Redox Regulation of Epigenetic Processes: A Role for Arsenic

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December 17, 2012

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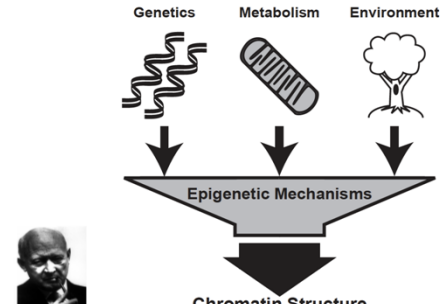
isrp
iowa superfund research program

What is epigenetics?



Conrad Waddington

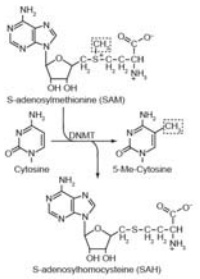
Cyr and Domann (2010) *ARS* PMID: 20919933

Epigenetic processes

- DNA Methylation of cytosine at CpG
- Modification of histone tails
- Non-coding RNA, miRNA
- Higher order chromatin structure

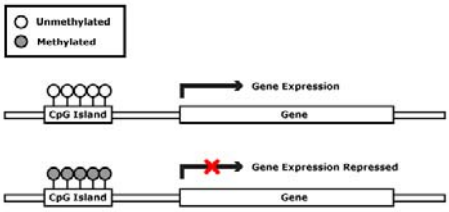
DNA methylation

- CpG di-nucleotides
  - 5'-CG-3'
  - Observed at only 1/5th their expected frequency
    - Clustered into promoter regions
- DNA methylation is heritable by the activity of DNA methyltransferases

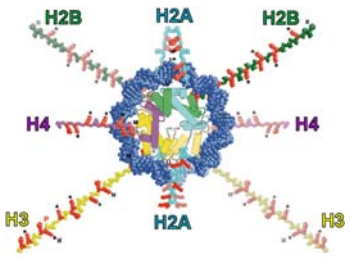


DNA methylation effects on gene expression

- Hypermethylation of CpG di-nucleotides is associated with gene silencing

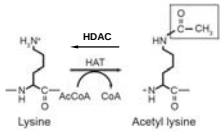


Histones

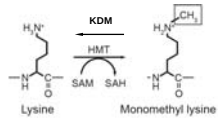


Histone modifications

- Histone acetylation (HAT / HDAC)



- Histone methylation (HMT / KDM)



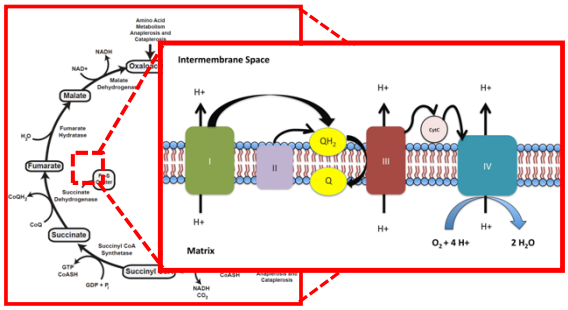
Histone modifications regulate gene transcription

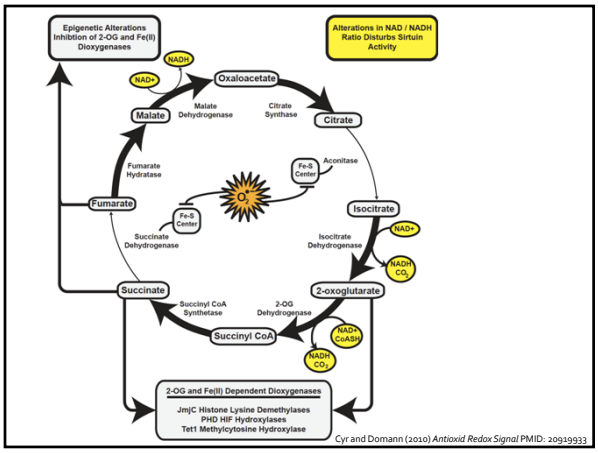
- Histone modifications modulate interactions between nucleosomes
  - Change chromatin structure
  - Allow, or block transcription
- Altered dynamically to change gene expression
  - Target for pharmacological epigenetic therapy
- Reversible, and redox senstive!

Overarching Hypothesis

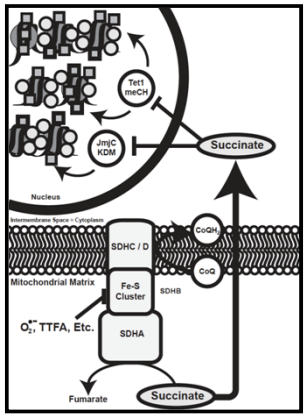
Metabolic perturbations lead to dysregulation of epigenetic programming

Mitochondrial Metabolism?

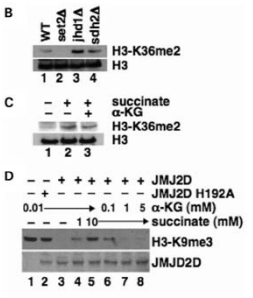




Cyr and Domann (2010) *Antioxid Redox Signal* PMID: 20919933



Cyr and Domann (2010) *Antioxid Redox Signal* PMID: 20919933



Cervera AM *et al.* (2009) *Mol Cancer* 8:89
Smith *et al.* (2007) *Hum Mol Genet* 16(24):3336-48

How does Arsenic affect Metabolism?

As (V): uncouples oxidative phosphorylation by competing with inorganic phosphate and blocking ATP synthesis (As+ADP -> unstable arsenate)

Gresser MJ, *J Biol Chem* 256(12): 5981-3

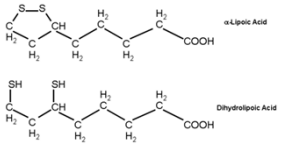
As (III): binds to sulfhydryl groups (R-SH), thus reacts with a variety of proteins and inhibits their activity (e.g: pyruvate dehydrogenase, glutathione reductase and thioredoxin reductase)

Arsenite inhibits succinate dehydrogenase.

Dwivedi, et al., *Toxicol Appl Pharmacol* 256(3): 241-48, 2011

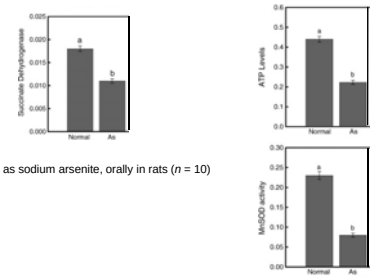
How does Arsenic affect Metabolism?

The citric acid cycle itself is impaired at the alpha-ketoglutarate dehydrogenase step; formation of succinyl coenzyme A is impaired. Both of these enzymatic steps require the active thiol, lipoic acid. Arsenic readily combines with sulfhydryl (-SH) groups, including those of lipoic acid.



http://www.drkaslow.com/html/arsenic.html

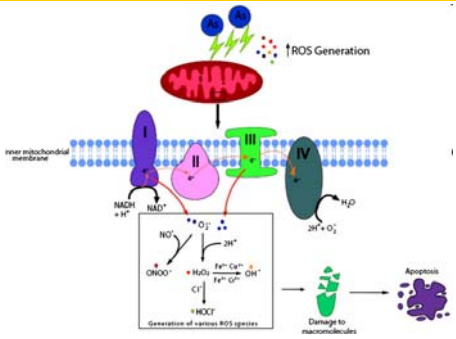
Arsenic Perturbs Mitochondrial Function



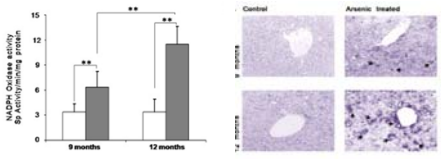
2.5 mg/kg, arsenic as sodium arsenite, orally in rats (n = 10)

Dwivedi, et al., *Toxicol Appl Pharmacol* 256(3): 241-48, 2011

Arsenic Perturbs Mitochondrial Function



Arsenic activates NADPH Oxidase

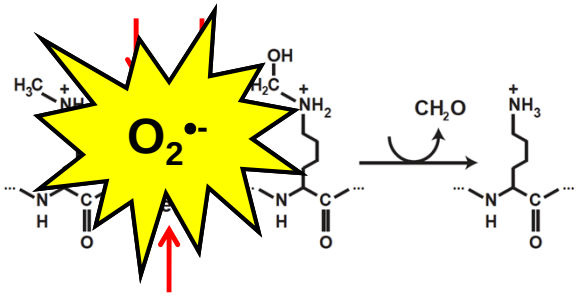


NADPH oxidase activity 4-hydroxynonenal adducts

NADPH Oxidase generates superoxide!

Ghatak S, Biswas A, Dhali GK, Chowdhury A, Boyer JL, Santra A. *Toxicol Appl Pharmacol.* 2011 Feb 15;251(1):59-69.

Jumonji demethylases, KDMs, catalyze the hydroxylation of methylated histones



Cyr and Domann (2010) *ARS* PMID: 20919933

Is DNA methylation reversible?

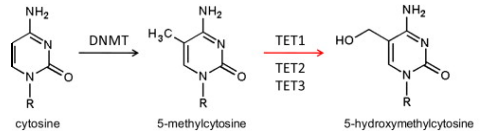
The Nuclear DNA Base 5-Hydroxymethylcytosine Is Present in Purkinje Neurons and the Brain

Skirmantas Kriaucionis and Nathaniel Heintz*

Despite the importance of epigenetic regulation in neurological disorders, little is known about neuronal chromatin. Cerebellar Purkinje neurons have large and euchromatic nuclei, whereas granule cell nuclei are small and have a more typical heterochromatin distribution. While comparing the abundance of 5-methylcytosine in Purkinje and granule cell nuclei, we detected the presence of an unusual DNA nucleotide. Using thin-layer chromatography, high-pressure liquid chromatography, and mass spectrometry, we identified the nucleotide as 5-hydroxymethyl-2′-deoxycytidine (hmdC). hmdC constitutes 0.6% of total nucleotides in Purkinje cells, 0.2% in granule cells, and is not present in cancer cell lines. hmdC is a constituent of nuclear DNA that is highly abundant in the brain, suggesting a role in epigenetic control of neuronal function.

Science 2009 324(5929):929-30, PMID: 19372393.

Tet proteins catalyze the hydroxylation of 5-methylcytosine

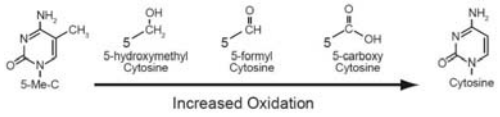


•Tet family of enzymes

-2-OG and Fe(II)-dependent dioxygenases

-Similar enzymatic mechanism to the prolyl hydroxylase domain and JmjC domain containing proteins

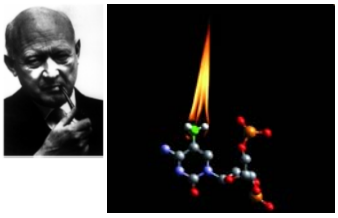
DNA Demethylation



5-methyl cytosine is a substrate for progressive oxidation

Hitchler and Domann, *Free Radic Biol Med.* 2009 PMID:19362589

"Burning off DNA methylation"



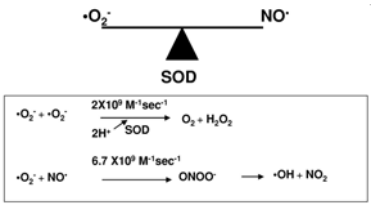
PMID:21998074

Metabolic epigenetics is (at least) a two-way street

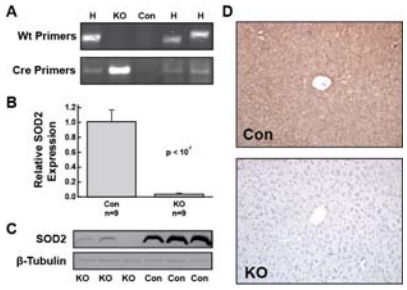
- ROS can alter epigenetic process to elicit changes in gene expression
- Genes encoding antioxidant enzymes can be targets of transcriptional repression by epigenetic mechanisms, eg. SOD3

Form & function of SODs

SOD1 **= CuZnSOD, cytosolic**
SOD2 **= MnSOD, mitochondrial**
SOD3 **= EcSOD, extracellular**



Generation of mice with Sod2$^{-/-}$ hepatocytes



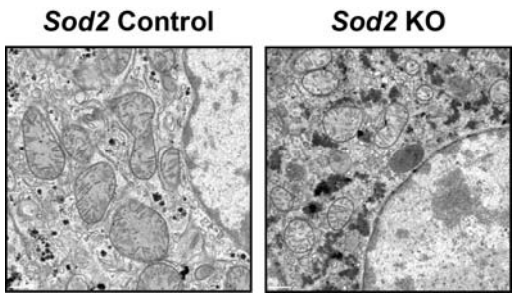
Cyr A, *et al. Liver Int.*, 2012, submitted

Rationale for Sod2 ko

MnSOD is ubiquitously expressed and functions exclusively in mitochondria

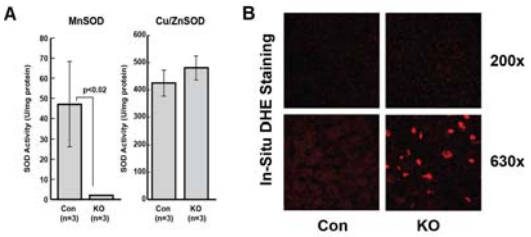
Knocking out the Sod2 gene encoding MnSOD is a highly effective way to induce mitochondrial oxidative stress

Morphology of Sod2 ko mitochondria is unremarkable



Cyr A, *et al. Redox Biology.*, 2012, submitted

Sod2-/- hepatocytes are under marked oxidative stress

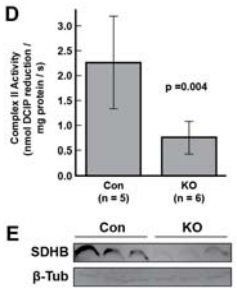


SOD Activity

DHE Staining

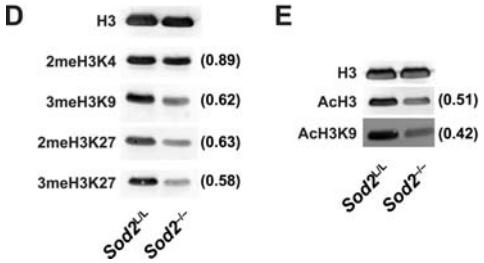
Cyr A, *et al. Redox Biology*, 2012, submitted

Complex II is inhibited in Sod2-/- hepatocytes

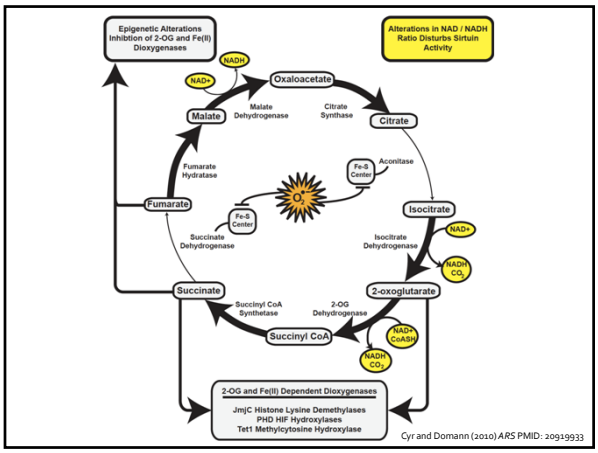


Cyr A, *et al. Redox Biology.*, 2012, submitted

Loss of Sod2 promotes changes in histone marks



Case A, *et al. Free Radic Biol Med.*, 2012, in press



Summary

- Arsenic promotes mitochondrial ROS stress bu a variety of mechanisms
- ROS can alter epigenetic process to elicit changes in gene expression
- Aberrant expression of genes encoding antioxidant enzymes can affect epigenetic control mechanisms

Metabolic Epigenetics

Enzyme		Target
PHD KDM TET	$O_2^{\bullet-}$	HIF-1a Histones 5-meC, hmC

Acknowledgements

Domann Laboratory

- Melissa Teoh, PhD
- Adam Case, PhD
- Tony Cyr
- Trent Place
- Sabine Vorrink
- Matt Fitzgerald

- NIH R01 CA115438