 사용됨을 알 수 있었다. 이러한 짝 단백질로는 저산소에 의한 유전자 발현에 관여하는 HIF-1a, 중뇌 발생에 관여하는 Sim 단백질이 그 예 이다. 다이옥신에 의하여 발현되는 유전자는 CYP1A1 및 다른 약물대사 효소들 외에는 그 타겟 유전자가 많이 밝혀지지 않아, 보다 체계적인 탐색이 요구된다. 기존에 밝혀진 유전자들의 발현과 다이옥신에 의하여 야기되는 다양한 독성과의 밀접한 상관성을 설명하기에는 부족하다. 따라서 다이옥신의 독성기전연구는 여러 범주에서 다각적인 접근이 필요하다. 즉 (1) AhR과 무관한 다이옥신의 영향 (2) AhR 전사활성화로 인한 독성 (3) AhR이 전사 활성화외적 기능에 의한 다이옥신의 독성 등으로 분리하여 연구되어야 한다. 즉 다이옥신 자체가 다른 신호전달체계 (Src pathway) 를 교란한다든지, CYP1A1 등에 의하여 매개되는 반응과정 중에 활성산소 혹은 발암성인 대사산물을 방출하여 간접적으로 상기한 다이옥신의 독성을 야기하는 경우도 많은 연구가 진행되어야 한다. 또한 최근에는 AhR이 세포 증식에 관련되는 유전자 p27Kip1 의 발현을 촉진하여 세포의 증식을 막거나, AhR이 직접Rb-1단백질과 결합하여 세포증식에 관여하는 등 AhR이 전사 활성화 인자가 아닌 세포성장 및 사멸에 관련될 가능성이 제기되고 있다. 다이옥신의 작용의 대부분 AhR기능을 조절함으로써 발생하며, 아직 밝혀지지 않은 AhR의 고유의 라이겐드를 모방함으로써 나타낸다고 받아들여지고 있다. AhR 결여 생쥐의 경우에서 밝혀진 바와 같이 AhR은 생리적으로 매우 중요한 단백질로 인식되며, AhR의 생리적 기능에 대한 연구는 주요한 생명현상의 발견뿐 아니라 다이옥신의 독성기전의 이해와 예방에 매우 중요한 결과를 제공할 것이다.

다이옥신 수용체의 작용기전
Molecular Mechanism of Dioxin Receptor

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Toxicodynamics of Dioxin

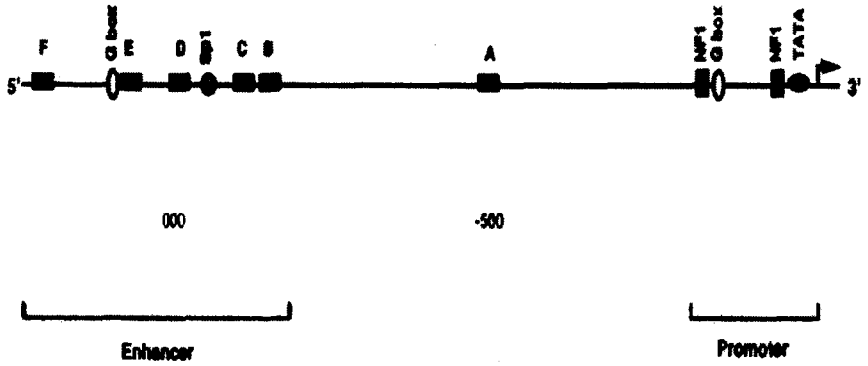
- Wasting: weight loss/depletion of adipose tissue
- Chloracne and epidermal changes
  hyperplasia, hyperkeratosis of interfollicular epidermis
- Hepatotoxicity: hepatomegaly:
  increase of metabolic enzymes
- Neurotoxic (Yusho): Polyneuropathy
- Infertility (male)
- Immuno-suppression: loss of lymphoid tissue
  decrease of humoral immunity
- Tumor promotion: 0.1-10㎍ g/kg TCDD
- Alterations in endocrine homeostasis:
  partial agonistic or antagonistic effects
  for steroid hormone receptor

Evidence for Dioxin Receptor (1)

• **Induction of aryl hydrocarbon hydroxylase by TCDD**
(AHH activity is catalyzed by cytochrome P450 1A1 (CYP1A1))

1. Poly aromatic hydrocarbons including TCDD, 3MC, benzo(a)pyrene, increase transcription of cytochrome P4501A1 gene (CYP1A1).
2. The enhancer region of CYP1A1 gene has dioxin-responsive elements (DRE; 5'-TNGCGTG-3')
3. Specific proteins bind this dioxin-responsive elements in response to dioxin.
4. Inbred mouse C57BL/6J (responsive), DBA/2J(nonresponsive) (polymorphism in responsiveness to TCDD)
→ cross breeding → responsive → allele Ah (dominant trait)

Regulatory Region of CYP1A1



Evidence for Dioxin Receptor (2)

• **Characterization, purification and cloning of AhR**

1. Poland and coworkers: ^{3}H-labeled TCDD binds a protein in cytosolic fraction of C57BL/6 mouse liver, saturably, reversibly, with high affinity and stereospecifically.
2. The TCDD bound cytosolic protein translocates to the nucleus.
3. Partially purified dioxin bound protein has DNA binding ability *in vitro*.
4. Purification and cloning of AhR by using ^{125}I-labeled photoaffinity ligand.
5. Proteins that make a complex with AhR: AhR makes a complex with 90kDa protein in cytosol and with 100kDa protein in DNA bound form.
6. Ah gene product: intracellular receptor protein that bound [^{3}H] TCDD Kd=10^{-10} M (TCDD most potent) Toxicity is linearly related with K_d

Evidence for Dioxin Receptor (3)

• **Factors required for activity of the AhR**

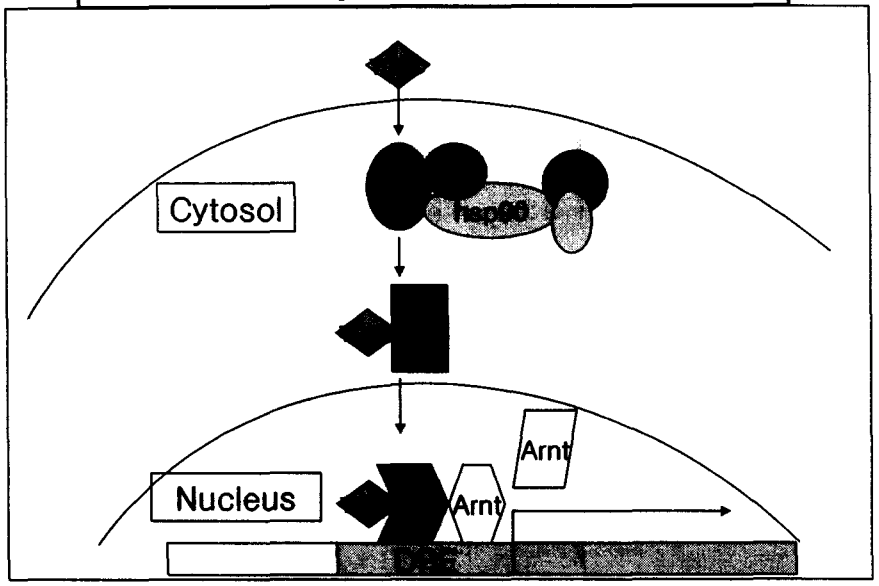
1. Unliganded AhR immunoprecipitates with Hsp90.
2. Hankinson *et al.* have isolated variant mouse hepatoma cell lines that fail to induce CYP1A1 by TCDD.

   Study of these variant cell lines reveals that several genes contribute to dioxin action.

   a. Defect in TCDD binding → AhR defective cells

   b. Defects in nuclear translocation and DRE binding of liganded receptor (AhR) → ??
3. Cloning of AhR nuclear translocator (ARNT) by selecting a gene that complements the defects variant hepatoma cells described (b)

Dioxin Receptor: Function of AhR



Roles of Hsp90 on the function of AhR

- Distinct roles of the molecular chaperone hsp90 in modulating dioxin receptor function via the basic helix-loop-helix and PAS domains
  C Antonsson, ML Whitelaw, J McGuire, JA Gustafsson and L Poellinger,
  Mol. Cell. Biol. (1995) 15: 756-765

  In Absence of ligand:
  (i) Folding of Ligand Binding Conformation
  (ii) Repression by Interference with DNA Binding/Dimerization

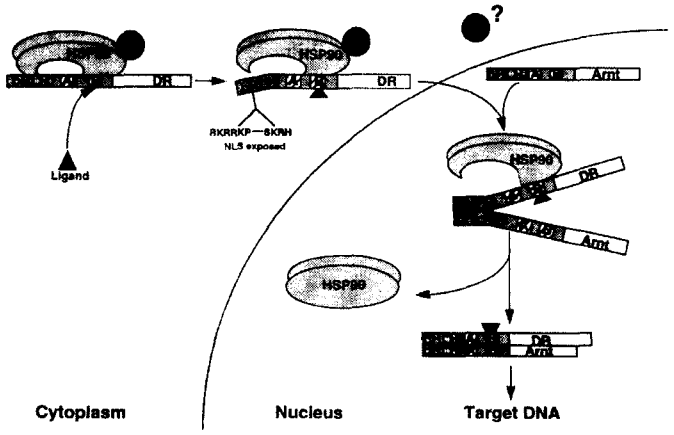
  In Presence of Ligand
  (iii) Folding of DNA Binding Conformation

- Other immunophilin-like proteins, AIP (or XAP2, Ara9) bind both Hsp90 and AhR; the binding of AhR to AIP stabilizes the the AIP-Hsp90-AhR complex

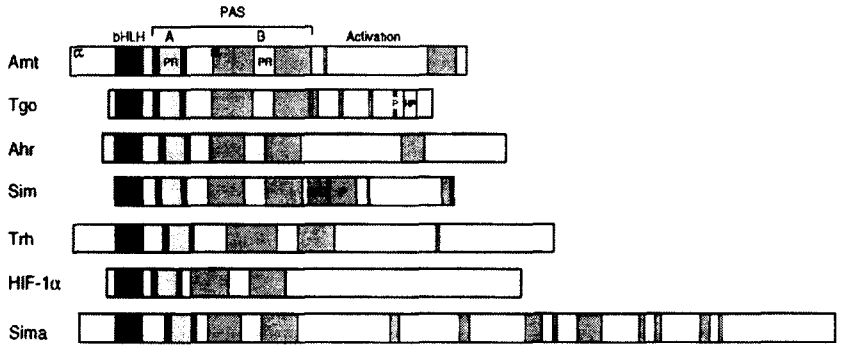
Molecular and Cellular Biology, August 1999, p. 5811-5822, Vol. 19, No. 8

Multiple Roles of Ligand in Transforming the Dioxin Receptor to an Active Basic Helix-Loop-Helix/PAS Transcription Factor Complex with the Nuclear Protein Arnt

Michael J. Lees and Murray L. Whitelaw*

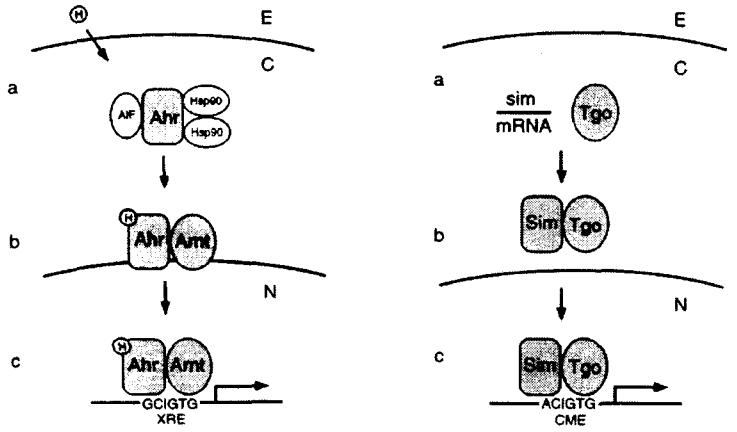


The structure of bHLH-PAS proteins



Crew et al. 1998 Gene& Dev

Mechanism of Action of bHLH-PAS Proteins



Crew et al. 1998 Gene& Dev

Structure and Function Relation of AhR and Arnt

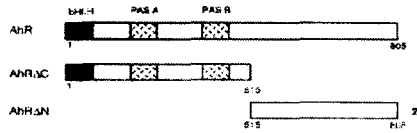
- **HLH and PAS domains mediate dimerization between AhR and Arnt.**
- **Two basic regions of AhR and Arnt make specific contact with DRE (5'-TNGCGTG-3').**
- **Dimerization is required for DNA binding of AhR and Arnt.**
- **PAS-B region of AhR binds to Dioxin.**
- **C-terminal regions of AhR and Arnt contains transactivation domains**

• *Mol Cell Biol* (1996)16:430-6

Dioxin-induced CYP1A1 transcription in vivo: the aromatic hydrocarbon receptor mediates transactivation, enhancer-promoter communication, and changes in chromatin structure.

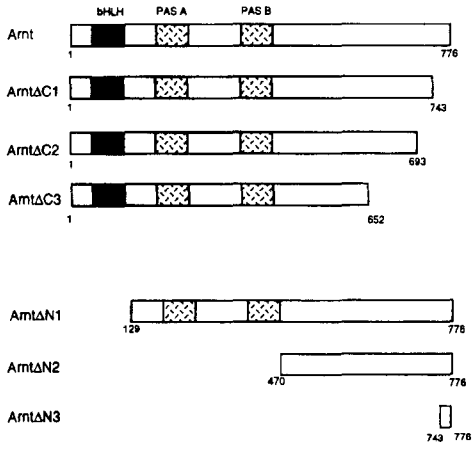
Hyunsung P. Ko, Steven T. Okino, Qiang Ma, and James P. Whitlock., Jr.

Structural Domains and Functional Properties of AhR



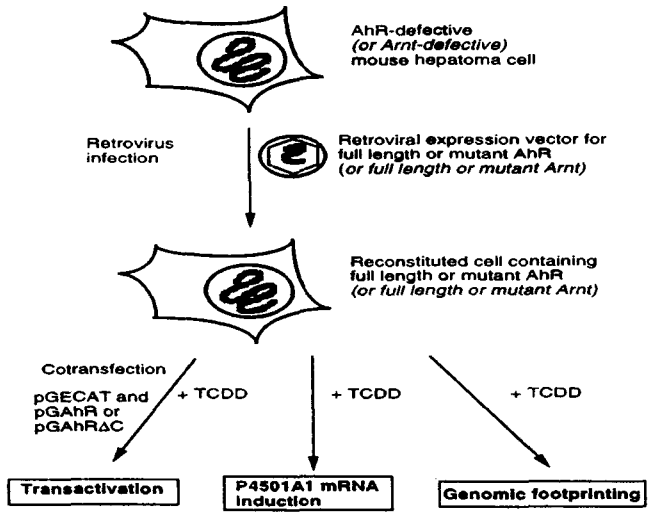
	Transactivation		Dimerization	
	DMSO	TCDD	DMSO	TCDD
AhR	2.5 ± 0.5	3.2 ± 0.6	21.5 ± 4.7	96.5 ± 11.7
AhRΔC	0.6 ± 0.1	0.8 ± 0.1	6 ± 1.8	109.0 ± 24.3
AhRΔN	278.5 ± 52.4	217.6 ± 33.8	N/A	N/A

Structural Domains and Functional Properties of Arnt

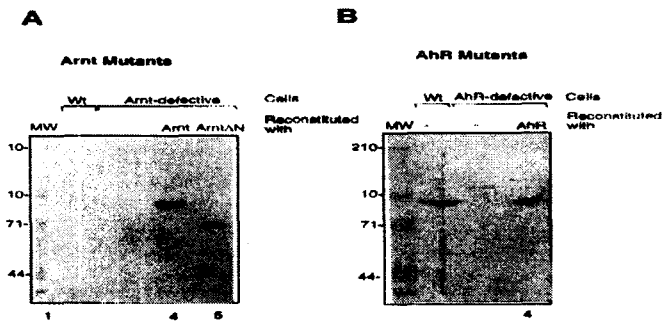


	Transactivation		Dimerization	
	DMSO	TCDD	DMSO	TCDD
Arnt	238.7 ± 88.5	250.0 ± 24.6	20.4 ± 5.2	121.1 ± 74
ArntΔC1	1.0 ± 0.3	1.7 ± 0.6	19.1 ± 13.0	172.5 ± 95.6
ArntΔC2	0.9 ± 0.3	2.4 ± 0.7	19.3 ± 12.8	169.5 ± 86.7
ArntΔC3	0.9 ± 0.2	1.3 ± 0.2	18.4 ± 4.3	176.1 ± 54.9
ArntΔN1	172.9 ± 8.3	277.7 ± 40.6	3.4 ± 0.6	16.6 ± 4.6
ArntΔN2	240.8 ± 55.4	236.5 ± 55.8	N/A	N/A
ArntΔN3	163.0 ± 70.7	169.7 ± 41.9	N/A	N/A

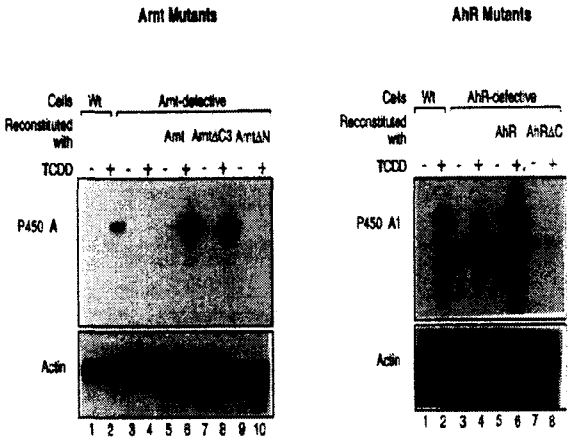
Reconstitution of AhR or Arnt Defective Cells



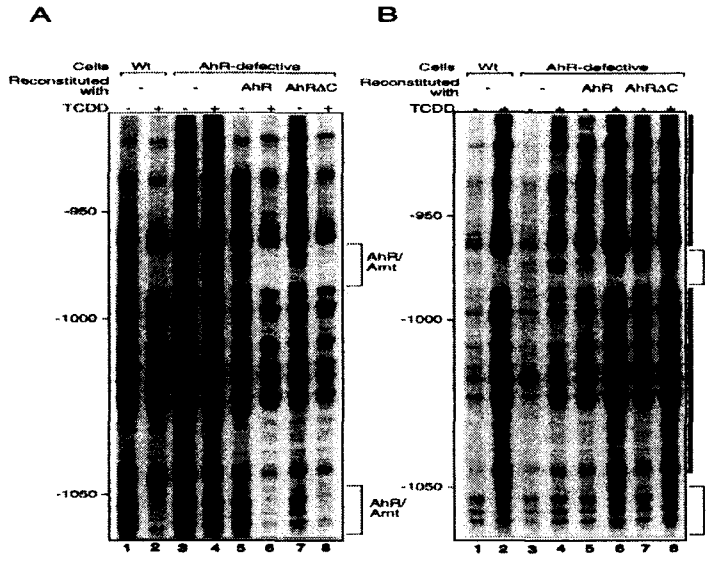
Reconstitution of AhR or Arnt defective cells with AhR and Arnt



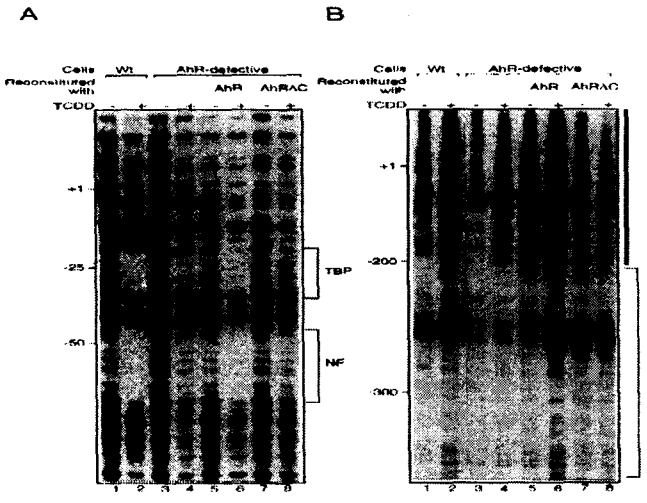
CYP1A1 gene expression in reconstituted cells

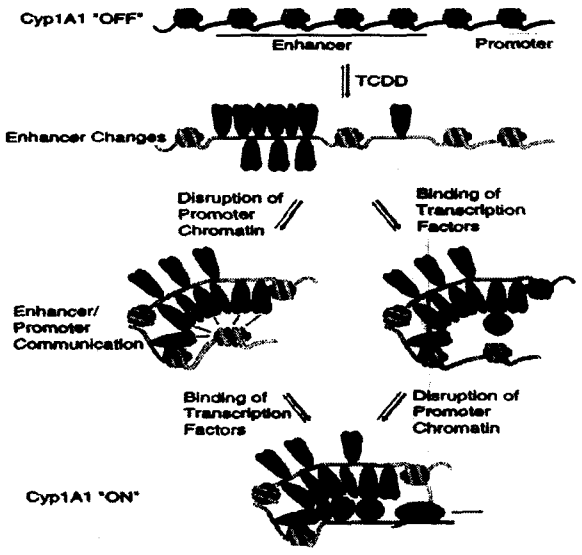


In vivo protein-DRE interaction at CYP1A1 enhancer



In vivo protein-DRE interaction at CYP1A1 promotere





Coactivators for AhR/Arnt

- *Gene Expr* (1999)8:273-86

Nuclear receptor coactivator SRC-1 interacts with the Q-rich subdomain of the AhR and modulates its transactivation potential.

Kumar MB, Perdew GH

- *J Biol Chem (1999) 274:22155-64*

Differential recruitment of coactivator RIP140 by Ah and estrogen receptor: Absence of a role for LXXLL motifs.

Kumar MB, Tarpey RW, Perdew GH

- *J Biochem (Tokyo) (1997)122:703-10*

CBP/p300 functions as a possible transcriptional coactivator of Ah recepto nuclear translocator (Arnt).

Kobayashi A, Numayama-Tsuruta K, Sogawa K, Fujii-Kuriyama Y

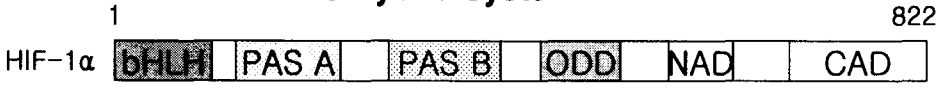
J. Biol. Chem. (1996) 271; 21262-21267

Induction of Phosphoglycerate Kinase 1 Gene Expression by Hypoxia

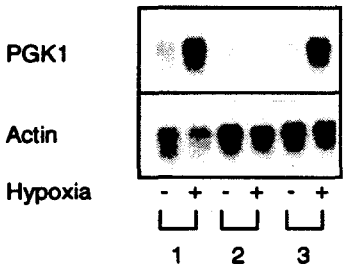
- ROLES OF ARNT AND HIF1-

Hui Li , Hyunsung P. Ko and James P. Whitlock Jr.

- **Purpose: To identify novel partner proteins for Arnt**
- **Method: Yeast Two Hybrid System**



- **Arnt is required in both dioxin and hypoxia-induced PGK1 expression**
  1. **Wt hepa1c1c7 cell**
  2. **Arnt defective hep1c1c7**
  3. **Arnt reconstituted**



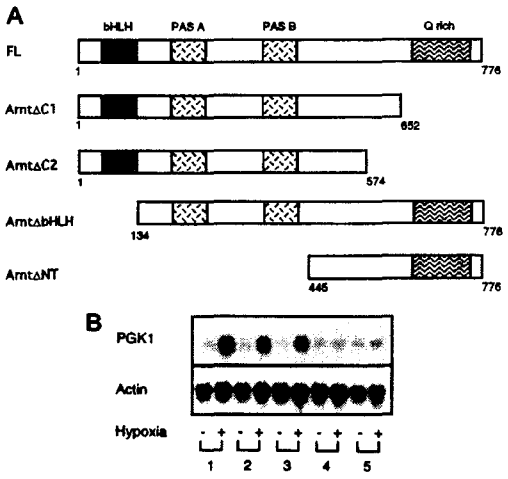
Other Partner Proteins for Arnt

- **Hypoxia-Inducible Factor-1α (HIF-1α)**

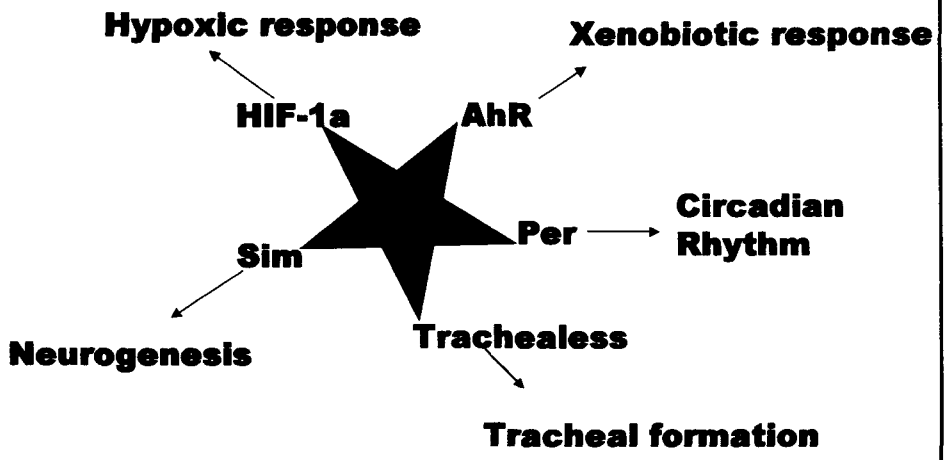
J. Biol. Chem. (1996) 271; 21262-21267
Induction of Phosphoglycerate Kinase 1 Gene Expression by Hypoxia ROLES OF ARNT AND HIF1
Hui Li , Hyunsung P. Ko and James P. Whitlock Jr.

- **Sim (single-minded) :mid brain development**
- **Per (period) : circadian rhythm**
- **Trachealess: tracheal development**

Hypoxia-Induced PGK in Arnt reconstituted cells



Arnt as a Common Partner Protein



Ligand-Induced Degradation of AhR

- *J Biol Chem (1999) 274:28708-28715*

Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytosplasmic proteasome following nuclear export.

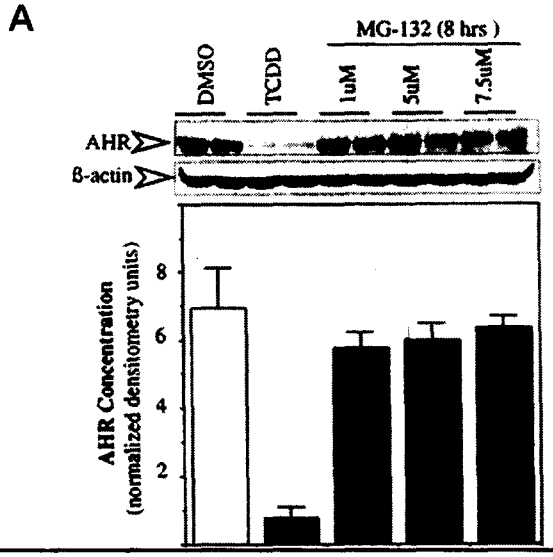
Davarinos NA, Pollenz RS

- *J Biol Chem* (2000) 275:8432-8

2,3,7,8-tetrachlorodibenzo-p-dioxin-induced degradation of aryl hydrocarbon receptor (AhR) by the ubiquitin-proteasome pathway. Role of the transcription activaton and DNA binding of AhR.

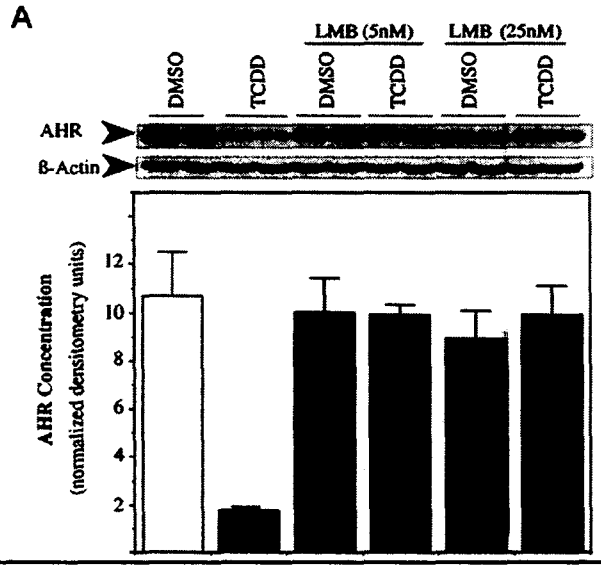
Ma Q, Baldwin KT

Protein level of AhR



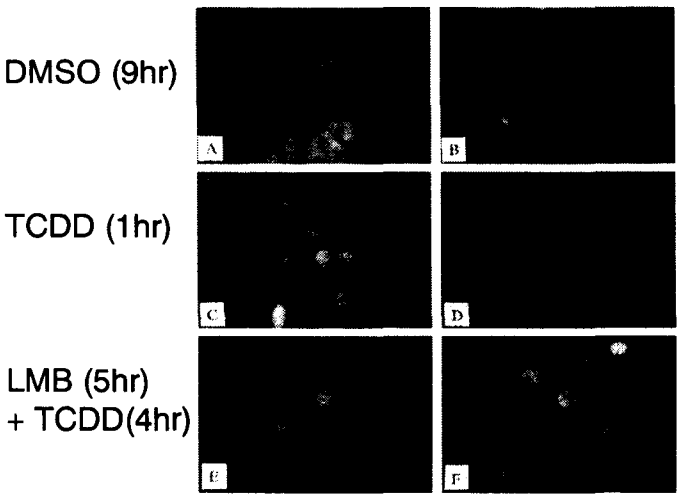
Davarinos & Pollenz
(1999) JBC

Inhibiotor of nuclear export (LMB) blocks the TCDD-induced degradation of AhR



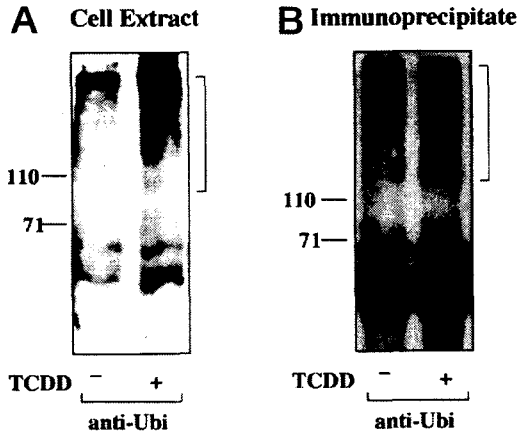
Davarinos & Pollenz
(1999) JBC

Subcellular localization of AHR in Hepa-1 cells exposed to LMB and TCDD.



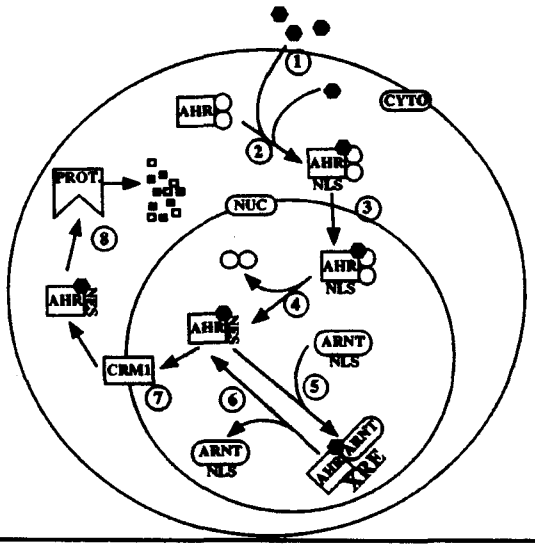
Davarinos & Pollenz (1999) JBC

Immunoblotting of ubiquitinated AhR



Ma & Baldwin JBC 2000

AhR-mediated signal tranduction



Davarinos & Pollenz

(1999) JBC

Knock-out mouse of AhR

***Science* (1995) 268:722-726**

Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor.

Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ

	Δ1/Δ1	Δ2/Δ2
Construct		
ES cells	J1 (129/SvJae)	R1 (129/SvJ × 129/Sv-$+^{p}$ $+^{Tyr+}$ Mgf^{Sl-J}/+)
Exon deleted	Exon 1	Exon 2
Recipient	C57BL/6N	C57BL/6J
Lethality		
Intra-uterine	None	None
Neonatal	40–50%	None
Adult	Normal	Normal
Growth (1–4 weeks)	Slower	Slower
Fertility	Decreased	Decreased
Liver		
Size	50% smaller (4 weeks)	25% smaller (3 weeks) (persists through life)
Lobule structure	Normal	n/a
Gross pathology/Histology	Portal tract fibrosis (3 weeks)	Pale, mottled, spongy (1 week/2 weeks)
	Centrilobular hypercellularity (3 weeks)	Extensive microvesicular fatty metamorphosis of hepatocytes (1 week/2 weeks)
	Inflammation of bile ducts (3 weeks)	Prolonged extramedullary hematopoiesis (1 week/3 weeks)
	Eosinophilia (3 weeks)	Mild portal region fibrosis (2 weeks)
	Glycogen depletion (3 weeks)	
P450 expression/activity		
Inducible		
Cyp1a1	Not inducible	n/a
Cyp1a2	Not inducible	Not inducible
Constitutive		
Cyp1a2	10% expression	25% expression
Ugt06	15% expression	n/a
Inducible EROD activity	n/a	Not inducible
AHR expression		
Protein	n/a	Not present
mRNA	Not present	Present at predicted size
Immunology		
Lymphoid histology	Smaller PALS (4 weeks)	Normal
Splenocyte numbers	20% (2–3 weeks)	Normal (2–3 weeks)
		150% (6 weeks)
	Normal (10–12 weeks)	
	50% (25–32 weeks)	
Lymphocyte subset proportions		
Spleen	Normal	Normal
Thymus	Normal	Normal

[1]Characteristics of *Ahr* null mice are given in comparison with $Ahr^{+/+}$ littermate controls.
[2]Age of mice at the time of the observed pathology is indicated in parentheses. When pathology is observed at several time points, the range is indicated by a dash (-). If pathology is observed to resolve with time, disappearance is indicated as denominator (/x).
[3]Periarterial lymphatic sheaths.
[4]Reported for some but not all mice.

AhR vs Cell Cycle

- ***J Biol Chem* (1998) 273:22708-13**

A direct interaction between the aryl hydrocarbon receptor and retinoblastoma protein. Linking dioxin signaling to the cell cycle.
Ge NL, Elferink CJ

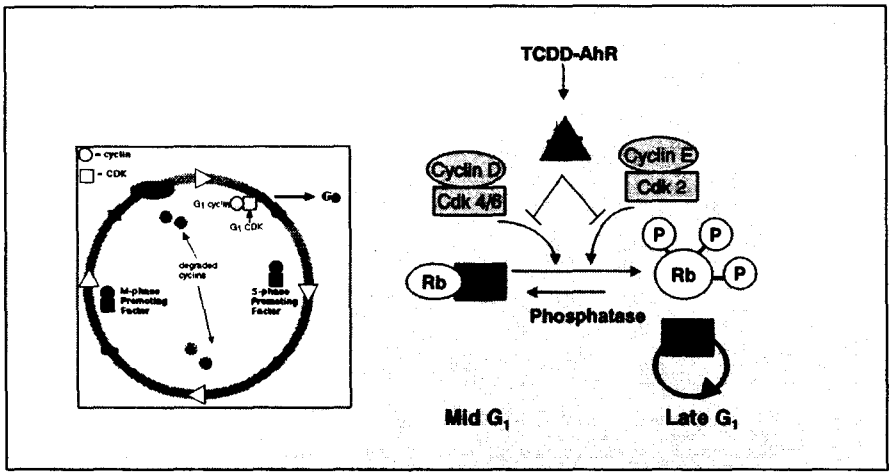
- ***Mol Pharmacol* (1998) 54:313-21**

The involvement of aryl hydrocarbon receptor in the activation of transforming growth factor-beta and apoptosis.
Zaher H, Fernandez-Salguero PM, Letterio J, Sheikh MS, Fornace AJ, Roberts AB, Gonzalez FJ

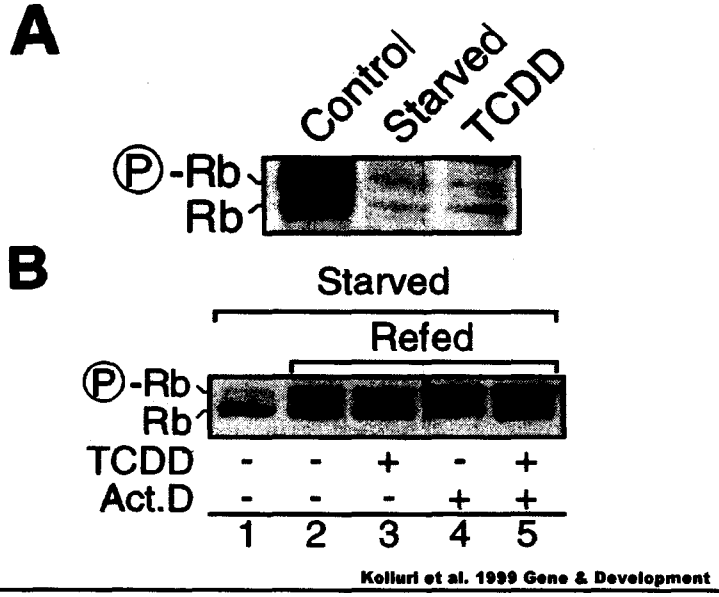
- ***Genes Dev* (1999) 13:1742-53**

p27(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells.
Kolluri SK, Weiss C, Koff A, Gottlicher M

Dioxin-induced p27^{Kip} inhibits the phosphoryltion of Rb

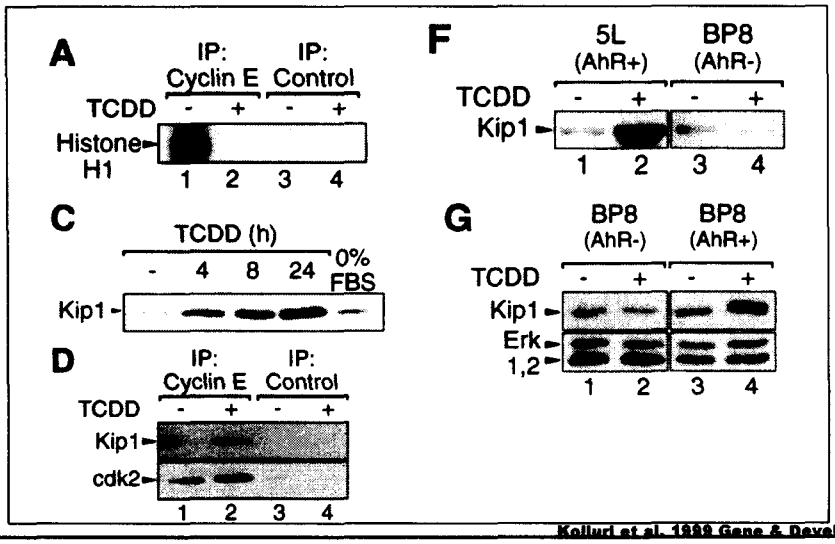


Transcription-dependent delay of cell cycle progression by TCDD



Kolluri et al. 1999 Gene & Development

Induction of the p27^{Kip1} cell cycle inhibitor during TCDD-dependent delay of cell cycle progression.



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Expression of *Kip1* antisense RNA impairs TCDD-induced inhibition of 5L cell proliferation

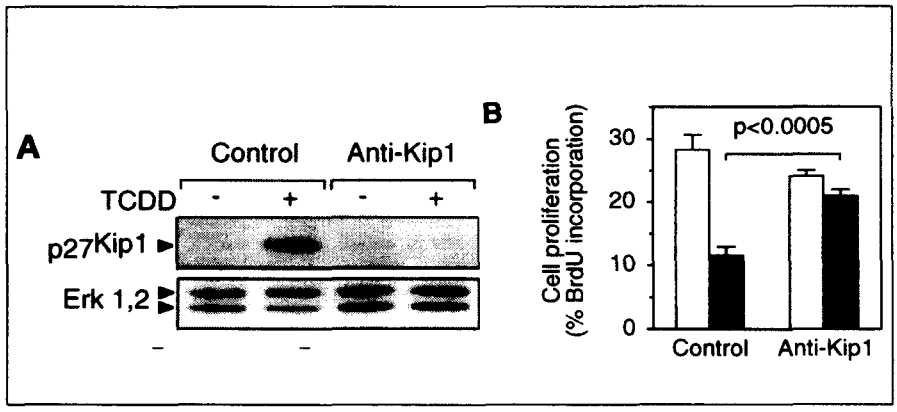

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Table 1. Effect of TCDD on proliferation of liver cells in vivo and in vitro

Duration of treatment (weeks)	Change in BrdUrd labeling index	References
In vivo		
30	Increase	Lucier et al. (1991)
2	Increase (periportal Decrease (pericentral)	Fox et al. (1993)
30	Increase (marginal)	Maronpot et al. (1993)
Up to 17	No significant change	Buchmann et al. (1994)
2, Prior to p.h.[a]	Decrease	Baumann et al. (1995)
Up to 16	No significant change	Stinchcombe et al. (1995)
30	Increase	Tritscher et al. (1995)
14	Decrease	Walker et al. (1998)
30,60	Increase	
Cell type	Effect on proliferation	References
In vitro		
5L hepatoma cells	Decrease	Göttlicher and Wiebel (1991)
Rat hepatocytes	Increase or decrease, depending on treatment regimen	Schrenk et al. (1992)
Mouse hepatocytes		Schrenk et al. (1994)
Rat hepatocytes	Decrease	Hushka and Greenlee (1995)
Rat hepatocytes	Increase or decrease, depending on treatment regimen and TCDD dose	Münzel et al. (1996)
WB-F344 cells		Köhle et al. (1999)

[a] p.h., partial hepatectomy

Toxicology Letters 112-113 (2000) 69-77

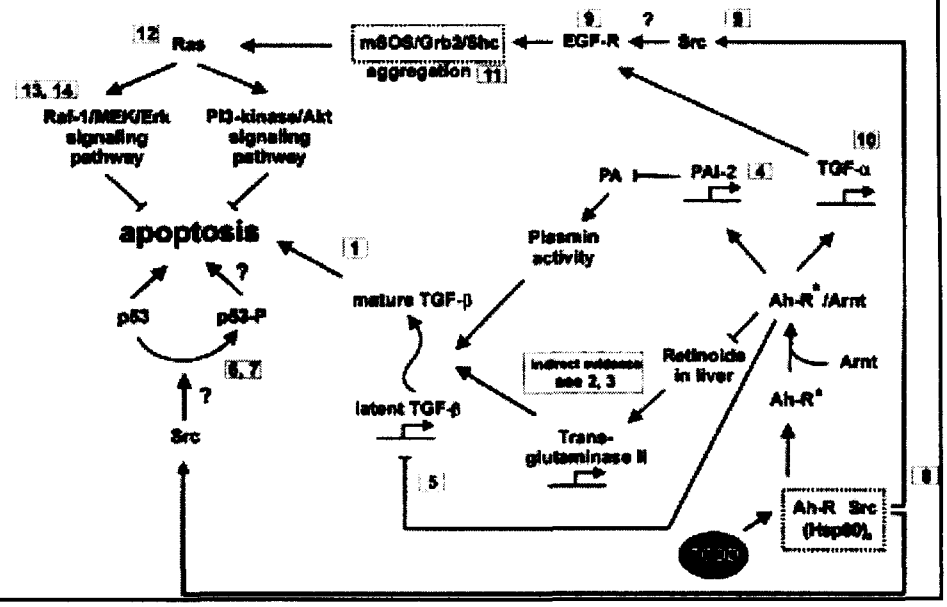
Ah receptor ligands and tumor promotion: survival of neoplastic cells

Michael Schwarz, Albrecht Buchmann, Stefan Stinchcombe, Arno Kalkuhl and Karl-Walter Bock

Table 2. Effect of TCDD on apoptosis of liver cells in vivo and in vitro

Species	Cell type	Apoptotic stimulus	Effect on apoptosis	References
In vivo				
Rat	Hepatocytes	'Spontaneous'	Suppression	Stinchcombe et al. (1995)
Mouse, TGFα transgenic	Hepatocytes	'Spontaneous'	No effect	Schrenk et al. (1997)
Mouse, c-myc transgenic (only in females)	Hepatocytes Schrenk et al. (1997)	'Spontaneous'	Suppression	
Mouse	Hepatocytes	CD95 (Fas/Apo-1)	No effect	Own (unpublished)
In vitro				
Rat	Primary hepatocytes	UV-light TGF-β1	Suppression No effect	Wörner and Schrenk (1996, 1998)
Mouse	Primary hepatocytes	Bleomycin TGF-β1	No effect No effect	Christensen et al. (1998)
Mouse	1c1c7 hepatoma	Ceramide	No effect	Reiners and Clift (1999)
Rat	FTO-2B hepatoma	TGF-β1	No effect	Own (unpublished)
Human	HepG2 hepatoma	UV-light	No effect	Own (unpublished)
Human	Primary keratinocytes	UV-light	No effect	Own (unpublished)

Possible mechanisms leading to suppression of apoptosis by TCDD



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