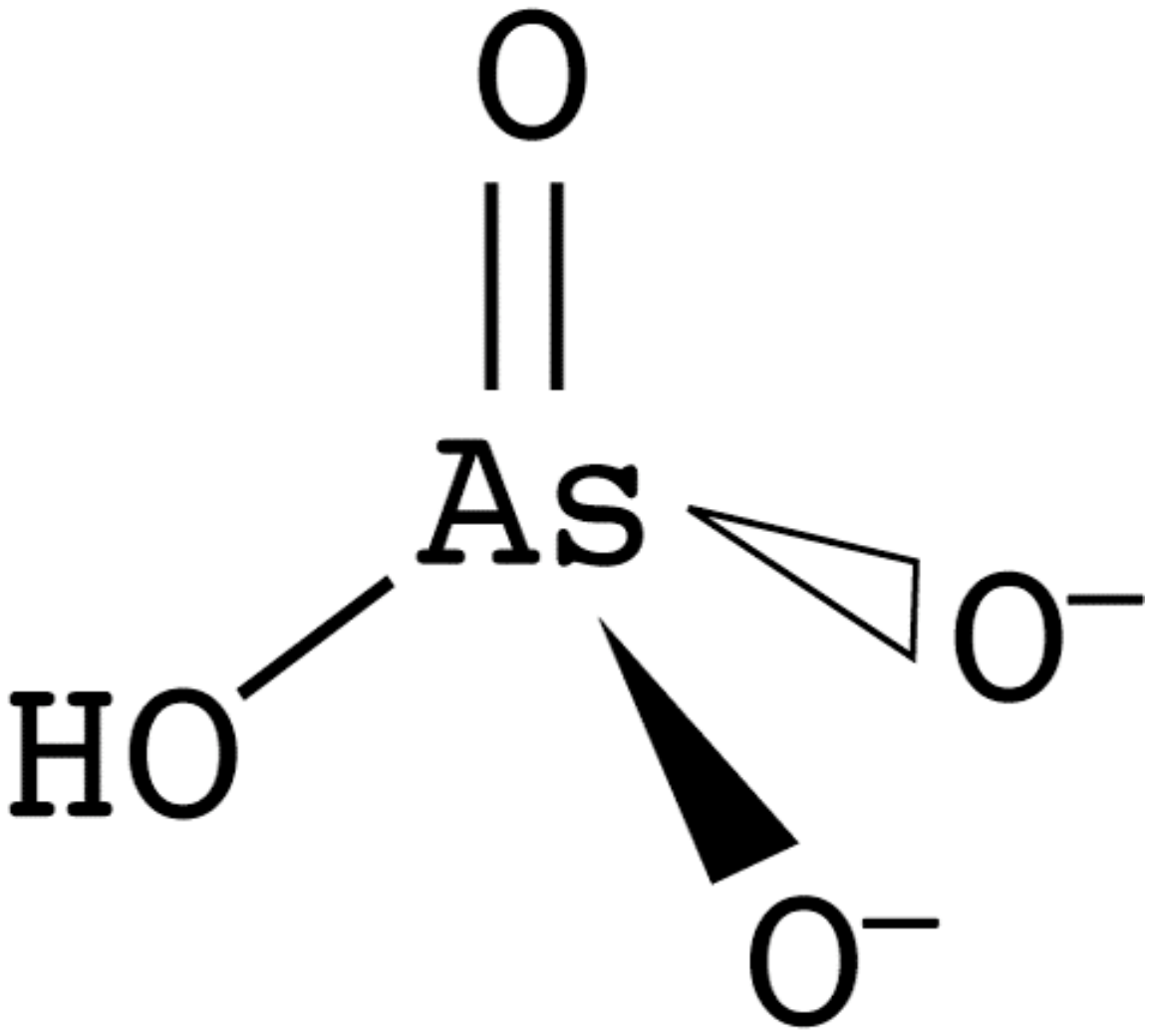
O
As
HO
O⁻
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CASE STUDY: Arsenate Pump.

Arsenic is a well known poison. It is an agent of murder. It is also an environmental poison, found in effluents such as pulp leachate, and a contaminant in drinking water.

It has also been used as a therapeutic. Originally to treat bacterial infections, for example, syphilis. Syphilis was a deadly STD (sexually transmitted disease) with enormous social consequences prior to the development of antibiotics in the 1940's

Even today, arsenicals remain a therapeutic for the Trypanosoma parasite - a blood parasite (eukaryotic) in sleeping sickness & Chagas disease.

In clinical settings, there were reports of arsenic-resistant bacterial strains. This was due to a conjugative R-factor (R773) isolated from an arsenic-resistant E. coli from a patient with a urinary tract infection[a].

In fact, in harsh environments, bacteria require resistance to many toxic metals[b].

[a] Rosen, BP, H Bhattacharjee & W. Su 1995 Mechanisms of metallo-regulation of an anion-translocating ATPase. J. Bioenerg. Biomemb. 27: 89-91. [b] Rosen BP 1999 Families of arsenic transporters. Trends Microbiol. 7: 207-212.

The Geofriends

EXCALIBUR
26 OCT 2005

AD EARLE

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SEATTLE POST-INTELLIGENCER
http://seattlepi.nwsource.com/national/1110AP_Arsenic_Death.html
Monday, November 7, 2005 · Last updated 11:28 a.m. PT

Wife pleads guilty in arsenic-poisoning

By AARON BEARD
ASSOCIATED PRESS WRITER

RALEIGH, N.C. -- A woman who was a scientist for a drug company admitted in court Monday that she conspired with her lover five years ago to fatally poison her husband, a pediatric AIDS researcher.

Ann Miller Kontz, 35, was sentenced to 25 to 31 1/2 years in prison after her lawyer read a statement saying she felt "a deep sense of remorse and regret" for Eric Miller's death.

"I will struggle for the rest of my life with how this could have happened," the statement said.

Authorities said Kontz, who worked at GlaxoSmithKline, was having an affair with a co-worker when her husband was poisoned by arsenic, a colorless and usually tasteless poison once common in ant and rat killers.

Under a plea deal, Kontz admitted conspiring with the co-worker, Derril Willard, and pleaded guilty to second-degree murder and conspiracy to commit first-degree murder. The two had access to arsenic at their laboratory, police have said.

Miller, a researcher at the University of North Carolina at Chapel Hill, died Dec. 2, 2000. He was 30.

Less than a month before he died, he went bowling with Willard and two others and fell ill about an hour after drinking a beer he complained was bitter, according to authorities.

He was hospitalized for a week but doctors failed to diagnose the poisoning, investigators said. Two weeks later, he again became violently ill after eating a meal prepared by his wife, investigators said. This time, doctors detected high levels of arsenic in his system, but they were unable to save him.

Willard committed suicide about a month after Miller died.

Lawyers discussed a possible plea agreement for several weeks, said District Attorney Colon Willoughby, who declined to give details about the negotiations.

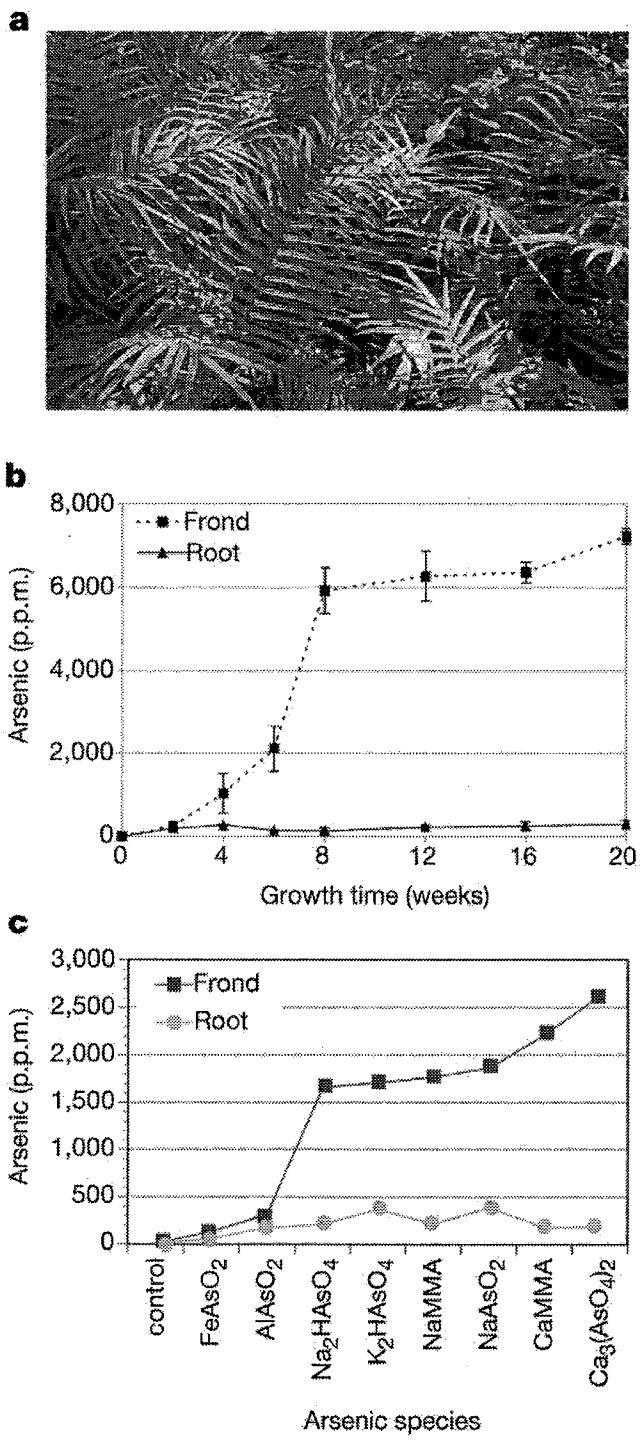
"We thought that this was in the family's and the community's best interest to resolve the case this way," he said.

The plea provided an abrupt end to a complicated case that included a fight over attorney-client privilege that reached the state Supreme Court.

That dispute ended with Willard's attorney revealing information implicating Kontz, which led to her indictment a few months later. In the statement, the lawyer revealed that Willard learned from Kontz that she had injected a syringe filled with an unnamed substance into Miller while he was hospitalized.

Kontz - who remarried after Miller's death - acknowledged in court Monday that she poisoned her husband at least twice before his death.

She and he had a daughter, Clare, who is now 5 years old.



a, Brake fern growing on an abandoned wood-preservation site contaminated with chromated copper arsenate (CCA); **b**, arsenic concentrations in brake fern after 20 weeks' growth in a CCA soil containing 97 p.p.m. As; and **c**, arsenic concentrations in brake fern after 18 weeks' growth in soil spiked with 50 p.p.m. As of various species. Brake fern plants grown in the laboratory were transferred to 2.5-litre pots (one plant per pot, with four replicates) containing 1.5 kg soil to determine arsenic-uptake changes with time and the arsenic species. NaMMA, monosodium methylarsonate; CaMMA, calcium acid methanearsonate.

Nature 409:579 (1 February 2001) A fern that hyperaccumulates arsenic

Lena Q. Ma1, Kenneth M. Komar, Cong Tu, Weihua Zhang, Yong Cai and Elizabeth D. Kennelley

Abstract: A hardy, versatile, fast-growing plant helps to remove arsenic from contaminated soils.

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GNN Genome News Network

Ferns Remove Arsenic from Soil and Water

By Kate Ruder
Posted: August 6, 2004

Six years ago researchers in Florida discovered ferns growing in soil contaminated with arsenic at an abandoned lumber yard. The ferns had been soaking up arsenic from the soil through their roots and storing it in their fronds.

Arsenic, which is poisonous to humans, is used to pressure treat lumber and to make semi-conductor chips. It was once also used to manufacture insecticides and chemical weapons, and it ranks number one on a list of substances to be removed from contaminated sites by the U.S. Department of Health and Human Services' Agency for Toxic Substances and Disease Registry.

The Florida discovery marked the first time a plant had been found to naturally take up arsenic in high concentrations. The fronds of *Pteris vittata*, or brake fern, can be clipped or the entire plant can be dug up and disposed of safely, a process that was patented by the Florida group in 2001.

"It was odd to identify a plant that has such useful characteristics that hadn't yet been discovered," says Bruce Ferguson, CEO of Edenspace, a Virginia-based company that now licenses the patent for the ferns and sells them commercially under the name "edenfern."

Today, the ferns are being used throughout the United States to remove arsenic from soil and drinking water. Edenspace, which specializes in a variety of plants to cleanup toxic substances, has twelve employees and reported $1.2 million in revenues last year.

Ferns in Washington, D.C.

This summer 2,800 edenferns are being planted in the nation's capital as part of a pilot project to remove arsenic from 600 acres near American University in the Northwest part of Washington, D.C. The area, called Spring Valley, includes residential and university property.

Spring Valley was once used by the US government for research and testing of chemical weapons during World War I, and remnants of these chemicals, including arsenic, are still thought to be underground. The US Army Corps of Engineers began to clean up the area in the 1990s, yet today there are more than 100 private properties that have contaminated soil waiting to be removed and replaced.

Residents, meanwhile, have voiced concern over the Corps removing or damaging big, old trees on their property in the process of digging up contaminated soil. In hopes of removing arsenic in a less destructive manner, the Corps of Engineers has planted the ferns at three locations in Spring Valley.

5

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"We've had positive reactions from residents [about using the ferns] so far, especially from people who have had concerns about their trees," says Ed Hughes of the Army Corps of Engineers in Baltimore, who is spearheading the cleanup effort.

The Corps plans to test the ferns for arsenic and then dispose of the ferns and fronds in airtight containers. If the arsenic levels are extremely high in the leaves, the plants are disposed of at a hazardous waste facility.

The plants pose an overall low risk and could be dangerous to children or animals only if consumed in large quantities, says Michael Blaylock, director of technology at Edenspace. In comparison to the ferns, household plants such as poinsettias and potato vines are more toxic to pets and people.

New Mexico Drinking Water

The ferns are also being used to remove arsenic from drinking water. In a recent pilot study in Albuquerque, New Mexico, the ferns significantly decreased the level of arsenic in samples of the city's drinking water.

Some varieties of the plant live hydroponically, or without soil, in the water. City workers set up a staircase of trays holding about 100 ferns with water filtering down from the top through the trays of ferns. About 450 gallons of water were pumped through the system daily.

The city of Albuquerque will probably never use the ferns on a large-scale because it uses chemicals to treat water supplies, as do most large cities.

But the study demonstrated that the low-cost technology could be feasible for the drinking water of rural communities in New Mexico and other parts of the western United States. Parts of the West have high levels of arsenic in drinking water because of naturally occurring volcanic rocks underground.

New strategies are needed to remove arsenic from drinking water cheaply and effectively for big and small cities in the United States. Under the Safe Water Drinking Act, the Environmental Protection Agency recently revised the standards for allowable limits of arsenic in drinking water. The new standards, which take effect in 2006, change the allowable level to ten parts per billion from 50 parts per billion.

In addition to the United States, the ferns could be used in small communities in developing countries such as Bangladesh, which has problems with arsenic in drinking water. The company recently made the ferns available royalty free to parts of the developing world, according to Ferguson.

Growing a Better Fern

The ferns have not been genetically modified, but they have been bred at Edenspace to have desirable traits. The brake fern, which is native to the Southeastern United States, tolerates sun surprisingly well for a fern.

Scientists at the company bred the ferns to be more adapted to cold weather, and they also bred larger

Page 4C of —

ferns that take up more arsenic. The ferns are now grown year round in Florida, and can be purchase online for $4.95 a piece, not including shipping.

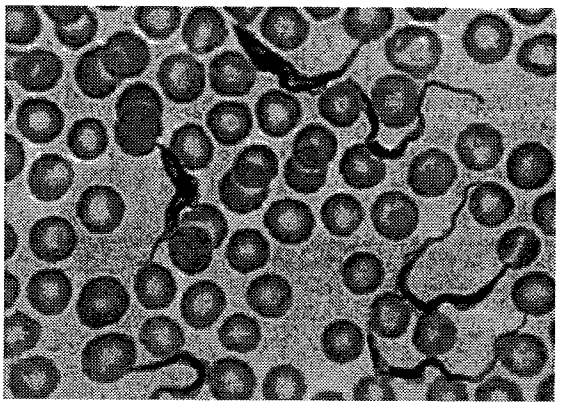
"Most people don't know we're around," says Ferguson. Most of his customers are well-versed in environmental issues. He suggests that homeowners might plant them under a deck with pressure-treated wood or in a yard where an old pile of lumber might have been.

"The ferns are easy to grow and inexpensive," he says. "And they look nice too."

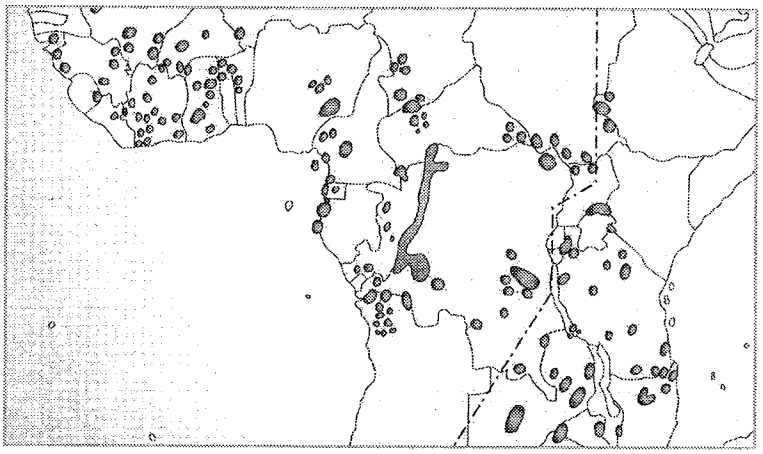
See Related GNN Article: Scientists modify plants to remove environmental toxins

Ma, L.Q. *et al.* A fern that hyperaccumulates arsenic. *Nature* 409, 579 (February 1, 2001).

Trypanosomes and red blood cells



Regions of trypanosomiasis incidence in Africa

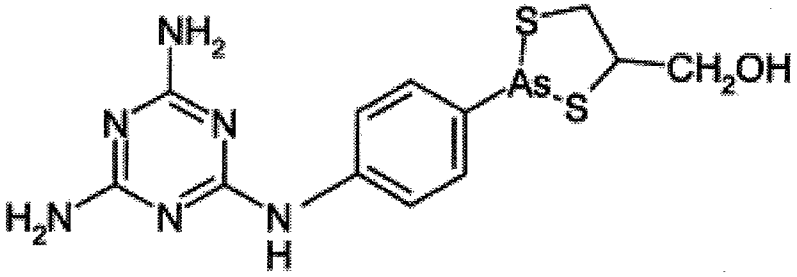


Drugs used to treat human African trypanosomiasis

Stage I (generalised infection)
First line: pentamidine
Second line: eflornithine or melarsoprol (arsenical)

Stage 2 (trypanosomes cross the blood-brain barrier)

Melarsoprol



First line: melarsoprol (arsenical)
Second line: eflornithine

Many toxic metals that bacteria are resistant to are cations: Such as Cd^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Tl^{+} etc. Resistance is often due to ATP-dependent cation pumps or M^{+}/H^{+} antiporters which extrude the metal(s) from the cytoplasm[c]. The arsenic resistant pump is fairly unique since this it is an anion rather than a cation.

In recent years, concerns about arsenic have grown because of its impact on human affairs. With groundwater wells used to supply drinking water, arsenic can enter the wells at concentrations high enough to cause chronic sub-lethal arsenic poisoning - primarily skin lesions called hyperkeratoses - hardened patches of skin - that can lead to skin cancer. This is a severe problem in India.

[c] Silver, S. (1996) Bacterial resistances to toxic metal ions - a review. Gene 179:9-19.

To begin, I think it's best to orient ourselves by looking at a periodic table.....

Arsenic is in the same 'family' as nitrogen and phosphorous...

GROUP V

N
P
As
(antimony) Sb
(bismuth) Bi

Indeed, some of its toxic properties in cells can be ascribed to its similarities to phosphorus

The group V compounds exist in a bewildering array of oxidation states:

	[-III]	[0]	[III]	[?]
P	PH_3	P	H_3PO_3	
As	AsH_3	As	$HAsO_2/AsO_2^-$ ($H_2AsO_3^-$)	$H_2O + AsO_2^- \rightleftharpoons H_2AsO_3^-$ arsenite
Sb	SbH_3	Sb	$SbO_2/SbO^+/SbO_2^-$	

	[V]	
P	H_3PO_4/PO_4^{3-}	
As	H_3AsO_4/AsO_4^{3-}	arsenate
Sb	Sb_2O_5	$Sb(OH)_6^-$

PERIODIC TABLE OF THE ELEMENTS

I	II											III	IV	V	VI	VII	VIII
1																	2
H																	He
1.0079																	4.0026
3	4											5	6	7	8	9	10
Li	Be											B	C	N	O	F	Ne
6.941	9.0122											10.811	12.011	14.007	15.999	18.998	20.180
11	12											13	14	15	16	17	18
Na	Mg											Al	Si	P	S	Cl	Ar
22.990	24.305											26.982	28.086	30.974	32.066	35.453	39.948
		Transition Metals															
		3	4	5	6	7	8	9	10	11	12						
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
39.098	40.078	44.956	47.88	50.942	51.996	54.938	55.847	58.933	58.69	63.546	65.39	69.723	72.61	74.922	78.96	79.904	83.80
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
85.468	87.62	88.906	91.224	92.906	95.94	[98]	101.07	102.91	105.42	107.87	112.41	114.82	118.71	121.75	127.60	126.90	131.29
55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Cs	Ba	*La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
132.91	137.33	138.91	178.49	180.95	183.85	186.21	190.2	192.22	195.08	196.97	200.59	204.38	207.2	208.98	[209]	[210]	[222]
87	88	89	104	105	106												
Fr	Ra	**Ac	Unq	Unp	Unh												
[223]	226.03	227.03	[261]	[262]	[263]												

Atomic weights are based on $^{12}C = 12$ and conform to the 1987 IUPAC report values rounded to 5 significant digits. Numbers in [ ] indicate the most stable isotope.

* Lanthanides	58	59	60	61	62	63	64	65	66	67	68	69	70	71
	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
	140.12	140.91	144.24	[145]	150.36	151.97	157.25	158.93	162.50	164.93	167.26	168.93	173.04	174.97
** Actinides	90	91	92	93	94	95	96	97	98	99	100	101	102	103
	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr
	232.04	231.04	238.03	237.05	[244]	[243]	[247]	[247]	[251]	[252]	[257]	[258]	[259]	[262]

PERIODIC TABLE

The redox rxns:

	E^o (Volts)
$As(s) + 3H^+ + 3e^- \rightleftharpoons AsH_3(g)$ [0] [-III]	-0.239
$HAsO_2 + 3H^+ + 3e^- \rightleftharpoons As(s) + 2H_2O$ [III] [0]	+0.247
$AsO_2^- + 2H_2O + 3e^- \rightleftharpoons As(s) + 4OH^-$ [III] [0]	-0.68
$H_3AsO_4 + 2H^+ + 2e^- \rightleftharpoons HAsO_2 + 2H_2O$ [V] [III]	+0.559
$AsO_4^{3-} + 2H_2O + 2e^- \rightleftharpoons AsO_2^- + 4OH^-$ [V] [III]	-0.67

Toxicity of soluble arsenates and arsenites are in the range 4-40 mg/kg body weight. (~ 200-2000 mg for a normal human) Lower dosages can cause embryo defects. In humans, it is normally methylated in the liver and excreted in urine. It is retained in hair and other ~~weight~~ non-recycled tissues.

ARSENIC CHEMISTRY HIGHLIGHTS

Periodic Table Group V

N	nitrogen
P	phosphorus
As	arsenic
Sb	antimony
Bi	bismuth

Relevant Oxidation States

[0]	[III]	[V]
P	H_3PO_3	H_3PO_4 / PO_4^{3-}
As	$HAsO_2 / AsO_2^- (H_2AsO_3^-)$*	H_3AsO_4 / AsO_4^{3-} (arsenate)
Sb	$SbO_2 / SbO^+ / SbO_2^-$	Sb_2O_5 $Sb(OH)_6^-$

*$H_2O + AsO_2^- \rightleftharpoons H_2AsO_3^-$
(arsenite)

Acid-Base

	phosphate		arsenate	
pK_1	~2.3	$H_2PO_4^-$	~2.3	$H_2AsO_4^-$
pK_2	~7.2	HPO_4^{2-}	~7.0	$HAsO_4^{2-}$
pK_3	~12.3	PO_4^{3-}	~11.5	AsO_4^{3-}

Redox

	midpoint potential
$H_3AsO_4 + 2H^+ + 2e^- \rightleftharpoons HAsO_2 + 2H_2O$	0.559 V
[V] [III]	
$AsO_4^{3-} + 2H_2O + 2e^- \rightleftharpoons AsO_2^- + 4OH^-$	-0.670 V
[V] [III]	

Note the pH dependence of the midpoint potentials. At neutral pH, catalyzed reduction/oxidation is straightforward.

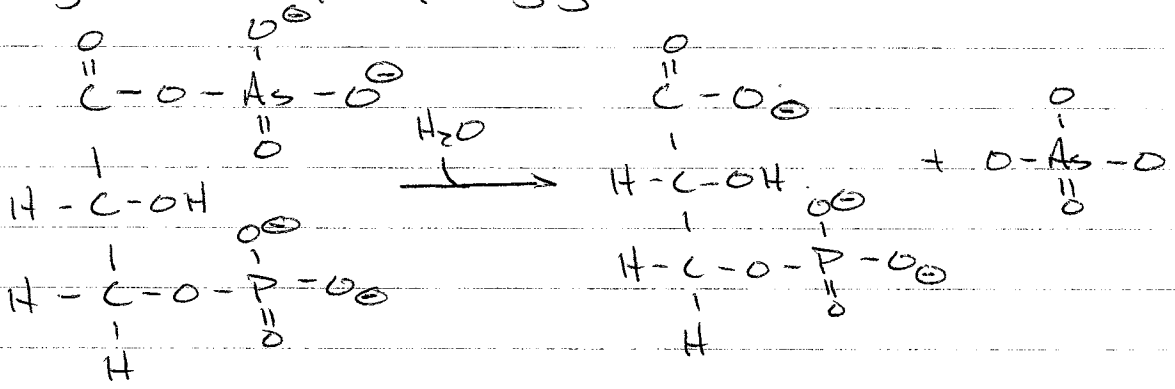
At least in terms of basic biochemistry, one mode of action is due to its similarity to phosphate in glycolysis:

competitive replacement by arsenate

3-phosphoglyceraldehyde + NAD^+ + P_i ⇌

3-phosphoglyceroyl phosphate + NADH + H^+

The 3-phosphoglyceroyl phosphate is non-enzymatically hydrolyzed to 3-phosphoglycerate.



3-phosphoglyceroylarsenate 3-phosphoglycerate

arsenite and antimony (AsO^- and SbO^+) can react with cysteines - dimercaptols are protective (in the test tube!)

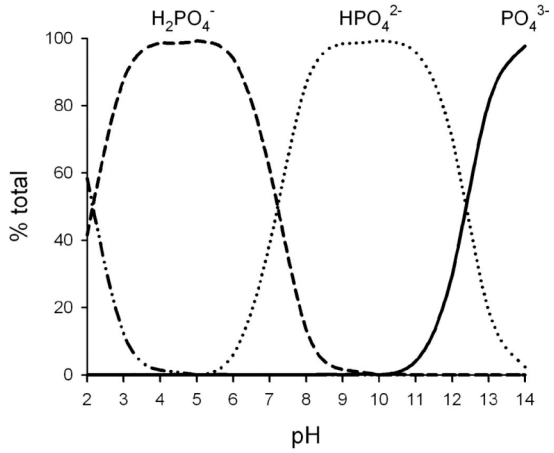
In vivo kidney and liver damage are commonly observed.

Now, in the context of the general families of ion pumps, an arsenate/arsenite pump is certainly out of place.

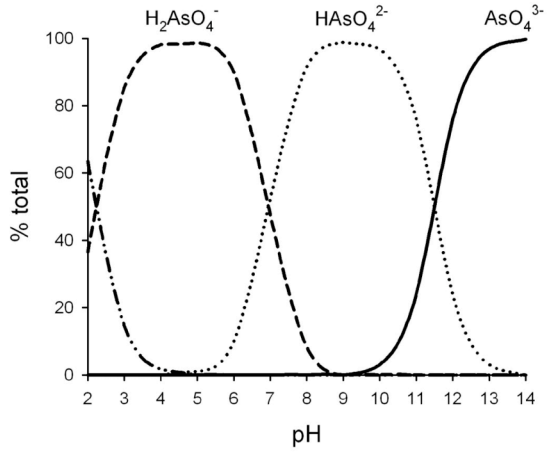
pH and redox potential (pe) are the most important factors controlling arsenic speciation.

Phosphate (A) and arsenate (B) speciation are shown as a function of pH for the (V) oxidation states. H_3PO_4 or H_3AsO_4 (dashed and dotted line), $H_2PO_4^-$ or $H_2AsO_4^-$ (dashed line), HPO_4^{2-} or $HAsO_4^{2-}$ (dotted line) and PO_4^{3-} or AsO_4^{3-} (solid line) are all indicated as a percentage of total P_i or As_i. The distribution curves in (A) and (B) show that As_i and P_i have similar charge and speciation under biologically relevant pH (Westall et al. 1976; Allison et al. 1991; Serkiz et al. 1996). Redox speciation is shown on a pe-pH diagram for aqueous arsenic species (C) in the systems P–O_2–H_2O and As–O_2–H_2O at 25°C and 1 bar total pressure. Arsenic (solid lines) and phosphorus (dashed line) species have been overlaid within the bounds of the O_2–H_2O redox couple (dotted lines). On such a diagram, phase boundaries represent the conditions at which the activities of the species on each side of the boundary are equal (Morel & Hering 1993; Smedley & Kinniburgh 2002). Under dysoxic conditions (pe≈0) and at neutral to mildly alkaline pH, the dominant As species is $HAsO_4$ suggesting that it would be present under conditions possibly relevant to the early evolution of life on Earth.

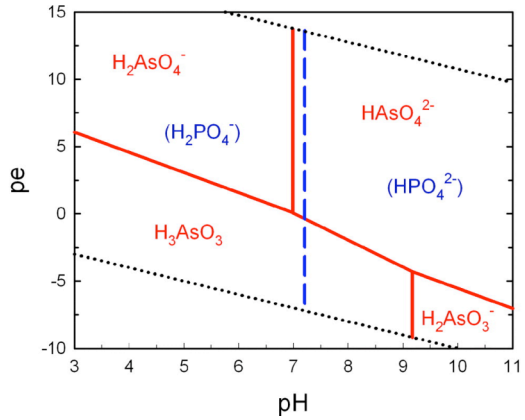
A



B



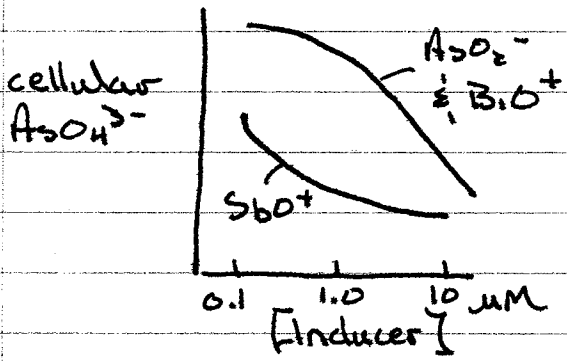
C



Source: Felisa Wolfe-Simon, Paul C.W. Davies and Ariel D. Anbar (2009) Did nature also choose arsenic? *International Journal of Astrobiology* 8:69-74

The initial characterization of arsenate resistance demonstrated induction and an exploration of the transport mechanism[d]

Induction of arsenate resistance was performed by pre-treating with SbO^+, AsO_2^- or Bi_1O^+. Then, arsenate added and cellular arsenate measured.



Induction by AsO_4^{3-} requires 100-250 μM

Mechanism of Arsenate Efflux

- The protonophore CCCP inhibits[1]
- Valinomycin had no effect[2]
- Independent of extracellular phosphate[3]
- Insensitive to pH[4]

(1) Thus, energy dependent

(2) anion efflux through a channel would be sensitive to $\Delta\Psi$ and thus valinomycin

(3) Thus, not a phosphate transporter

(4) Thus, not a H^+/AsO_4^{3-} antiporter

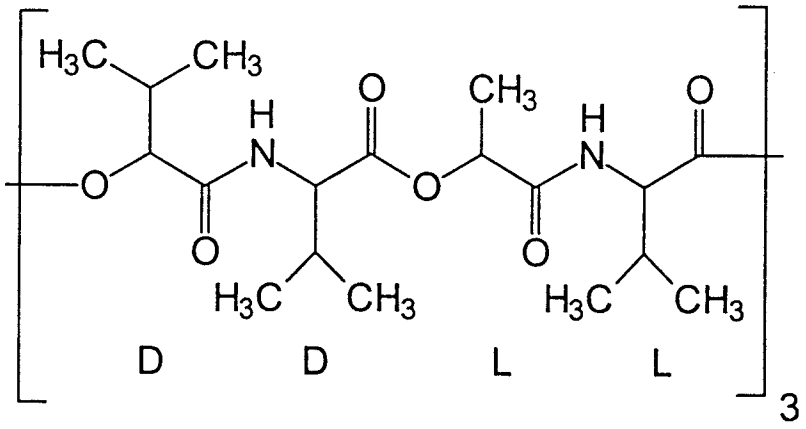
(d) Silver S, D Keach 1982 Energy dependent arsenate efflux: the mechanism of plasmid-mediated resistance. Proc. Natl. Acad Sci. 79: 6114-6118.

Valinomycin, *Streptomyces fulvissimus*

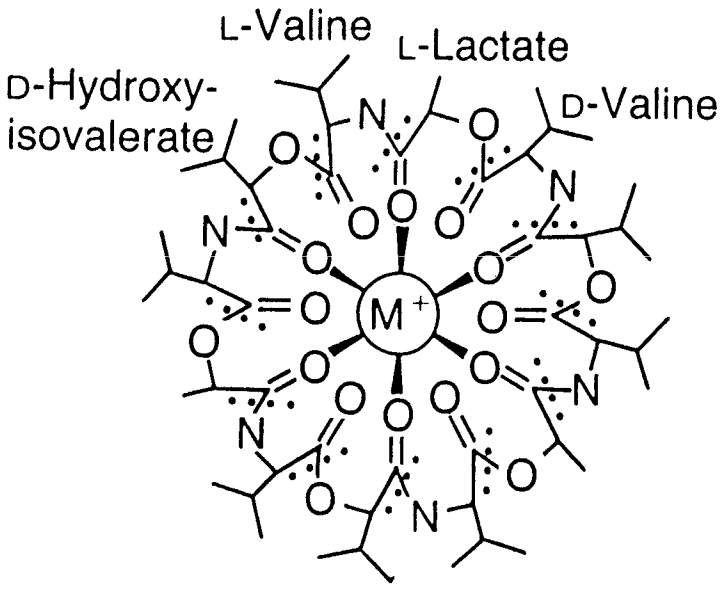
White solid. A cyclododecadepsi-peptide ionophore antibiotic. Potassium ionophore of the mobile ion-carrier type that transports alkali metal ions across artificial or biological lipid membranes. Induces K^+ conductivity in cell membranes at concentrations as low as 10^{-8} M. Often used in membrane electrode systems for determining K^+ concentration. Uncouples oxidative phosphorylation by binding to sites on membranes rich in sulfhydryl groups. Induces apoptosis in murine thymocytes. Also reported to inhibit NGF-induced neuronal differentiation. *Purity: ≥93% by HPLC. Ion specificity:* $Rb^+ > K^+ > Cs^+ > Ag^+ > NH_4^+ > Na^+ > Li^+$. *Soluble in acetic acid,* $CHCl_3$, *DMSO, or ether.* RTECS YV9468000, CAS 2001-95-8, $C_{54}H_{90}N_6O_{18}$, M.W. 1111.3.

Ref.: *Merck Index* **12,** 10047; Harada, H., et al. 1994. *Biochim. Biophys. Acta* **1220,** 310; Luvisetto, S., et al. 1994. *Biochim. Biophys. Acta* **1186,** 12; Orlov, V.N., et al. 1994. *FEBS Lett.* **345,** 104; Deckers, C.L., et al. 1993. *Exp. Cell Res.* **208,** 362.

Risk and Safety Statements: **R: 26/27/28; S: 22-36/37/39-45**



B Valinomycin



Proc Natl Acad Sci U S A **79:** 6114-6118 (1982) [83065128]

Energy-dependent arsenate efflux: the mechanism of plasmid-mediated resistance.

S. Silver & D. Keach

Plasmid-mediated resistance to arsenate, arsenite, and antimony(III) is coordinately induced by arsenate, arsenite, antimony(III), and bismuth(III). Resistance to arsenate was recently shown [Silver, S., Budd, K., Leahy, K.M., Shaw, W.V., Hammond, D., Novick, R.P., Willsky, G.R., Malamy, M.H. & Rosenberg, H. (1981) J. Bacteriol. 146, 983-996] to be due to decreased accumulation of arsenate by the induced resistant cells. We report here that decreased net uptake results from accelerated efflux of arsenate by induced plasmid-containing cells of Staphylococcus aureus and Escherichia coli. The efflux system in S. aureus was inhibited by nigericin, monensin, and proton-mobilizing uncouplers; efflux was unaffected by valinomycin. The mechanism of arsenate efflux in S. aureus was apparently not by chemiosmotic coupling to the membrane electrical potential or pH gradient. The intracellular efflux system was inhibited by low pH and mercurials (reversible by mercaptoethanol). The efflux rate was relatively independent of external pH or phosphate level and showed a sigmoidal pattern of concentration dependence.

Proc Natl Acad Sci U S A **79**: 6119-6122 (1982) [83065129]

Energetics of plasmid-mediated arsenate resistance in Escherichia coli.

H. L. Mobley & B. P. Rosen

Plasmid R773, which codes for resistances to arsenate, arsenite, and antimony, was introduced into Escherichia coli strain AN120, a mutant deficient in the H+-translocating ATPase of oxidative phosphorylation. Cultures depleted of endogenous energy reserves were loaded with 74AsO3-4, and arsenate efflux was measured after dilution into medium containing various energy sources and inhibitors. Rapid extrusion of arsenate occurred when glucose was added. Arsenate was extruded both against and down a concentration gradient. In this strain glucose allows formation of both ATP via substrate-level phosphorylation and an electrochemical proton gradient (or protonmotive force) via oxidation of the products of glycolysis. When oxidation was inhibited by cyanide, glucose metabolism still produced arsenate efflux. Energy sources such as succinate, which supplies a protonmotive force but not ATP, did not result in efflux. Measurement of intracellular ATP concentration under each set of conditions demonstrated a direct correlation between the rate of efflux and ATP levels. Osmotically shocked cells lost the ability to extrude arsenate; however, no arsenate-binding activity was detected in osmotic shock fluid from induced cells. These results suggest that the arsenate efflux system is coupled to cellular ATP rather than an electrochemical proton gradient, possibly by an arsenate-translocating ATPase.

Using E. coli to examine the energetics of arsenate efflux is useful, because there are unc mutants which lack the F_1F_o ATP synthetase. This makes it possible to differentiate between $\Delta\mu H^+$-dependent, and ATP-dependent arsenate efflux (1):

Ars efflux

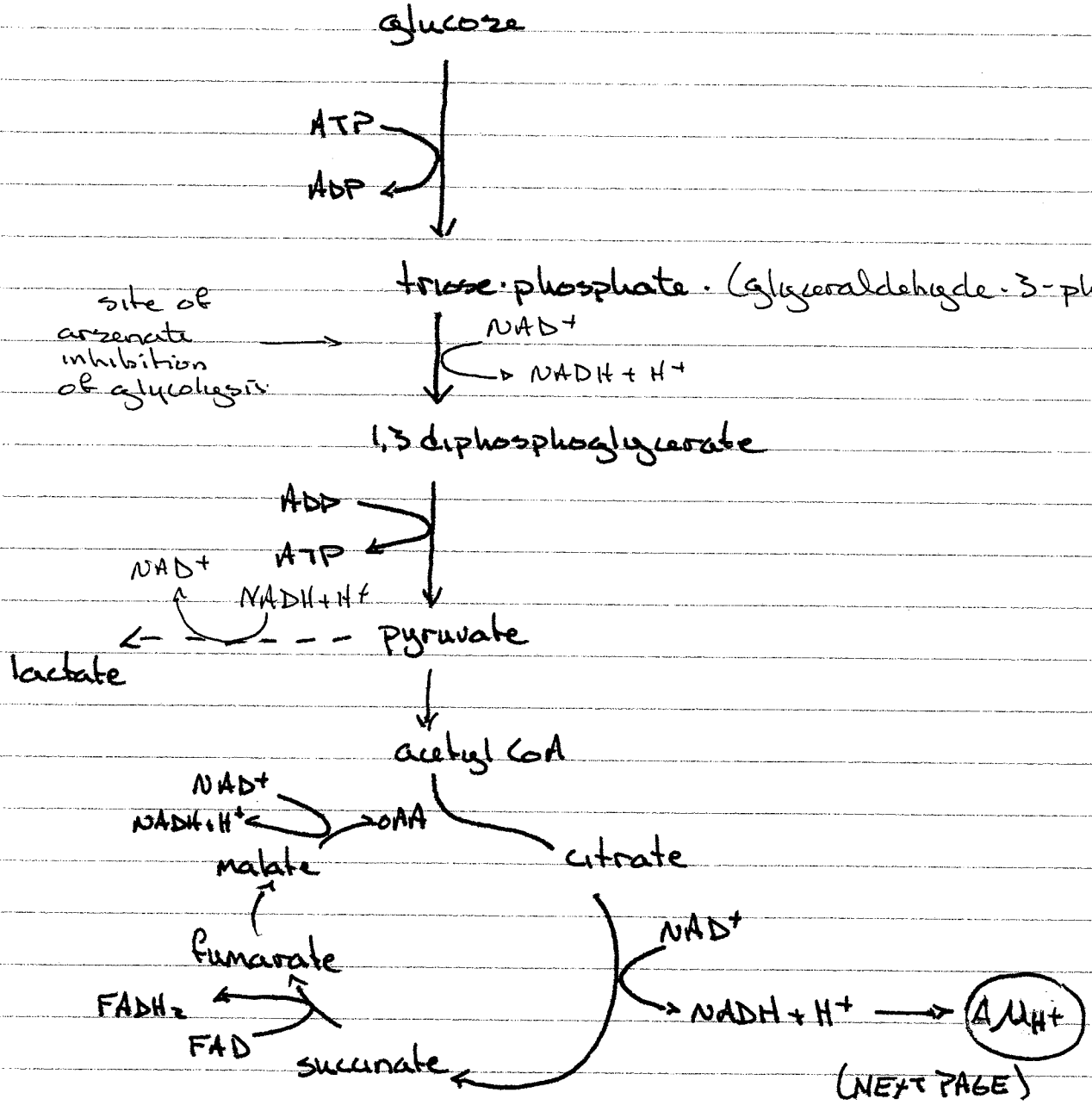
-glucose	-	
+glucose	+	substrate-level ATP-production & $\Delta\mu H^+$
+glucose + CN^- (cyanide)	+	substrate-level ATP production only.
+succinate	-	$\Delta\mu H^+$, no ATP production

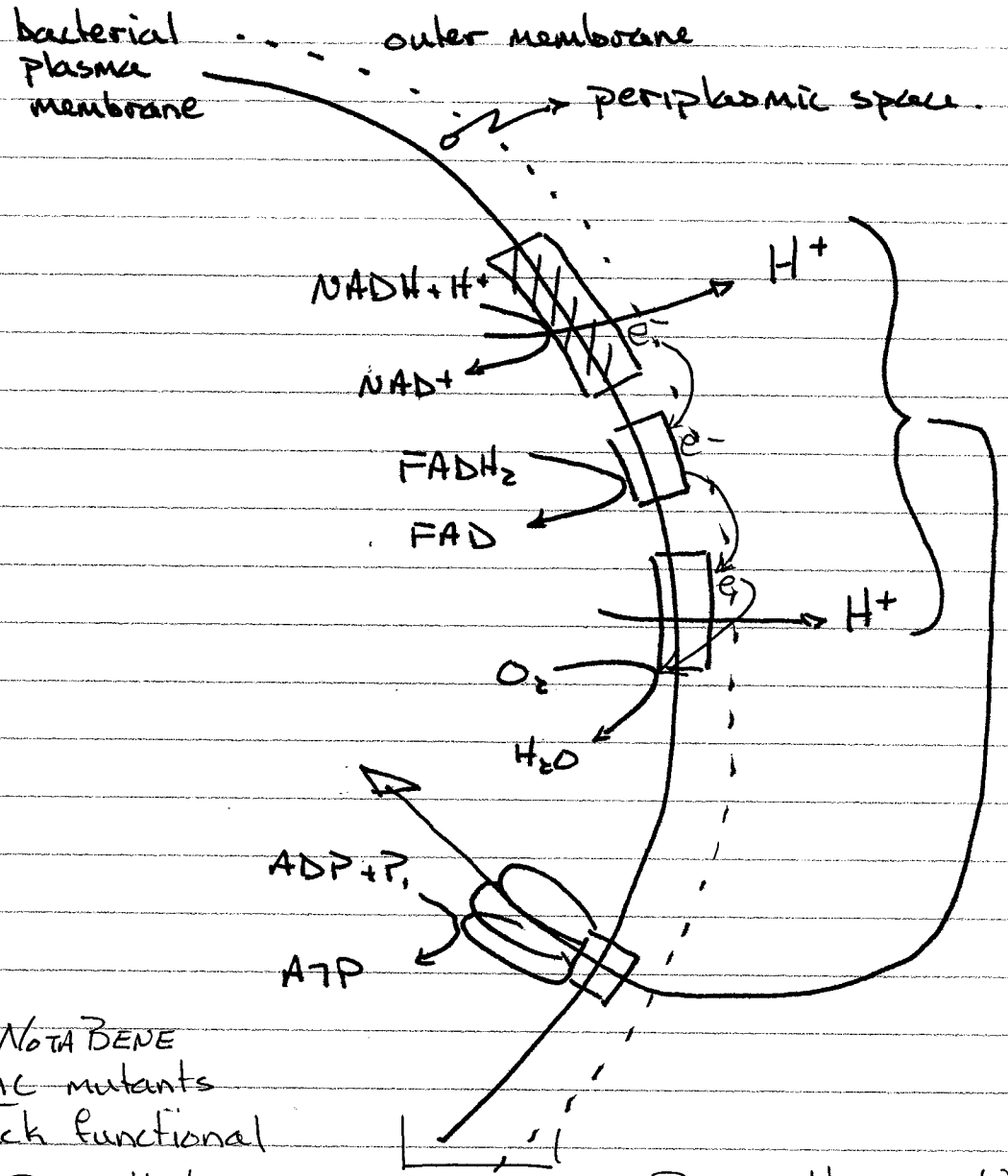
These experiments at the whole cell (in bacterio) level indicate that ATP is required for arsenate efflux. Is it an arsenate-ATPase?

With the plasmid conferring arsenate resistance in hand, it became possible to identify the genes involved, and their role.

(1) Mobley HL & BP Rosen 1982 Energetics of plasmid-mediated arsenate resistance in Escherichia coli. Proc Natl. Acad. Sci. 79 6119-6122.

Overview of Metabolism.



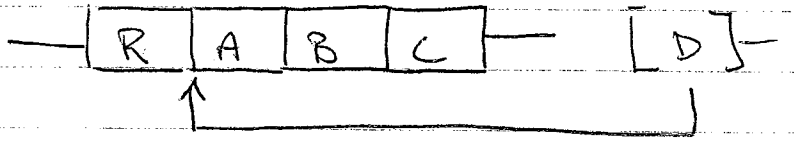


$$\Delta\mu_{H^+} = \frac{RT}{\lambda}\Delta pH + F\Delta\Psi$$

2.303

NOTA BENE
unc mutants lack functional ATP synthetase
In unc, ATP synthesis can occur in glycolysis (with glucose substrate) but only at substrate-level phosphorylation upstream of succinate.

The ars operon



The arsR gene produces a 13.2 kDa protein. When cloned into a promoter probe vector, the arsR gene confers arsenite induction. ⓓ
It is a typical transcription regulator, with a 'helix-turn-helix' motif that binds to the DNA. In addition, there are 3 cysteine residues which form the binding site for arsenite (III) - creating a metal-thiol cage which can also bind antimoney Sb (III).

Some ars operons also contain arsD, another 13 kDa transcriptional regulator.

ArsR
~~Both regulators are~~ is normally transcribed and binds to DNA to repress transcription. Upon binding to arsenate, transcription is turned on. ArsD, when present, apparently acts to prevent overexpression of the functional gene, ArsB, which is toxic when expressed at high levels.

ⓓ San Francisco, MJD, CL Hope, JB Owolabi, LS Tisa and BP Rosen 1990 Identification of the metalloregulatory element of the plasmid-encoded arsenical resistance operon. Nuc. Acids Res. 18:619-624.

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* Bacterial regulatory proteins, arsR family signature *
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The many bacterial transcription regulation proteins which bind DNA through a 'helix-turn-helix' motif can be classified into subfamilies on the basis of sequence similarities. One of these subfamilies, which we call arsR, groups together proteins that seem to dissociate from DNA in presence of metal ions. These proteins are listed below.

- arsR from various plasmids (such as R773, pSX267, pI258). ArsR acts as a transcriptional repressor of an arsenic resistance operon (ars).
- smtB from Synechococcus PCC 7942. SmtB is a transcriptional repressor of the smtA gene that codes for a metallothionein.
- cadC from plasmid pI258 and from Bacillus firmus OF4. CadC is a protein required for full cadmium-resistance.

It has been shown [1] that there could be an helix-turn-helix (H-T-H) region in the central part of these proteins. An interesting feature of this putative H-T-H region is that it contains, at its N-terminal extremity, one perfectly conserved cysteine residue and another one which is found in arsR and cadC but not in smtA and at its C-terminal extremity at least one and generally two histidine residues. We believe [2] that these residues could be involved in metal-binding (zinc in smtB, metal-oxyanions such as arsenite, antimonite and arsenate for arsR, and cadmium for cadC). Binding of a metal ion could induce a conformational change that would prohibit the protein from binding to DNA. Such a mechanism is highly suitable for regulatory systems that act to regulate the transcription of proteins involved in metal-ions efflux and/or detoxification.

The signature pattern for these proteins span the entire helix-turn-helix region.

- Consensus pattern: C-x(2)-D-[LIVM]-x(6)-[ST]-x(4)-S-[HR]-[HQ]
- Sequences known to belong to this class detected by the pattern: ALL.
- Last update: October 1993 / First entry.

[1] Morby A.P., Turner J.S., Huckle J.W., Robinson N.J.
Nucleic Acids Res. 21:921-925(1993).
[2] Bairoch A.
Nucleic Acids Res. 21:2515-2515(1993).

Identification of the metalloregulatory element of the plasmid-encoded arsenical resistance operon

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ABSTRACT

The regulatory region of the plasmid-encoded arsenical resistance (*ars*) operon was cloned as a 727-bp *Eco*RI-*Hin*dIII fragment. When cloned into a promoter probe vector this fragment conferred arsenite inducible tetracycline resistance in *Escherichia coli*, indicating that the fragment carried a regulatory gene, the *arsR* gene. A single region corresponding to −35 and −10 promoter recognition sites was identified. The transcriptional start site of the mRNA was determined by primer extension. The sequence has an open reading frame for a potential 13,179 Da polypeptide, termed the ArsR protein. The fragment was cloned into a temperature regulated expression vector. A protein with an apparent molecular mass of about 12 kDa was induced by either temperature or arsenite. This protein was purified and used to produce antibodies specific for the ArsR protein.

INTRODUCTION

The salts of arsenic and antimony are toxic to bacteria. The arsenical resistance (*ars*) operon of resistance plasmid R773 encodes an oxyanion pump, the first member of a new family of ion-translocating ATPases (1−3). In *Escherichia coli* this system catalyzes extrusion of arsenite, antimonite, and arsenate. Resistance results from lowering of the intracellular concentration of these toxic oxyanions (4−6). The nucleotide sequence of the structural genes of the operon has been reported (1). There are three structural genes, and the product of each has each been identified (1,2,7). The *arsA* and *arsB* gene products are sufficient to form a pump for arsenite and antimonite, the (+III) oxidation states of the metals (8), while the ArsC protein is postulated to be a modifier subunit which increases the substrate specificity to include arsenate, the (+V) oxidation state of arsenic (2,8,9).

Oxyanion resistance is inducible in the conjugative R-factor R773 but constitutive in the recombinant plasmid pUM3 (10). In pUM3 expression of the structural genes of the operon is dependent on the tetracycline P1 promoter of pBR322. In this report we describe the cloning of the *ars* operon with an intact regulatory region. Features of the regulatory region were identified, including the transcriptional start site and the product of the fourth gene, *arsR*. The *arsR* gene product, the ArsR protein, was subcloned, overexpressed and purified.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophage

The *E. coli* strains and plasmids used in this study are described in Table 1. Cells were grown in LB medium (11). Where required ampicillin (40 μg/ml), kanamycin (40 μmg/ml), tetracycline (35 μg/ml) and arsenite (1 mM) were added to the growth medium. When used as a noninhibitory inducer, arsenite was added to 50 μM. Procedures for manipulating DNA were as described by Maniatis *et al.* (11). Plasmid pWSU1 was constructed from plasmid pUM1, which is inducible for arsenical resistance (10). The 33-kb plasmid pUM1 was digested completely with *Eco*RI and partially with *Hin*dIII. The fragments were ligated into pBR322 which had been digested with both *Eco*RI and *Hin*dIII. Transformed cells were screened for inducible arsenite resistance, resulting in the isolation of the 9.4-kb plasmid pWSU1 (Fig. 1). The restriction map differs from that of pUM3 only by the presence of the 0.73-kb *Eco*RI-*Hin*dIII fragment. For expression studies this fragment was excised from pWSU1 and inserted into the multiple cloning site of plasmid pKK175-6 (12) to create plasmid pWSU2, into pCP40 (13) to create pWSU3, and into plasmid pT7-5 (14) to create plasmid pWSU4. The fragment was also cloned into plasmids pUC18 and pUC19 and phages M13mp8 and M13mp9 (15) for sequencing with *E. coli* strain JM103 used as host. A *Hin*cII-*Hin*dIII digest of the 0.73-kb fragment cloned into M13mp9 was also used for sequencing.

DNA sequencing

The nucleotide sequence was determined by the dideoxy chain termination method of Sanger *et al.* (16) in both M13 and pUC plasmid derivatives using the enzyme Sequenase (United States Biochemicals). The primer for M13 derivatives was the M13 universal primer. In addition the M13 reverse primer was used with pUC18 and pUC19 derivatives. Analysis of the nucleotide sequence was performed using GENEPRO 4.20 (Riverside Scientific, Seattle, WA).

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The ArsA gene encodes a 63 kDa protein. It is an oxyanion-stimulated ATPase, with a K_m (ATP) of 0.13 mM. and a pH_{opt} of 7.5 ~ 7.8. Oddly, the substrate is apparently not the usual Mg·ATP, but instead, $2 Mg^{2+} \cdot ATP$.

As is the case for the ArsR protein, the binding site for As(III) is a metallo-thiol cage composed of three cysteines.

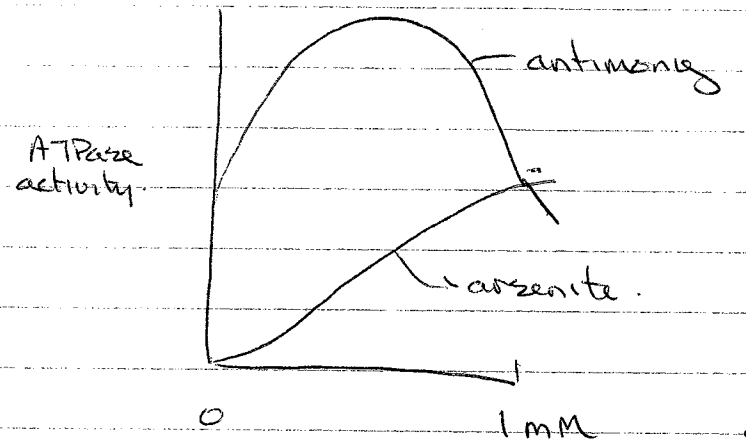
There is no doubt that binding of ATP & As(III) causes conformational changes in the protein ⓔ.

trypsin digestion	⟶	30 kDa product
+ ATP	⟶	partial protection
+ antimony	⟶	no protection
+ ATP + antimony	⟶	complete protection.

ATP probably binds at 2 nucleotide binding domains, one at the N-terminal, the other at the C-terminal. In fact, mutations at the N-terminal site cause: a) sensitivity to arsenite; b) no arsenite extrusion; c) no oxyanion-stimulated ATPase activity or ATP binding ⓕ

ⓔ J. Biol. Chem. 264: 17349-17354 [1989]
ⓕ J. Biol. Chem. 265: 7832-7836 [1990]

The activity of the ArsA gene product is oxyanion-stimulated ATPase activity.



In $ArsA^-$/$ArsB^-$ mutants there is no arsenate efflux.

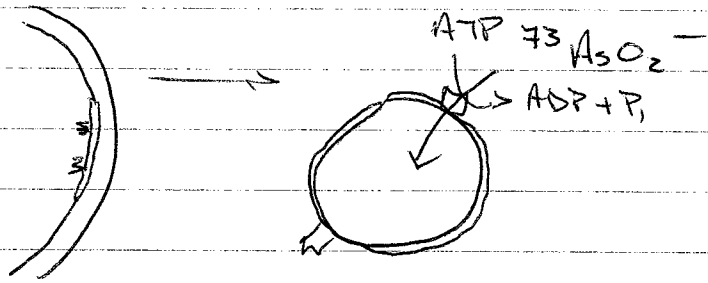
In $ArsB^-$ mutants, the ArsA ATPase activity (that is, oxyanion-stimulated activity) is found in the cytosol, not on the bacterial inner membrane

In addition, since ArsB is membrane bound, based on hydropathy plots, this suggests that ArsB is the membrane anchor for ArsA. (9)

So, it is easy to hypothesize that, analogous to the F_1/F_o ATP synthetase story, the ArsB protein would be the equivalent of the F_o, although it would be unique in the sense of being an oxyanion channel. Aspects of this hypothesis can be easily examined in E. coli, using a variety of mutants

(9) Tisa LS & BP Rosen 1990 Molecular characterization of an anion pump. The ArsB protein is the membrane anchor for the ArsA protein. J. Biol. Chem. 265: 190-194.

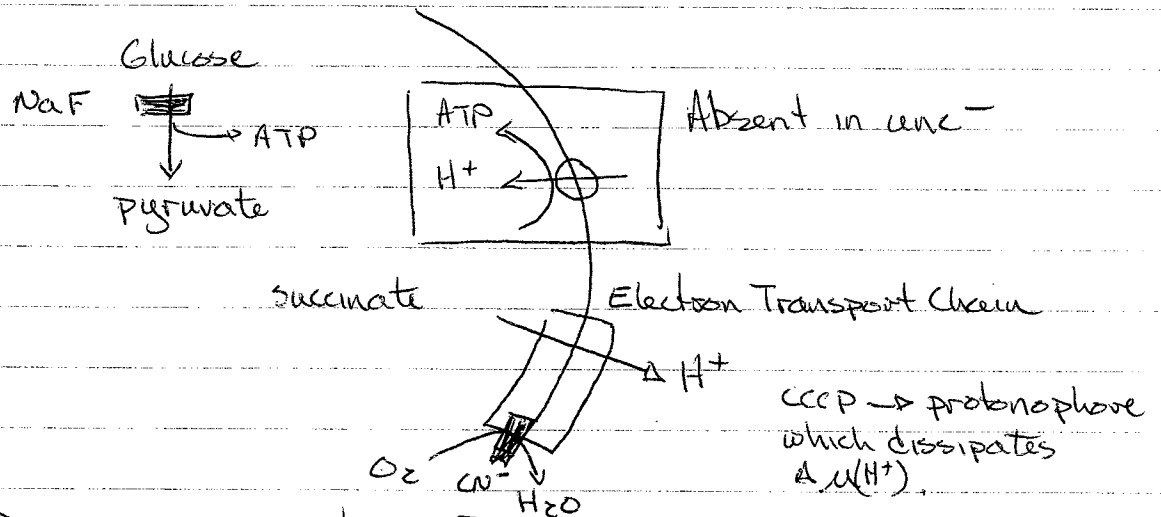
First, arsenite transport can be demonstrated in vitro. E. coli cells are broken apart, and everted vesicles isolated:



Arsenite uptake is only observed when both the ArsA & ArsB proteins are expressed.(h)

mutants

To probe the role of ArsB, a set of mutants were produced in unc$^-$ (an E. coli strain lacking the F_1/F_o ATP synthetase).



(h) Dey, S, D Dou & BP Rosen 1994 ATP-dependent arsenite transport in everted membrane vesicles of Escherichia coli. J. Biol. Chem. 269: 25442 - 25446. ATP required (no other nucleotide, incl. GTP, CTP & UTP worked), was not sensitive to known inhibitors of other ATPase transporter families.

ARSENATE HANDOUT

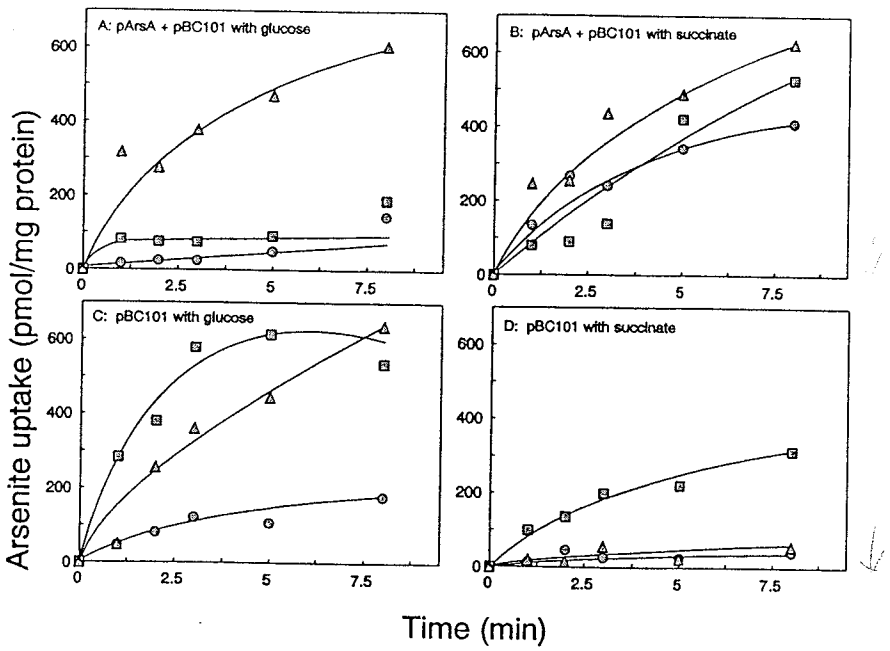


FIG. 3. Energetics of ArsB-mediated arsenite transport. Cultures were grown in TEA medium supplemented with sodium succinate. Accumulation of $^{73}AsO_2^-$ was measured in deenergized cells bearing both plasmids pBC101 (*arsBC*) and pArsA (*arsA*) (A and B) or only plasmid pBC101 (C and D). Either 20 mM glucose (A and C) or 20 mM sodium succinate (B and D) was added as an energy source. For each panel, no inhibitor (●), 20 mM KCN (■), or 10 mM NaF (▲) was added.

SOURCE DEY S., BP ROSEN 1995 Dual mode of energy coupling by the oxyanion-translocating ArsB protein. J. Bacteriol. 177 385-389.

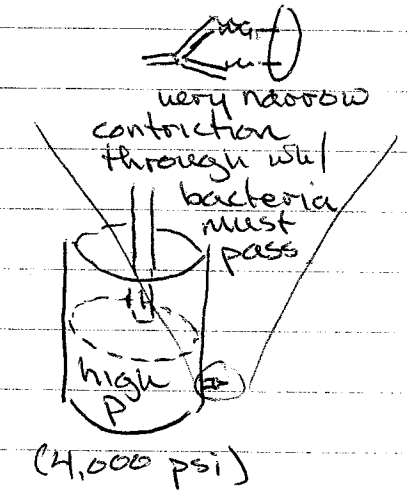
TABLE 1. ATP content and O_2 consumption in *E. coli* LE392Δ*uncIC*

Energy source[a]	O_2 consumption (nmol/mg/min)	ATP content (nmol/mg)
Endogenous	20	0.31
10 mM glucose	583	3.16
10 mM glucose + 10 mM KCN	17	3.61
10 mM glucose + 10 mM NaF	ND[b]	0.58
10 mM glucose + 10 μM CCCP	ND	2.75
10 mM succinate	107	0.27
10 mM succinate + 10 mM KCN	50	0.22
10 mM succinate + 10 mM NaF	ND	0.18
10 mM succinate + 10 μM CCCP	ND	0.47

[a] Cells were grown in TEA medium supplemented with 0.15% sodium succinate to induce succinoxidase activity.
[b] ND, not determined.

In addition to the indirect in bacteria experiments, it is essential to obtain direct biochemical evidence to corroborate the existence of an ATP-dependent Arsenic pump. (1)

To do this, membrane vesicles must be isolated from the cytoplasmic membrane of E-coli.



Induced E. coli suspension

↓ lysed by passage through a French Press

homogenate

↓ centrifuge at 100,000 x g for 60 minutes

pellet

↓ resuspend

membrane vesicles

Assay for ATP-dependent $^{73}As(III)$ uptake.

(1) Dey S, D Dexian & BP Rosen 1994 ATP-dependent arsenite transport in everted membrane vesicles of Escherichia coli. J. Biol. Chem. 269 25442-25446

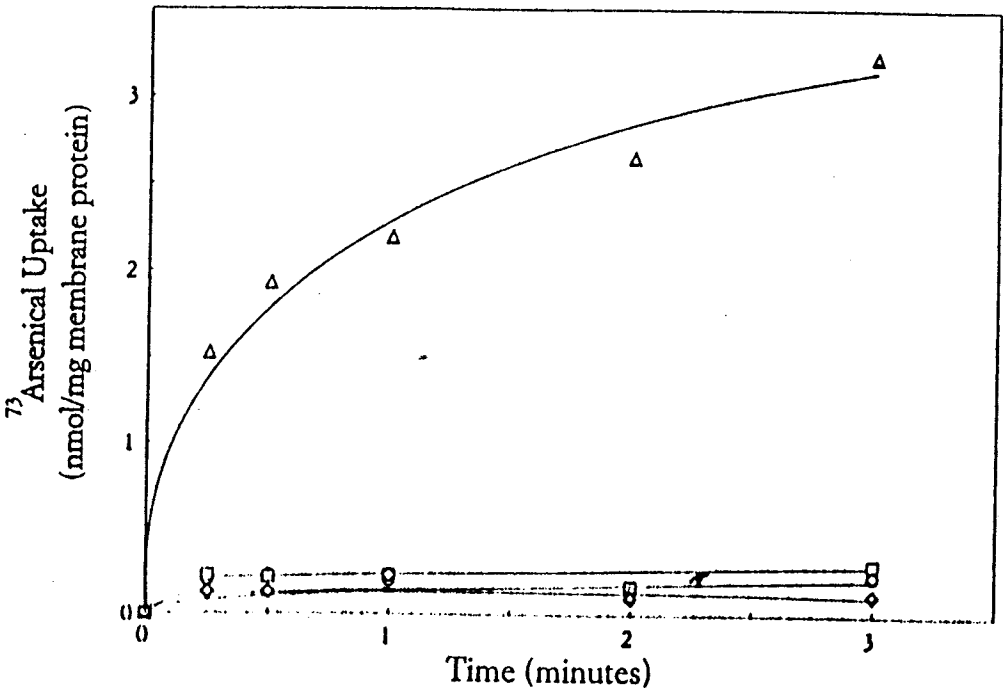


FIG. 1. **Uptake of $^{73}AsO_2^-$ or $^{73}AsO_4^{3-}$ into everted membrane vesicles.** Everted membrane vesicles prepared from cells of *E. coli* strain HB101 bearing either plasmids pJUN4 (*arsAB2, arsC*) and pArsA (*arsA*) (▲, ●) or vector plasmids pBR322 and pACYC184 (■, ♦) were assayed for uptake of $^{73}AsO_2^-$ (▲, ■) or $^{73}AsO_4^{3-}$ (●, ♦), as described under "Material and Methods." Each assay contained 5 mM ATP and an ATP regenerating system, and the reaction was started by addition of 5 mM $MgCl_2$.

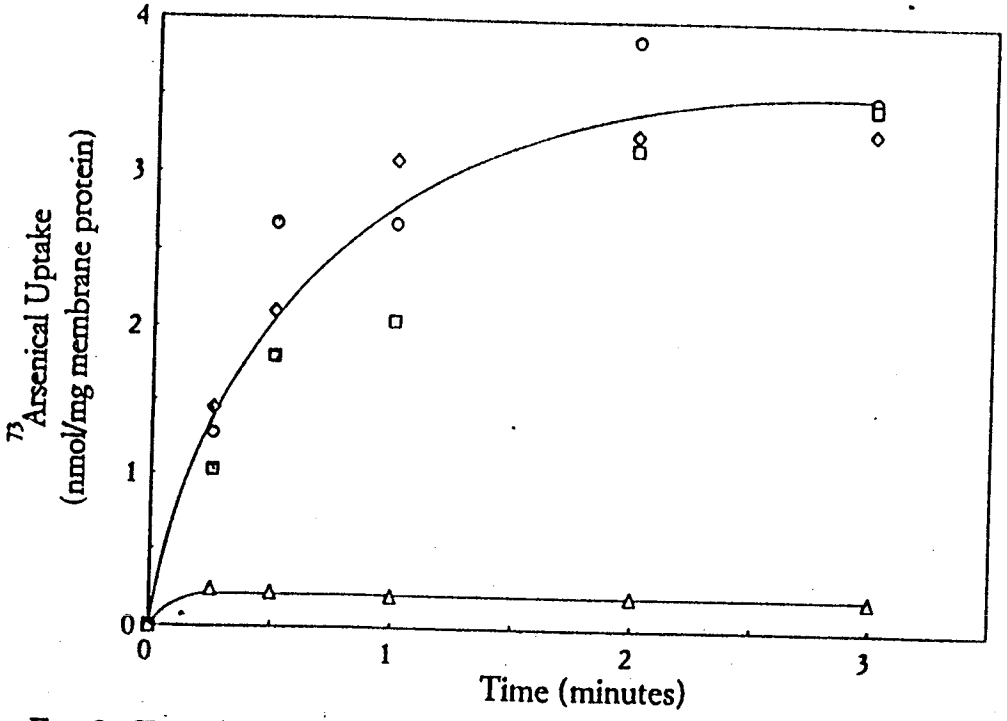
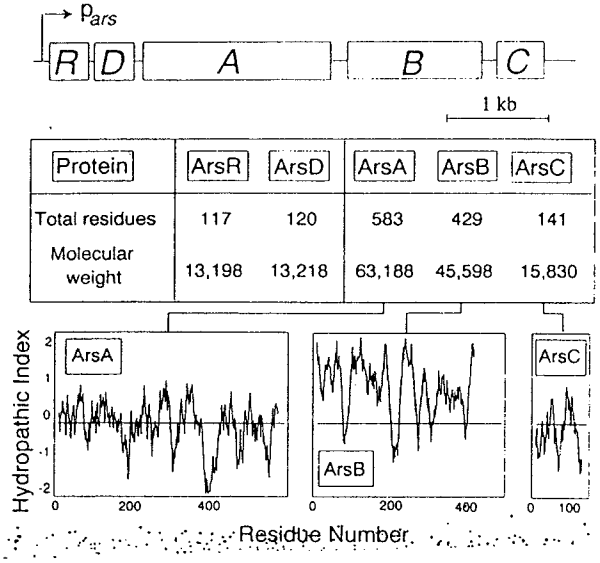
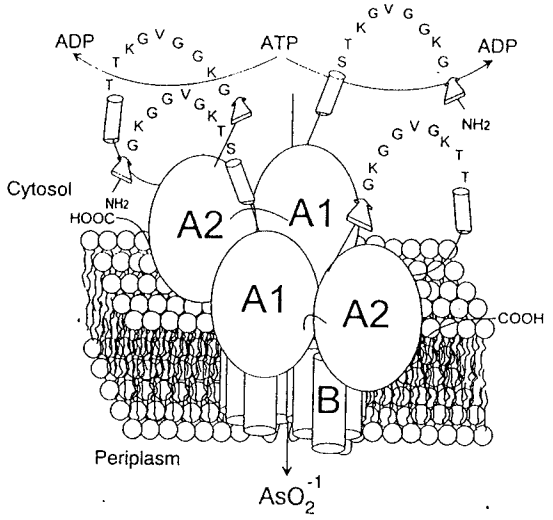


FIG. 3. **Effect of ATPase inhibitors.** $^{73}AsO_2^-$ uptake in everted membrane vesicles prepared from cells of *E. coli* HB101 bearing both plasmids pJUN4 (*arsAB2*) and pArsA (*arsA*) was assayed in absence of inhibitors (■) or in the presence of 0.1 M of sodium orthovanadate (○), sodium azide (♦), or *N*-ethylmaleimide (▲). The vesicles were preincubated with inhibitors and ATP for 2 min prior to addition of $^{73}AsO_2^-$ and $MgCl_2$.





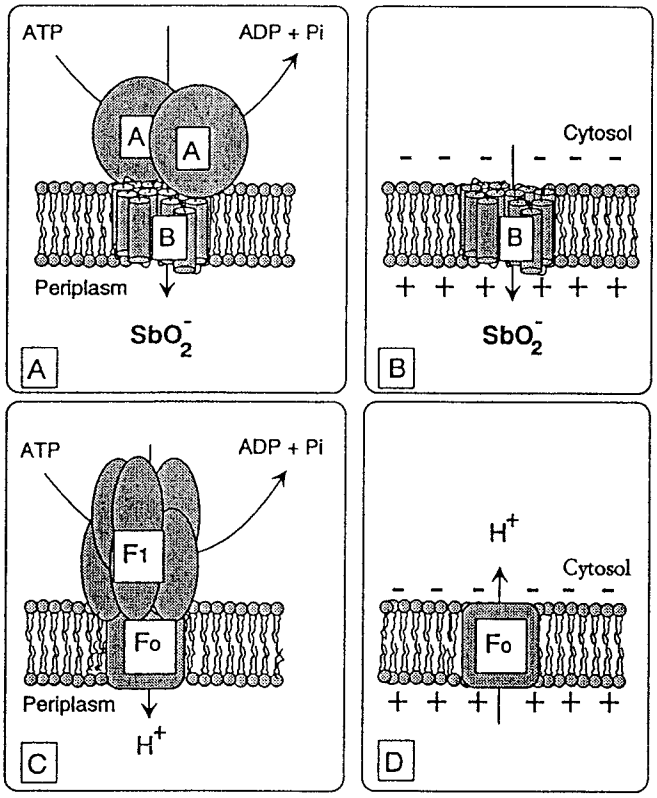


FIG. 5. Dual mode of energy coupling of ion transport systems. The components of ion transport systems such as the arsenite pump (A and B) or the H^+-translocating F_0F_1 (C and D) can function as either (A and C) primary ATP-driven pumps or secondary $\Delta\psi$-coupled porters (B and D), depending on association of the catalytic subunits with the intrinsic membrane subunits.

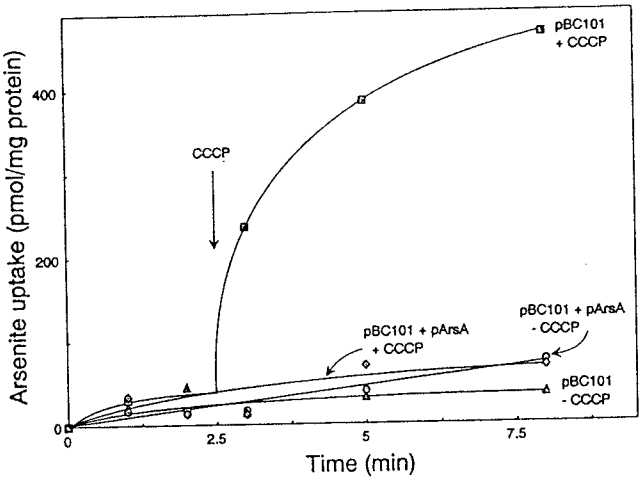


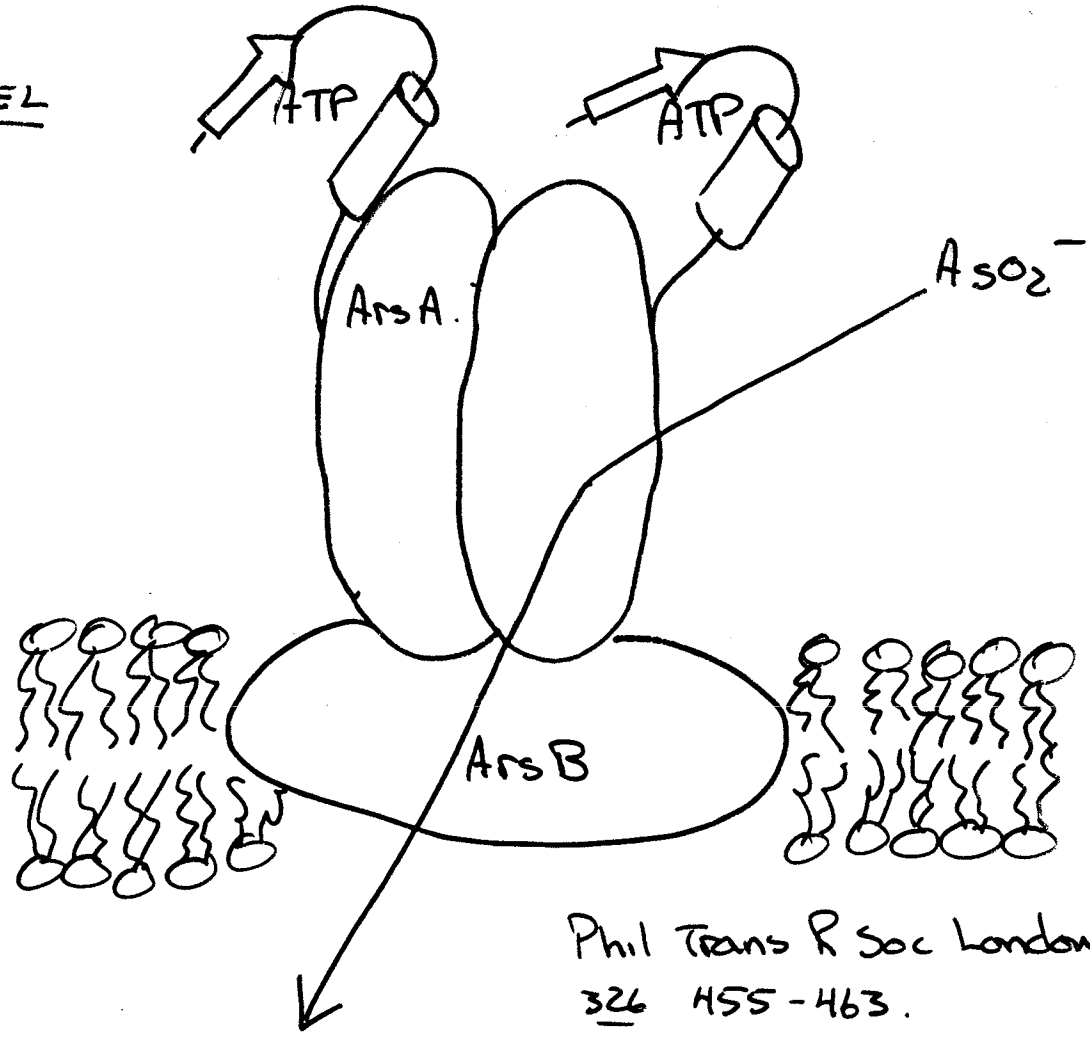
FIG. 4. Effect of uncoupler on ArsB-mediated arsenite transport. Cultures were grown in TEA medium supplemented with sodium succinate. The cells were depleted of endogenous energy reserves, and accumulation of $^{73}AsO_2^-$ was measured following addition of 20 mM glucose to cells bearing both plasmids pArsA (*arsA*) and pBC101 (*arsBC*) or 20 mM sodium succinate to cells bearing only plasmid pBC101. At 2.5 min, 10 μM CCCP was added to cells bearing both plasmids or just plasmid pBC101.

SUMMARY

protein	ArsR	ArsA	ArsB	ArsC
total residues	117	583	429	141
Mw	13.2 kDa	63.2	45.6	15.8
est pI	9.8	6.1	9.8	5.9
function	regulatory	ATPase*	Anion Channel	Recognition of arsenate

nb nucleotide-binding domains similar to and overall homology to nifH gene of nitrogenase (N_2-reductase)(which also has ATPase as part of its function).

MODEL



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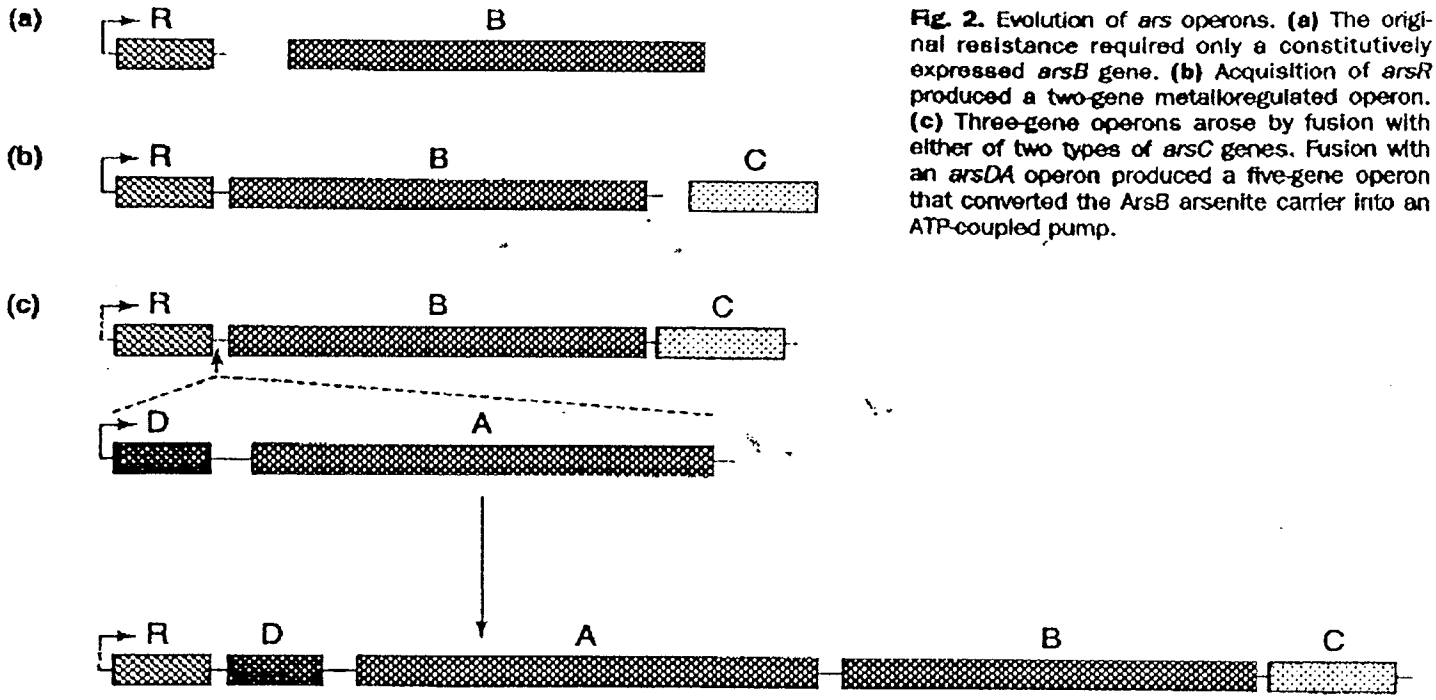


Fig. 2. Evolution of *ars* operons. **(a)** The original resistance required only a constitutively expressed *arsB* gene. **(b)** Acquisition of *arsR* produced a two-gene metalloregulated operon. **(c)** Three-gene operons arose by fusion with either of two types of *arsC* genes. Fusion with an *arsDA* operon produced a five-gene operon that converted the ArsB arsenite carrier into an ATP-coupled pump.

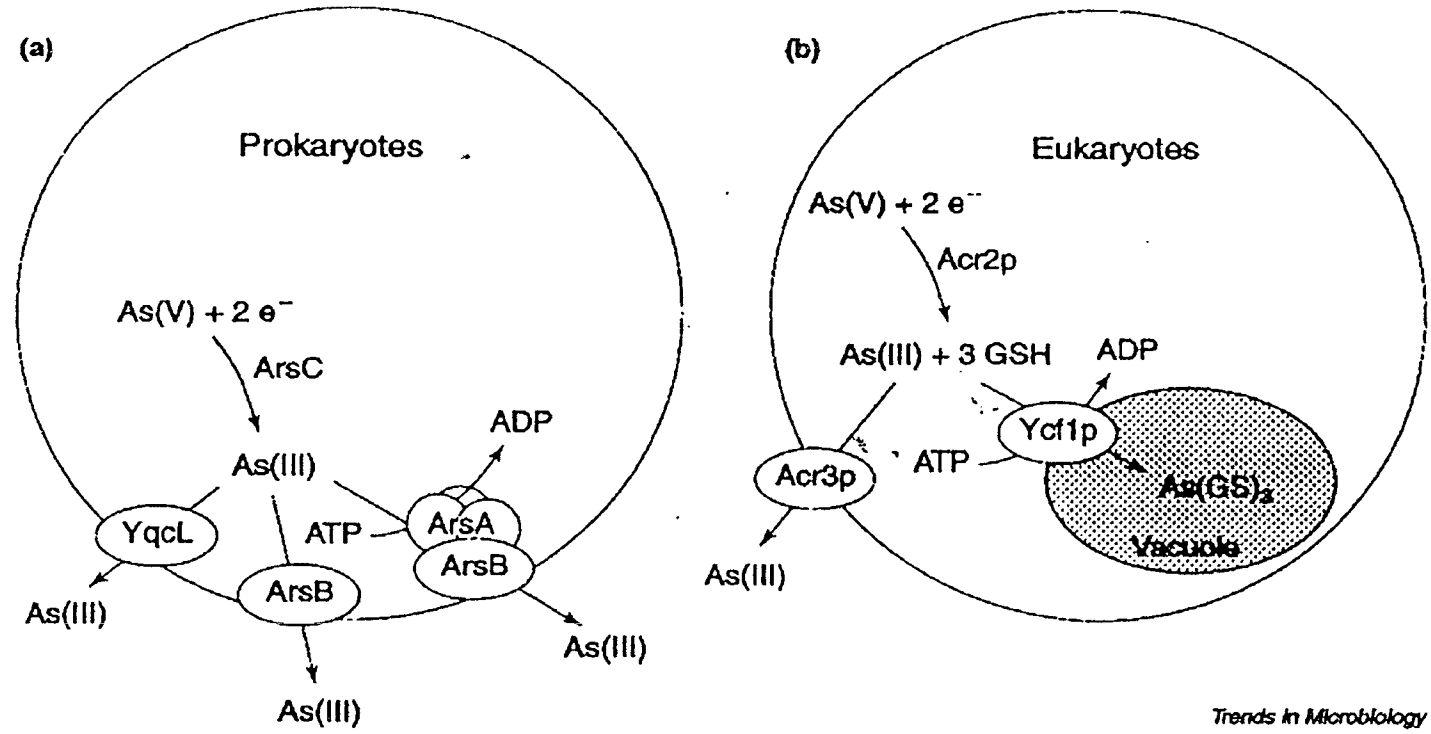


Fig. 1. Arsenical detoxification mechanisms in prokaryotes and eukaryotes. In bacteria or *Saccharomyces cerevisiae*, one of three arsenate reductases ($ArsC_{ec}$, $ArsC_{pm}$ or Acr2p) transforms As(V) to As(III), which is then removed from the cytosol. **(a)** Bacteria have two types of arsenite transporters, ArsB or YqcL (or, as in *Bacillus subtilis*, both). ArsB has a dual mode of energy coupling, functioning as a secondary carrier or, in a complex with ArsA, as an ATP-coupled arsenite pump. **(b)** *S. cerevisiae* also has two types of arsenite transporters: Acr3p, a YqcL homolog, or Ycf1p, a member of the ABC superfamily. Acr3p is a secondary carrier located in the plasma membrane that extrudes arsenite from the cells. Ycf1p pumps $As(GS)_3$ into the vacuole. In both cases, removal of arsenite from the cytosol confers resistance.

To understand how effective the Ars AB arsenate pump is. That is, how well it can remove arsenate from the cytoplasm, we need to consider the energetics.

Our starting point is to consider the arsenate efflux as a vectorial chemical reaction.

$$ATP + AsO_4^{3-}{}_{inside} \rightleftharpoons ADP + P_i + AsO_4^{3-}{}_{outside}.$$

$AsO_4^{3-}{}_{inside} \rightsquigarrow As_i$

$AsO_4^{3-}{}_{outside} \rightsquigarrow As_o$

At equilibrium, the total Gibbs free energy is the sum of the Gibbs free energy for ATP hydrolysis and the chemical potential for "As".

$$\Delta G_{TOT} = n \cdot \Delta\mu_{As} + \Delta G_{ATP} = 0 \quad \text{(equilibrium)}$$

n: stoichiometry ($=1$ in our case)

now, $\Delta G_{ATP} = \Delta G^{\circ} + 2.3\,RT \log_{10} \dfrac{[ADP][P_i]}{[ATP]}$ (products / reactant)

$\Delta G^{\circ} \rightsquigarrow$ The standard Gibbs free energy will vary with $[Mg^{2+}]$ and pH. It is in the range of 7 - 10 kcal/mole.

$$\Delta\mu_{As} = \#\, 2.3\,RT \log_{10} \frac{[As_i]}{[As_o]} + zF\Delta\Psi$$

z: valence

Since $\Delta G_{TOT} = 0$, we can equate the two (ΔG_{ATP} & $\Delta\mu_{As}$)

$$\Delta G^\circ + 2.3RT \log_{10} \frac{[ADP][P_i]}{[ATP]} = 2.3RT \log_{10} \frac{[As_i]}{[As_o]} + zF\Delta\Psi$$

Now, there are real complications associated with the use of energetics. First, the reactants and products are more complex than "just" ADP, P, & ATP, and we are often challenged to get accurate quantitation of concentrations in the cytoplasm.
Second, we don't know the stoichiometry.
If two "As" are transported:

$$ATP + 2As_i \rightleftharpoons ADP + P_i + 2As_o$$

The equilibrium $\frac{\text{products}}{\text{reactants}}$ is

$$\frac{[ADP][P_i][As_o]^2}{[ATP][As_i]^2}$$

Finally H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ or AsO_4^{3-} ?
Which?
It makes a big difference in the equilibrium concentrations of As_i at a given ΔG_{ATP}.

The energetics
Even so, it offers insight into the relative ability of the bacterial cell to exclude As.

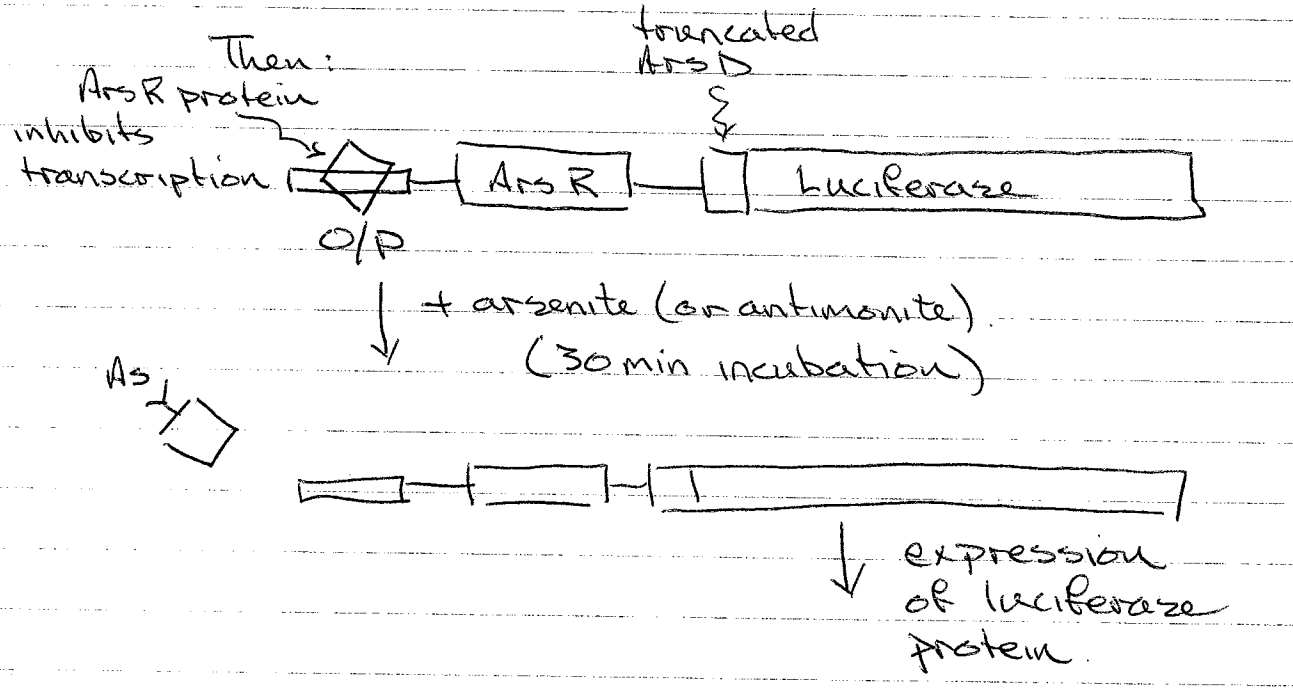
Arsenic-sensing: the bioengineering angle.

With a clear understanding of the Ars operon, there is a clear path to applying that knowledge in these biotechnological applications

In particular, it becomes possible to create an arsenic bio-sensor.

To do this, a plasmid is constructed:

pRLUX is a circular plasmid which includes an ori sequence (for plasmid replication) and amp (conferring resistance to ampicillin for use in transformation (selection).



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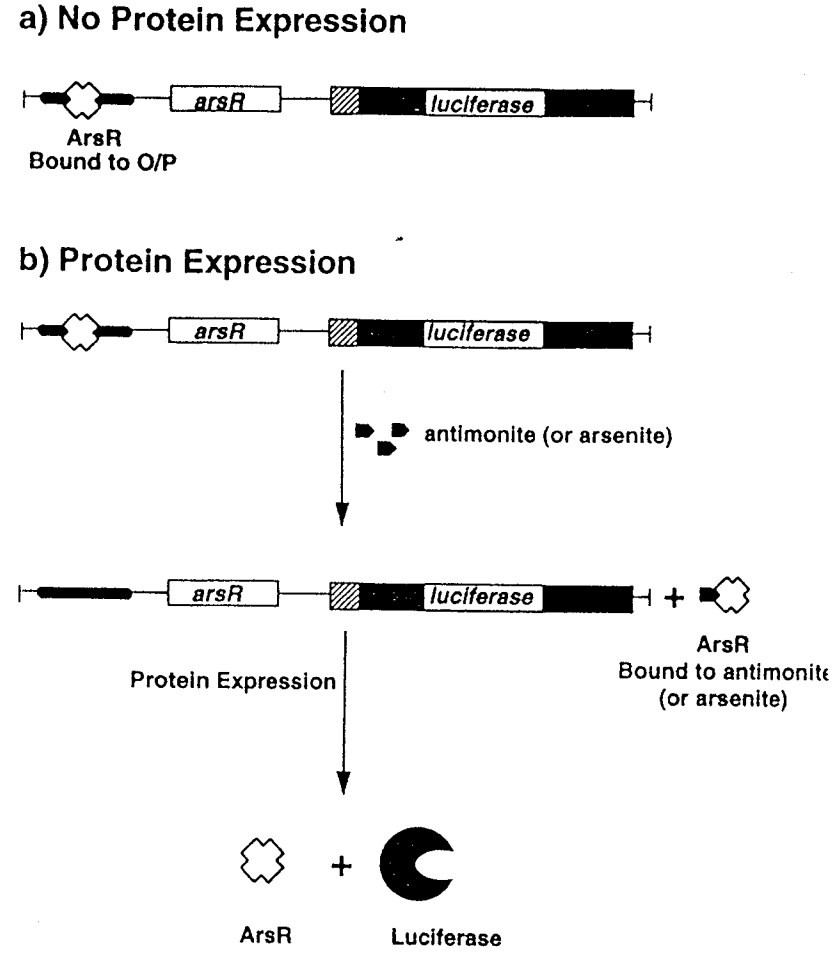


Figure 2. Schematic representation of the interactions between the operator/promoter (O/P) of the *ars* operon in plasmid pRLUX and ArsR. (a) In the absence of antimonite (or arsenite), no luciferase is being produced. (b) The presence of antimonite (or arsenite) results in the subsequent expression of luciferase.

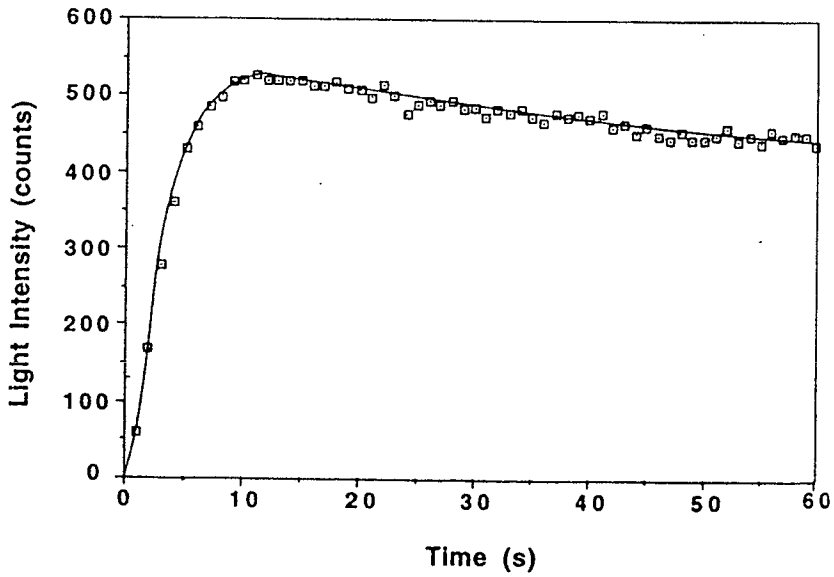


Figure 3. Bioluminescence emission of bacteria with pRLUX plasmid. A volume of 100 μL of 63 μM decanal was injected into a solution containing 50 μL of bacteria in 250 μL of Tris–EDTA buffer.

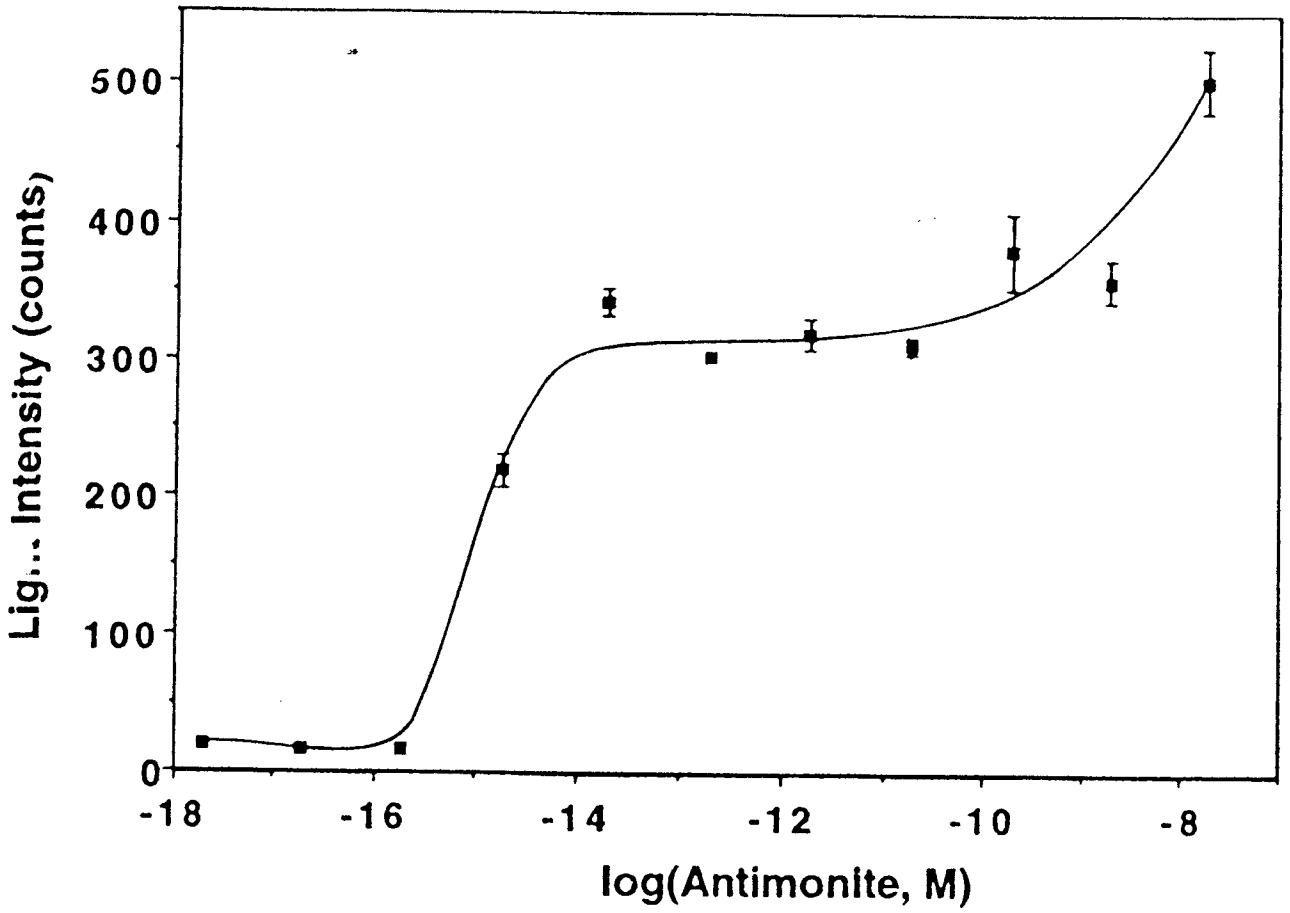
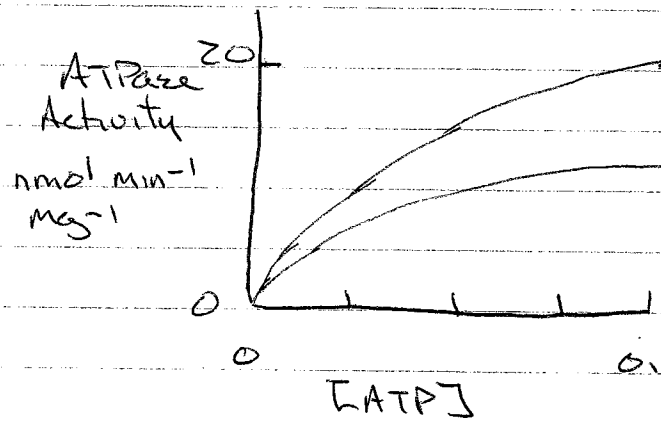


Figure 4. Dose–response curve for antimonite performed after the bacteria with the pRLUX plasmid were incubated with potassium antimonyltartrate standard solutions for 30 min. A volume of 100 μL of 63 μM decanal was injected into 50 μL of bacteria in 250 μL of Tris–EDTA buffer. The bioluminescence signal was integrated over a period of 3 s and has been corrected with respect to the blank. Data are the average $\pm$ 1 standard deviation ($n = 3$).

Human arsenite pumps ?

In a surprising development Kuroda-Hardee et al. (1998)* reported a arsenite-stimulated ATPase in humans.

The gene was cloned based on sequence homology with the ArsA gene. The sequence codes for a 37 kDa protein. Unlike bacteria, the standard molecular tools for deducing function by deleting the gene are not available for humans. Instead, the protein was isolated as a GST-fusion protein so its function could be examined



	K_m^{ATP}	V_{max}
+100 μM Na-arsenite	0.33	31
-arsenite	0.22	17

~ 2-fold stimulation
[compared to 10- to 20-fold for ArsA ATPase]

whether it really functions as an arsenic pump remains to be seen: An ArsB homolog is yet to be found

*Kuroda-Hardee B, D Heath, S Aebi, & SB Howell 1998 Biochemical characterization of the human arsenite-stimulated ATPase (hASNA-1). J. Biol. Chem 273: 22173-22176.

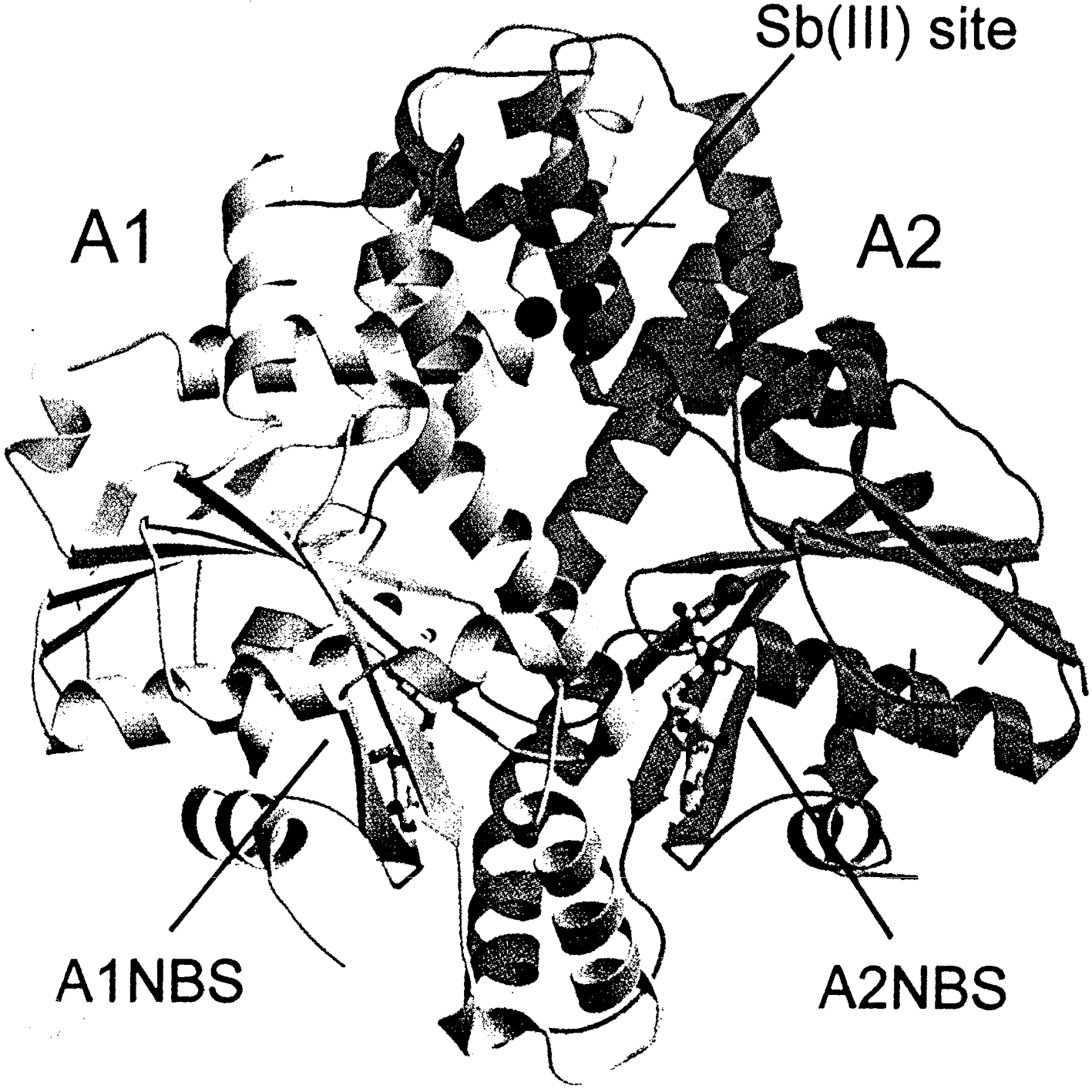
Structural Characterization of the ArsA protein.

The most recent advance in characterization of the arsenate pump is x-ray crystallography of ArsA.[1] Needless to say, structural characterization of ArsB - the putative oxyanion channel would be more enlightening for a membrane transport class; but, far more difficult to accomplish.

The ArsA protein exhibits a 'pseudo' two-fold axis of symmetry. The N- & C- termini align such that the two nucleotide binding domains 'face' each other.

There appear to be multiple Sb(III) binding sites. In fact, the x-ray crystallographic data reveal that As/Sb coordination relies not just on cysteine S-groups, but also coordination with histidine groups.

[1] Zhou T, S Radaev, BP Rosen & DL Gatti 2000 Structure of the ArsA ATPase: the catalytic subunit of a heavy metal resistance pump. EMBO J 19 4838-4845



C

There is a structural 'hint', if you will, of how the ATP hydrolysis is coupled to transport of the Sb(III) / As(III).

The two sites (ATP- & As(III) -binding) are distant from one another in the enzyme, but there are two elongate amino-acid sequences which extend from the binding site for ATP to the binding region for Sb/As.

A intuitive explanation for 'pumping' would require:

a) ATP-binding/hydrolysis modifying the affinity of As/Sb binding, and,
b) A conformational change which would result in the well-defined vectorial release of As/Sb. So that entry into an ArsB protein channel would be assured.

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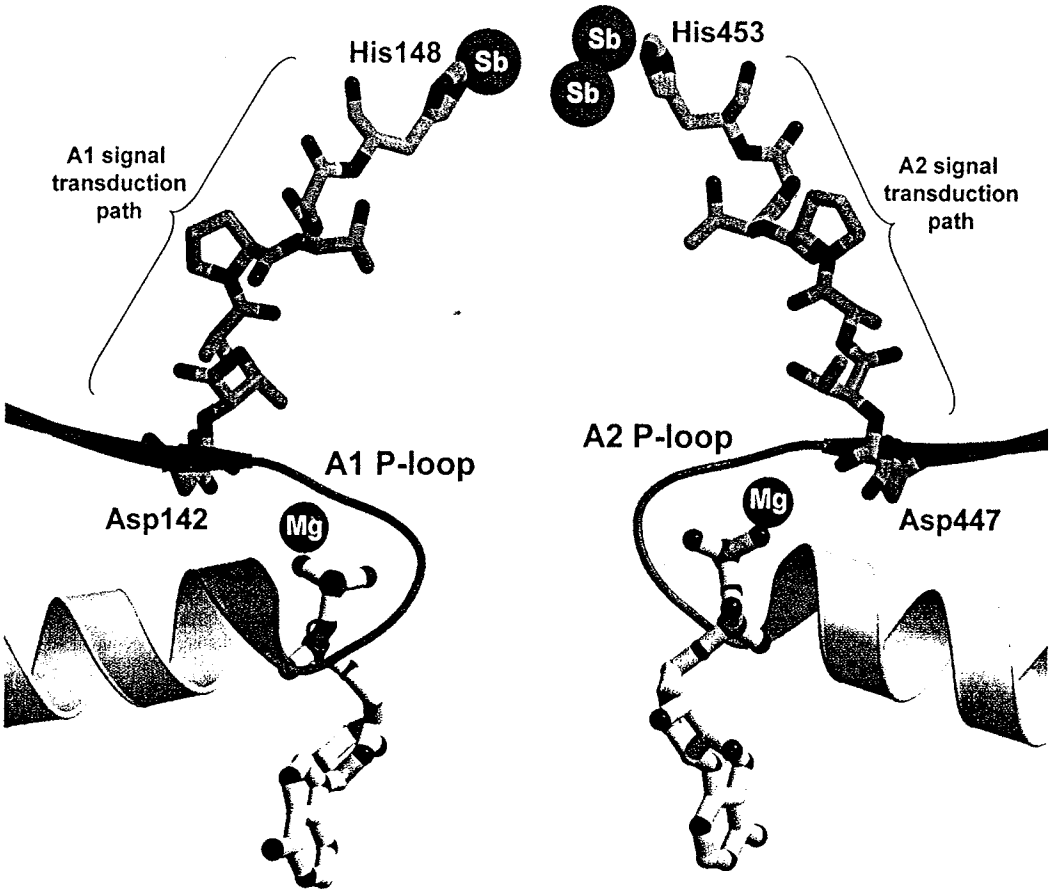


Fig. 5. The signal transduction pathway. Two stretches of seven residues with the identical sequence $D_{142/447}TAPTGH_{148/453}$ connect the A1 and A2 NBS to the metal-binding site. Strands (dark orange), helices (ivory) and P-loops (chartreuse) are drawn as ribbons. The nucleotides bound in the two NBSs are shown as ball-and-stick models colored according to atom type (phosphorus, yellow; oxygen, red; nitrogen, blue). The DTAPTGH sequences are shown as stick models with cyan bonds. Sb(III) (blue) and Mg^{2+} (hot pink) are shown as space-filling models. Generated with MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merritt and Murphy, 1994).

In the latest structural characterization, there are hints, but only hints, of similarities to the ABC transporter family.

The similarities between the AosAB & P-glycoprotein (An ABC member):

1) Both are ATPases/activated by the substrates they pump. (Also true of the K-ATPase Na K ATPase (P-types) etc.)
2) Both have two similar consensus nucleotide binding sites (okay. many enzymes bind nucleotides)
3) Both have 12 membrane-spanning α-helices. (?? - what proof?)

[Zhou et al., 2000]

It's a very far stretch. What is true is similar function - toxin-pumping

VARIATIONS on a THEME...

Although arsenate resistance appears to involve

1) arsB - the channel alone.

or

2) arsA & B - An ATP-dependent oxyanion pump

In most bacteria, there are other variations on a theme which involve other transport processes.

In the legume symbiont Sinorhizobium meliloti, there is a cluster of four open reading frames:

ORF

1 (arsR) SMc02647: homologous to arsR (regulatory)

2 (aqpS) SMc02648: homologous to aquaporins. aquaporins are a family of proteins which transport water across membranes.

with homology to the bacterial glycerol facilitator (GlpF), yeast aquaglyceroporin (Fps1p) and mammalian aquaglyceroporin (AQP9)

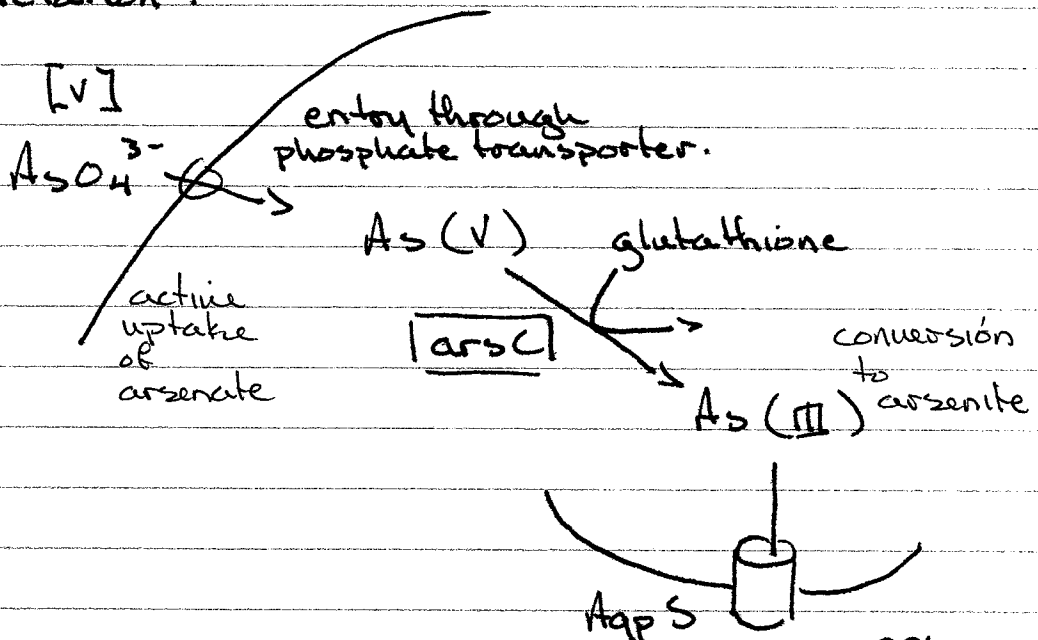
3 (arsC) SMc02649: homologous to arsC (arsenate reductase)

4 (arsH) SMc02650: homologous to NADPH-dependent flavin mononucleotide reductase class of enzymes.

Mutant.	Growth on sodium arsenite	sodium arsenate
Wildtype	0.95	0.70
Δaqp S	0.80	0.55
Δ ars C	0.80	0.40
Δ ars H	0.10	0.25

When the aqp S & ars C genes are expressed in an E. coli mutant lacking the native ars operon, the two genes confer arsenate resistance but not arsenite resistance.

Interpretation:



Yang H-C, J Cheng, TM Finan, BP Rosen, H Bhattacharjee (2005) Novel pathway for arsenic detoxification in the legume symbiont Sinorhizobium meliloti. J. Bacteriol. 187: 6991-6997.