

**2005 Superfund Basic Research Program  
Annual Meeting**

**Chromate and  
Polycyclic Aromatic Hydrocarbon Interactions**

*(Transcriptional Repression by Chromium-Chromatin Interactions)*

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## **Why study mixtures of PAHs and metals?**

**As, Cr, B(a)P and PCBs are in USEPA top 20 hazardous substance priority list**

**9,727 Superfund sites with PAH contamination;  
2,539 have also Cr; 2,274 have also As**

**1,200 sites in National Priority List; 40% have Cr/As and PAHs.**

**Is there enough time in the combined lifetimes  
Of all biological scientists to study all mixtures?**

**275 CERCLA compounds**

**37,675 binary combinations**

**3,428,425 ternary combinations**

**233,132,900 quaternary combinations**

**...**

**175,000 biological scientists (Latest NSF estimates)**

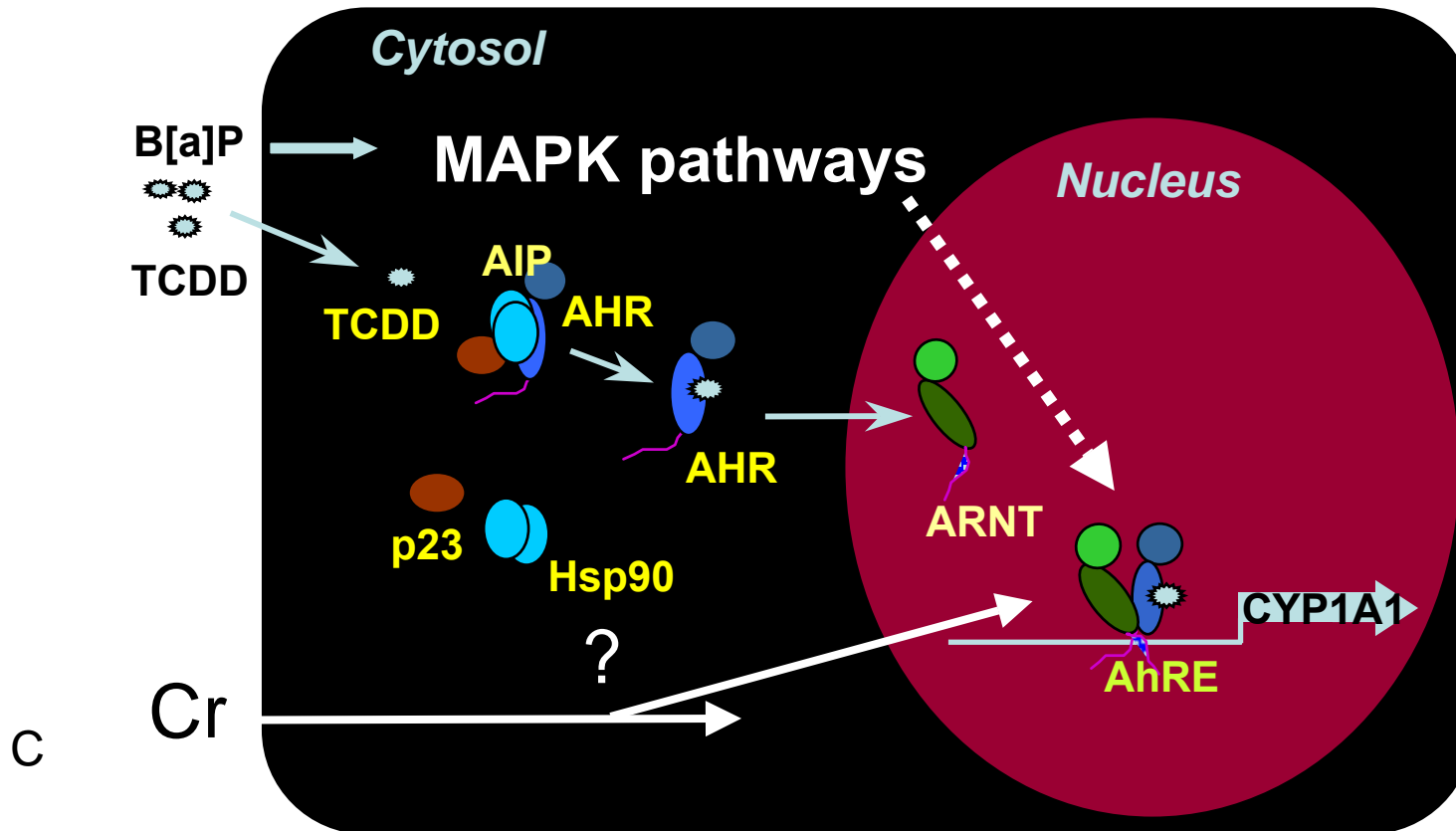
**To study complex mixtures we need  
a different experimental paradigm**

**For binary mixtures:**

**Cross-talking signaling pathways**

***How does one member of the mixture  
modify the effect of the other?***

# Ah Receptor-Dependent Transactivation and Target Gene Expression in the presence of Cr



Mouse Hepa-1 cells

# Cr inhibits inducible gene expression

- Cr-DNA crosslinks
- Cr-DNA adducts

*Zhitkovich (2005) Chem.Res.Tox. 18:3-11*

- Disruption of transcriptional complexes
  - MT, PEPCK, but not actin

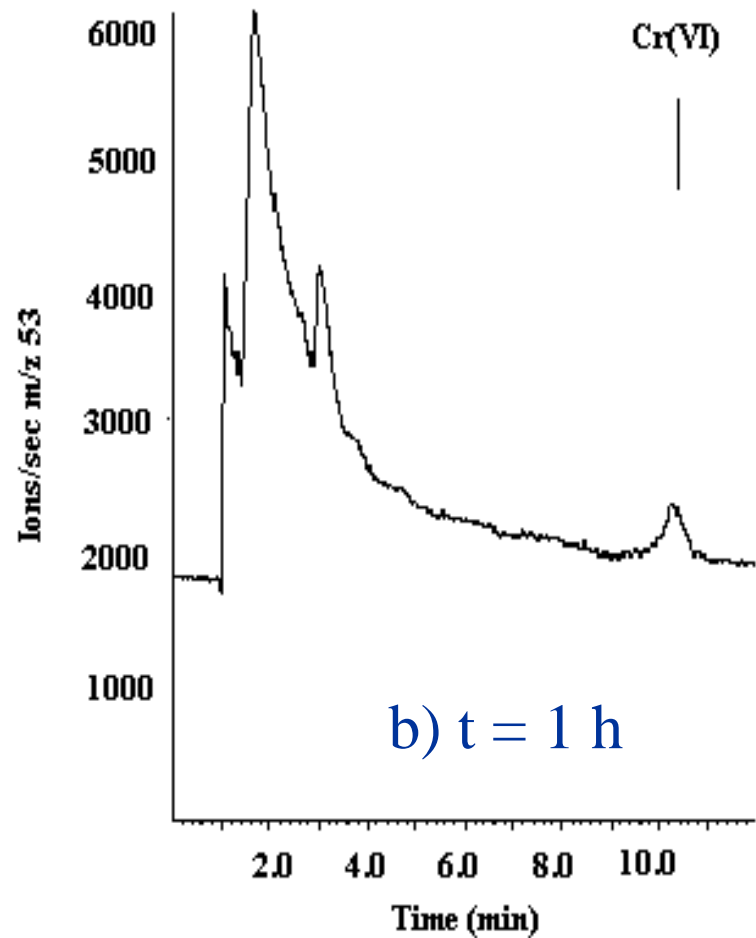
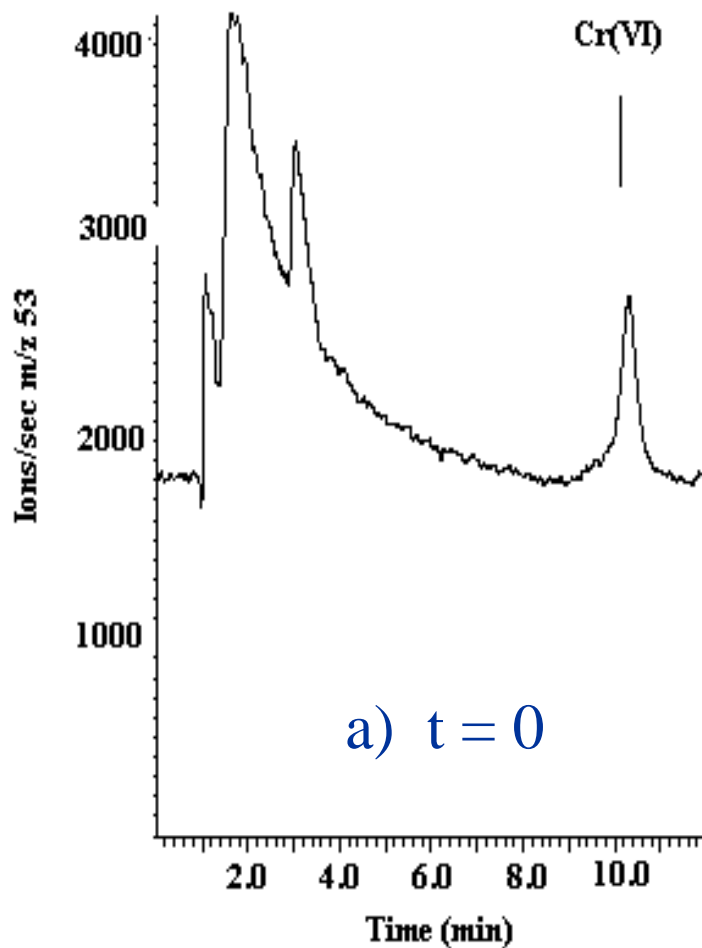
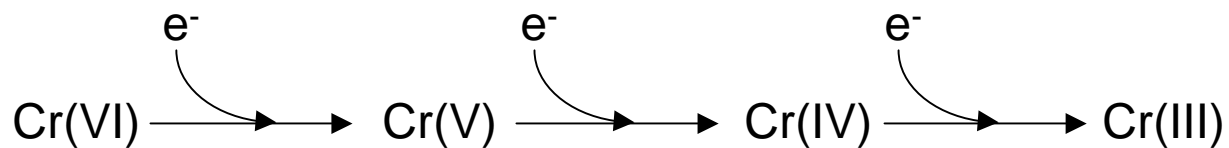
*Hamilton et al (1989) Mol.Carcinog. 2:274-286*

- Blocks binding of p300 to NFkB p65

*Shumilla et al (1999) J.Biol.Chem. 274:36207-36212*

- **Chromium Speciation and Distribution**
- **Transrepression of AHR-inducible Genes by Chromium**
- **Epigenetics of Chromium-Chromatin Interactions**

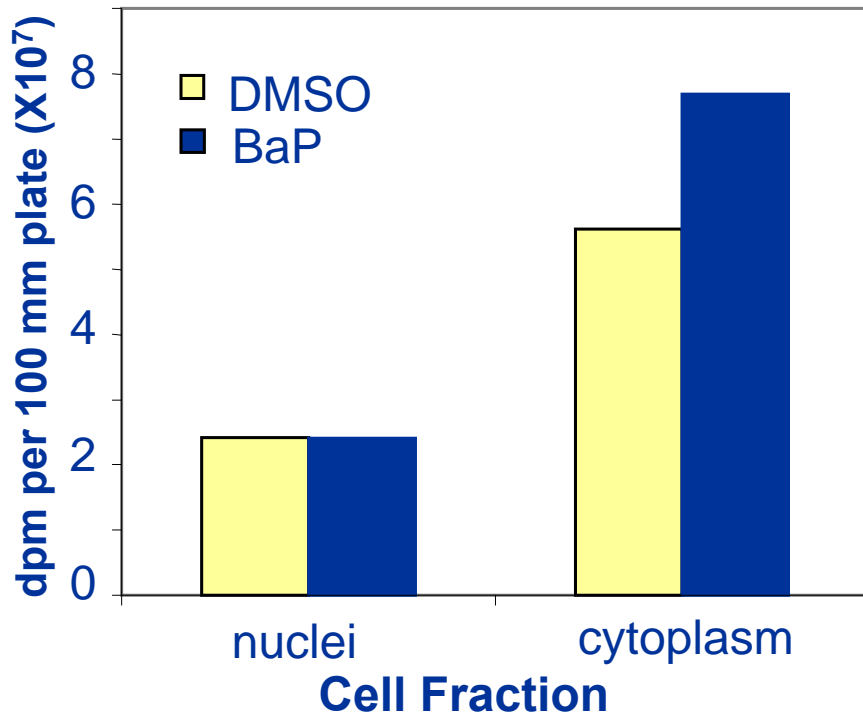
# Chromium speciation by HPLC/ICP-MS



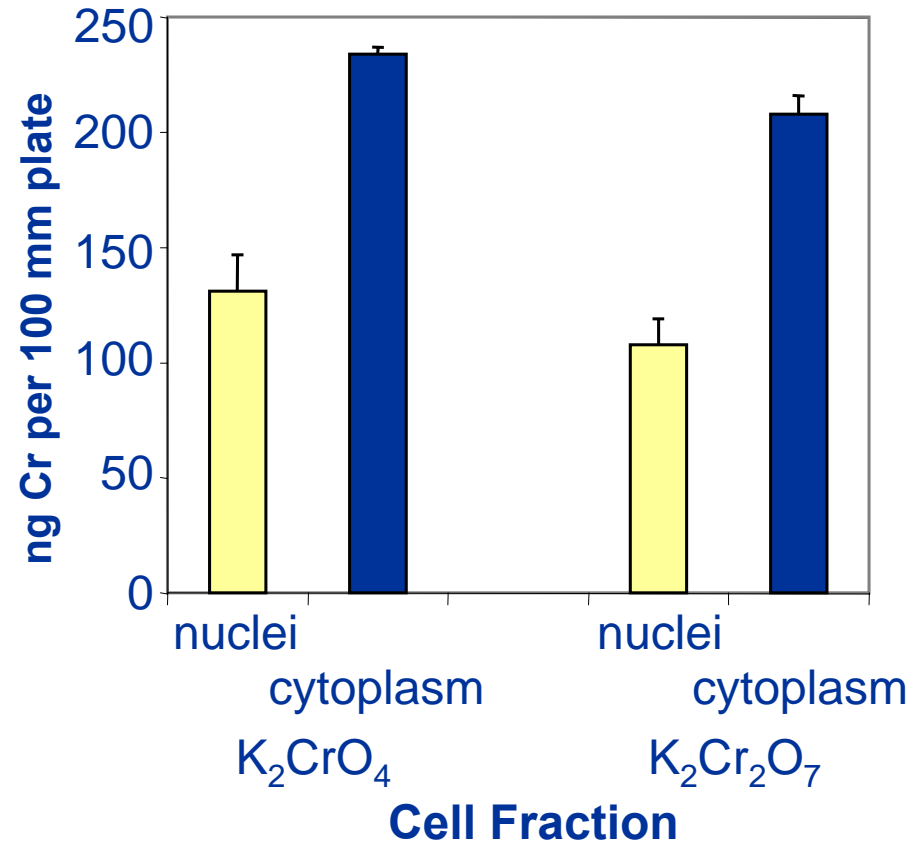


# Chromium distribution to nuclei and cytoplasm

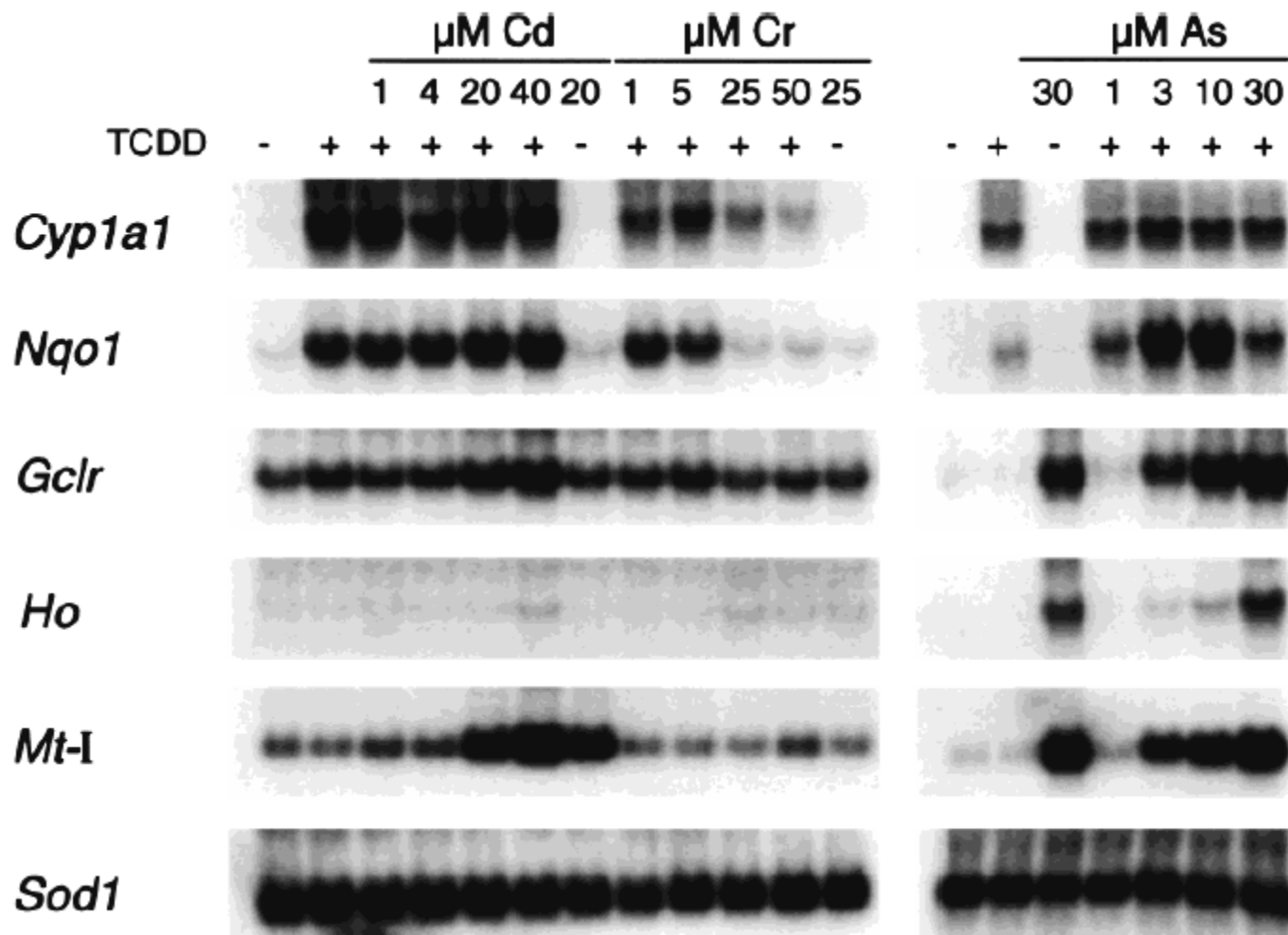
## $^{51}\text{Cr}$ Uptake



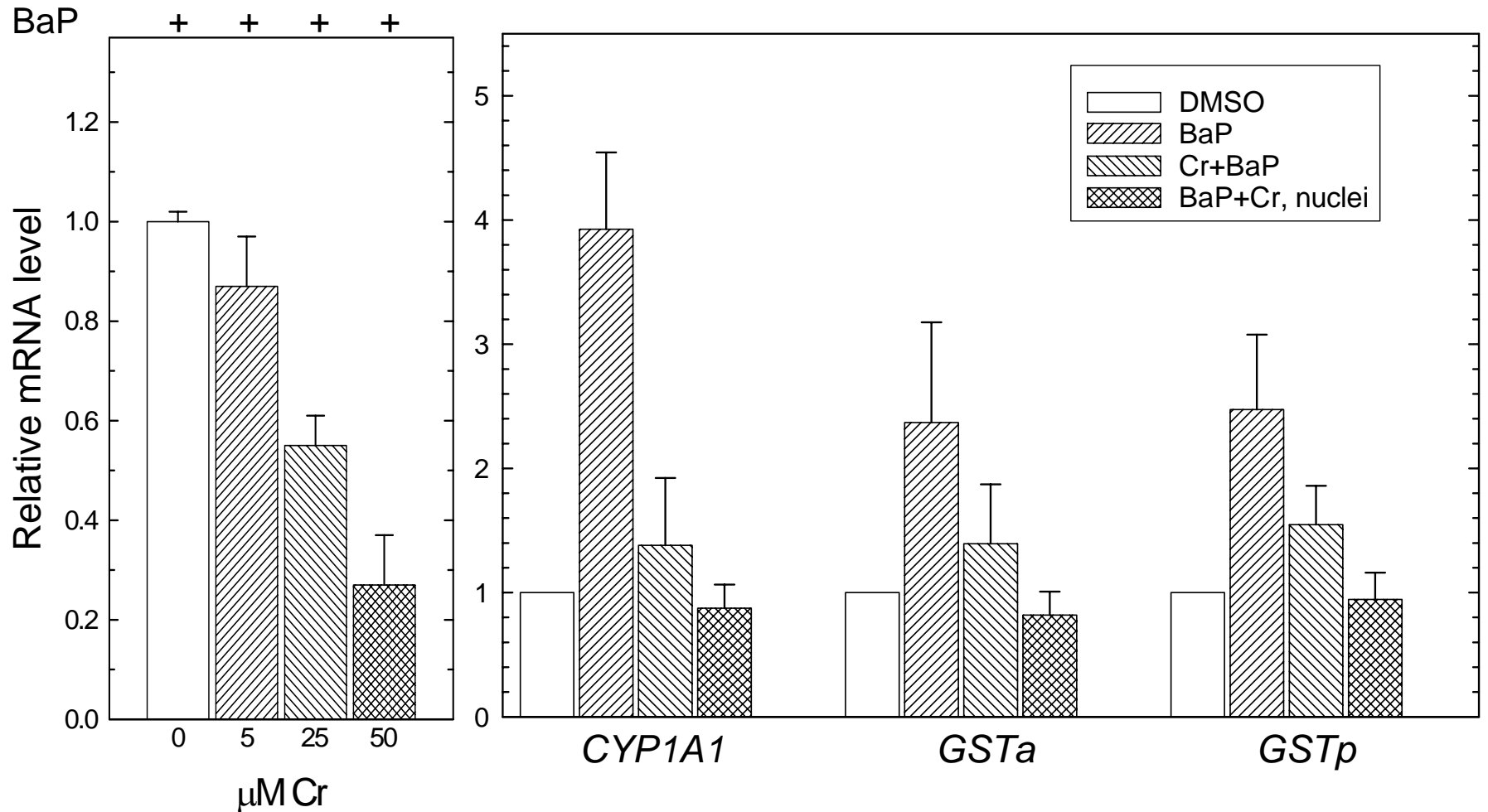
## ICP-MS



## As, Cd and Cr affect AHR-dependent gene expression differently in Hepa-1 cells

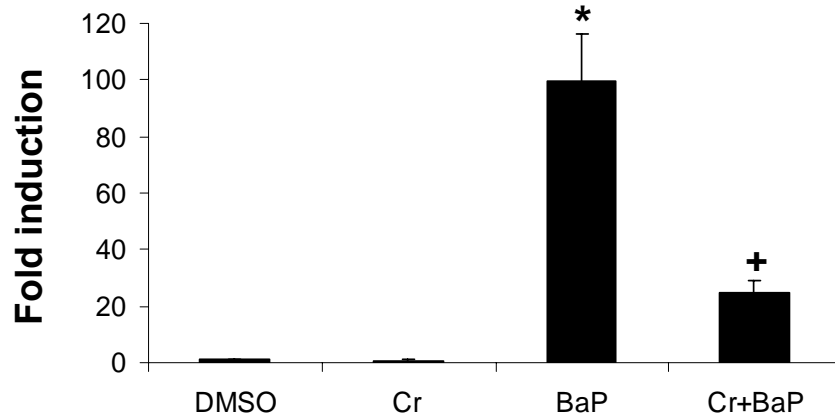


# The effect of Chromium on AHR-dependent gene expression is transcriptional



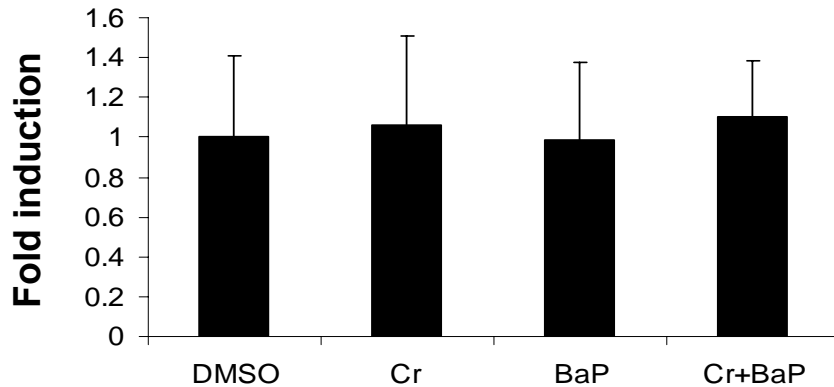
# Cr represses only inducible gene expression

## B[a]P-induced CYP1A1 mRNA in Human HepG2 cells

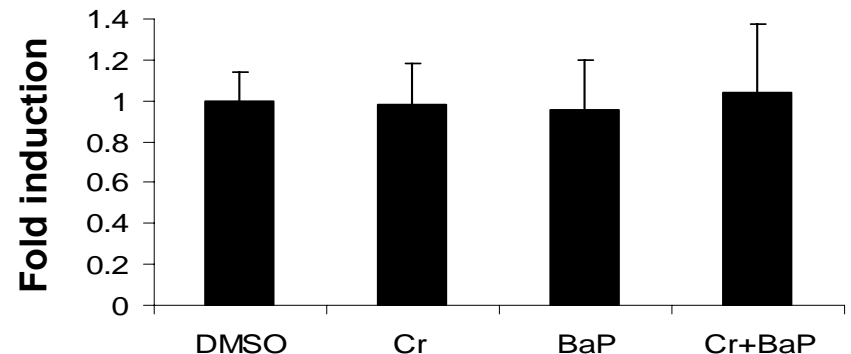


BaP: Benzo[a]pyrene (5  $\mu$ M, 8h)  
Cr: chromium (50  $\mu$ M, 9h)  
Cr+BaP: Cr (50  $\mu$ M, 1h) + BaP (5  $\mu$ M, 8h)  
\*  $p < 0.01$  Vs DMSO, +  $p < 0.01$  Vs BaP

### CYP1A1

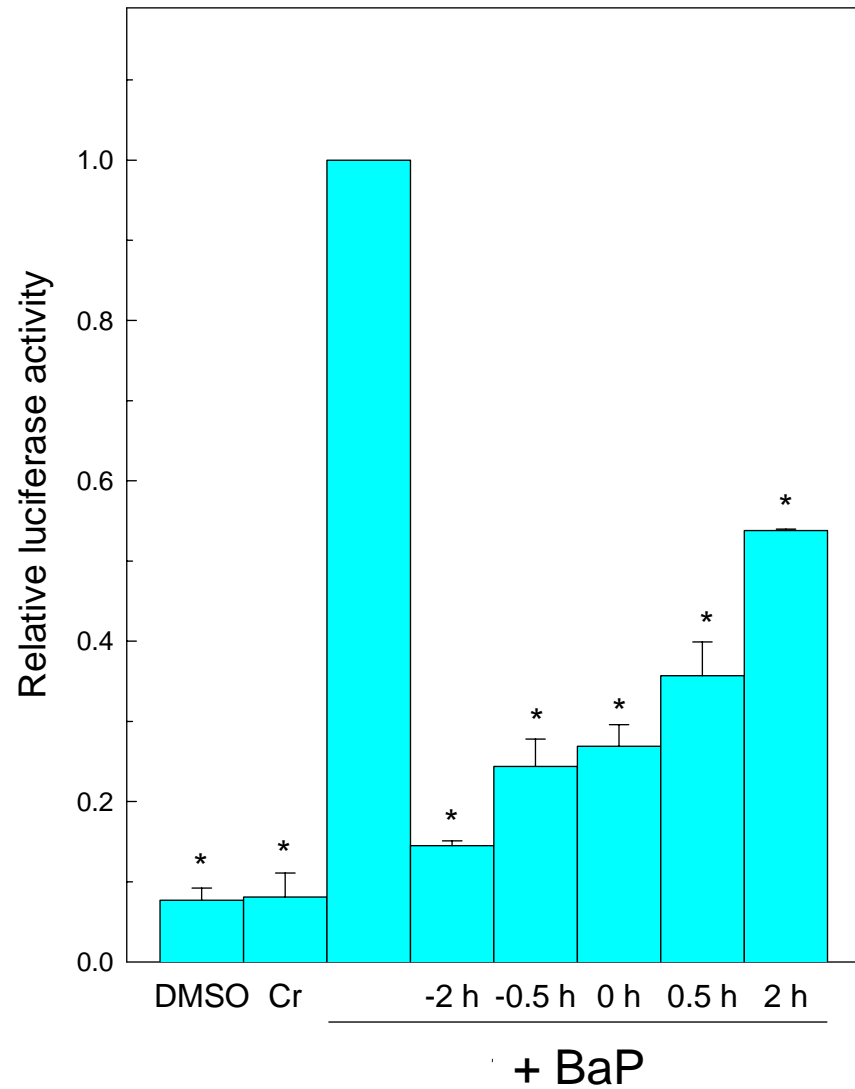


### CYP1A2

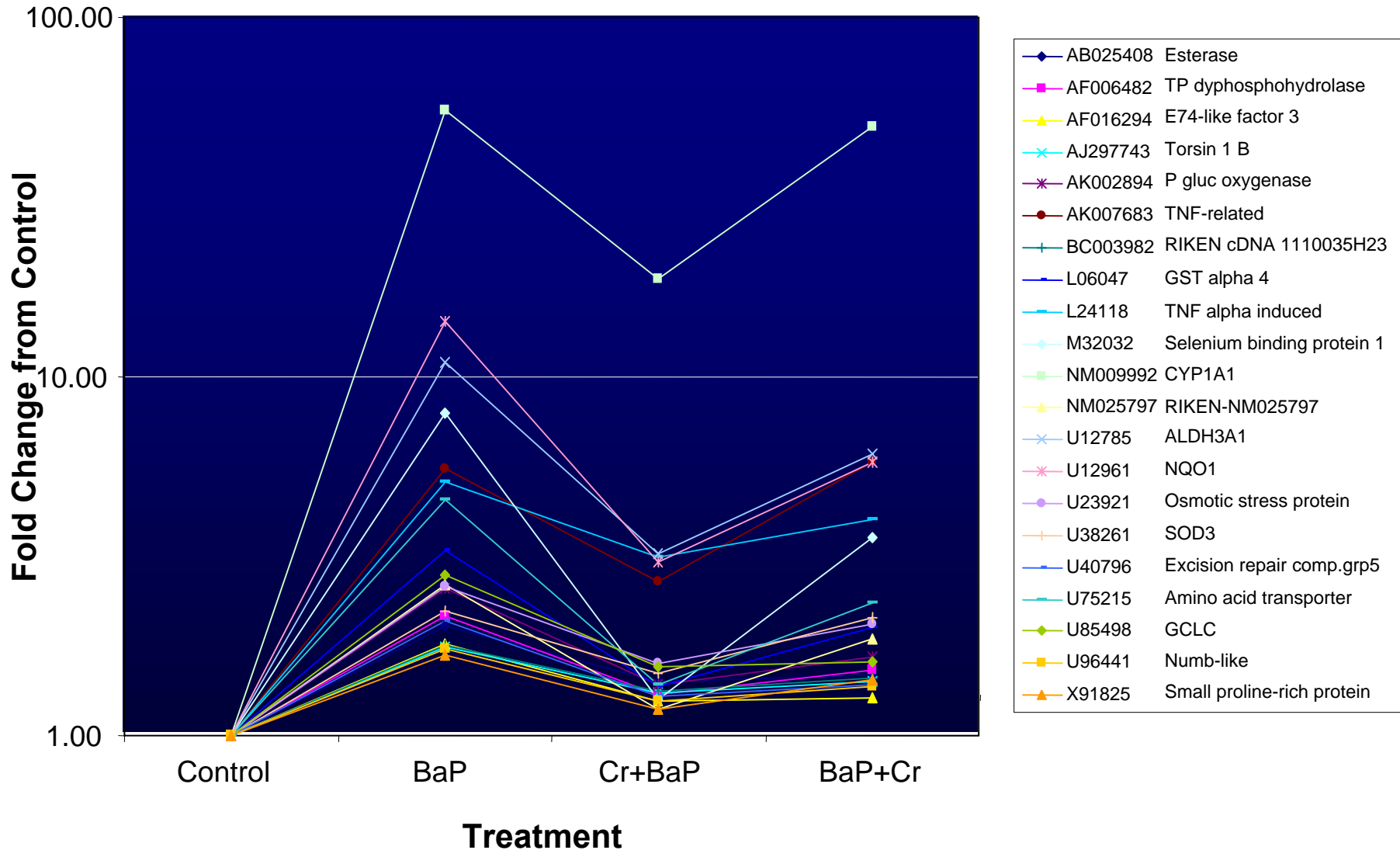


### CYP1B1

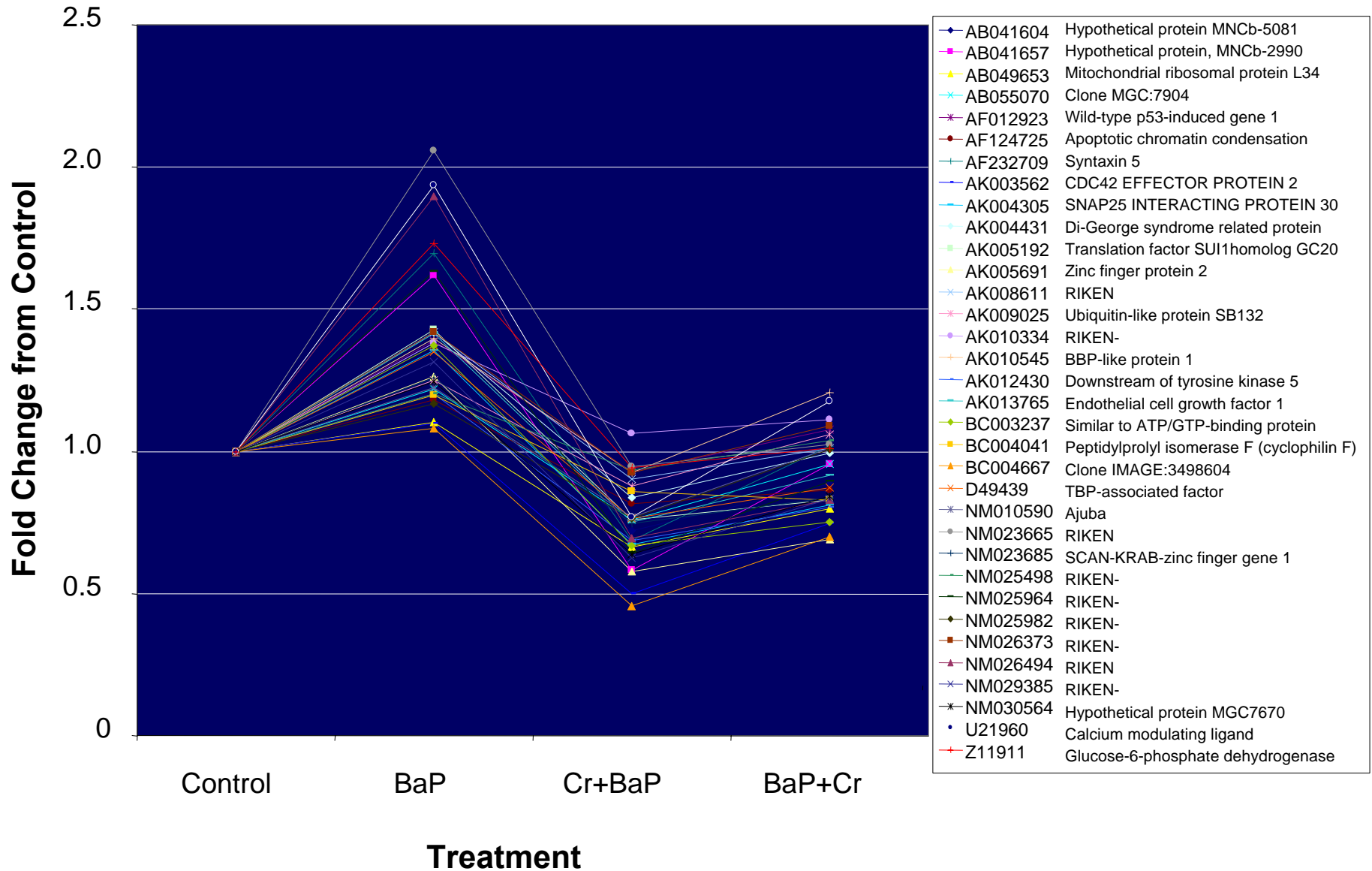
# Cr is less effective if added after AHR activation



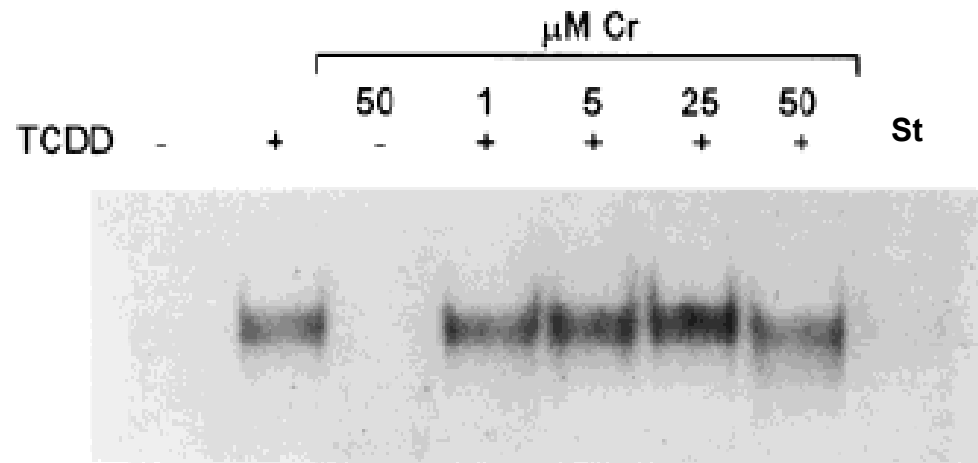
# Cr affects the expression profiles of BaP-induced genes



# Profiles of Other BaP-Induced Genes



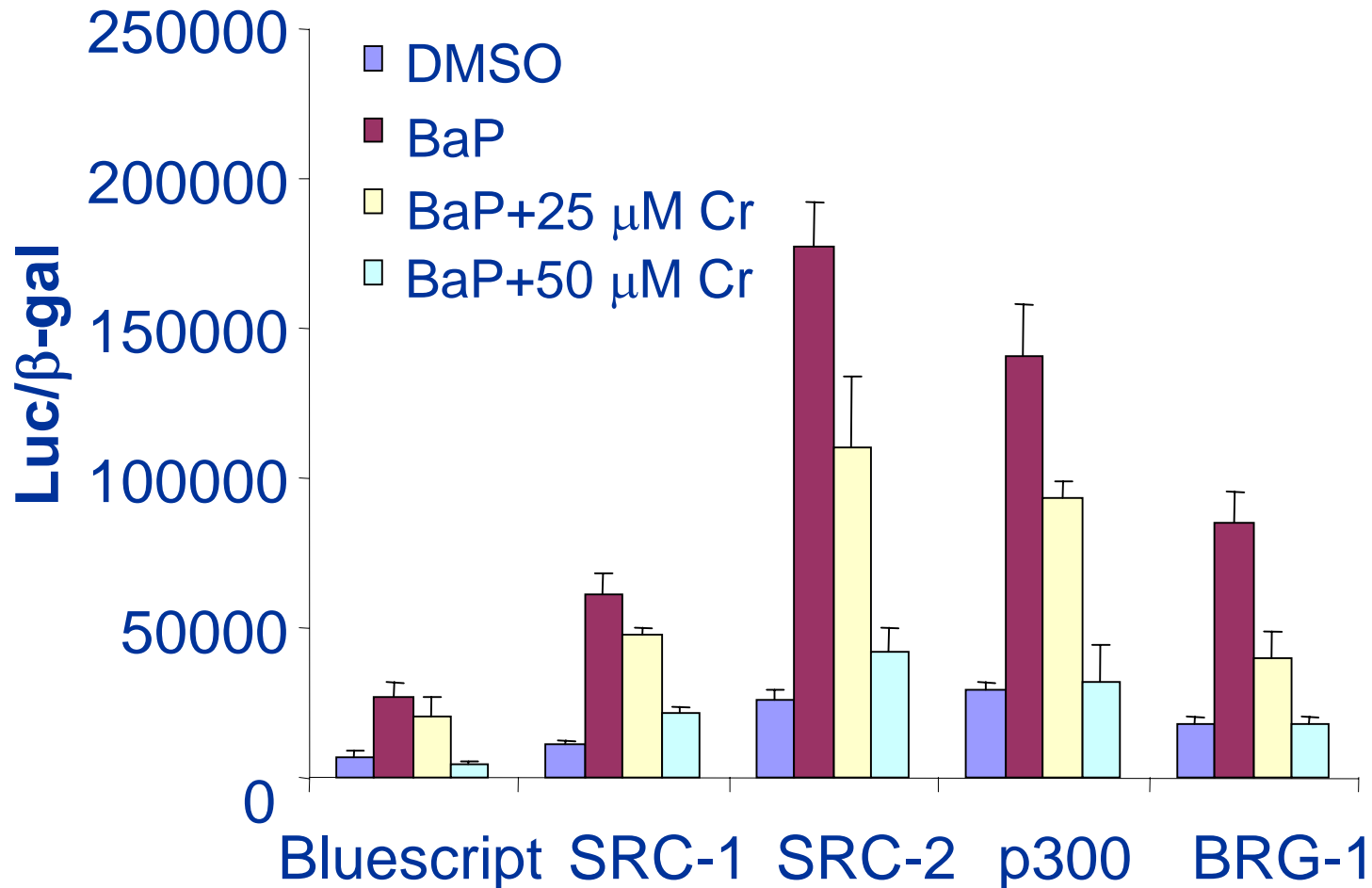
# TCDD activates the AHR to a DNA-binding configuration in the presence of Cr



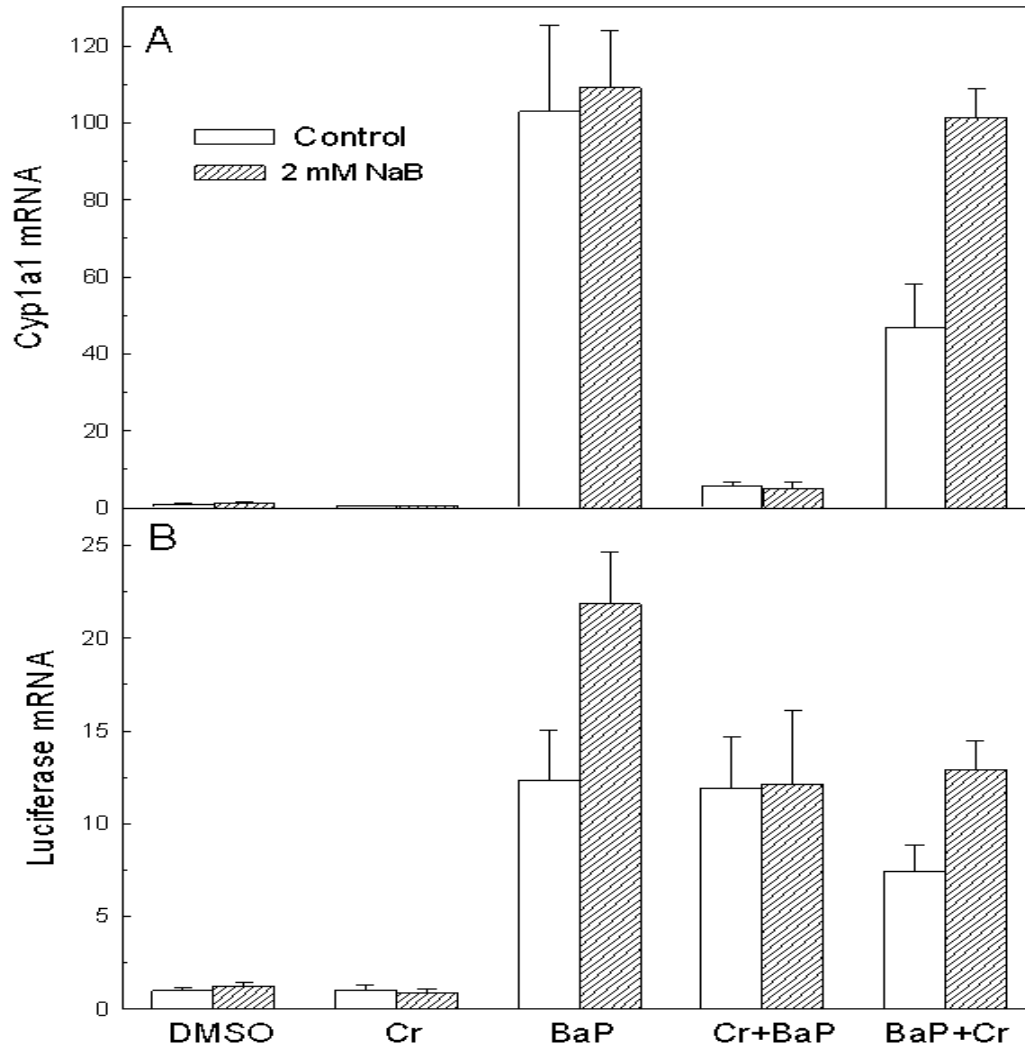
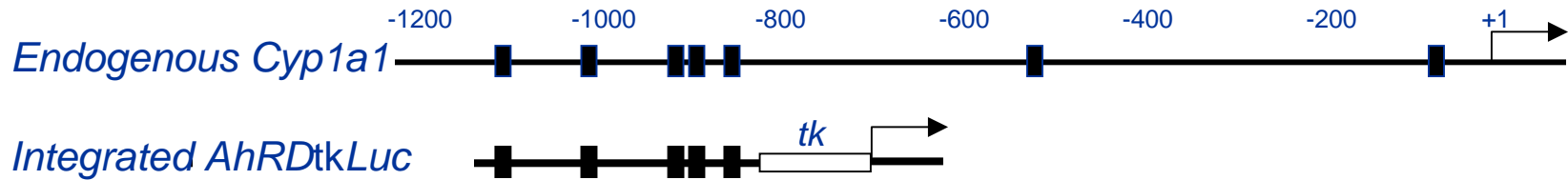
*In the presence of Cr AHR binds to DNA but transcription is inhibited*



## Several co-regulators increase BaP-induced gene expression and their effect is blocked by Cr

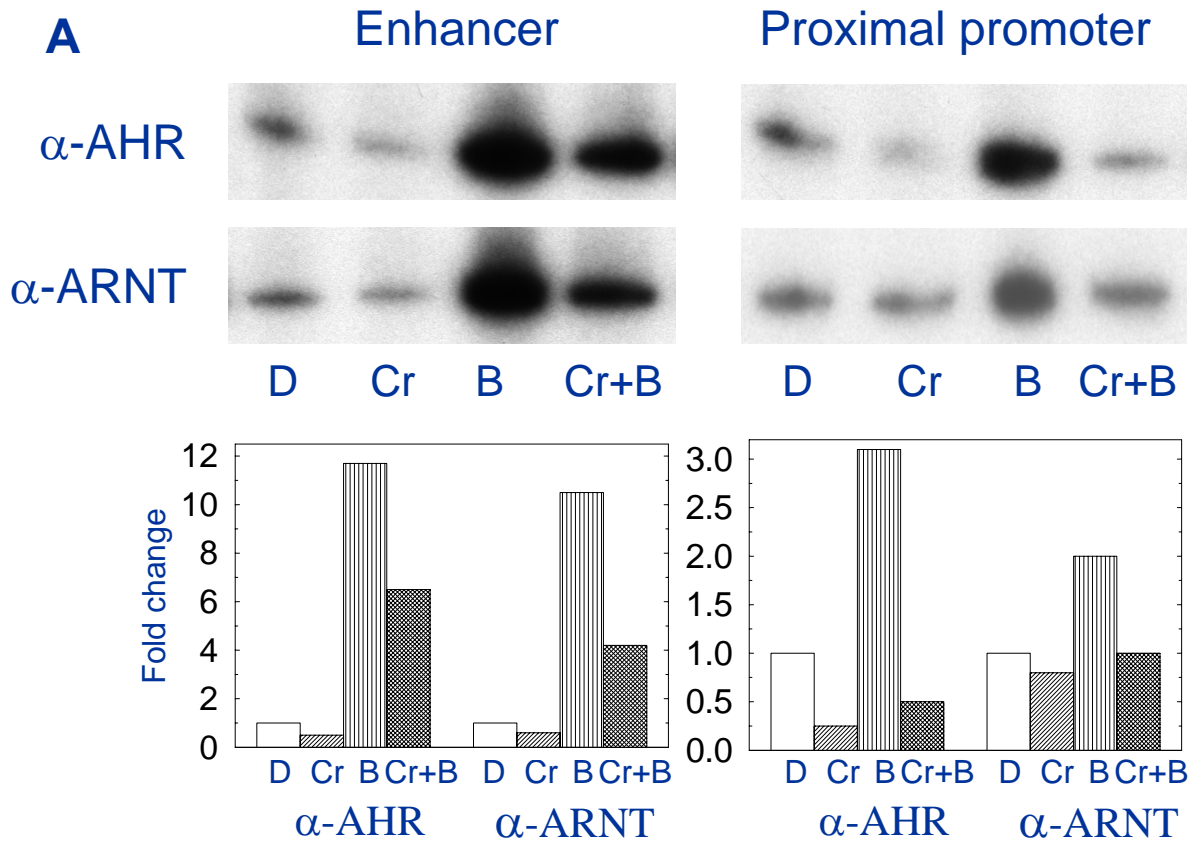
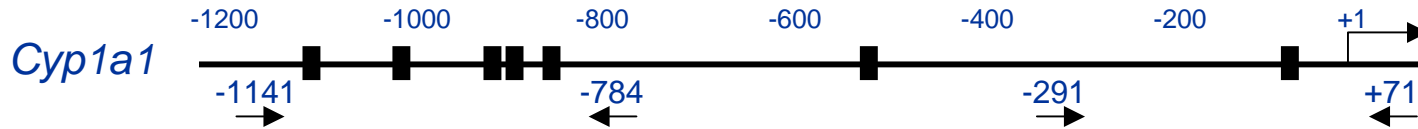


***Chromium interferes with co-activator recruitment and chromatin structure***

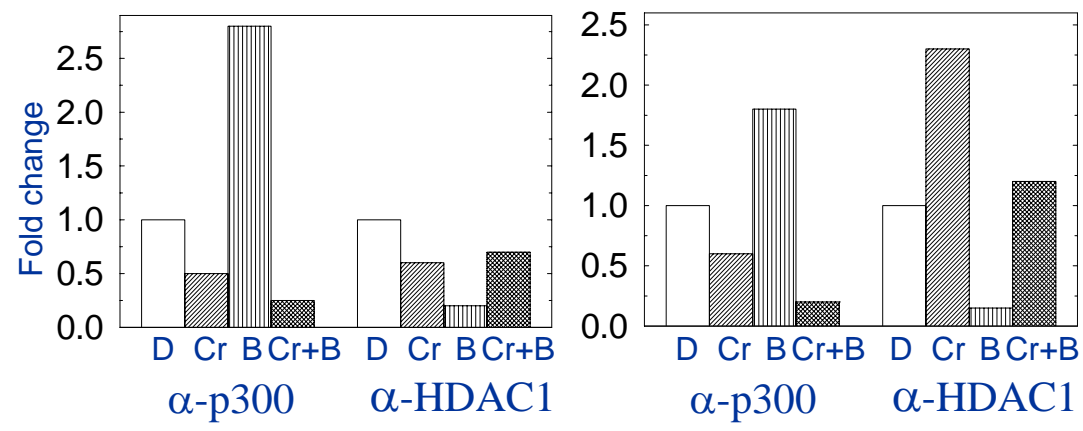
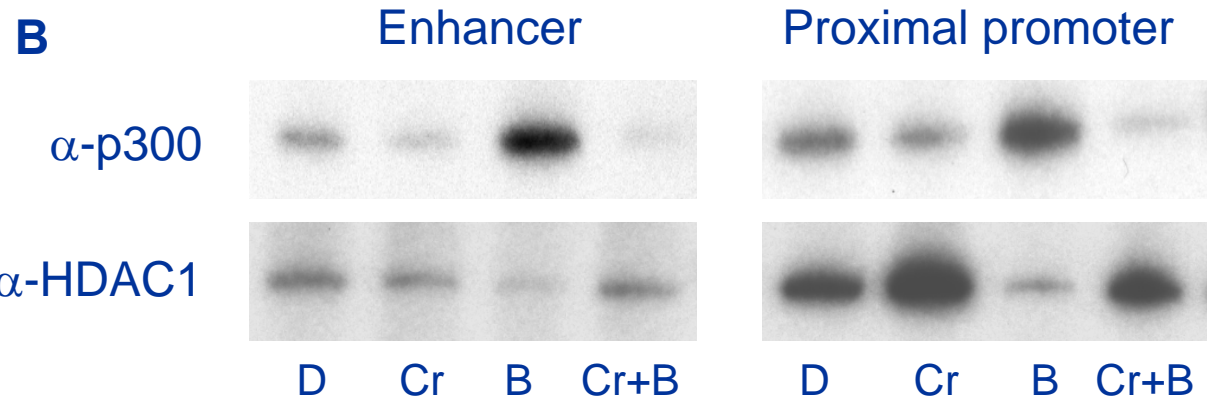
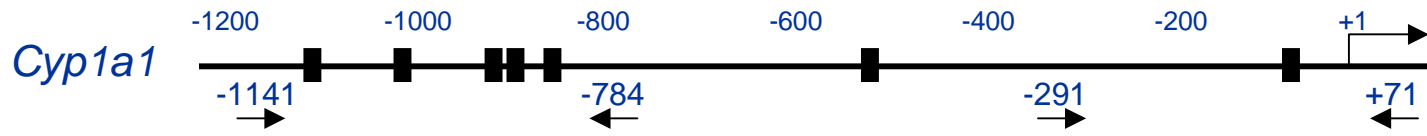


**NaB blocks the Inhibition by Cr of BaP-induced gene expression .... But inhibition takes place at the level of the immediate promoter**

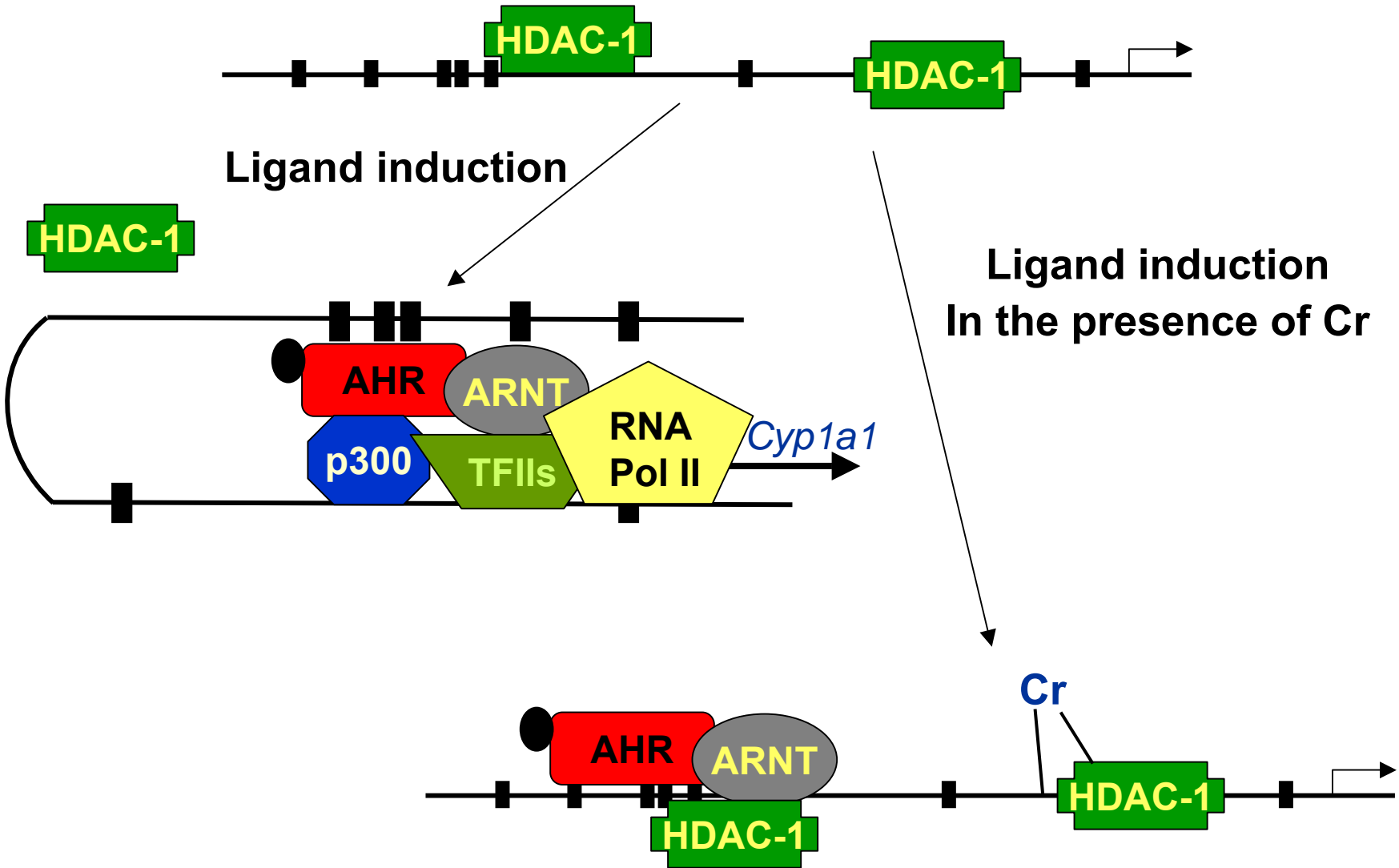
# ChIP for AHR and ARNT



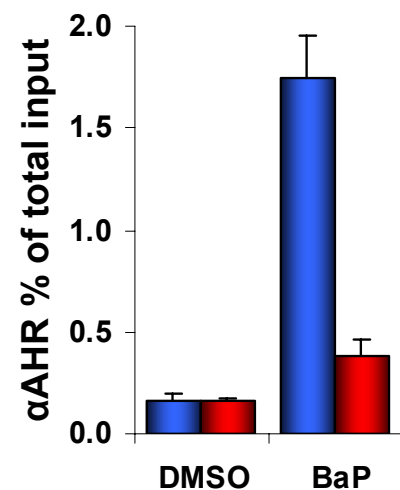
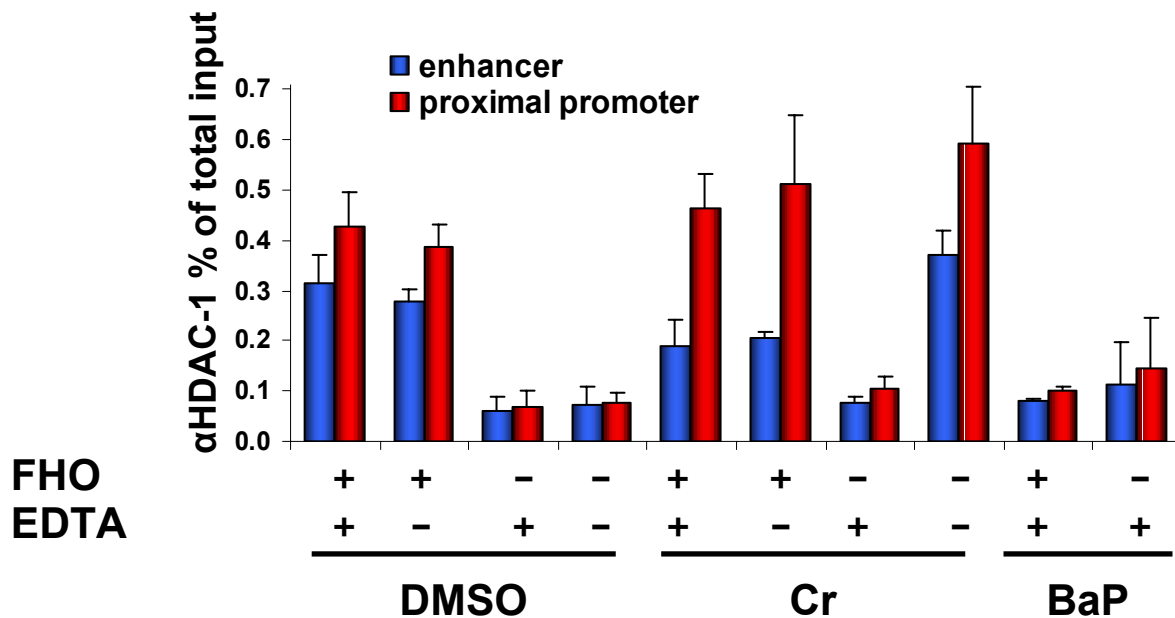
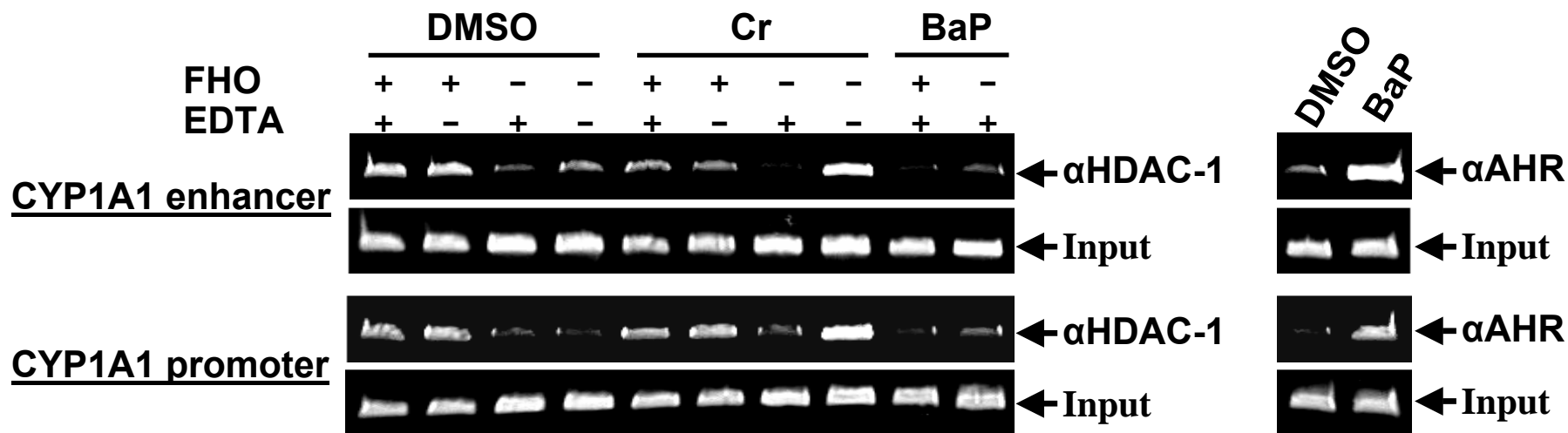
# ChIP for p300 and HDAC-1



# Effect of Cr on AHR-inducible genes

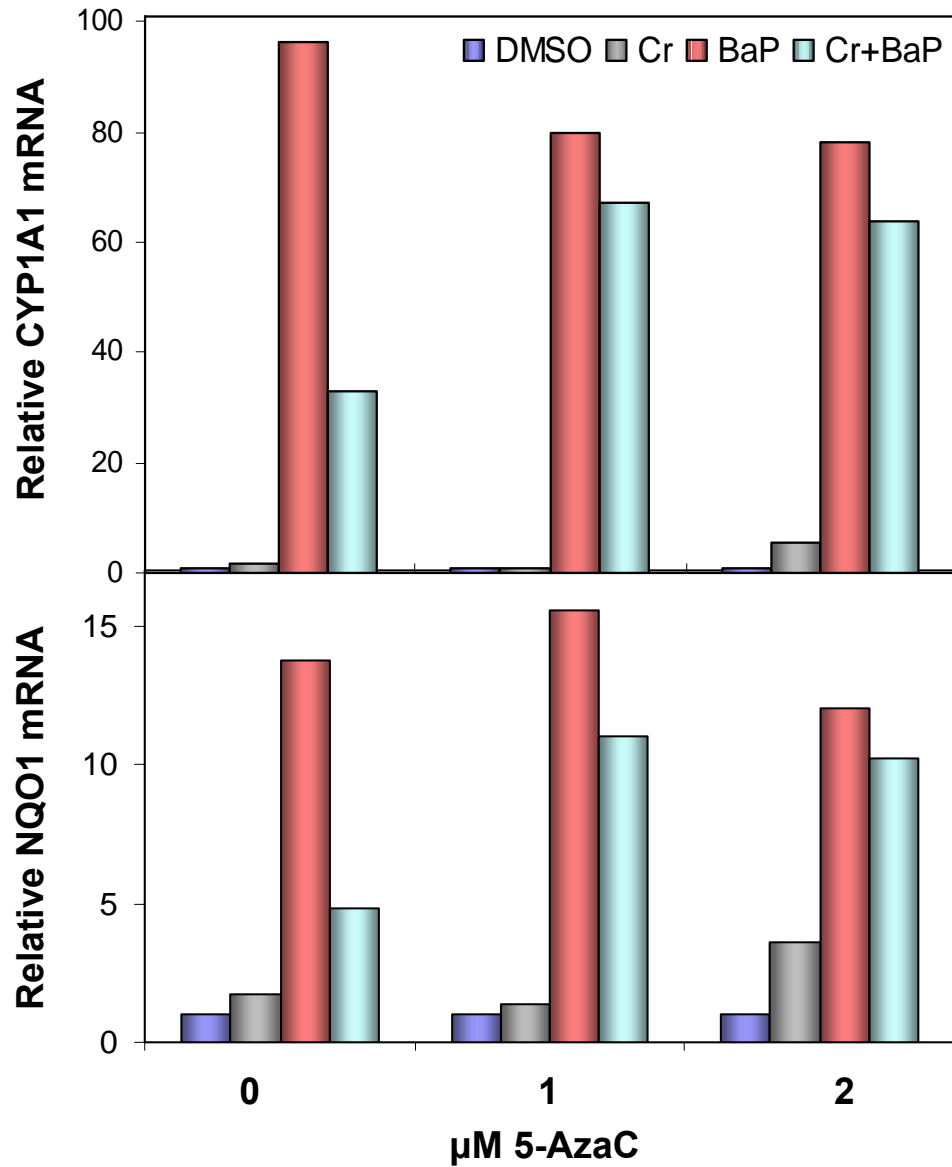


# Cr crosslinks HDAC-1 to the Cyp1a1 promoter in Hepa-1 cells



50  $\mu$ M Cr, 2h; 5  $\mu$ M BaP, 2h  
n=3

# DNA demethylation by 5-azacytidine inhibits Cr effects



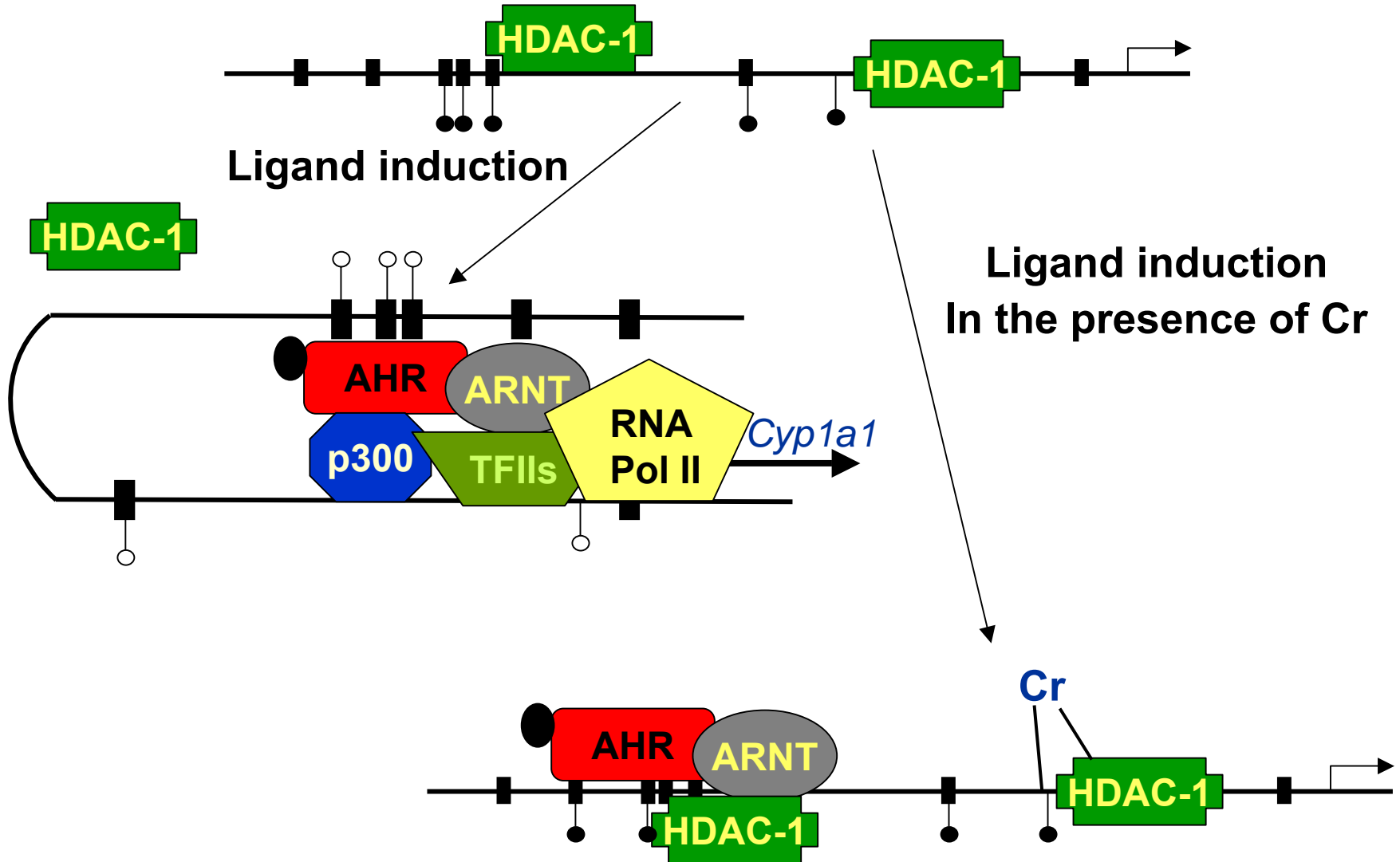
5AzaC: 56h

BaP: 5 μM, 8h

Cr: 50 μM, 9h

Cr+BaP: Cr (1h) + BaP (8h)

# Effect of Cr on AHR-inducible genes





# Conclusions and Questions

Cr inhibits *BaP inducible* gene expression by keeping HDAC-1 bound to chromatin and derailing methylation/demethylation patterns in promoters

Does chromium affect epigenetic imprinting?

How generalized is this mechanism?



**Kathy Tepperman**



**Yu-Dan Wei**



**Ming-ya Huang**



**Michael Schnekenburger**

***(R01 ES10807, P42 ES04908)***

# GSH depletion or replenishment does not affect significantly inhibition by Cr of the expression of an AHR reporter

