SUPERFUND
Basic Research Program

The University of Cincinnati - SBRP

Health Effects and Biodegradation of Complex Mixtures
P.I.: Dr. Paul Bishop

Arsenic and Cellular Responses to DNA Damage

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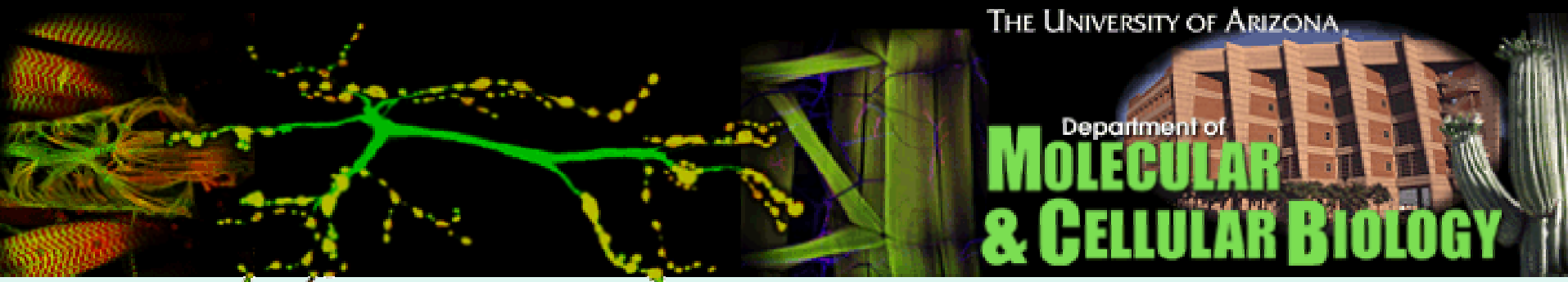

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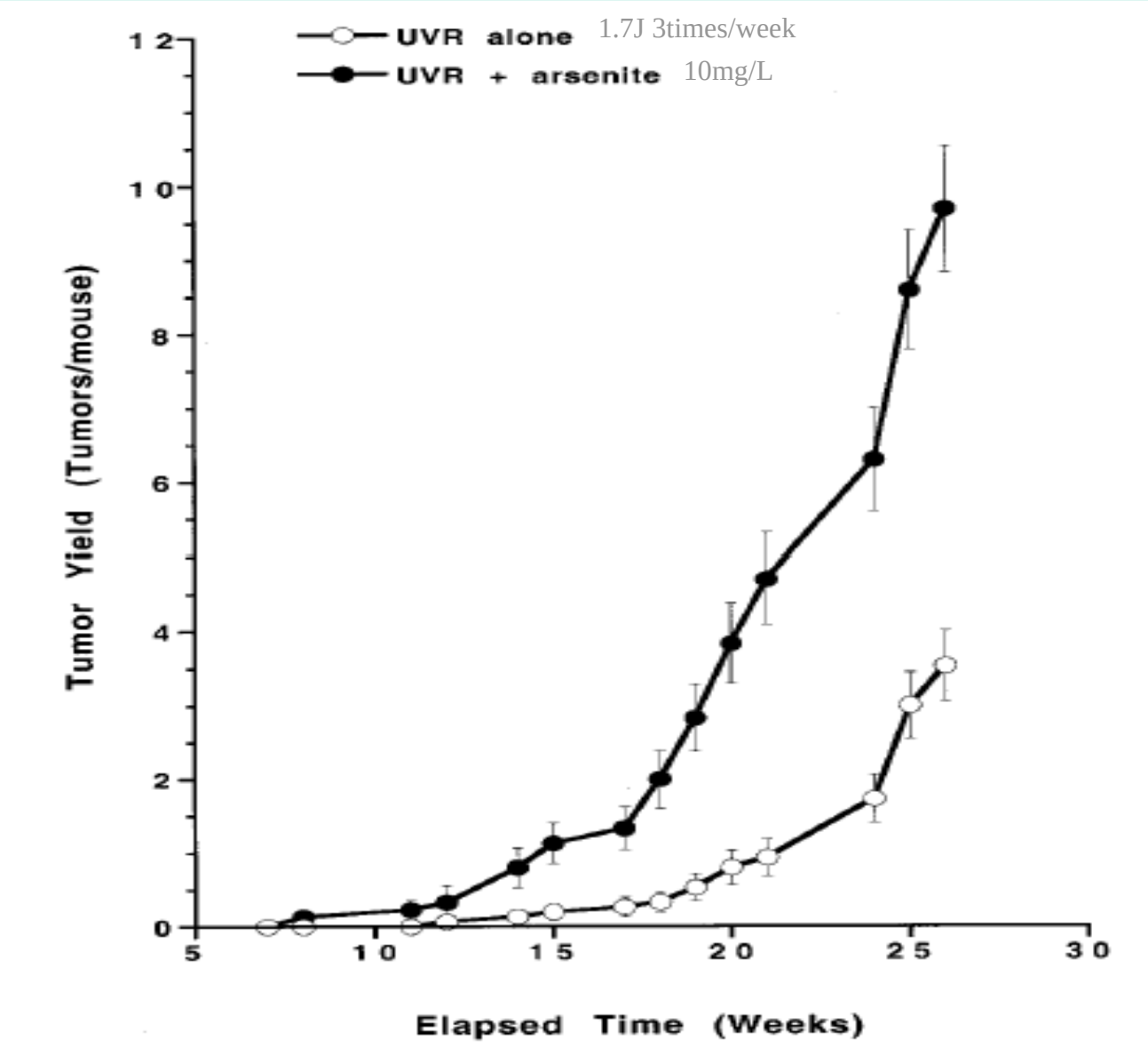
Arsenic Carcinogenicity

- **Well-established human carcinogen: skin, lung, bladder, kidney cancer**
- **Exposure pathway: ingestion, inhalation, dermal**
- **Negative in most animal carcinogenesis bioassays.**

BACKGROUND

- **A naturally occurring metalloid; frequently found in ground water**
- **Human exposures from: smelting of metals, combustion of fuels, use of pesticide**
- **Inorganic (O, Cl, S): arsenite(+3) and arsenate(+5)**
- **Metabolized to methylated forms**

Arsenite is a Co-carcinogen with UV for Mouse Skin



Rossman TG et al Toxicology and Applied Pharmacolgoy (2001) 176, 64-71

Mechanism of Carcinogenicity

- Arsenic is poorly mutagenic, but clastogenic
- Co-mutagenic with other mutagens
  - UV (Hartmann and Speit, 1996)
  - MNU (Li and Rossman, 1989)
  - Benzo(a)pyrene (Maier *et al.*, 2002)

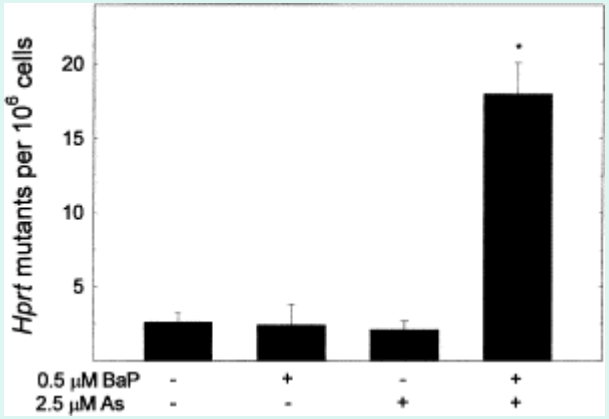
Hypotheses

Arsenic potentiates the action of other mutagens by:

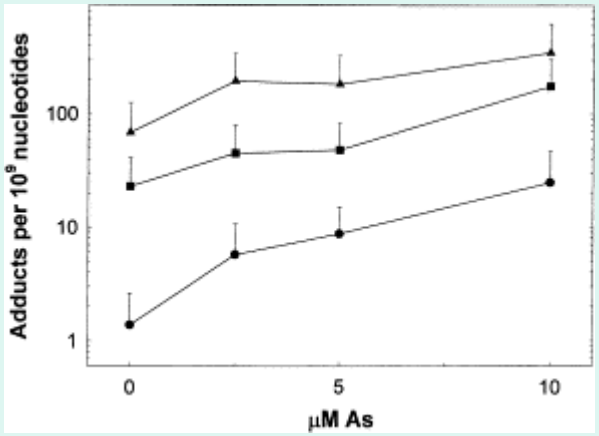
- **Inhibiting DNA repair**
- **Interfering with cell cycle checkpoints**
- **Altering gene expression**

Arsenic enhances mutagenesis and BaP adduct formation in mouse Hepa-1 cells

A. Maier, B. L. Schumann, X. Chang, **G. Talaska** and **A. Puga**



24 h treatment, 6-TG selection



1.0 uM BaP

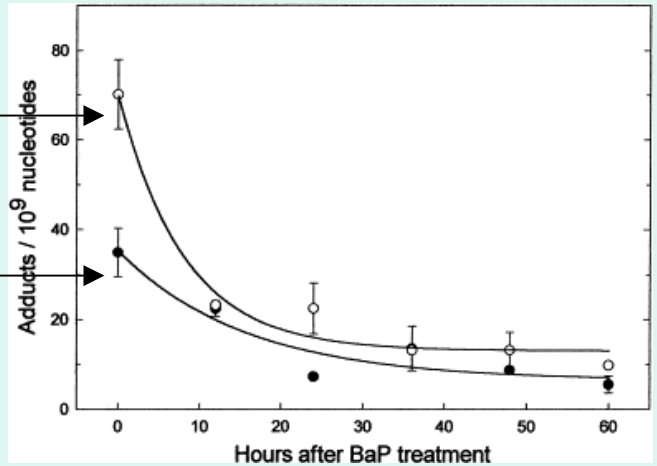
0.5 uM BaP

0.1 uM BaP

BaP adducts after 24 h treatment

0.5 uM BaP + 2.5 uM As

0.5 uM BaP

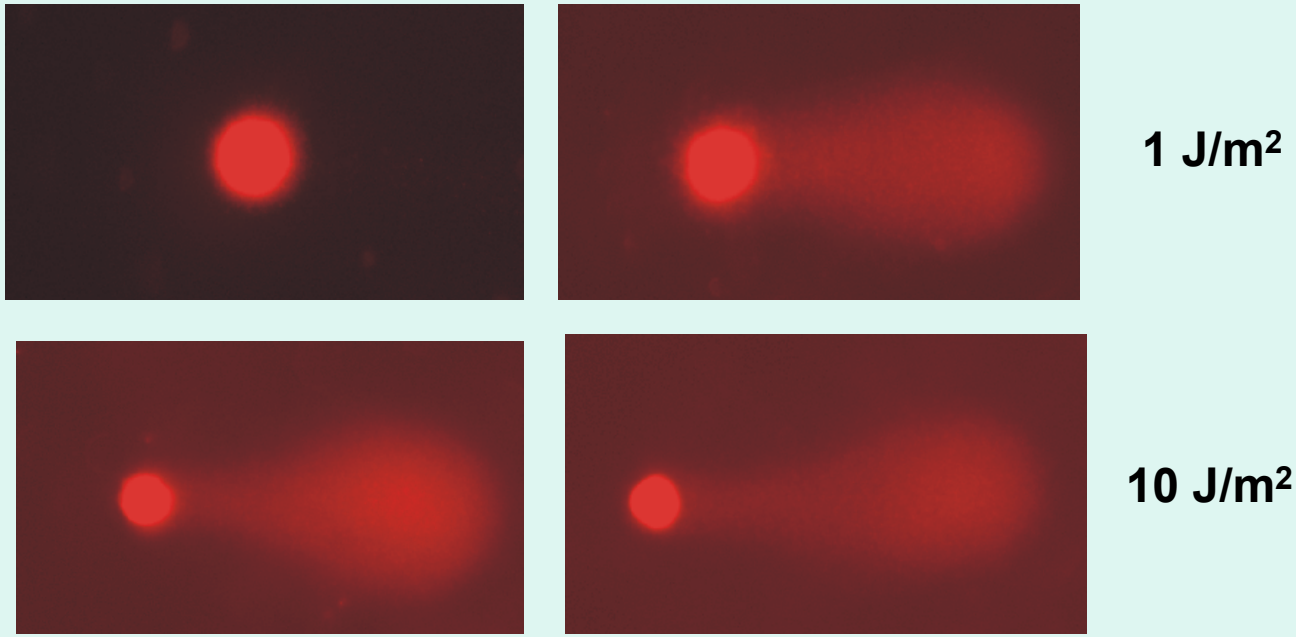


24 h pretreatment

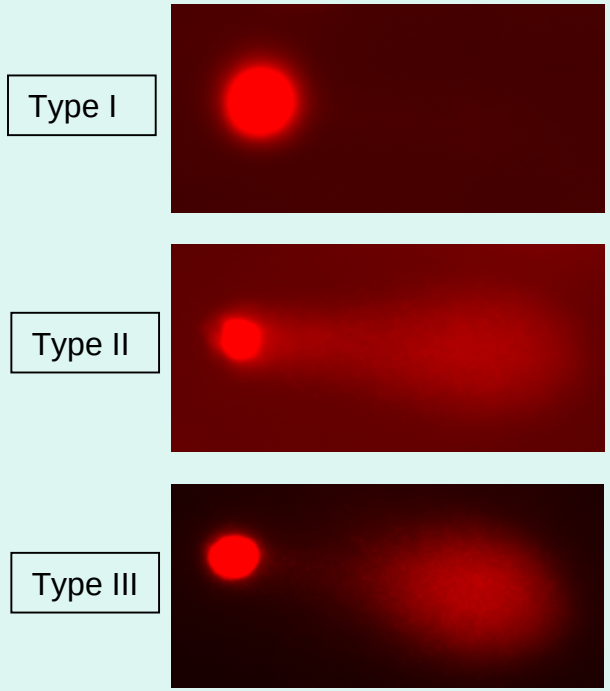
Does arsenite interfere with UV photoproduct removal?

Measure UV photoproducts with comet assay

1. **Embed cells in agarose**
2. **Treat cells with T4 endonuclease**
3. **Electrophorese**



Comet Assay with T4 Endonuclease



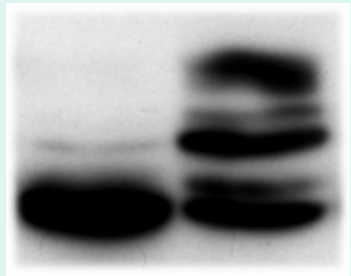
Type I: Without tails or with tail length less than 2 diameters.
Type II: The tails are connected with the nuclear, with tail width less than 2 diameters.
Type II: The tails are detached from the nuclear, with tail width more than 2 diameters.

		UV	As	UV+As
4h	I	0	100	0
	II	1	0	4
	III	99	0	96
8h	I	1	100	0
	II	13	0	0
	III	86	0	100
16h	I	4	100	0
	II	68	0	0
	III	29	0	100

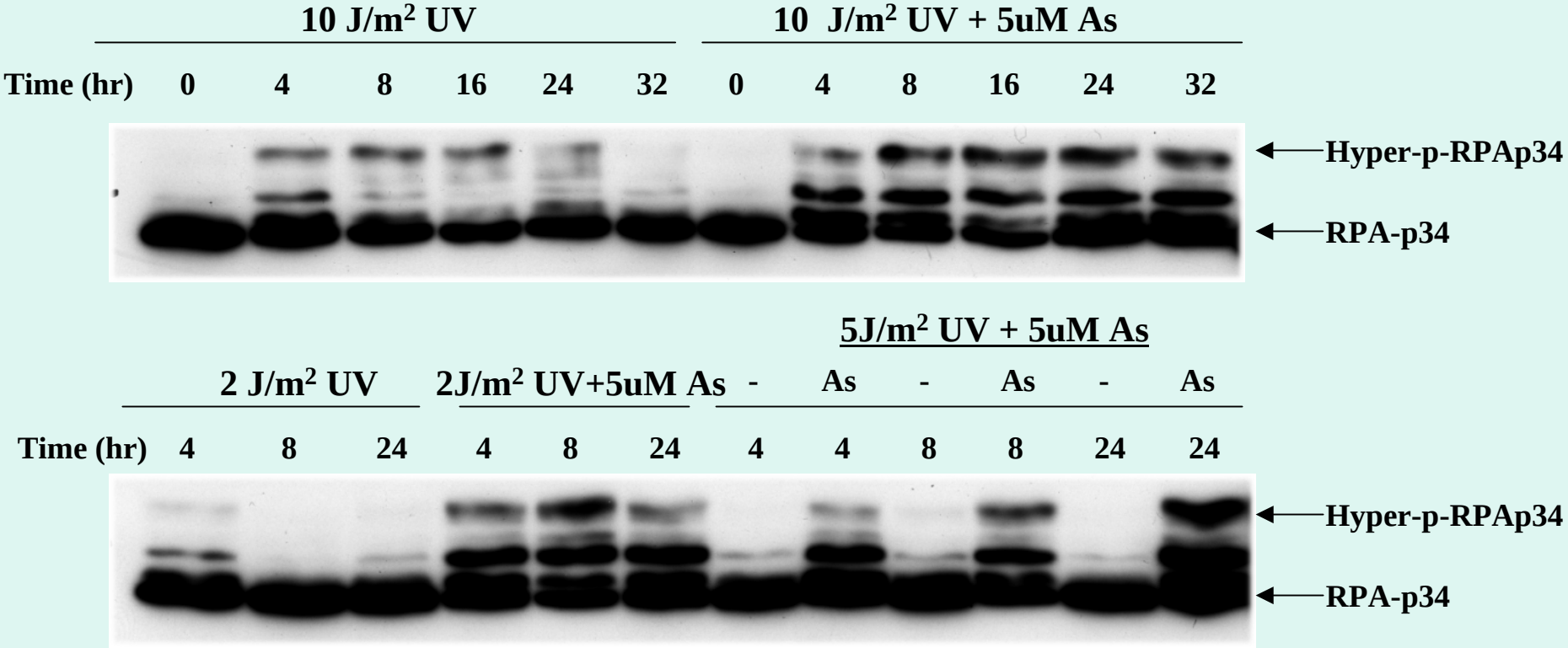
Does DNA damage signaling persist after UV in the presence of arsenite?

RPA-p34 phosphorylation as a DNA damage marker

- RPA: single-stranded DNA binding protein; 3 subunits: p70, p34 and p14
- RPA-p34 is phosphorylated in response to DNA damage: IR, UV, BPDE, etc.

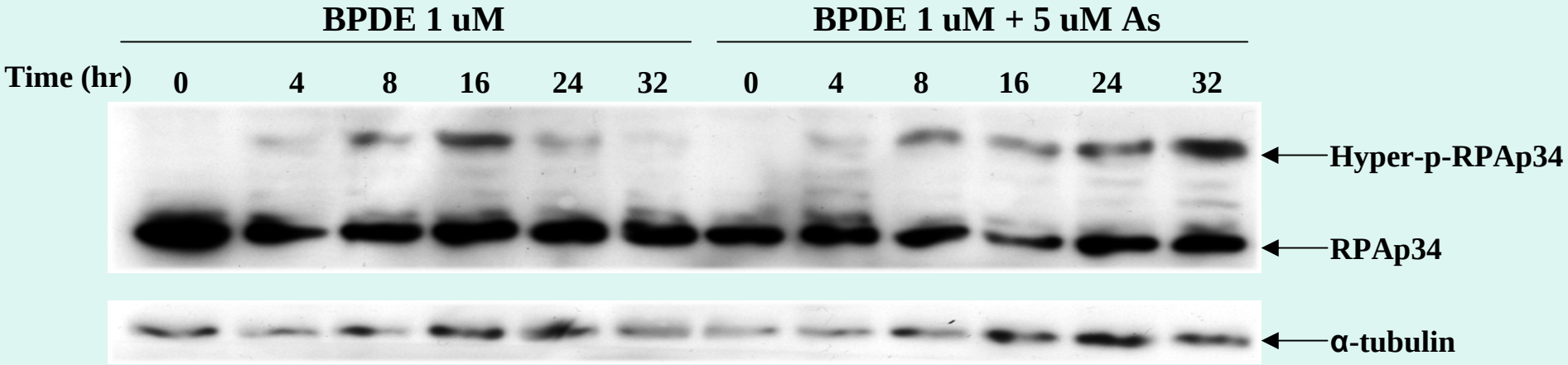


Arsenic enhanced and prolonged RPA phosphorylation upon UV irradiation



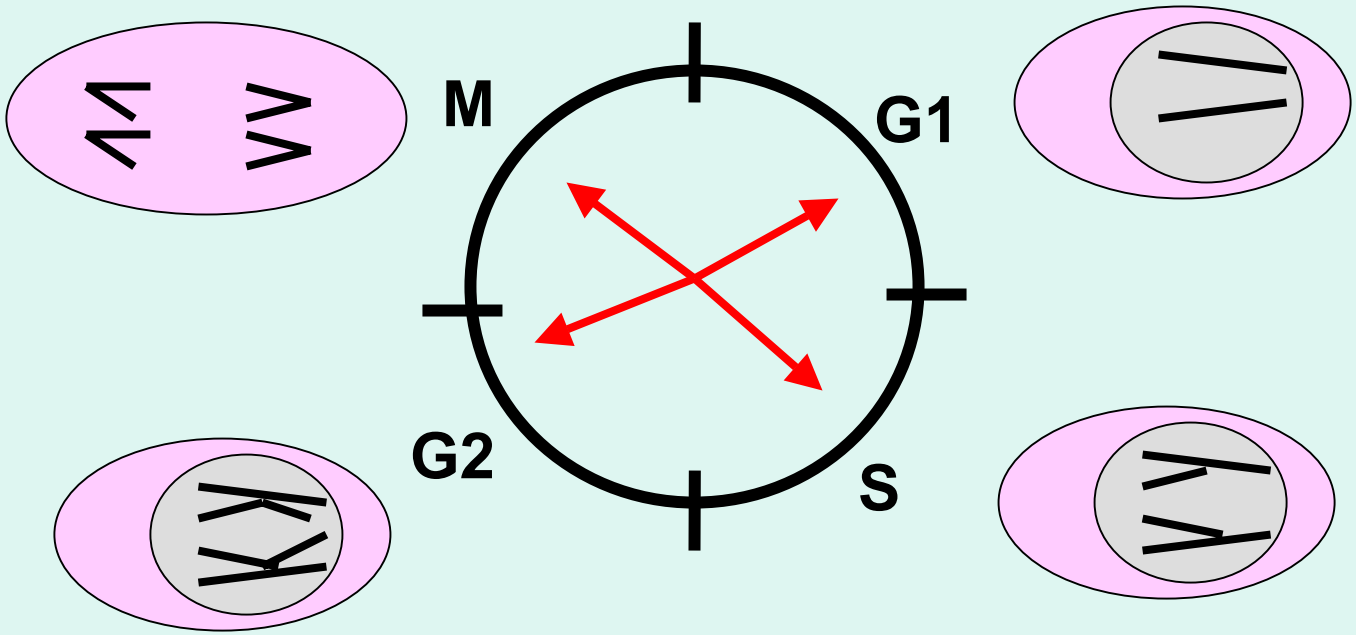
Hela cells were synchronized at G_1/S phase boundary with 3 uM aphidicolin for 17 hours. After release, cells were treated with 5 uM As for 3 hours, then with 2, 5, or 10 J/m^2 UV in PBS. Complete medium with or without arsenic was added back.

Arsenic enhances RPA phosphorylation upon BPDE treatment

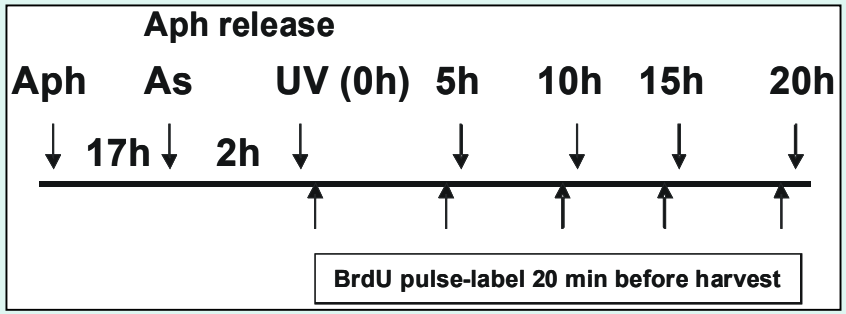


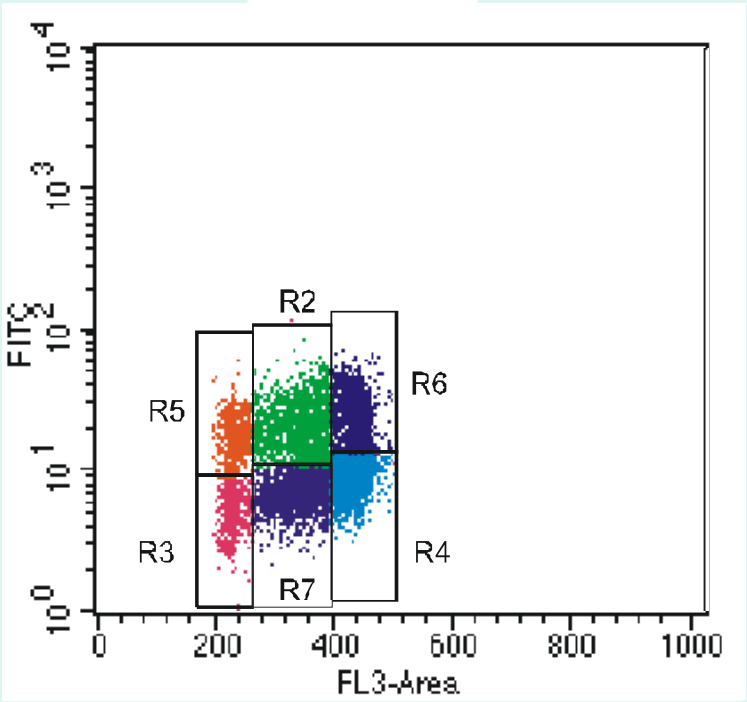
Hela cells were preincubated with 5uM As for 3 hours, then treated with 1uM BPDE in PBS for 30 minutes at 37C. Then fresh complete medium with or without arsenic was added back.

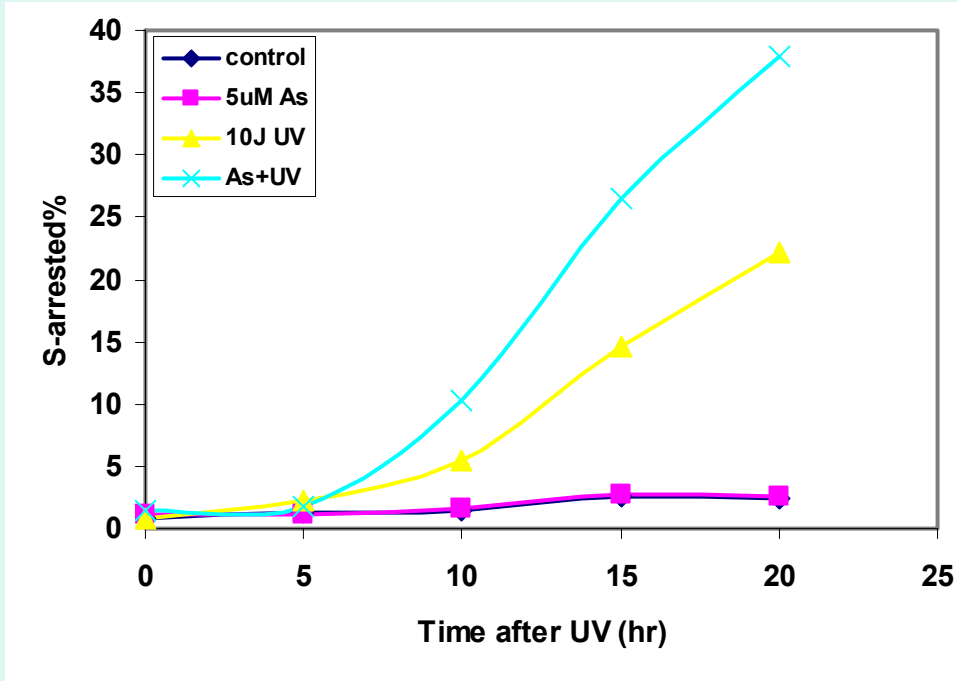
Does arsenic interfere with cell cycle checkpoints?



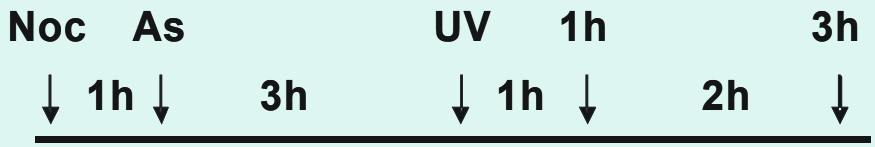
S-phase checkpoint remains intact in arsenic-treated cells

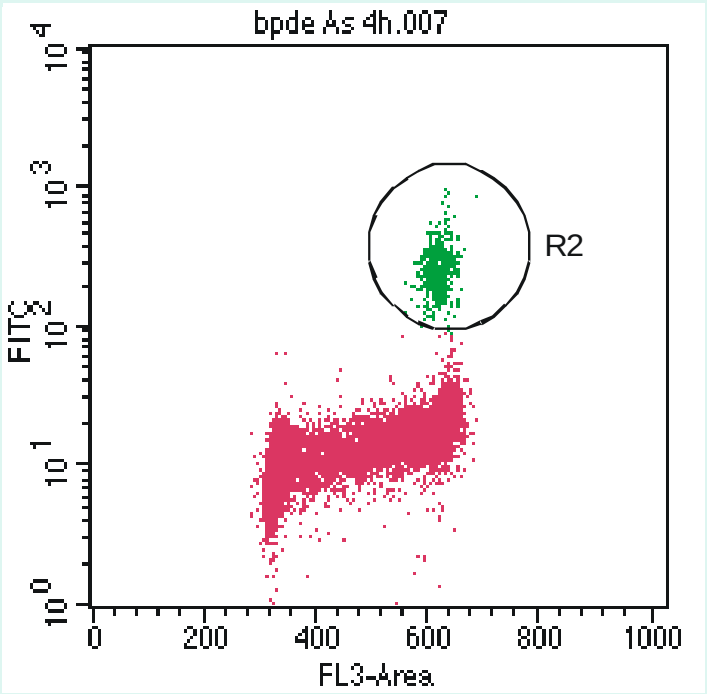


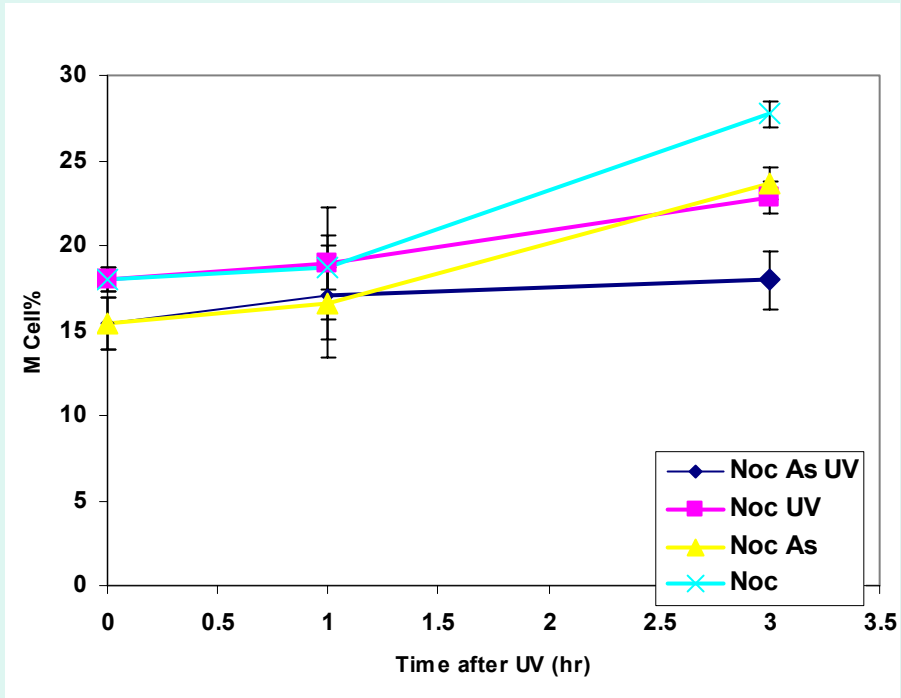




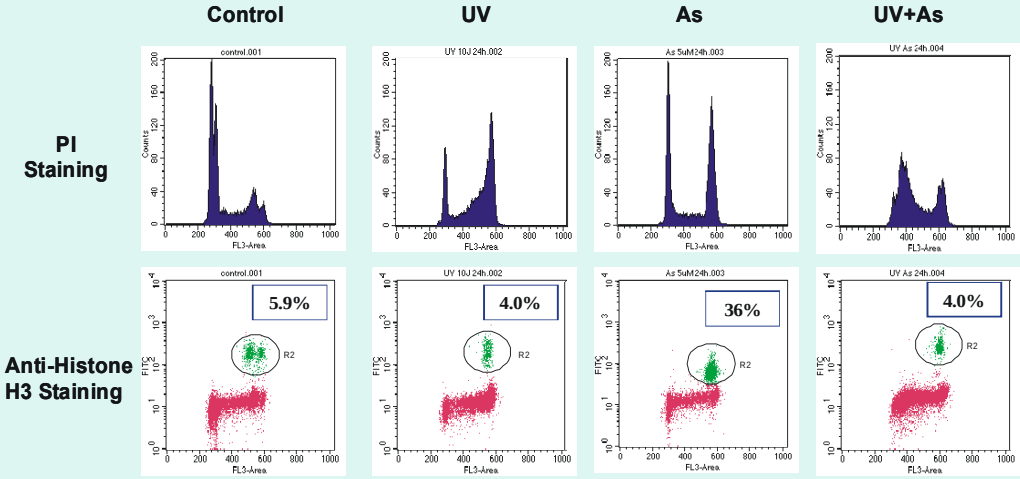
G2/M checkpoint remains intact in arsenic-treated cells

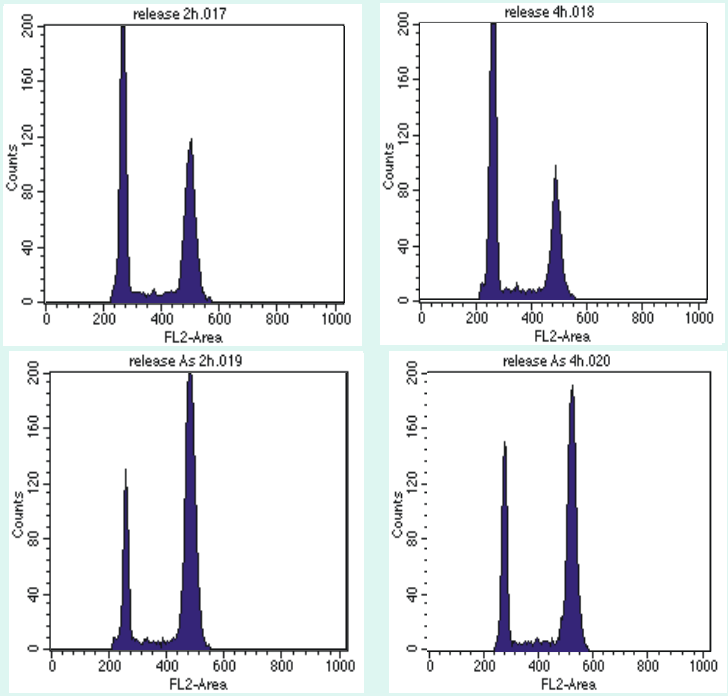






Arsenic alone arrests cells in M-phase



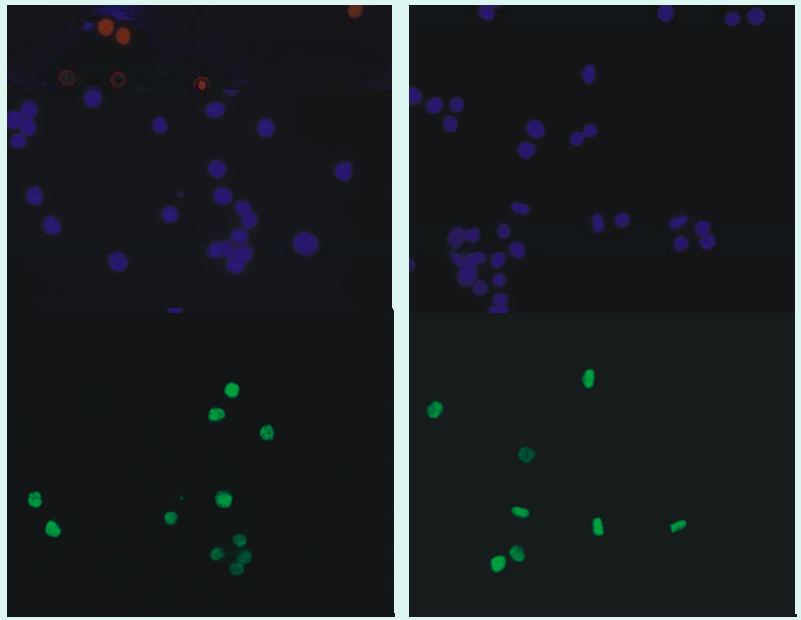


Noc 0.6 uM

As 5 uM

DAPI

p-His H3 (mitotic Marker)



Does arsenic alter gene expression?

Puga and Xia

Measure up-regulation and down-regulation of gene expression by microarray analysis

5 uM BaP

2 uM As

5 uM BaP + 2 uM As

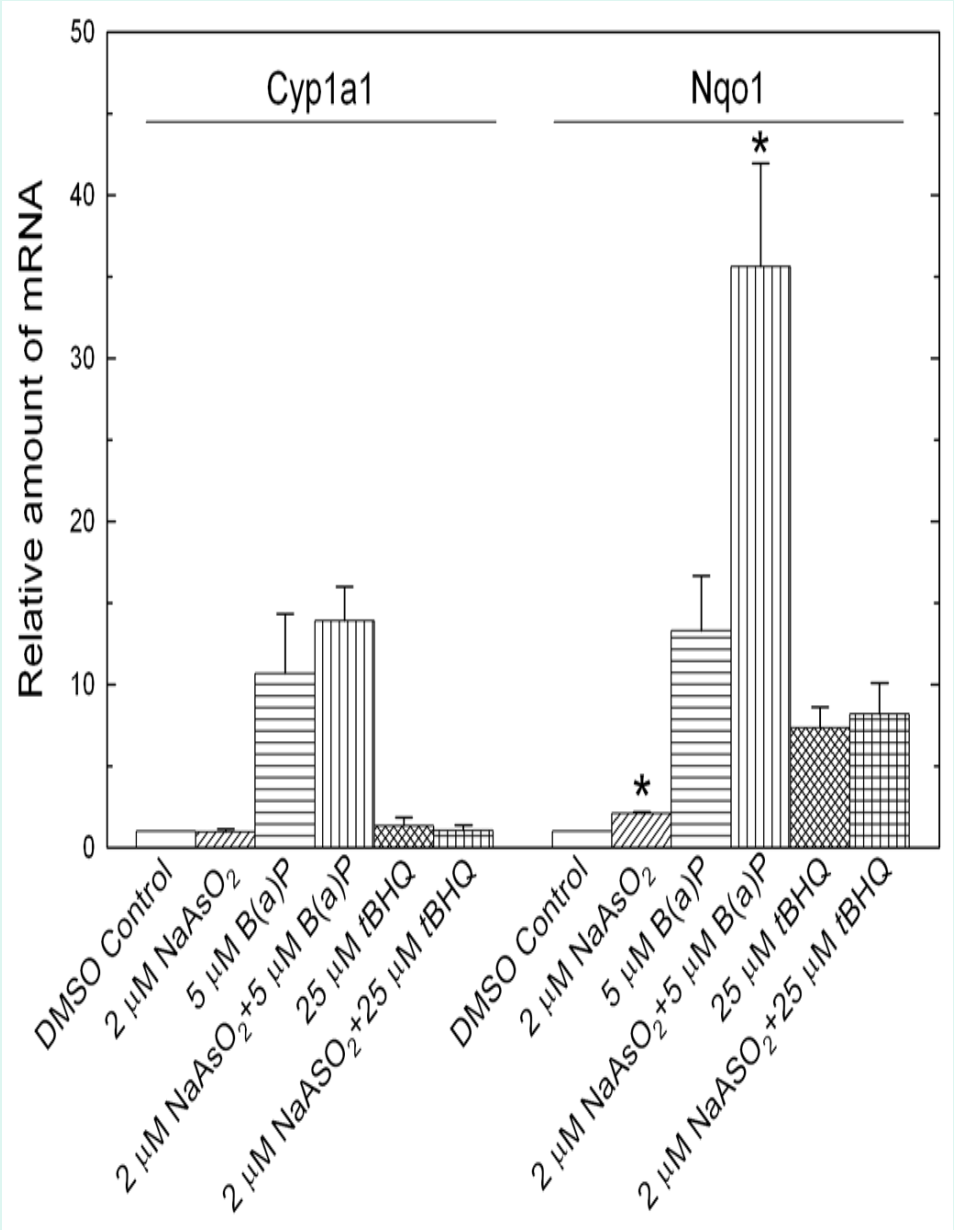
Puga, Xia

Table 1. Genes that arsenite and BaP deregulate additively or synergistically

			5 μM BaP		2 μM As		5 μM BaP+2 μM As			
Accession	Symbol	Gene name	Change	*p*-value	Change	*p*-value	Change	*p*-value	GO Process	GO Function
U85713	Plcb1	Phospholipase Cβ	-3.16	0.0001	1.17	0.09150	-2.12	0.0002	Intracellular signaling	PI phospholipase
X57413	Tgfb2	Transforming growth factor β-2	-2.08	0.00001	-1.18	0.09535	-4.17	0.00000	Cell differentiation	Growth factor
X98014	Siat8E	Sialyltransferase 8E	-1.81	0.00891	-2.24	0.00023	-4.24	0.00001	Protein glycosylation	Sialyltransferase
AF118855	Tprt	Trans-prenyltransferase	-1.57	0.00972	-2.33	0.00037	-2.20	0.00022	Unknown	Transferase
U52524	Has2	Hyaluronan synthase 2	-1.46	0.00549	-2.40	0.00000	-4.25	0.00000	Protein glycosylation	Transferase
U79550	Snai2	Snail homolog	-1.37	0.00298	-1.15	0.08507	-2.15	0.00000	Development	DNA binding
AB024565	Hs6s2	Heparan sulfate 6-O-sulfotransferase 2	-1.33	0.00037	-1.48	0.00000	-2.02	0.00000	Protein sulfation	Transferase
AF054623	Fzd1	Frizzled 1	-1.33	0.00005	-1.32	0.00002	-2.21	0.00000	Receptor signaling	G-protein coupled receptor activity
U89924	Ppp31r3c	Protein phosphatase 1 inhibitory subunit	-1.30	0.00026	-1.39	0.00002	-2.22	0.00000	Glycogen metabolism	Protein phosphatase
AF038507	Cip7	RIKEN cDNA 2010204K13 gene	-1.28	0.01026	-1.52	0.00010	-2.49	0.00000	Unknown	Unknown
AF117709	Sfrp4	Secreted frizzled-related protein 4	-1.25	0.07195	-1.18	0.15614	-2.46	0.00000	Receptor signaling	G-protein coupled receptor activity
U25096	Klf2	Kruppel-like factor 2 (lung)	-1.24	0.14524	-1.97	0.00007	-2.46	0.00001	Transcription regulation	DNA binding
Z29532	Fst	Follistatin	-1.21	0.00013	-1.22	0.00002	-1.87	0.00000	Differentiation	BMP/TGFb inhibitor
U31758	Hdac2	Histone deacetylase 2	-1.20	0.03066	-1.12	0.09363	-2.03	0.00000	Chromatin remodeling	Histone deacetylase
X99572	Figf	C-fos induced PDGF family member	-1.18	0.02416	-1.29	0.00069	-1.87	0.00000	Angiogenesis	Growth factor
AJ131395	Col14a1	Procollagen α1, type XIV	-1.09	0.11044	1.18	0.00186	-1.63	0.00000	Cell adhesion	Extracellular matrix tensile strength
X61455	Napb	N-ethylmaleimide sensitive attachment protein	-1.03	0.72960	-1.18	0.07939	-2.10	0.00001	Transport	Intracellular transport
Z48781	Efnb1	Ephrin B1	-1.01	0.80959	-1.27	0.00021	-1.72	0.00000	Receptor signaling	Ephrin receptor binding
AF174535	Sqrd	Sulfide quinone reductase	1.01	0.94770	1.00	0.99359	1.47	0.00037	Electron transport	Disulfide oxidoreductase activity
U67611	Taldo-1	Transaldolase-1	1.01	0.85902	1.07	0.20978	1.65	0.00000	Pentose-phosphate shunt	Aldehyde-lyase activity
AF093853	Prdx6	Peroxiredoxin 6	1.02	0.53778	1.16	0.00153	1.53	0.00000	Lysosomal lipid catabolism	Antioxidant activity
U20780	Ube2	Ubiquitinating enzyme-E2	1.08	0.30923	1.14	0.06032	1.55	0.00002	Protein degradation	Ubiquitin ligase
U27014	Sdh1	Sorbitol dehydrogenase-1	1.09	0.37171	1.22	0.01999	1.69	0.00002	Detoxification. Stress response	Alcohol dehydrogenase
AF062071	Za20d2	Zinc finger protein-2	1.09	0.15158	1.27	0.00040	1.59	0.00000	Transcription regulation	DNA binding
Y13479	Ogg1	8-oxoguanine DNA-glycosylase-1	1.11	0.02220	1.20	0.00014	1.57	0.00000	Base-excision repair	DNA damage repair
U65636	Psmb4	Proteasome subunit β-4	1.13	0.00415	1.25	0.00001	1.61	0.00000	Ubiquitin dependent protease	Endopeptidase
AF051324	Scap2	Src-associated phosphoprotein	1.14	0.00001	1.28	0.00000	1.77	0.00000	Negative regulation of proliferation	Unknown
AF220134	Trim16	Tripartite motif protein	1.21	0.03397	1.06	0.42146	1.68	0.00001	Unknown	Zinc ion binding
X06683	Sod1	Superoxide dismutase-1	1.21	0.00287	1.34	0.00003	1.72	0.00000	Reactive oxygen detoxification	Superoxide radical dismutation
U71205	Rit1	RAS-like protein	1.24	0.00045	1.40	0.00000	1.82	0.00000	GTPase signal transduction	GTP binding
X80502	Siat8c	Sialyltransferase 8 (alpha-2, 8-sialytransferase) C	1.27	0.00416	1.47	0.00002	1.75	0.00000	Protein glycosylation	Sialyltransferase
U40930	Sqstm1	Sequestosome-1	1.30	0.00000	1.69	0.00000	2.53	0.00000	Transcription cofactor	Binds to ubiquitin. Oxidative stress
U95607	Dnajb3	Heat shock protein 40, DNAJ-homolog	1.50	0.00037	1.24	0.01118	2.72	0.00001	Heat shock response	ATP binding during oxidative stress
U23921	Osp94	Osmotic stress protein	1.50	0.00065	1.39	0.00137	4.48	0.00000	Protein folding	ATP binding during oxidative stress
X56824	Hmox1	Heme oxygenase-1	1.65	0.28623	11.45	0.00009	20.52	0.00007	Heme oxidation	Oxidoreductase
U95053	Gclm	Glutamate-cysteine ligase modulatory subunit	1.69	0.00372	2.08	0.00124	4.49	0.00001	Glutathione biosynthesis	Glutamate-cysteine ligase
U85498	Gclc	Glutamate-cysteine ligase catalytic subunit	1.81	0.00000	1.39	0.00017	2.70	0.00000	Glutathione biosynthesis	Glutamate-cysteine ligase
U12961	Nqo1	NAD(P)H quinone oxidoreductase-1	4.62	0.00000	1.24	0.01811	9.24	0.00000	Electron transport	Oxidoreductase
U12785	Aldh3a1	Aldehyde dehydrogenase-3A1	6.74	0.00003	1.13	0.47355	9.75	0.00002	Oxidative metabolism	Aldehyde dehydrogenase activity

Table 2. Genes deregulated by arsenite and not by B[a]P

			5 μM BaP		2 μM As		5 μM BaP+2 μM As			
Accession	Symbol	Gene name	Change	*p*-value	Change	*p*-value	Change	*p*-value	GO Process	GO Function
X15378	Mpo	Myeloperoxidase	-1.29	0.01101	-1.65	0.00001	-1.84	0.00000	Oxidative stress response	Oxidoreductase
X69619	Inba	Inhibin-βA	-1.16	0.00663	-1.64	0.00000	-1.71	0.00000	Cell growth	Growth factor activity
U50413	Pik3r1	Phosphatidylinositol 3-kinase regulatory subunit	-1.11	0.24442	-1.31	0.00317	-1.71	0.00001	Intracellular signaling	PI3 kinase
X62934	Tacr1	Tachykinin receptor 1	-1.10	0.24510	-1.67	0.00003	-2.00	0.00000	G-protein signaling pathway	G-protein coupled receptor activity
U39738	Pak3	P21 (CDKN1A)-activated kinase-3	1.00	0.95994	1.42	0.00000	1.42	0.00000	Protein phosphorylation	Cell cycle arrest. Kinase activity
AF173681	Txnip	Thioredoxin interacting protein	1.02	0.62063	1.38	0.00000	1.50	0.00000	Oxidative stress response	Binds to thioredoxin
AF213389	Abcc1b	ATP-binding cassette-C-6	1.04	0.44732	1.51	0.00000	1.64	0.00000	Transport	ATP binding during oxidative stress
AF055664	Dnaja1	Heat shock protein 40, DNAJ-homolog	1.07	0.43305	1.42	0.00022	1.63	0.00001	Heat shock response	ATP binding during oxidative stress
AF225959	Adrm1	Adhesion regulating molecule-1	1.11	0.02244	1.41	0.00000	1.53	0.00000	Cell adhesion	Protein binding



Arsenic enhances the BaP-induced up-regulation of phase 2 enzymes

Hypotheses

Arsenic potentiates the action of other mutagens by:

- **Inhibiting DNA repair** **YES**
- **Interfering with cell cycle checkpoints** **NO**
- **Altering gene expression** **MAYBE**