Biochemical Determinants Governing Redox Regulated Changes in Gene Expression and Chromatin Structure

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The Central Dogma of Molecular Biology
Oxidative Stress

Biological Response

Anti-oxidants

Pro-oxidants
Redox Regulation of Gene Expression

Compensatory changes in gene expression in response to metabolic and environmental cues that directly or indirectly perturb cellular redox homeostasis
Transcription Factors

- Proteins that bind DNA (gene) in a sequence-specific manner
- Recruit other proteins to the site of DNA binding including RNA synthetic machinery
- Resulting interactions cause a change in the rate of transcription initiation of the affected gene
- This leads to a change in the steady state level of RNA (and protein) from the gene
OxyR and Sox R/S Systems

- Prokaryotic
- $\text{H}_2\text{O}_2$ and $\text{O}_2^\cdot$ sensitive, respectively

Gisela Storz

Bruce Demple
OxyR is Activated by $\text{H}_2\text{O}_2$ Induced Disulfide Formation


$\text{H}_2\text{O}_2$

SoxR is Activated by $O_2^\cdot$ - Mediated Disruption of an Fe/S cluster

SoxR dimer

2Fe-2S cluster

Cellular resistance to:
- Superoxide stress
- NO$\cdot$-generating macrophages
- Multiple antibiotics
- Organic solvents
- Heavy metals

SoxR

SoxR$^*$

soxS
Speaking of Fe/S clusters…

Post-transcriptional regulation is another way to change RNA and protein levels in cells.

One important known mechanism for post-transcriptional regulation in eukaryotic cells involves Fe/S clusters.
IRE & IRP, A Classical Tale

- **Iron Responsive Elements (IRE)**
  - Regulate Ferritin mRNA translation
  - Regulate Transferrin Receptor mRNA stability
  - Effects on other Iron utilizing proteins

- **Iron Responsive Proteins (IRP1/2)**
  - Cytosolic aconitase
  - Bind IREs
  - Contain Fe/S Clusters
  - Iron Sensitive
  - Superoxide sensitive
IREs are RNA Stem-Loops

IRPs bind IREs to control translation and RNA stability

Eukaryotic Transcription Factors

- Modular structures
- Some require ligands
  - Nuclear hormone receptors
Examples of Redox Regulated Mammalian Transcription Factors

- **AP-1**
  - Ref-1 & Thioredoxin

- **Egr1**
  - Zinc fingers, most common motif in the human proteome

- **HIF-1α / ARNT**
  - $\text{O}_2$
  - $\text{Fe}^{+2}$
  - $\alpha$-ketoglutarate
  - Ascorbate

- **PAS** (Per/Arnt/Sim) Domain Proteins (NADPH & NADH sensitive)
AP-1 (activator protein-1) activity is controlled by reversible cysteine oxidation

Zinc Fingers are a common redox sensitive DNA binding motif

Alberts et al., Molecular Biology of the Cell, 4th Edition
HIF-1α is Post-Translationally Regulated

O₂, Fe²⁺, α-KG, Asc
HIF-1α is O₂ sensitive

PAS Domain Proteins are Sensitive to Reduced NADPH

All of these are wonderful examples of redox regulated transcription factors,

BUT …

what good will they do if their DNA binding sites are inaccessible?
For Example, DNA Methylation can Block the Binding of Transcription Factors

Overview of Cytosine Methylation

- 5-methyl cytosine – the 5th base
- CpG dinucleotides
- Distribution of CpG in the genome
- Cytosine methylation patterns
- DNA Methyltransferases (DNMTs)
• The only modified base found in the human genome.

• Occurs in the nucleotide doublet 5’- CpG - 3’

• Propagated in somatic tissue by CpG methyltransferase.

• 5-methylcytosine is necessary for organism viability.

• CpG islands are frequently associated with the promoter and 5’end of genes.

• CpG hypermethylation associated with transcriptional silencing
DNA Methylation and Cancer

Cancer cells have less methylated cytosine than normal cells

Nevertheless some regions of the cancer cell genome become aberrantly hypermethylated

Cytosine methylation is associated with gene silencing
Genes become inappropriately turned off or on by alterations in mammalian genomic DNA methylation patterns.

Methylated DNA is associated with a repressive chromatin structure.

Many tumor suppressor genes are inactivated by aberrant cytosine methylation.
Aberrant CpG Methylation Leads to Tumor Suppressor Gene Silencing in Human Cancers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tumors with methylation</th>
<th>Gene</th>
<th>Tumors with methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>Retinoblastoma</td>
<td>VHL</td>
<td>Renal carcinoma</td>
</tr>
<tr>
<td>p16/INK4A</td>
<td>Most common solid tumors</td>
<td>p15/INK4B</td>
<td>Acute leukemia, Burkitt lymphoma</td>
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<td>p27/KIP</td>
<td>Pituitary cell line</td>
<td>h-MLH1</td>
<td>Colon</td>
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<td>E-cadherin</td>
<td>Bladder, breast, colon, liver tumors</td>
<td>BrCA1/2</td>
<td>Breast Cancer</td>
</tr>
<tr>
<td>WT-1</td>
<td>Wilms tumors</td>
<td>maspin</td>
<td>Breast Cancer</td>
</tr>
</tbody>
</table>

Distribution of methylated CpG in Normal Cells

- Methylated CpG
- Unmethylated CpG
Distribution of methylated CpG in Cancer Cells

Methylated CpG

Unmethylated CpG
How do these aberrant methylation patterns emerge?

DNA methyltransferases (DNMTs) are upregulated in cancer cells.

DNMTs require the metabolite S-adenosyl methionine.

Cancer cells often display symptoms of oxidative stress.
Is DNA Methylation Redox Sensitive?

Anti-oxidants

Pro-oxidants

Biological Response

GSH

Compensatory increase
Overview of one carbon metabolism featuring the SAM cycle

* Note the metabolic link to cysteine and thus glutathione (GSH) synthesis
Hypothesis

Perturbations in one carbon metabolite pools cause the aberrant DNA methylation patterns observed in human cancer and other pathobiological states.
Methylated DNA is associated with a repressive chromatin structure

What’s Chromatin?

Located in cell nucleus

DNA and its associated proteins

DNA exists on nucleosomes composed of histone proteins

One histone octamer contains 2 subunits each of H2A, H2B, H3, H4
Chromatin Structure and Organization

NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000X SHORTER THAN ITS EXTENDED LENGTH.

Essential Cell Biology, by Alberts et al., 1998, Garland Publishing Inc
short region of DNA double helix

“beads-on-a-string” form of chromatin

30-nm chromatin fiber of packed nucleosomes

(A) 50 nm
Nucleosome Structure

Nucleosome Tails are Post-Translationally Regulated

N-terminal tails: signaling platforms?  Nucleosome core

H2A/H2B tails

H2A Ac-NH$_1$SGRGKQGGKARAKAKTRSSRAGL ···

H2B NH$_3$-PEPSKSAPAPKGS1KAATTKAQKKGKRRKRKRSRK ···

H3 NH$_3$-ARTKQTARKSTGGKAPRKQLATKAARKSAP···

H4 Ac-NH$_1$SGRGKGGKGLGKGGAKRHRKVLR ···

$\uparrow$ = Acetyl-Lysine

● = Phospho-Serine

DNA
Modifications to Nucleosomes

- Acetylated (Lys)
- Methylated (Lys, Arg)
- Phosphorylated (Ser)
- Ubiquitinated (Lys)
- ADP-ribosylated
- ?
The "Histone Code"
HATs, HDACs, and HMTs

- **Histone Acetyltransferase (HAT)**
  - Acetyl-CoA is the co-factor
- **Histone Deacetylase (HDAC)**
- **Histone Methyltransferase (HMT)**
  - SAM is the cofactor

- Determinants of the chromatin architecture, or “epigenetic landscape”
Epigenetics

A heritable change in phenotype that is independent of a change of genotype.

- RNA Editing
- RNA Interference
- Histone Modification
- 5-methylcytosine

Holliday Hypothesis- ca. 1975
Chromatin Structure Governs DNA Accessibility

Plasticity of the epigenetic state

Georgopouos K, *Nature Reviews Immunology* 2, 162, 2002
Some transcription factors function through chromatin remodeling
One Type of Histone Deacetylase, Sir2, Yields a Unique Product

An Increase in Sir2 Extends Lifespan

Summary

• Cells respond to redox challenges with compensatory responses
  – Direct transcription factor activation
  – Alterations in mRNA translation
  – DNA methylation
  – Histone modifications
  – Higher ordered chromatin structure
    • Chromatin Accessibility!!!!