Studies on Intracellular Trafficking of Metals and Huntingtin Associated Cargos

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Intracellular trafficking of metals/metalloids is well regulated

Periodic Table of the Elements

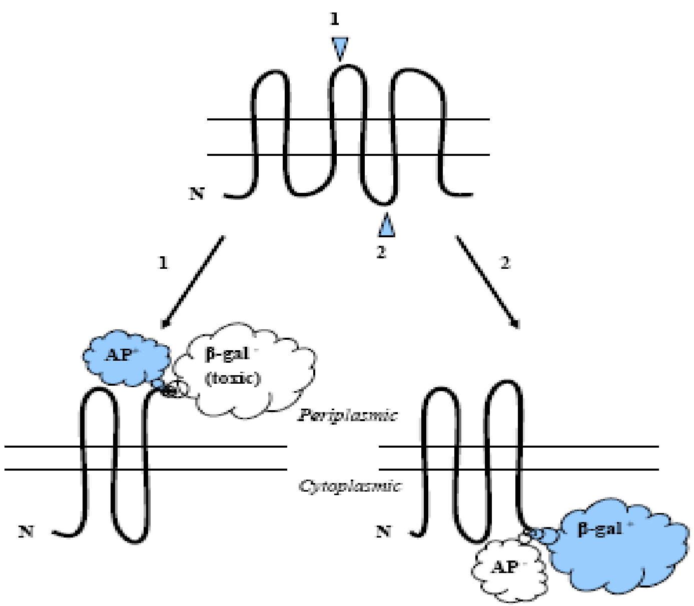
	IA	IIA	IIIB	IVB	VB	VIB	VIIB	VII			IB	IIB	IIIA	IVA	VA	VIA	VIIA	0
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	57 *La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	89 +Ac	104 Rf	105 Ha	106 Sg	107 Ns	108 Hs	109 Mt	110 110	111 111	112 112						

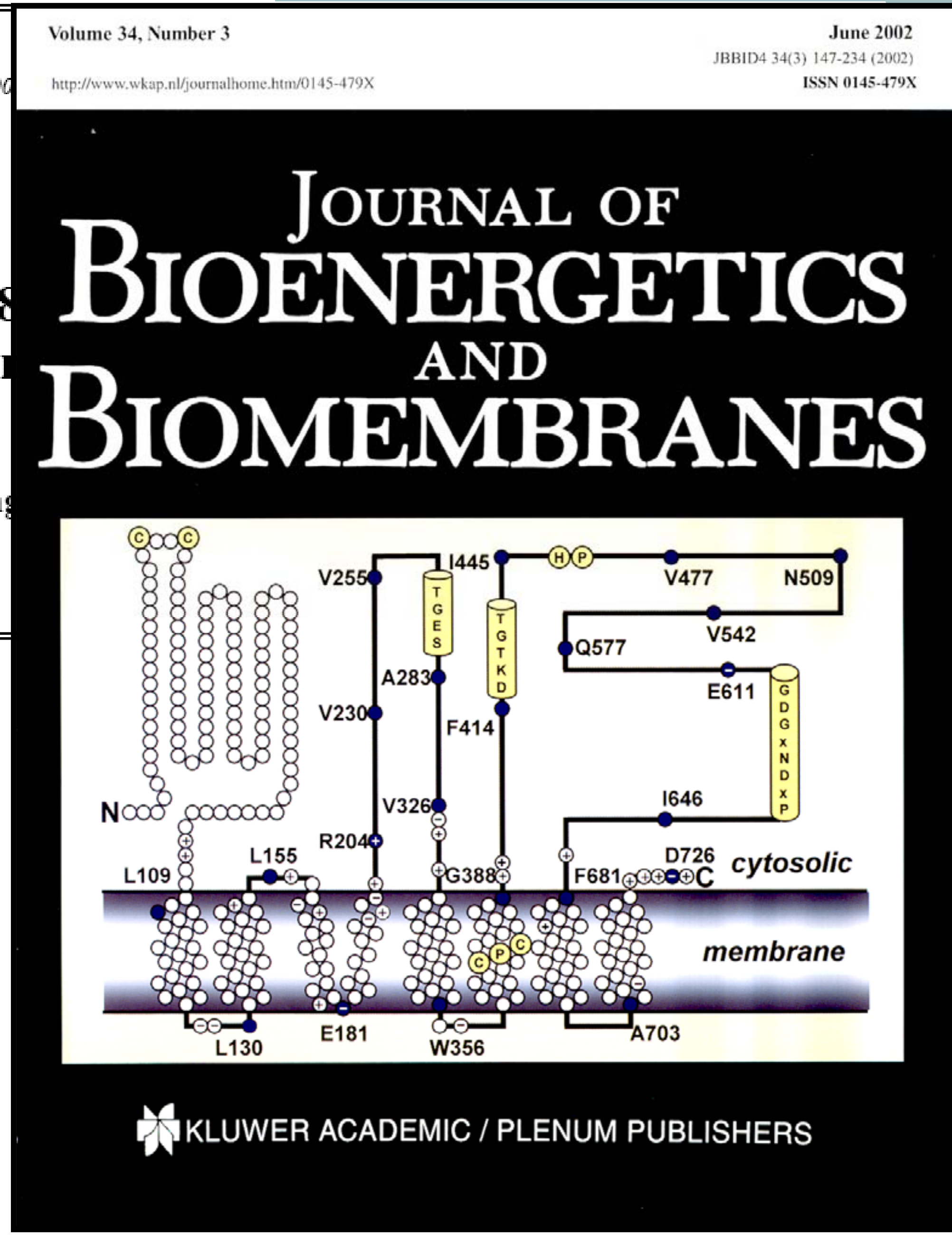
* Lanthanide Series	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
+ Actinide Series	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

Journal of Bioenergetics and Biomembranes, Vol. 34, No. 3, June 2002 (© 200

Membrane Topology of the pl258 Cd(II)/Pb(II)/Zn(II)-Translocati

Kan-Jen Tsai,[1,4] Yung-Feng Lin,[2] Marco D. Wong
Hsueh-Liang Fu,[2] and Barry P. Rosen[3]

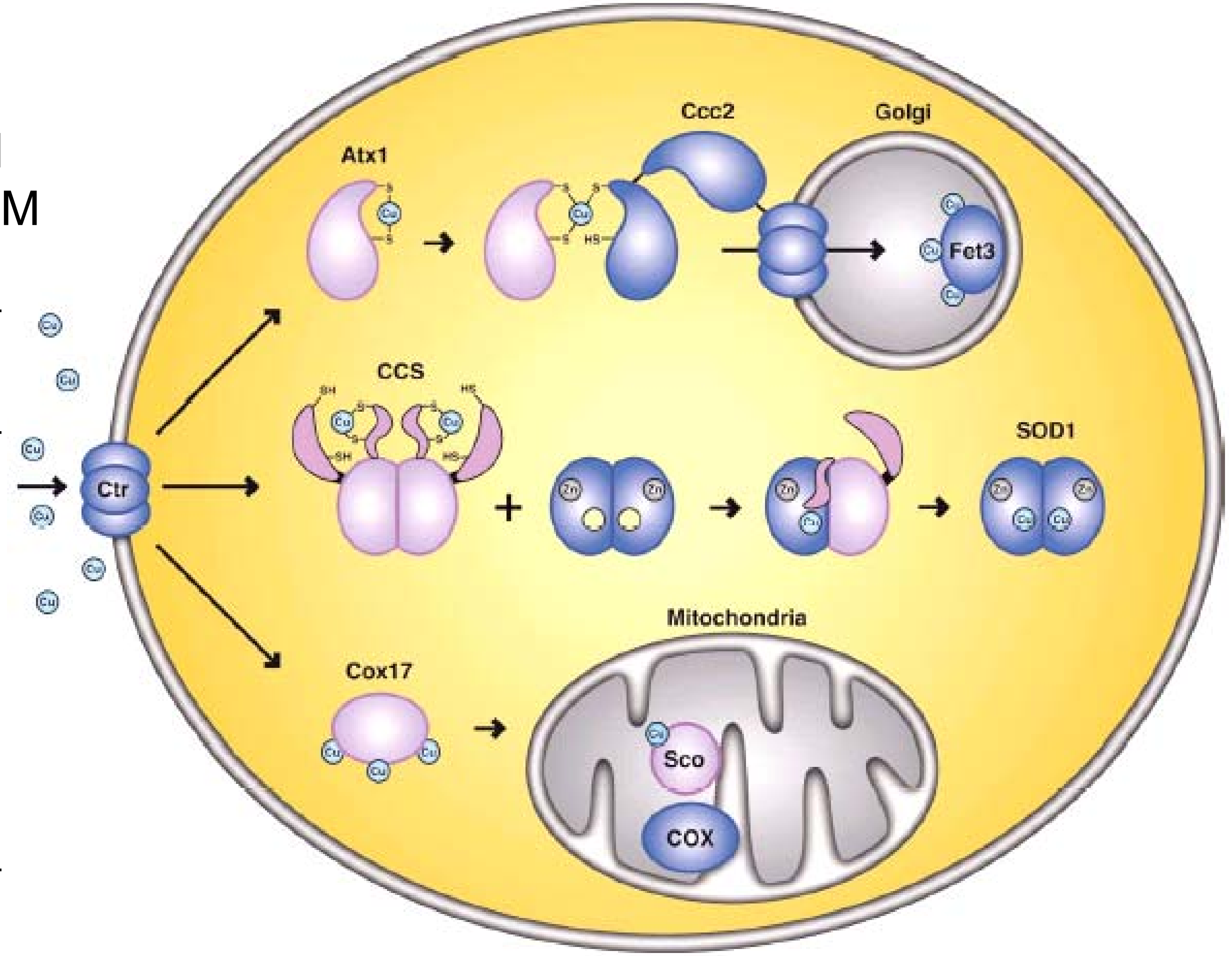




Metallochaperones
金屬陪伴子

Kd of SOD1 for Cu: 10^{-15} M
Cytoplasmic free Cu: 10^{-18} M

Metal	**Metallo-chaperone**	Target Protein
Cu	**CCS**	SOD1
	Cox17	Sco1
	Atx1	Ccc2
	Hah1 (Atox1)	ATP7A ATP7B
	CopZ	CopY CopA
Ni	**UreE**	urease
Fe	**Frataxin**	iron-sulfur clusters heme



Rosenzweig, *Science*, 2002

Arsenic is the most prevalent environmental toxic substance

Department of Health and Human Services
ATSDR Agency for Toxic Substances & Disease Registry

Home › CERCLA 2007 CERCLA Substance List

2007 CERCLA Priority List of Hazardous Substances

2007 RANK	SUBSTANCE NAME	TOTAL POINTS	2005 RANK	CAS #
1	ARSENIC	1672.58	1	007440-38-2
2	LEAD	1534.07	2	007439-92-1
3	MERCURY	1504.69	3	007439-97-6
4	VINYL CHLORIDE	1387.75	4	000075-01-4
5	POLYCHLORINATED BIPHENYLS	1365.78	5	001336-36-3
6	BENZENE	1355.96	6	000071-43-2
7	CADMIUM	1324.22	8	007440-43-9
8	POLYCYCLIC AROMATIC HYDROCARBONS	1316.98	7	130498-29-2
9	BENZO(A)PYRENE	1312.45	9	000050-32-8
10	BENZO(B)FLUORANTHENE	1266.55	10	000205-99-2

http://www.atsdr.cdc.gov/cercla/07list.html

Total points: Toxicity + Frequency of occurrence + Potential for human exposure

Arsenic chaperone

nature Vol 443|26 October 2006

RESEARCH HIGHLIGHTS

Hear, hear

Cell **127**, 277–289 (2006)
Researchers have uncovered a novel mechanism underlying inherited deafness.

Christine Petit of the Pasteur Institute in Paris, France, and her colleagues studied the mouse equivalent of a protein known to be defective in some people who are profoundly deaf. They found that the protein, otoferlin, is sited at a key location within the inner hair cells (pictured) of the cochlea.

These cells transform sound into signals that trigger auditory nerves to fire. Sacs of neurotransmitters are anchored to the inner side of membranes of these hair cells. They fuse with the membrane to release their contents, activating neighbouring nerve endings. Otoferlin is essential for fusion.

PLANETARY SCIENCE

Frosted Earths

Astrophys. J. **650**, L139–L142 (2006)
Recent observations have shown that some small stars called M dwarfs host icy planets that are roughly ten times more massive than the Earth. How are these 'super-Earths' made?

Planet formation around dwarf stars is different to that around Sun-like stars. This is because the dwarfs fade and shrink during the process, pulling in the 'snow line', which separates regions of icy-planet formation from those of rocky-planet formation.

Grant Kennedy of the Australian National University in Weston Creek and his team have concocted a theoretical model of this process, showing that it favours the rapid appearance of middleweight icy planets. As the contracting snow line moves like a cold front over small rocky protoplanets, they become thickly ice-coated before coagulating through collisions to make super-Earths.

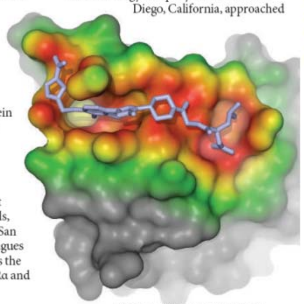
DRUG DISCOVERY

Target practice

Proc. Natl Acad. Sci. USA **103**, 15422–15427 (2006)
An analysis of how one small molecule interrupts a protein–protein interaction may help researchers to design new drugs.

Protein–protein interactions are promising drug targets, but researchers have struggled to find footholds for small molecules in flat protein–protein interfaces. Jim Wells, then at Sunesis Pharmaceuticals in San Francisco, California, and his colleagues studied a small molecule that blocks the interaction of two proteins — IL-2Rα and IL-2, involved in conveying immune signals.

This small molecule binds within a crevice of IL-2 (pictured below). They found that it targets many of the same contact points as does IL-2Rα, despite having a different structure. This is possible because IL-2 is very flexible. The finding shows that small molecules do not need to structurally mimic the proteins they displace.



STEM CELLS

Grown naturally

Nature Biotechnol. doi:10.1038/nbt1259 (2006)
Researchers have edged a step closer to making cells that might cure diabetes.

Diabetes occurs when 'beta' cells in the human pancreas fail to make enough of the hormone insulin. Making functional beta cells from human embryonic stem cells might cure this deficit, but it has proved difficult.

A team led by Emmanuel Baetge at the biotechnology company Novocell in San Diego, California, approached the problem by trying to coax human embryonic stem cells through the stages of normal fetal pancreatic development. The stem cells did develop into cells that produce high levels of insulin, but not in response to the body's normal chemical triggers.

CELL BIOLOGY

A chaperone for arsenic

Proc. Natl Acad. Sci. USA **103**, 15617–15622 (2006)
Arsenic is flushed through biological systems with the help of a protein that clings to the toxic metal and guides it to a cellular-scale pump, a new study finds.

Researchers are driven to understand arsenic toxicity because the metal contaminates water supplies in areas such as Bangladesh and West Bengal. In this study, Barry Rosen of Wayne State University in Detroit, Michigan, Adrian Walmsley of Durham University, UK, and their team identify a protein, ArsD, in bacterial cells that picks up arsenite ions from the cell's cytoplasm. ArsD then liaises with an enzyme to activate the cell's efflux pump. The protein is therefore acting as a metallochaperone — the first to be described for arsenic.

GEOLOGY

Deep impact

Earth Planet. Sci. Lett. doi:10.1016/j.epsl.2006.09.009 (2006)
A painstaking survey of rocks from around the globe has provided new information about the nature of a meteorite impact 65 million years ago, which may have triggered the mass extinction that wiped out the dinosaurs.

The impact would have been most devastating if the meteorite hit at a shallow

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CELL BIOLOGY

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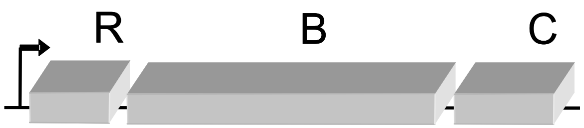
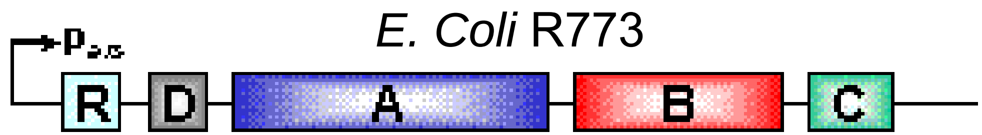
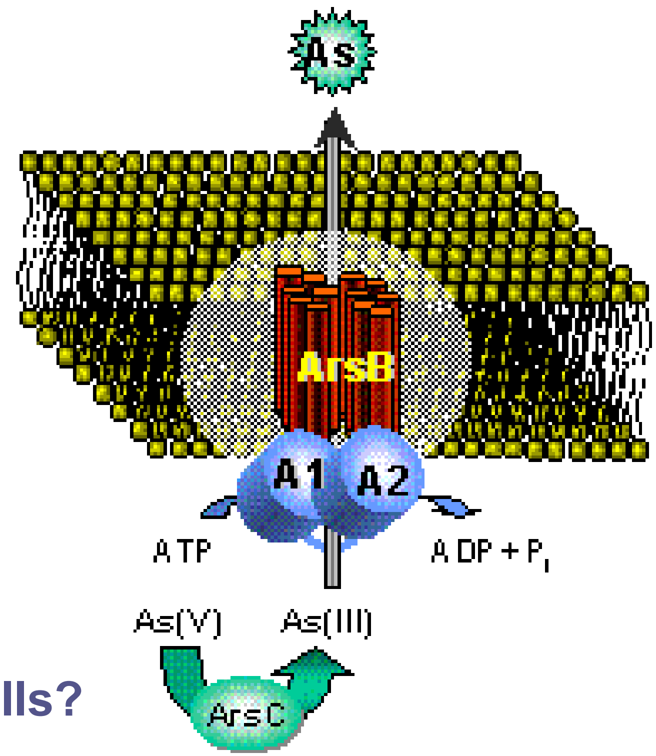
ars operons in bacteria



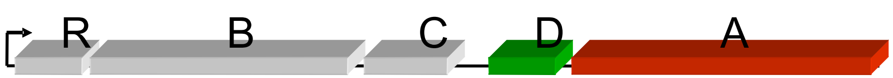
Many bacteria



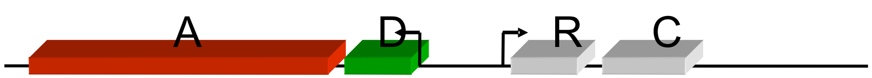
Protein	ArsR	ArsD	ArsA	ArsB	ArsC
Total residues	117	120	583	429	141
Mass (Da)	13,198	13,218	63,188	45,598	15,830



Klebsiella, Acidiphilium, Salmonella and Listeria sp.



Bacillus and Sinorhizobium sp.

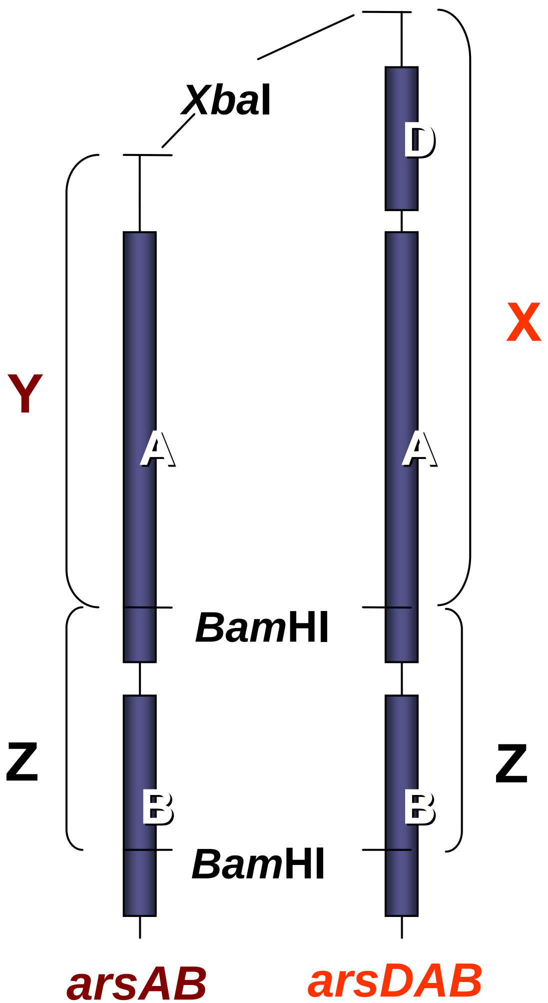


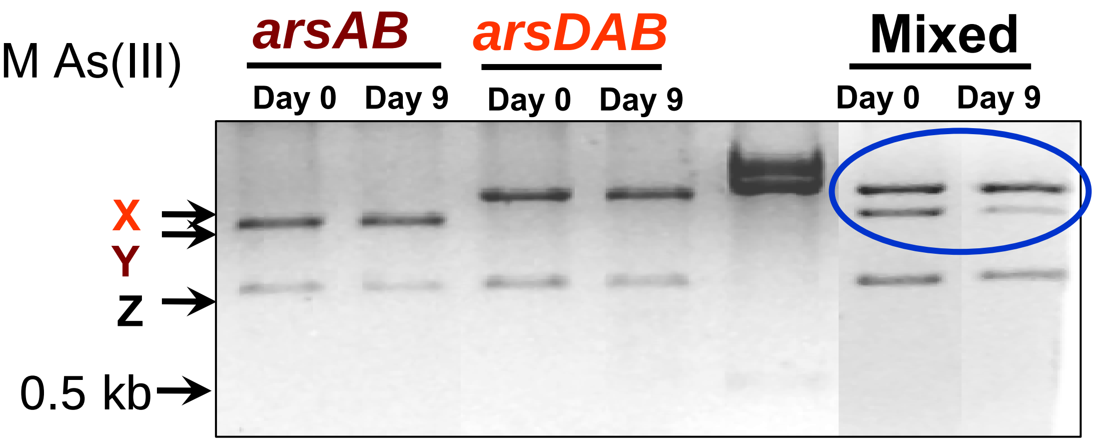
Halobacterium sp.

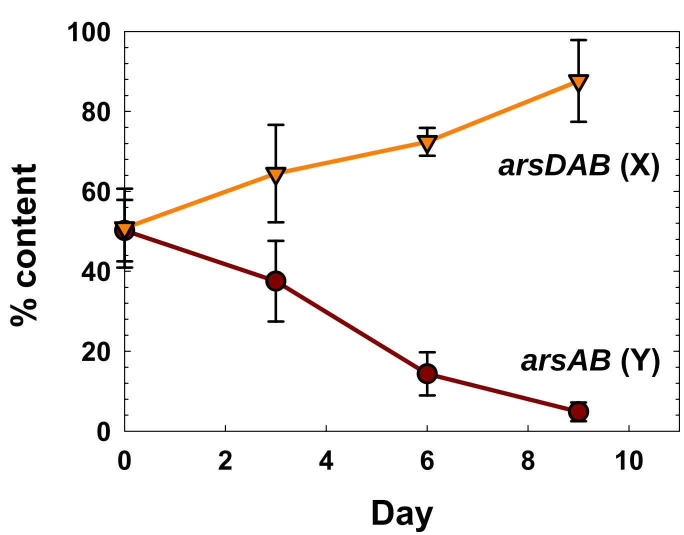
What advantage of ArsD to provide for cells?

ArsD confers a competitive advantage to cells growing in environmental concentrations of arsenic.

E. coli (Δ*ars*) / pSE380 / 10 μM As(III)

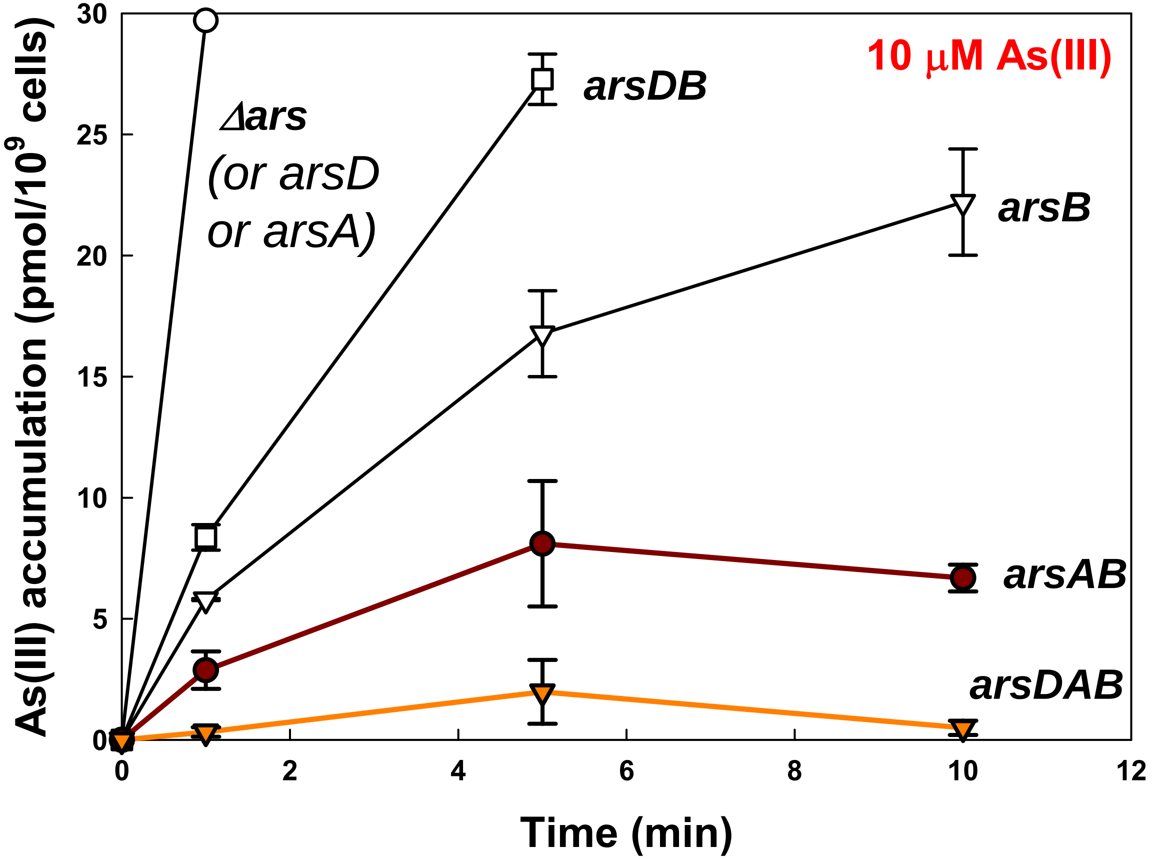


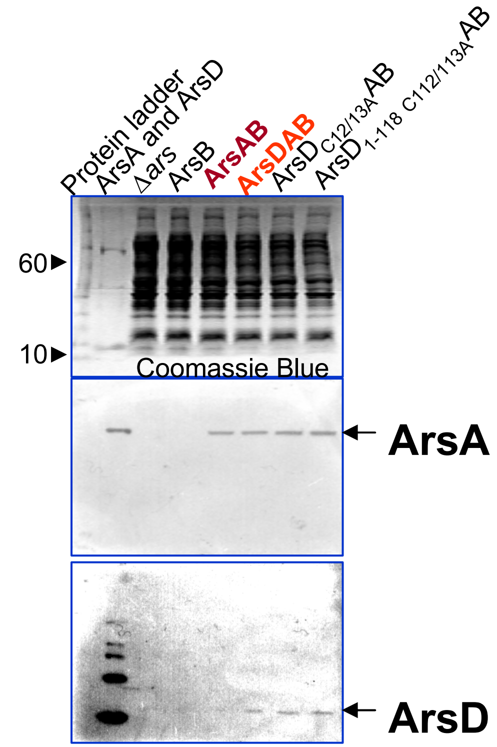


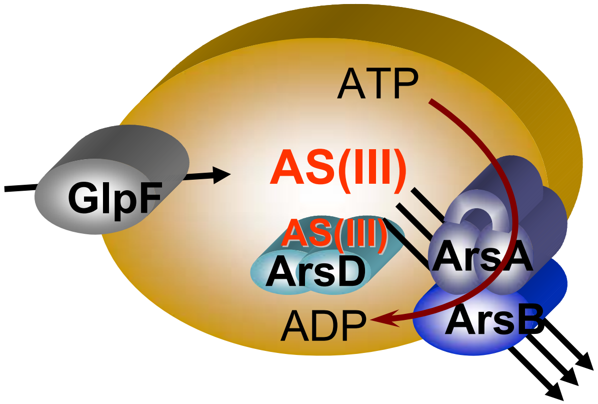


ArsD increases the efficiency of the pump to lower intracellular As(III) through working with ArsA

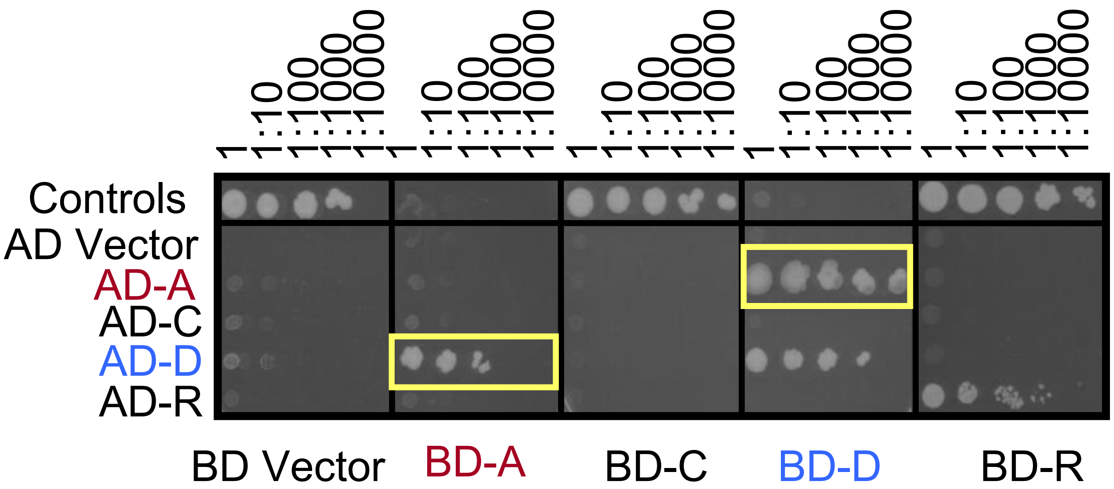
- pACYC184 (P_{BAD}; ***arsD***) + pSE380 (P_{TRC}; ***arsB*** or ***arsAB***)
- *E. coli* AW3110 (Δ*ars*)
- 10 μM As(III)
- [As] by ICP-MS



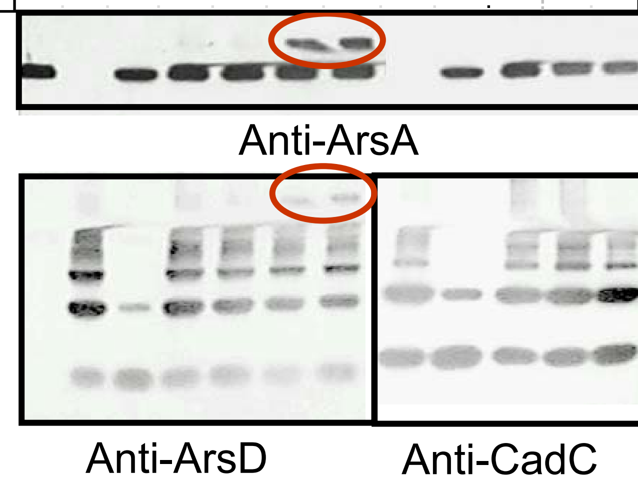




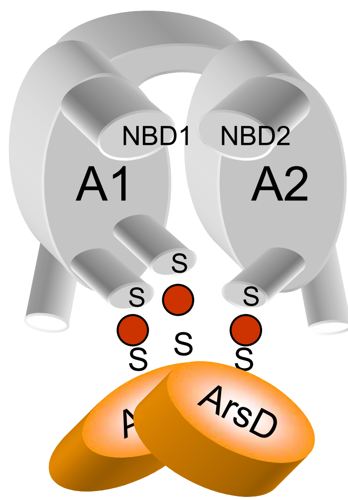
ArsD and ArsA interact



	1	2	3	4	5	6	7	8	9	10	11	12
ArsA	+		+	+	+	+	+		+	+	+	+
ArsD		+	+	+	+	+	+					
CadC								+	+	+	+	+
Sb(III)					+		+					+
MgCl2						+	+				+	+
ATP						+	+				+	+
bBBr	+	+		+	+	+	+	+		+	+	+

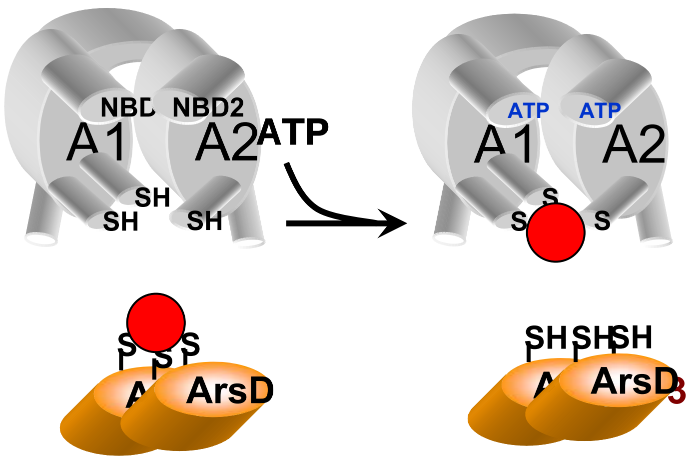


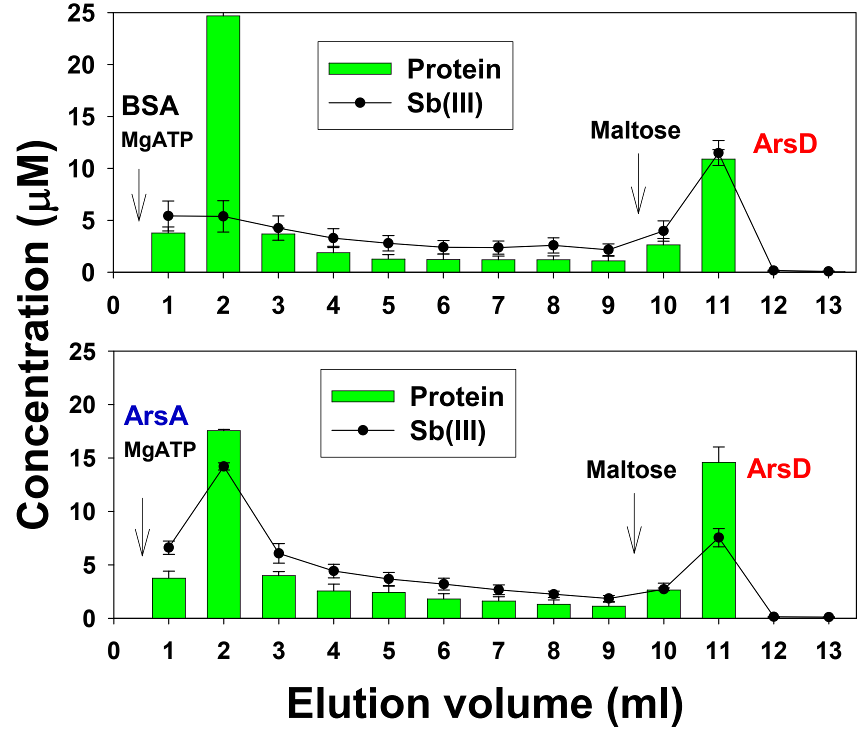
- Physically
- Through metal binding sites
- ArsA in nucleotide-bound form

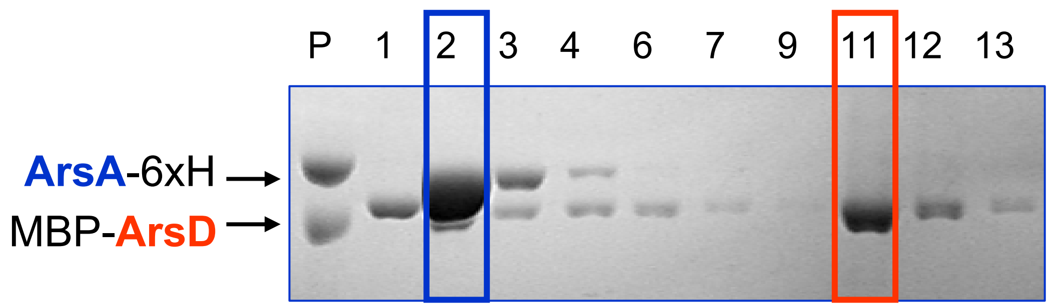


ArsD transfers metalloid to ArsA

- MBP-ArsD + Sb(III) + amylose column
- + ArsA (or BSA)

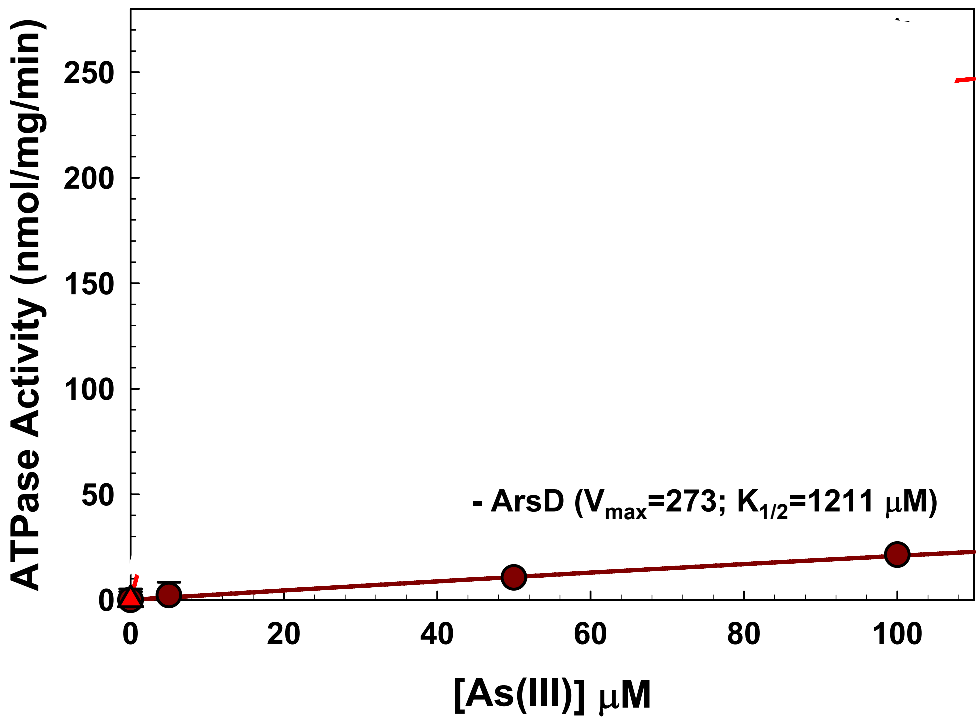


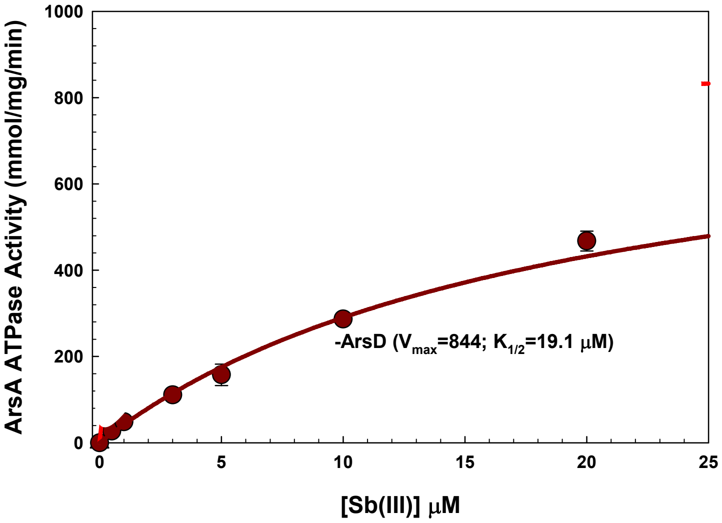


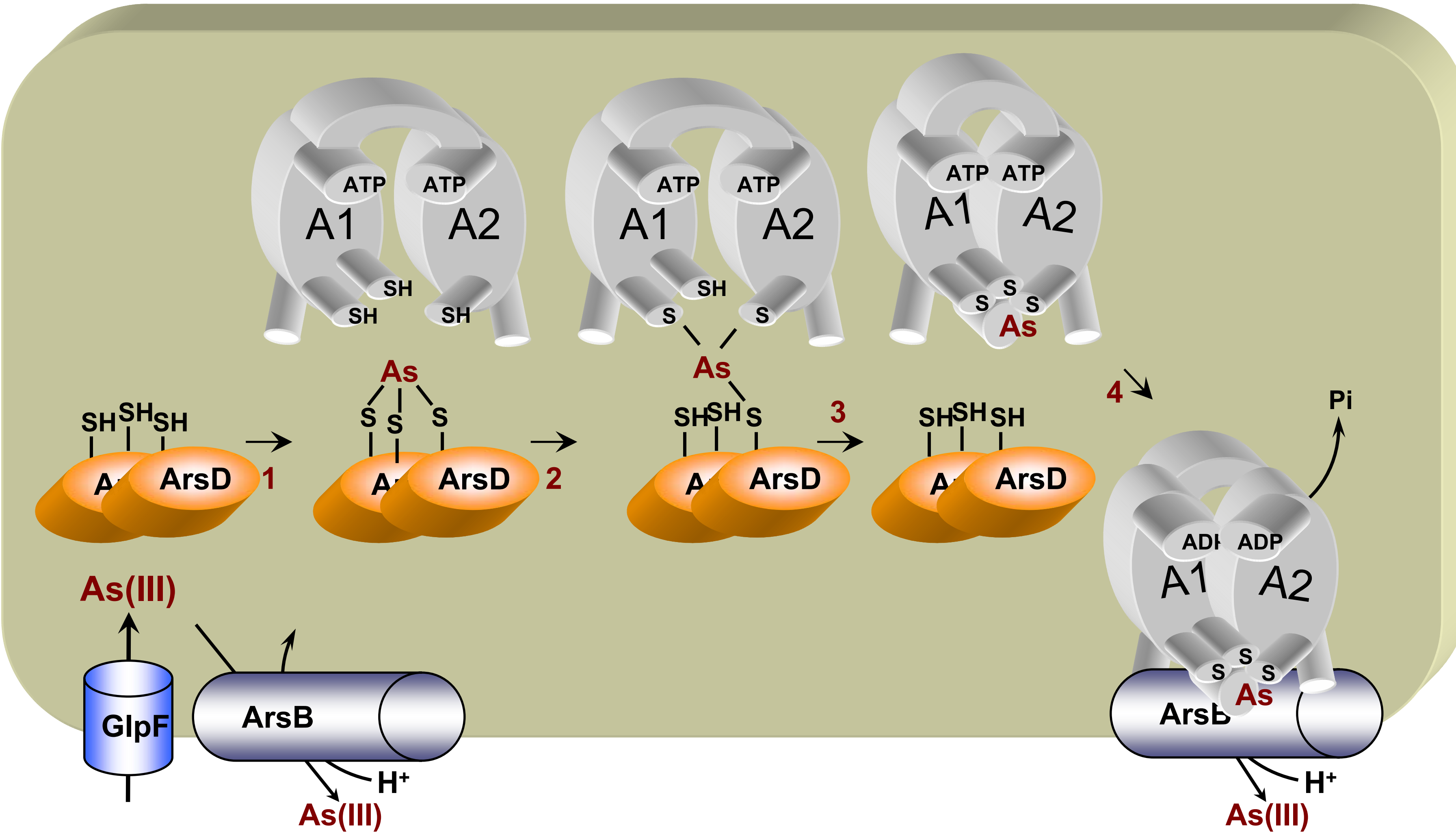


Kd (μM)	As (III)	Sb(III)
ArsA	~1200	~20
ArsD	~20	~1.5

ArsD increases the affinity of the ATPase for metalloids







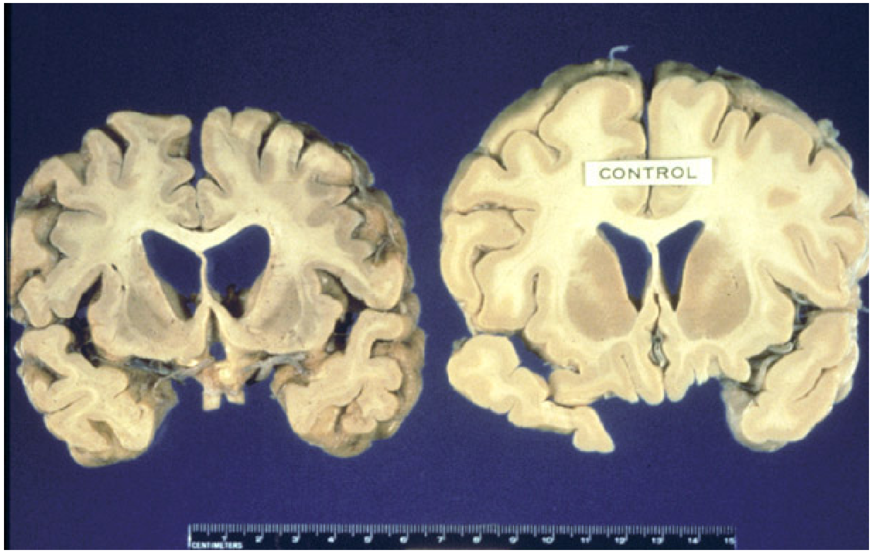
Intracellular trafficking of molecules is well regulated and important to health.

Detroit, MI

Impairment of intracellular trafficking leads to neuropathology

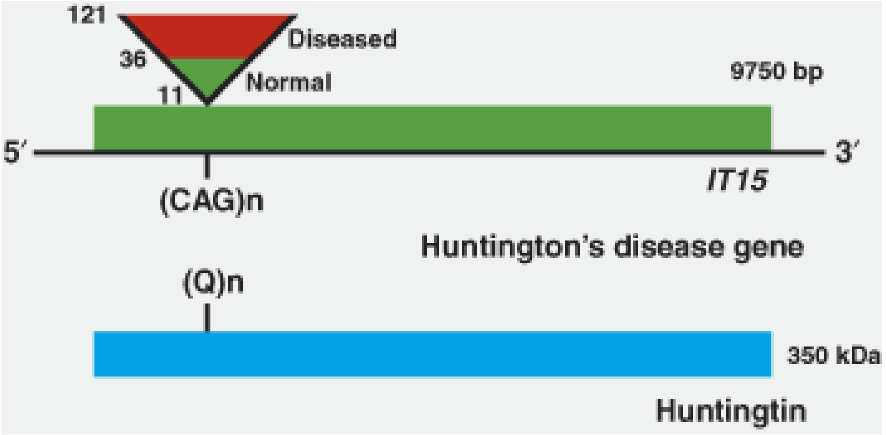
Neurodegenerative diseases and dysfunction of trafficking

Disease	Protein	Dysfunction	Prevalence
Huntington's disease	Htt	Dynein/dynactin Adaptor	1/10,000~ 300,000
Alzheimer's disease	Tau APP	Microtubule associated protein Kinesin-1 adaptor	1/10~100 (old>young)
Parkinson's disease	α-synuclein Parkin PINK1 DJ-1	Microtubule-associated protein Maintenance of mitochondria	1/300~3000
Amyotrophic lateral sclerosis (ALS)	$p150^{Glued}$ SOD1	Motor associated protein Mitochondrial enzyme	1/10,000~ 50,000

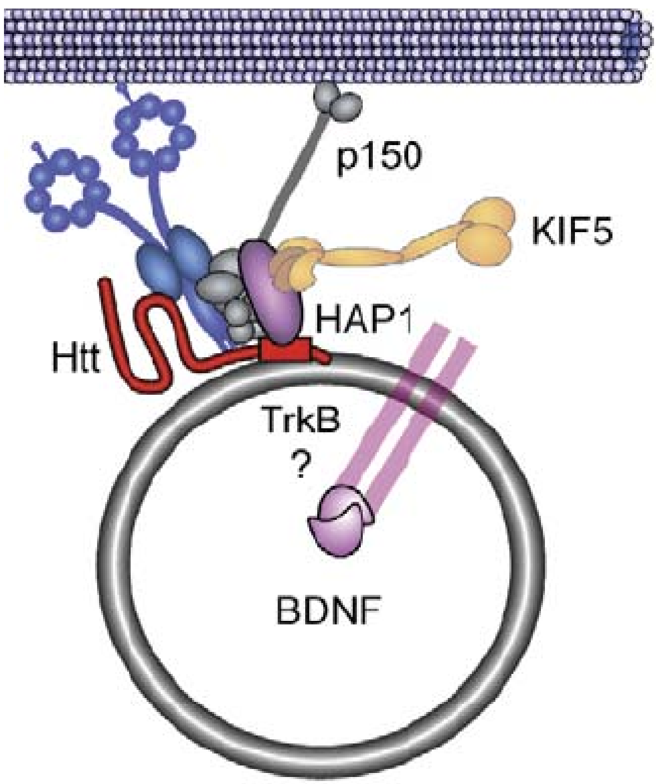


Trafficking pathology in Huntington disease (HD)

- HD has a single genetic cause, a well-defined neuropathology, and informative pre-manifest predictive genetic testing.
- Mutant Huntingtin (mHtt) retards HAP1 and inhibits HAP1 trafficking.
- It fails to transport BDNF efficiently.
- It interferes microtubule-based transport of mitochondria and reduces ATP level in synaptosome.



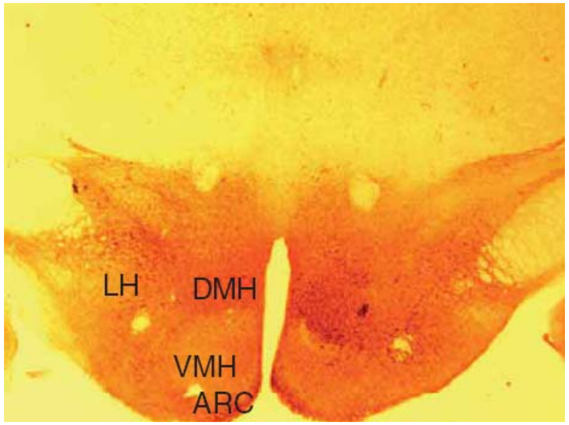
Hap1 and Htt in trafficking



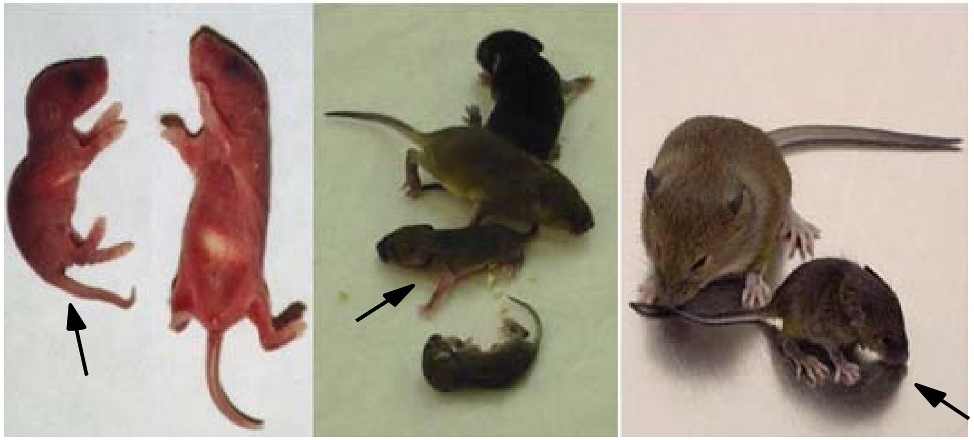
Salinas S et al, *Curr Opin Cell Biol* 2008

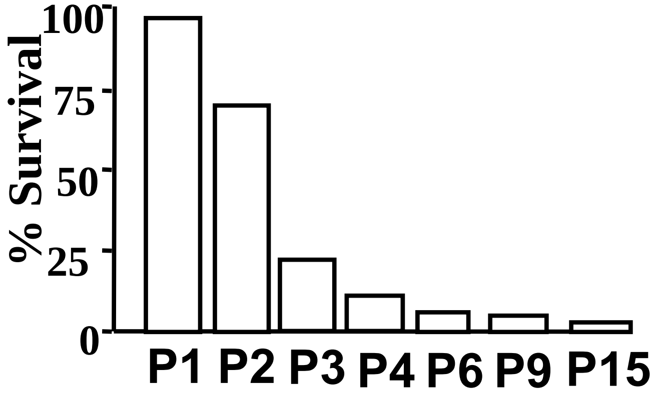
Hap1-interacting proteins	Function	References
Huntingtin	Scaffold protein[c]	1995
P150Glued	Microtubule-dependent transporter	1997, 1998
Rho-GEF Kalirin-7 (Duo)	GDP–GTP exchange factor	1997
Hrs	Vesicular trafficking	2002
$GABA_A$ receptor	Membrane receptor	2004
$IP_3$1 receptor	Membrane receptor	2003
NeuroD	Neuronal transcription factor	2003
Kinesin light chain (KLC)	**Microtubule-dependent transport**	2006
Androgen receptor (AR)	**Membrane receptor**	2006
14-3-3	**Protein trafficking complex assembly**	2007
TBP	**Transcription factor**	2007
AHI1	**Intracellular trafficking**	2008
proBDNF	**Neurotrophin precusor**	2009
KIF5	**Microtubule-dependent transport**	2009

HAP1 expression in hypothalamus



P3 **P10** **P15**

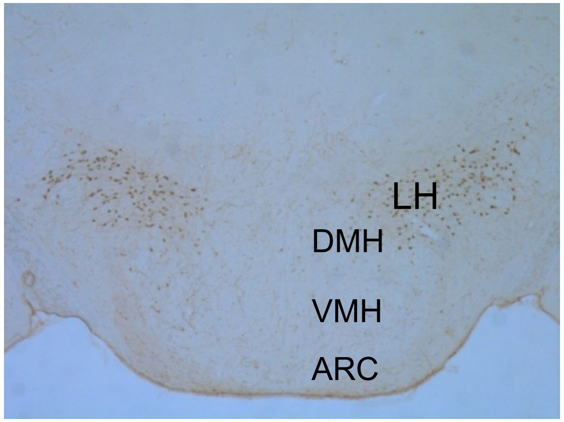




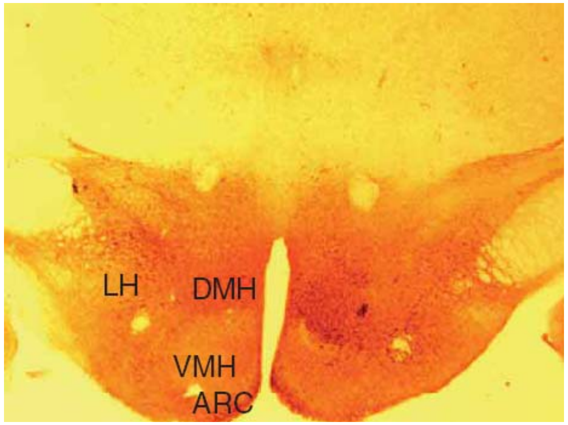
Li S *et al., J. Neurosci*, 2003

Orexin neurons are located in the lateral hypothalamic area (LHA) and project to most parts of the brain

Orexin expression



HAP1 expression

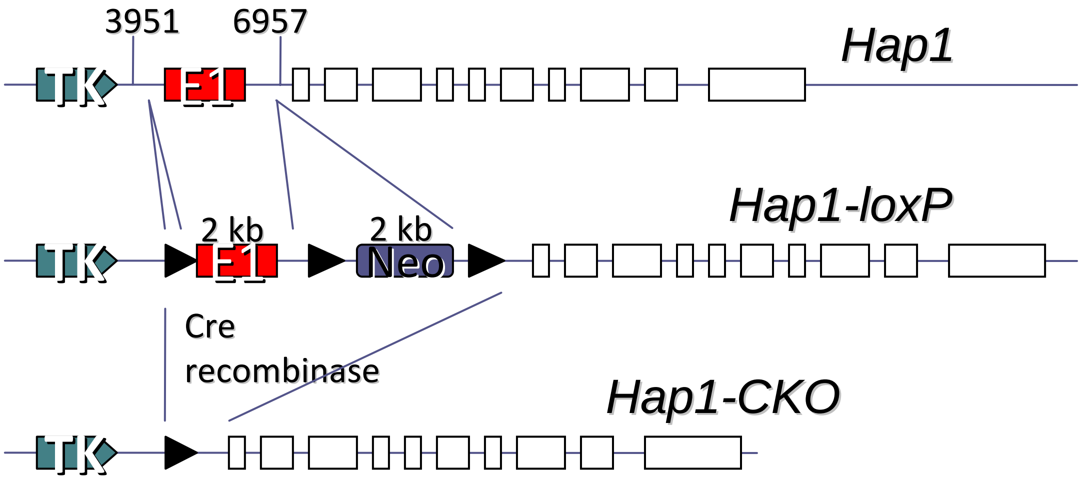


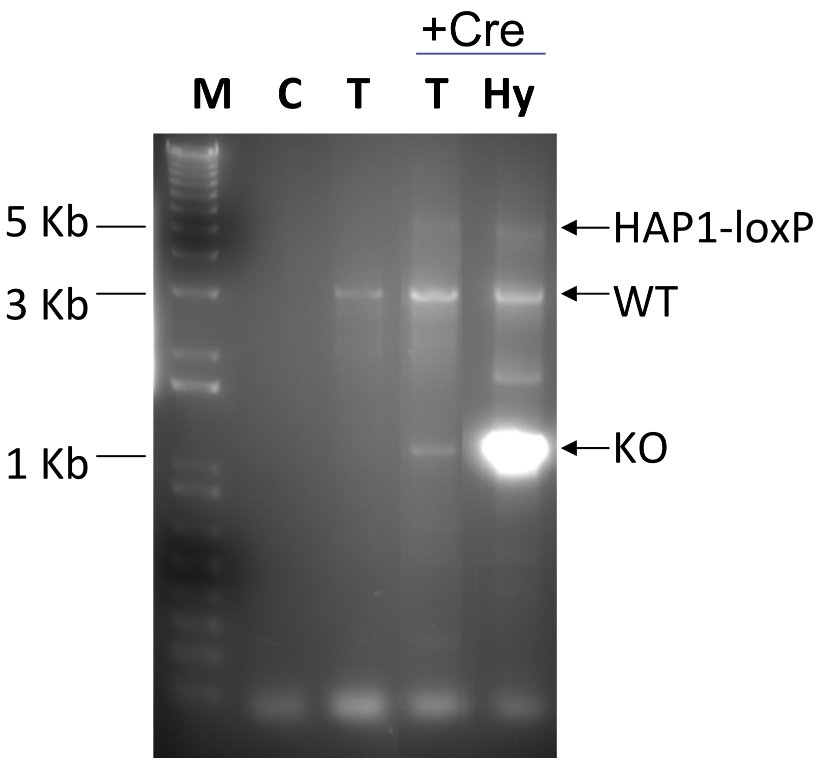
Orexin neuronal function:

Feeding
Locomotor Activity
Sleep/wakefulness

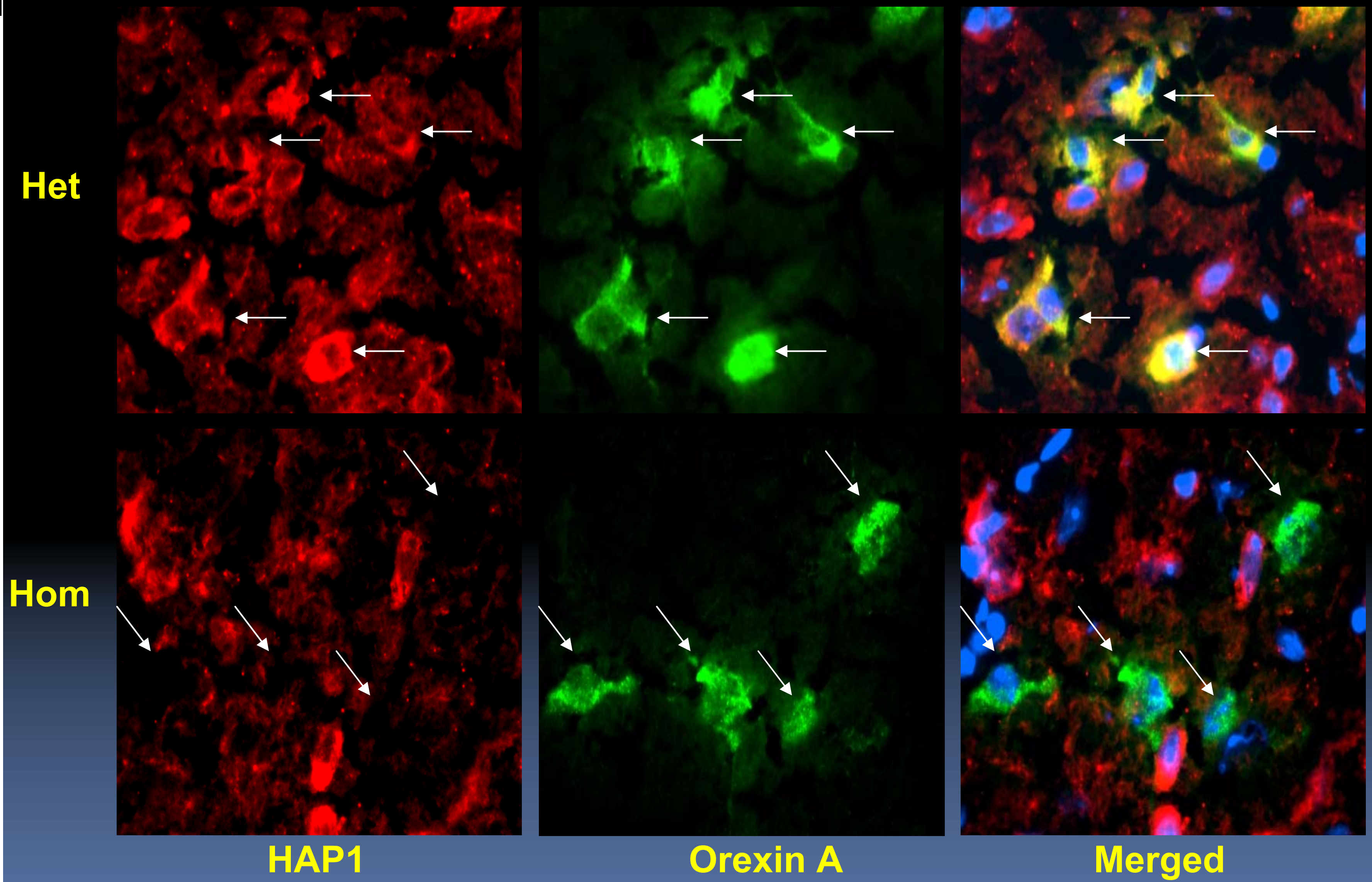
My study focuses on orexin neurons because of their importance and availability of orexin-Cre mice

Cre-loxP system and the Orexin-Hap1 conditional knockout mouse

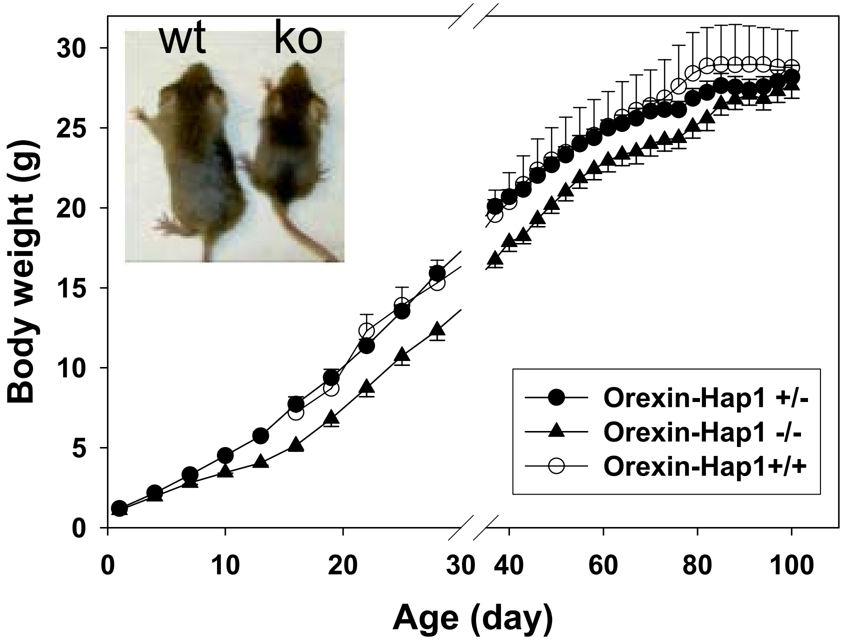


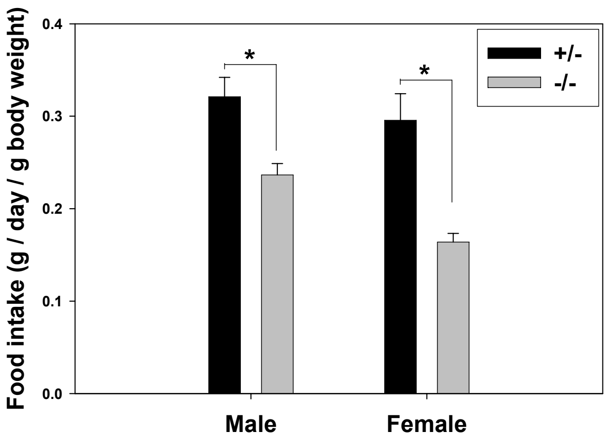


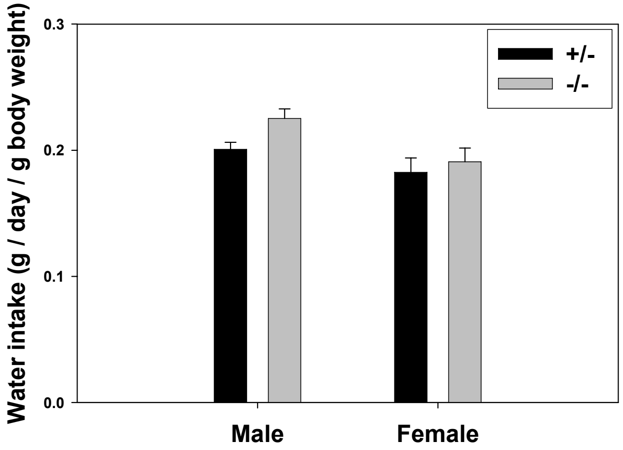
Homozygous orexin-Hap1 knockout selectively depletes HAP1 in orexin neurons



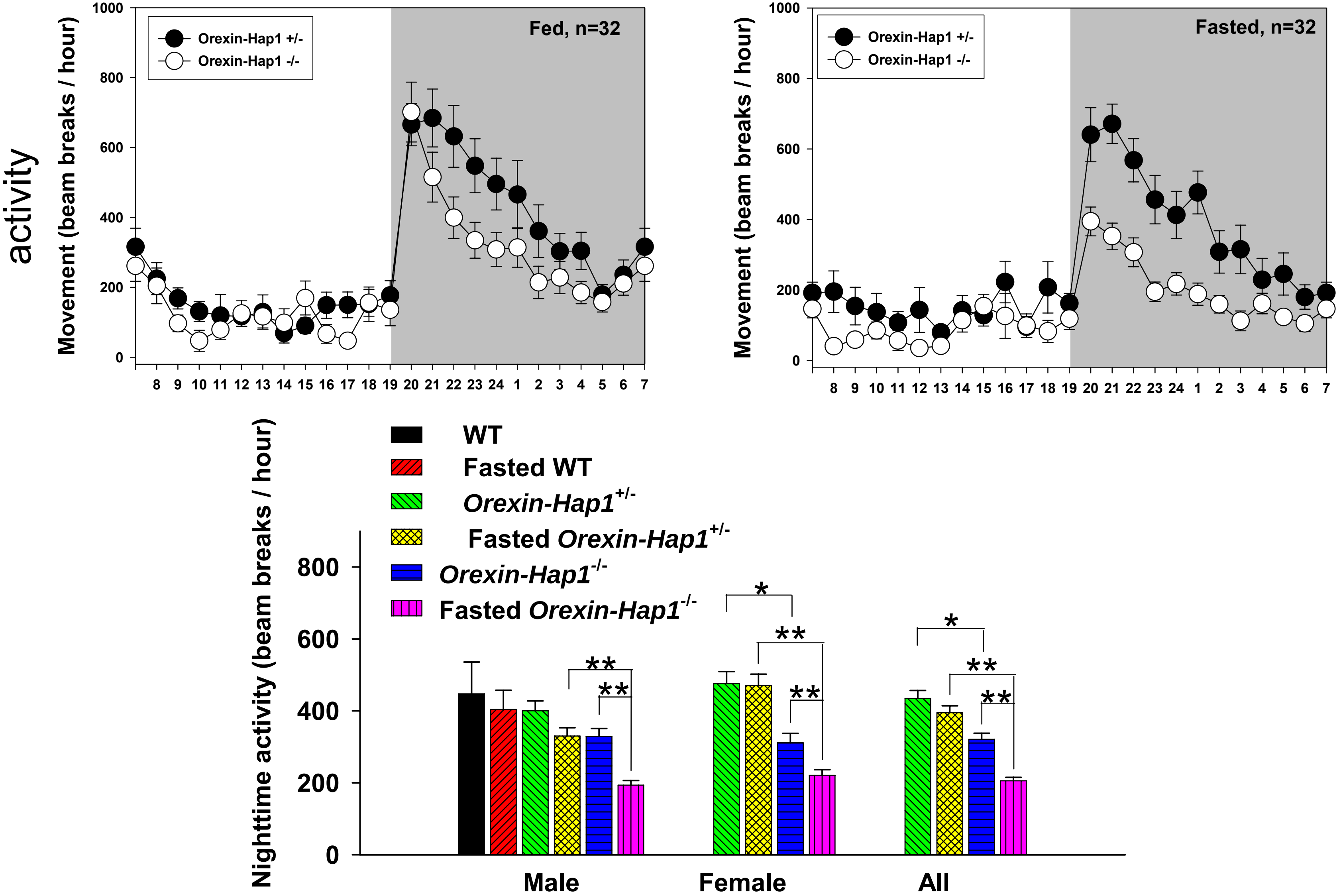
Reduced body weight and food intake in Orexin-Hap1 KO mice







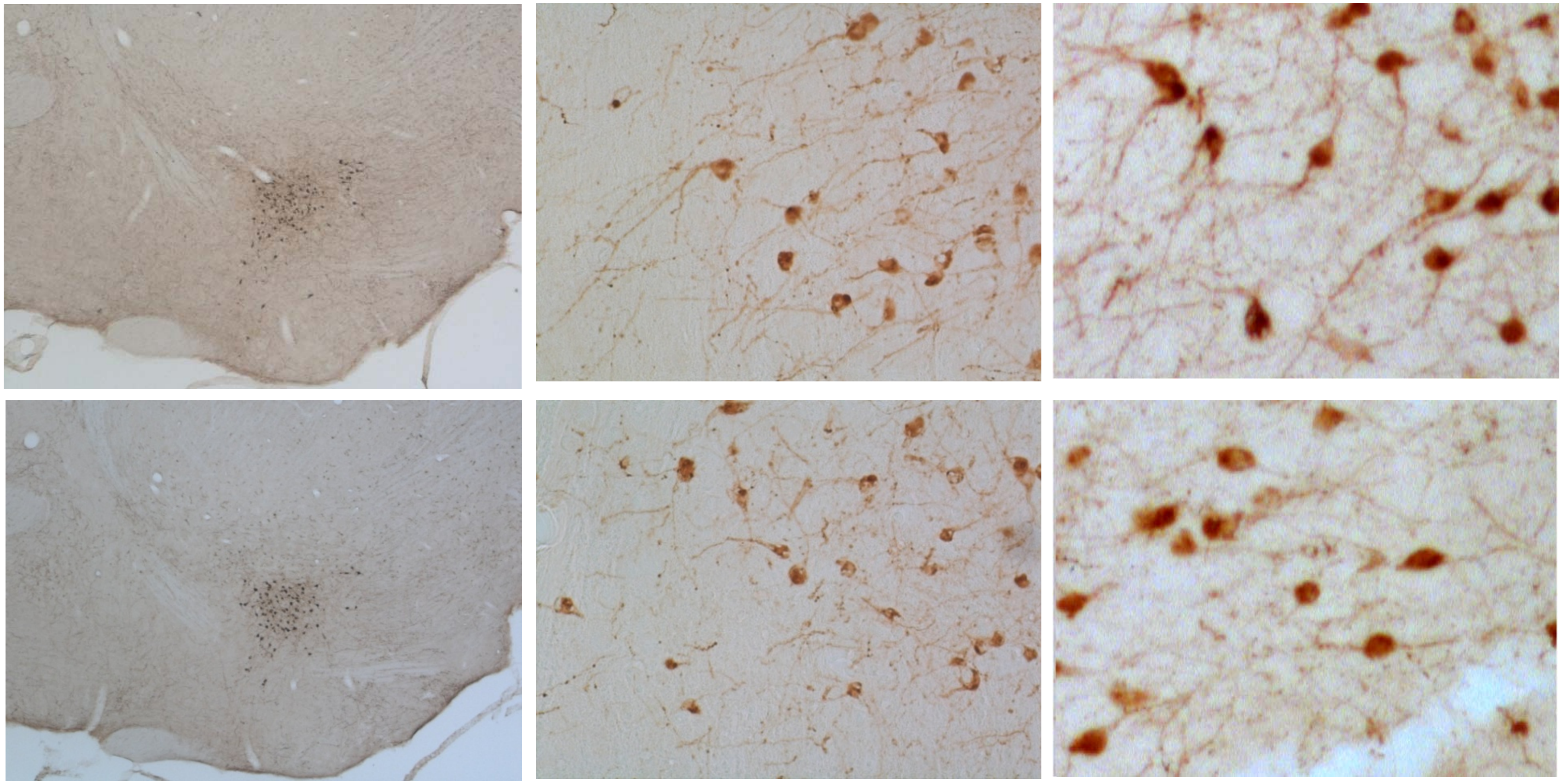
Decreased locomotor activities in Orexin-HAP1 KO mice



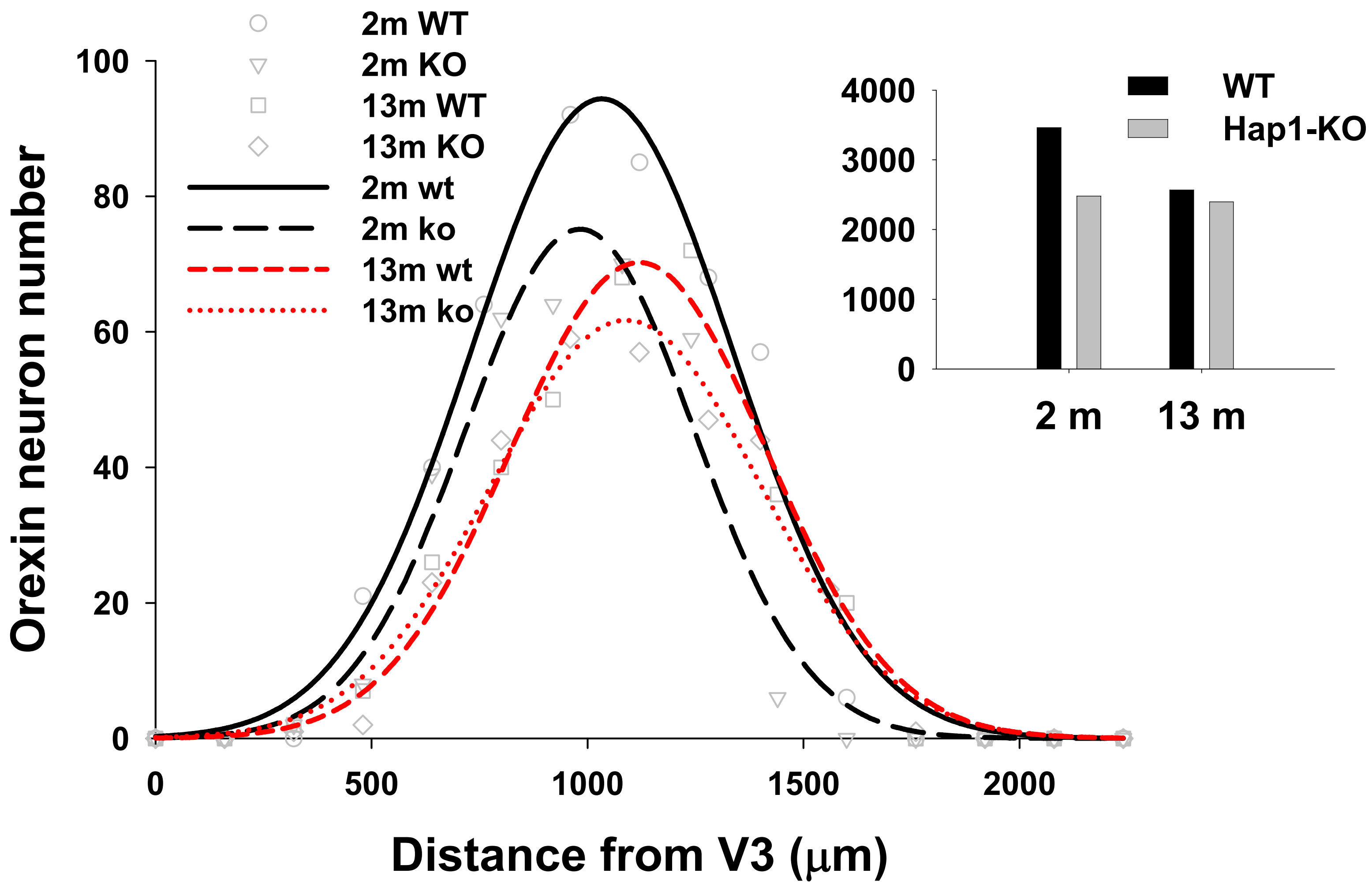
Impaired orexin neuronal processes in Hap1 KO mouse brain

(+/–)

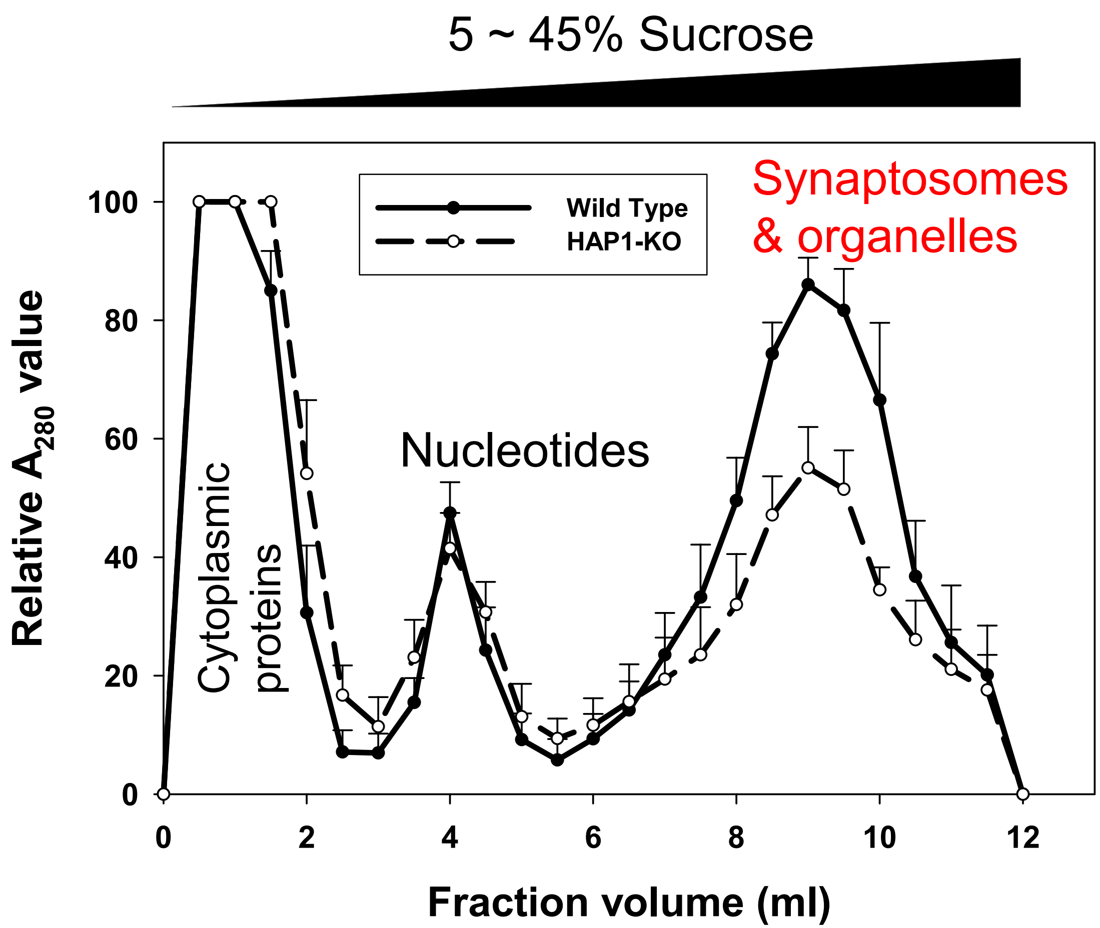
(–/–)



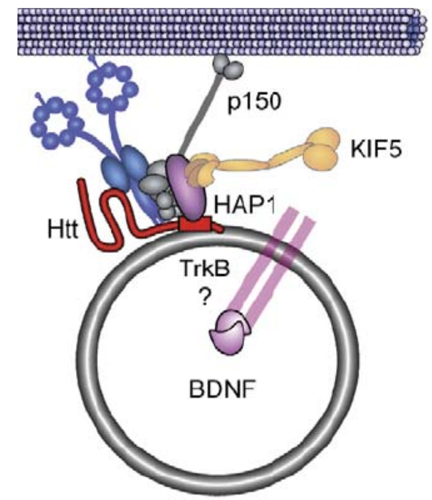
Reduced orexin neuron population in Orexin-Hap1KO mouse brain

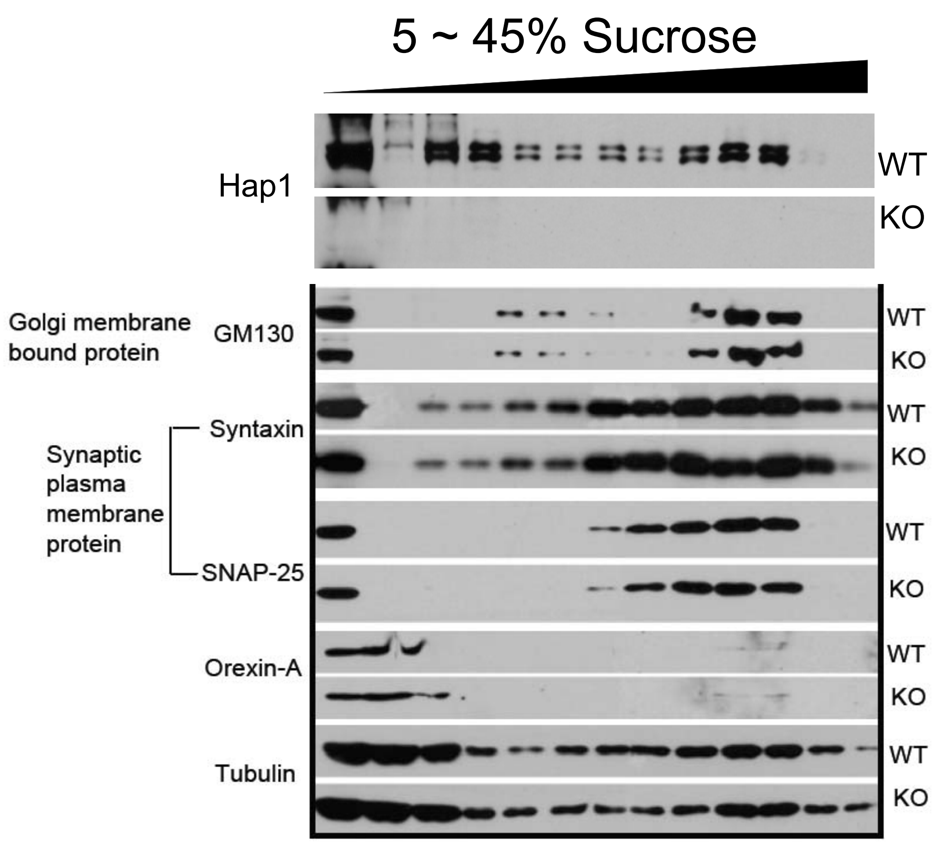


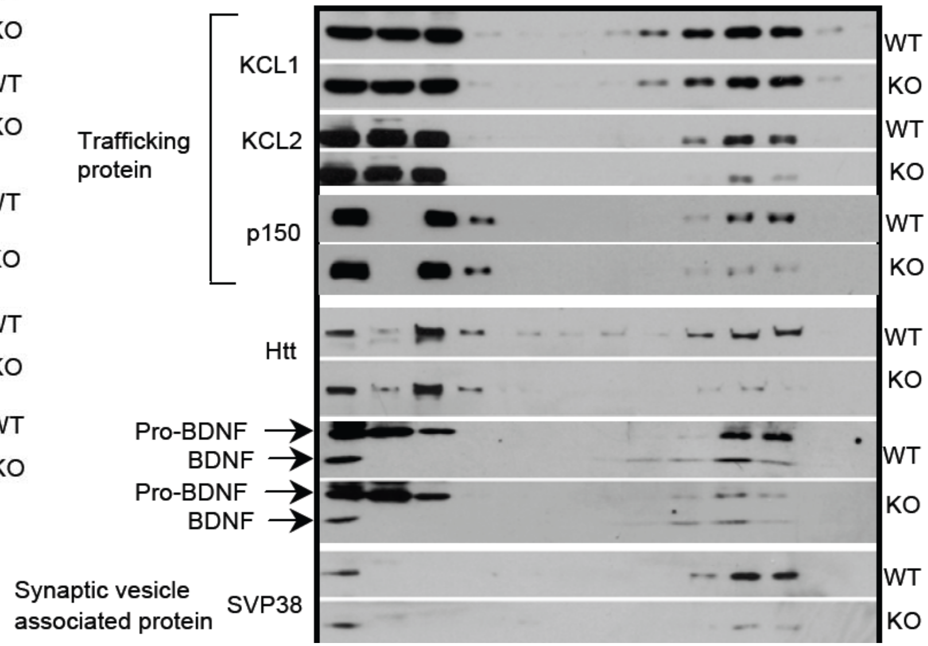
Mouse brain fractionation in sucrose gradient



Loss of Hap1 alters the distribution of trafficking proteins and cargos







HAP1 deficiency

↓

Intracellular trafficking impairment

↓

Neuronal development defect or degeneration

↓

Neuropathology

Atlanta, GA

Summary

- Intracellular trafficking of certain metals/metalloids is well regulated by metallochaperones.
  - No arsenic chaperon has been identified in eukaryotes.
  - Other small molecules may also require chaperones intracellularly.
- Impairment of intracellular trafficking by HAP1 deficiency leads to neuropathology.
  - Exact function of HAP1 is still not clear.
  - Regulation of HAP1-partner interactions would be a key to the regulation of intracellular trafficking.
  - There could be unknown HAP1 partners.

Human Genome Project

  - http://www.ornl.gov/sci/techresources/Human_Genome/project/journals/insights.shtml
- The total number of genes is estimated at 25,000, much lower than previous estimates of 80,000 to 140,000.
- Functions are unknown for more than 50% of discovered genes. ---- Last modified: Friday, October 09, 2009

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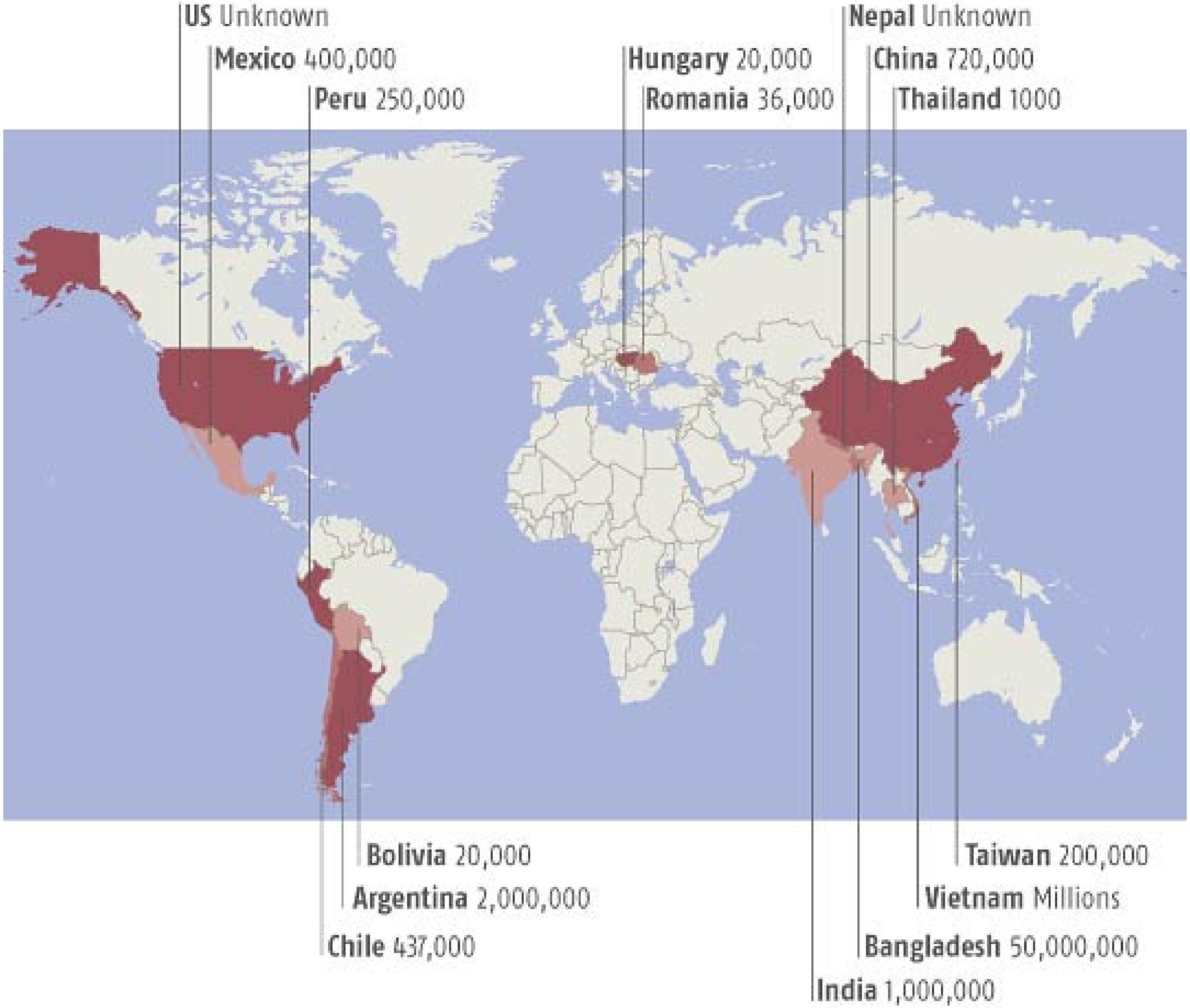
Conclusive thoughts

- I was like a boy playing on the sea-shore, and diverting myself now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me. *Isaac Newton*

- Science is an imaginative adventure of the mind seeking truth in a world of mystery. *Sir Cyril Herman Hinshelwood (1897-1967) English chemist. Nobel prize 1956.*

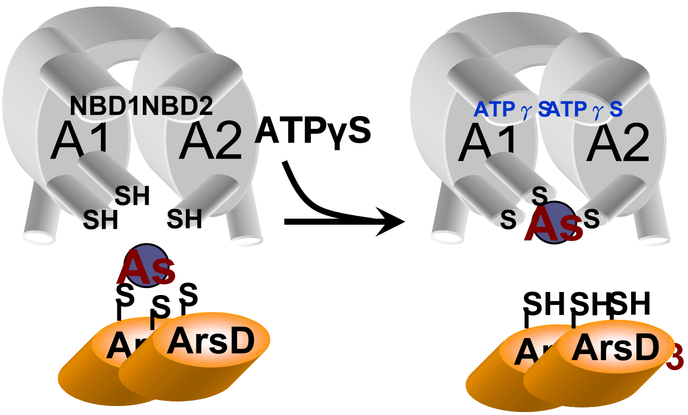
MASS POISONING

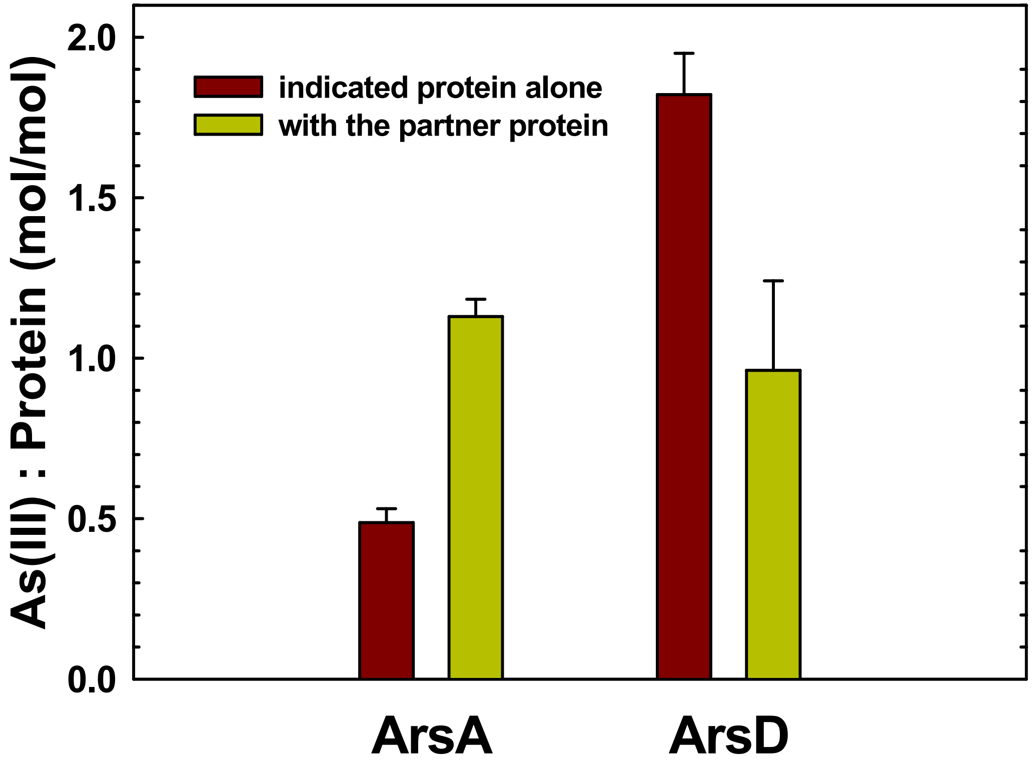
Number of people at risk from arsenic contamination



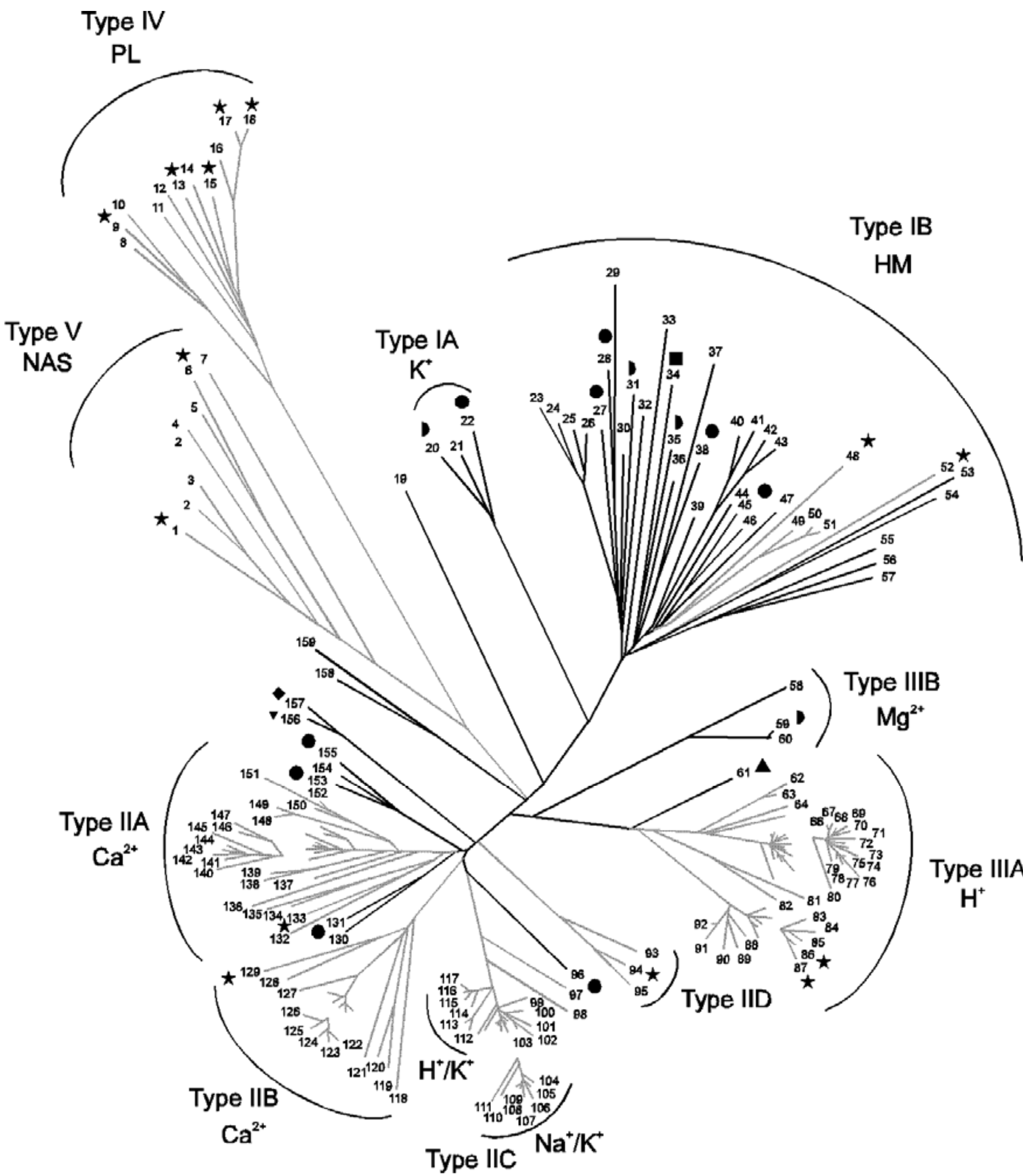
ArsA binds more metalloid in the presence of ArsD

- As(III) + MBP-ArsD + ArsA-6xHis + MgATPγS
- Amylose or Ni resin + Gel filtration column





These data are consistent with transfer of metalloid from ArsD to ArsA.



P-Type ATPases

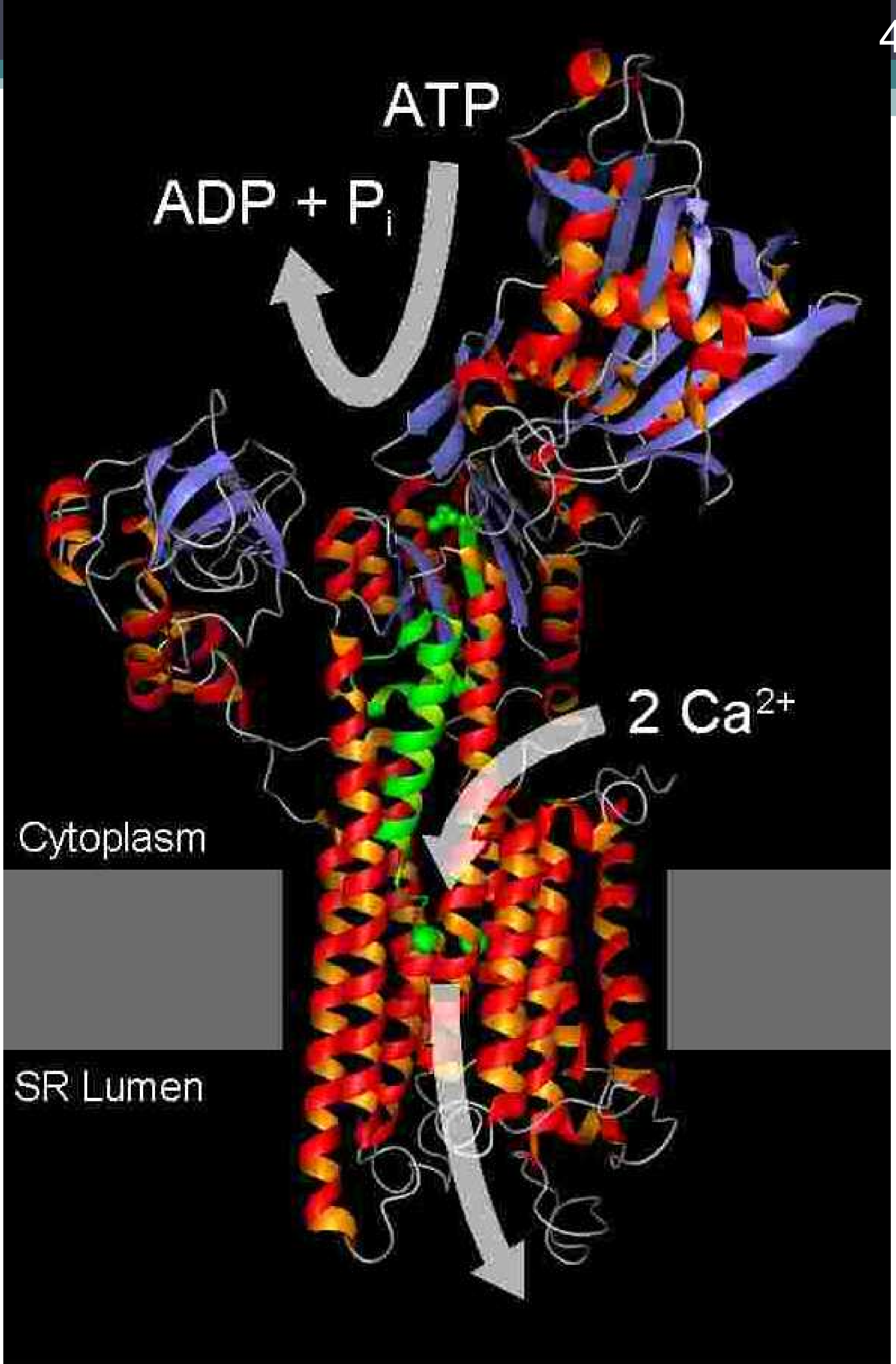
- They are a large group of ion pumps.
- They catalyze auto-phosphorylation of a key conserved aspartate residue within the pump.

➢ Type IB:

- Cu^{+}, Ag^{+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Co^{2+}.
- They are key elements for metal resistance and metal homeostasis in a wide range of organisms.

A Checklist for Future Research
from the Human Genome Project

- Exact gene number, exact locations, and functions
- Gene regulation
- DNA sequence organization
- Chromosomal structure and organization
- Noncoding DNA types, amount, distribution, information content, and functions
- Coordination of gene expression, protein synthesis, and post-translational events
- Interaction of proteins in complex molecular machines
- Predicted vs experimentally determined gene function
- Evolutionary conservation among organisms
- Protein conservation (structure and function)
- Proteomes (total protein content and function) in organisms
- Correlation of SNPs (single-base DNA variations among individuals) with health and disease
- Disease-susceptibility prediction based on gene sequence variation
- Genes involved in complex traits and multigene diseases
- Complex systems biology, including microbial consortia useful for environmental restoration
- Developmental genetics, genomics


ATP
ADP + P$_i$
2 Ca^{2+}
Cytoplasm
SR Lumen

Neurodegenerative diseases (examples)