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

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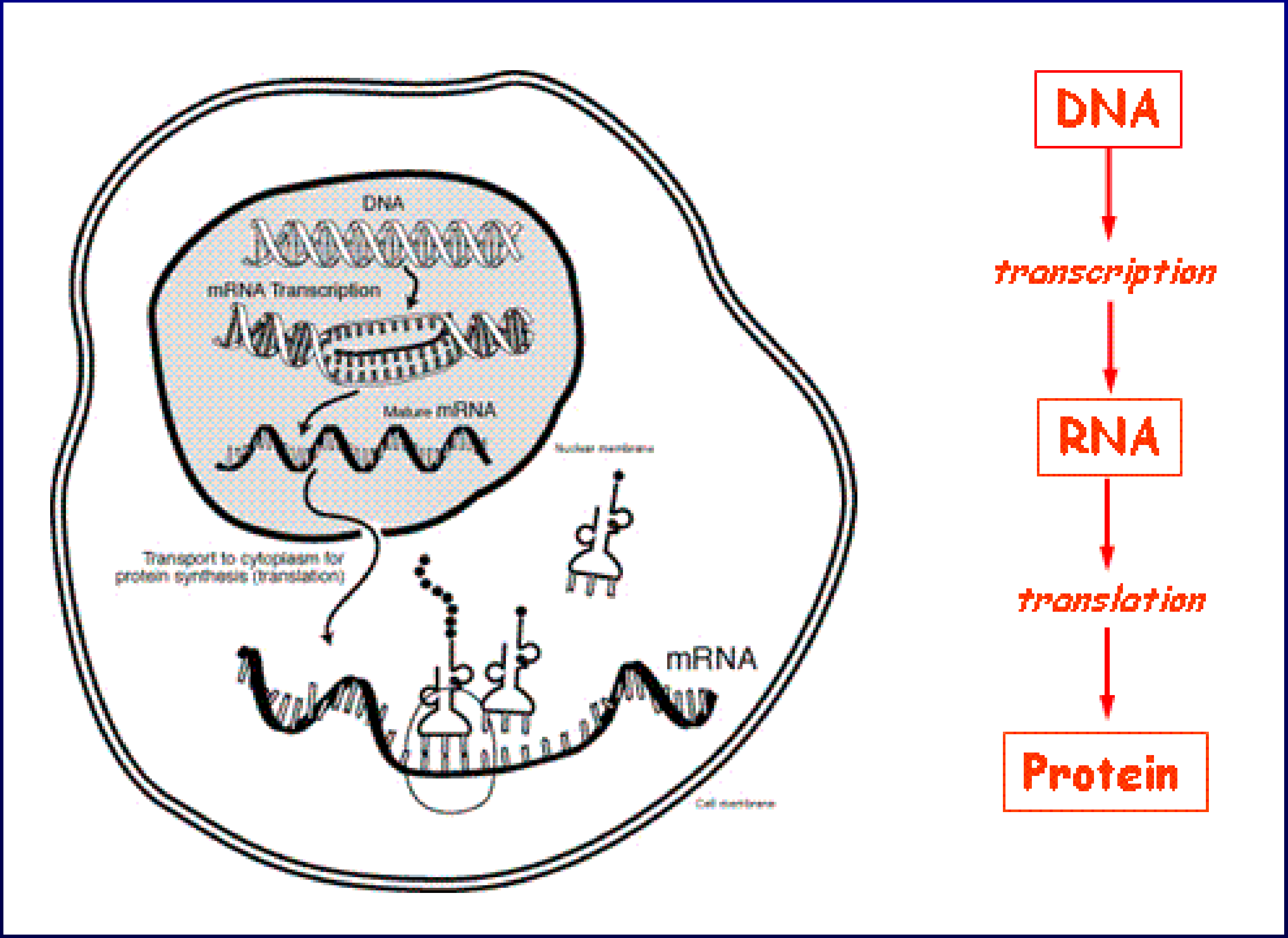
The Central Dogma of Molecular Biology



Oxidative Stress

→ Biological Response

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

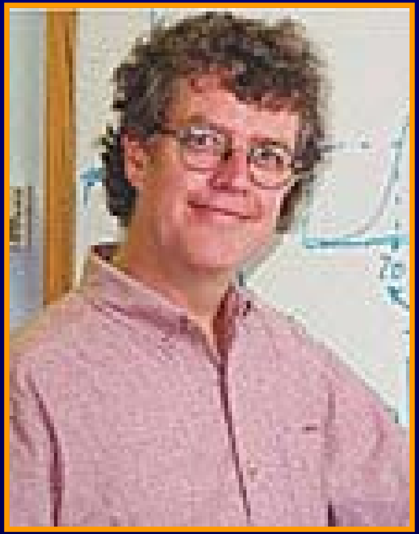
Transcription Factors

- 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

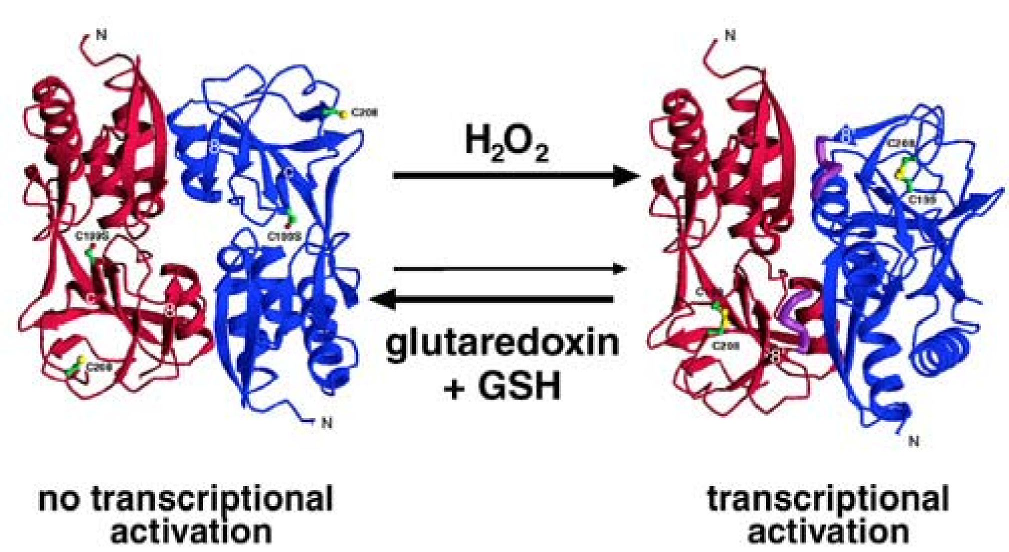
- Prokaryotic
- H_2O_2 and $O_2^{\bullet -}$ sensitive, respectively

Gisela Storz

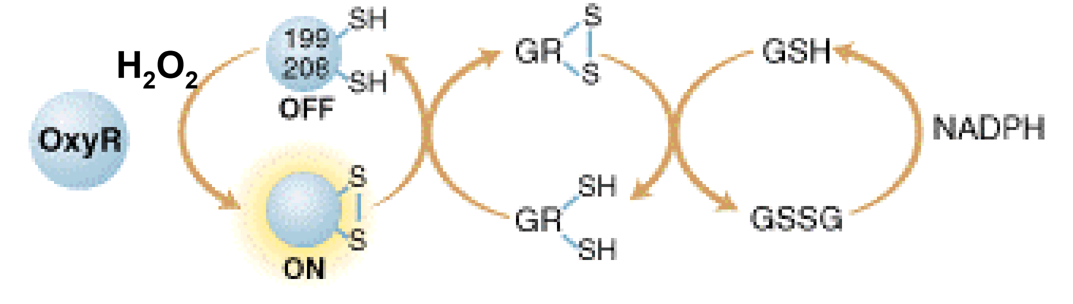


Bruce Demple

OxyR is Activated by H_2O_2 Induced Disulfide Formation

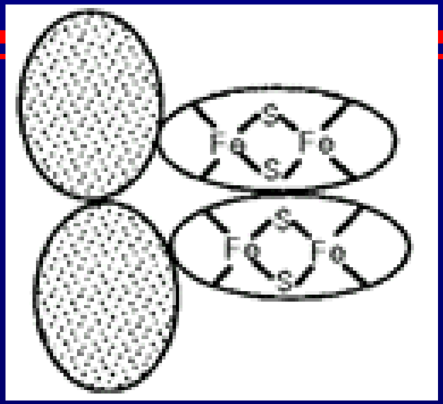


Storz G, http://eclipse.nichd.nih.gov/nichd/cbmb/segr/segr.html

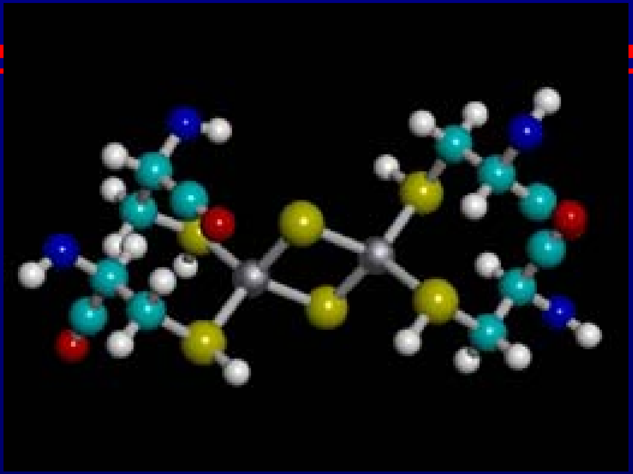


Science, Vol 279: 1655, 1998

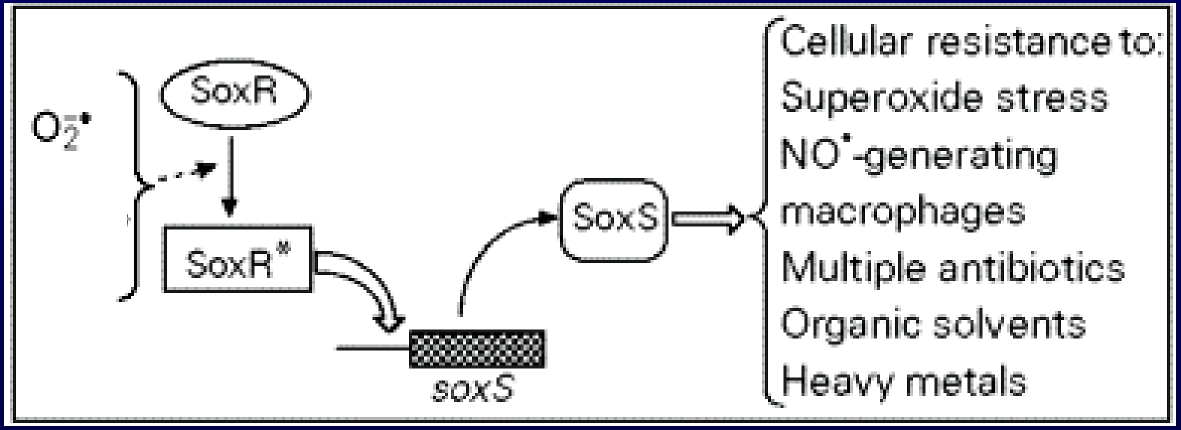
SoxR is Activated by $O_2^{\bullet -}$ Mediated Disruption of an Fe/S cluster



SoxR dimer



2Fe-2S cluster



Speaking of Fe/S clusters…

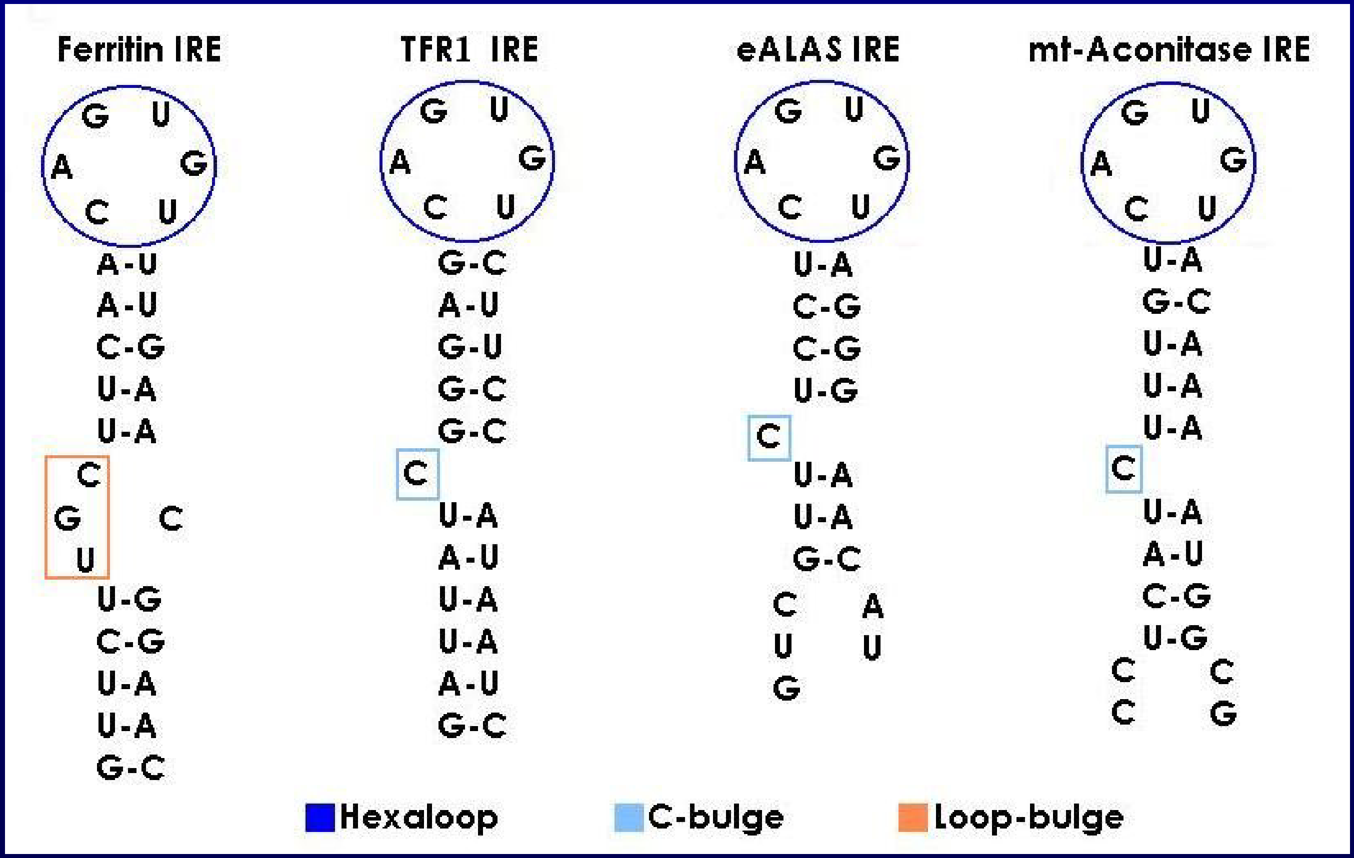
Post-transcriptional regulation is another way to change RNA and protein levels in cells

One important known mechanism for post-transcriptional regulation in eukaryotic cells involves Fe/S clusters

IRE & IRP, A Classical Tale

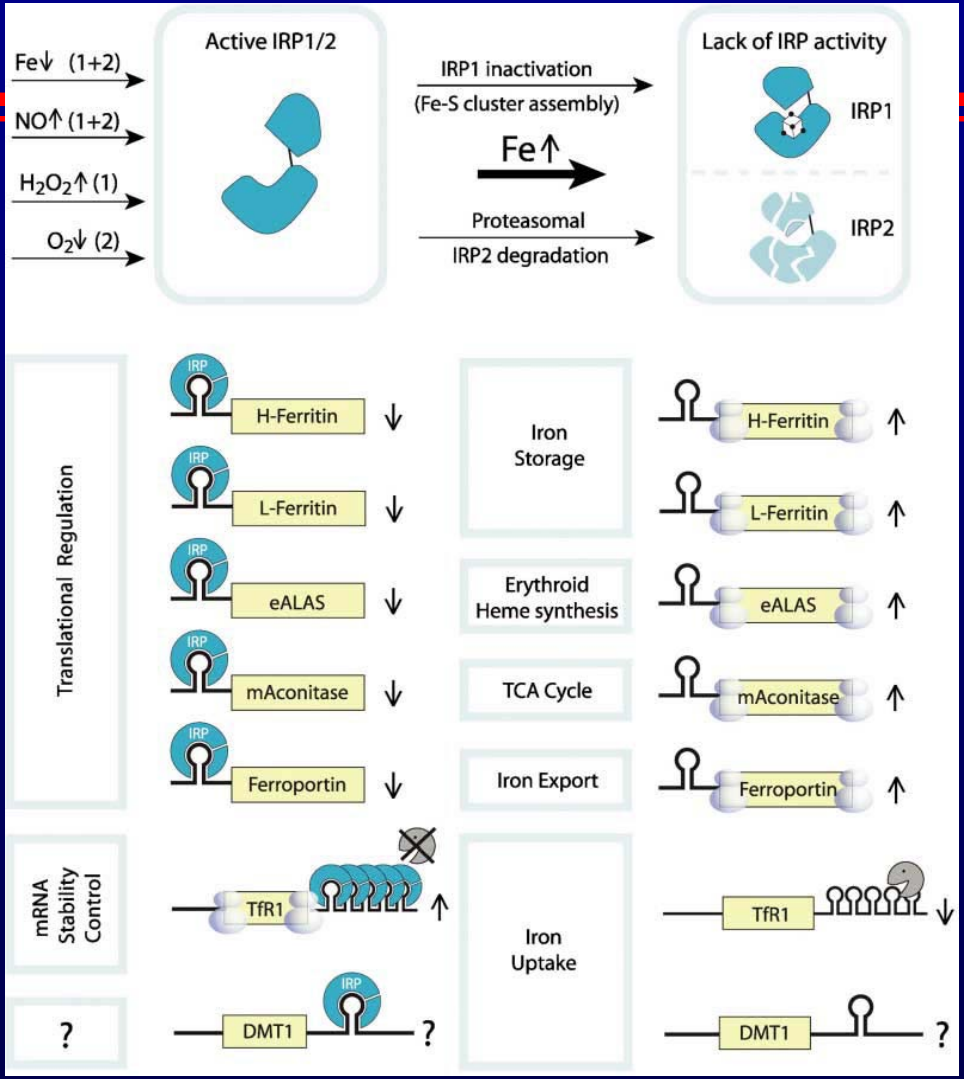
- **Iron Responsive Elements** (IRE)
  - Regulate Ferritin mRNA translation
  - Regulate Transferrin Receptor mRNA stability
  - Effects on other Iron utilizing proteins
- **Iron Responsive Proteins** (IRP1/2)
  - Cytosolic aconitase
  - Bind IREs
  - Contain Fe/S Clusters
  - Iron Sensitive
  - Superoxide sensitive

IREs are RNA Stem-Loops



http://www.embl-heidelberg.de/ExternalInfo/hentze/maykapage2005.html

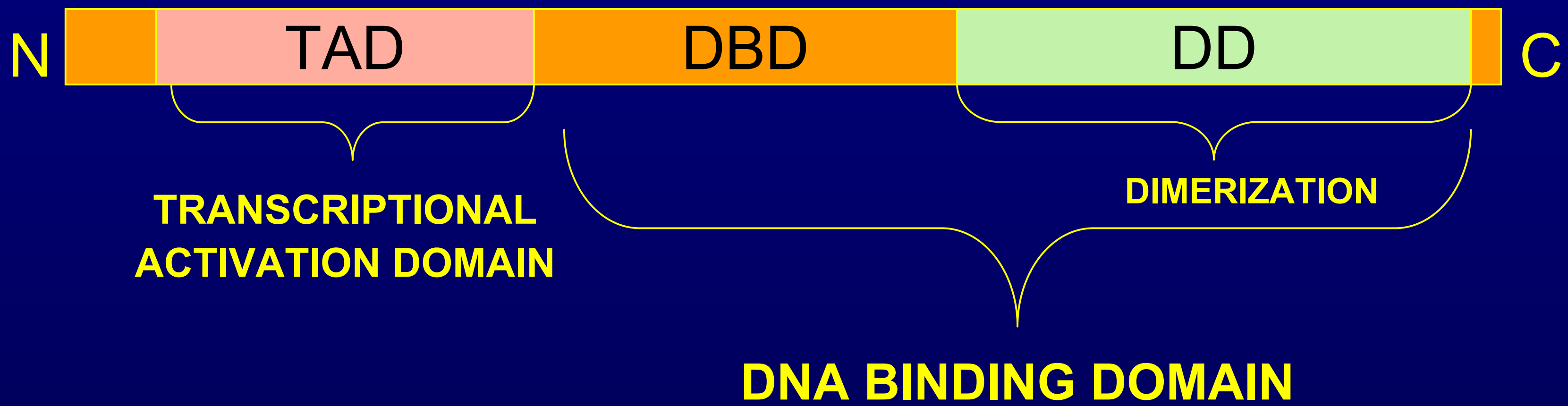
IRPs bind IREs to control translation and RNA stability



http://www.embl-heidelberg.de/ExternalInfo/hentze/maykapage2005.html

Eukaryotic Transcription Factors

- Modular structures

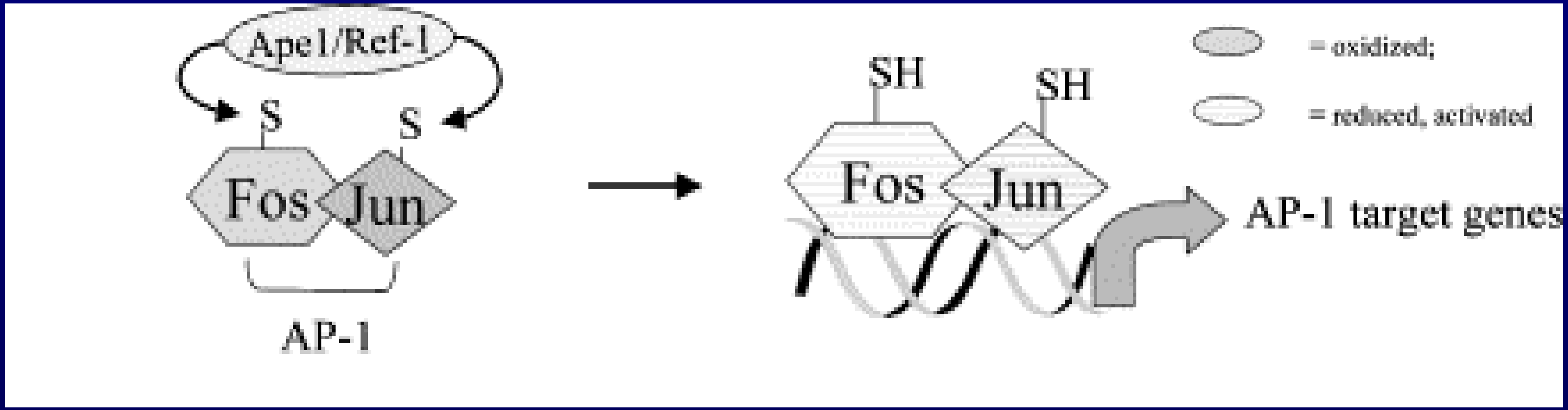


- Some require ligands
  - Nuclear hormone receptors

Examples of Redox Regulated Mammalian Transcription Factors

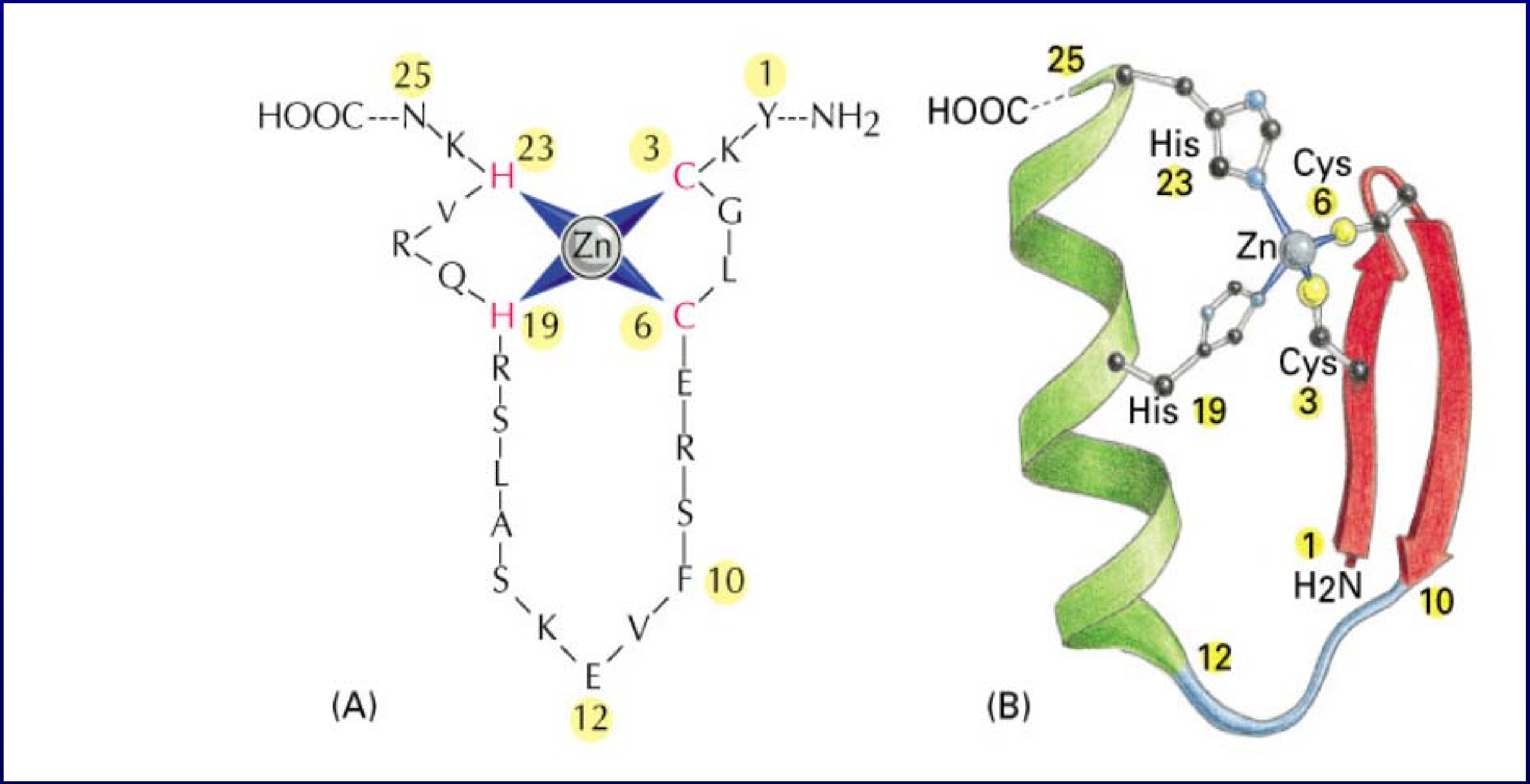
- **AP-1**
  - Ref-1 & Thioredoxin
- **Egr1**
  - Zinc fingers, most common motif in the human proteome
- **HIF-1**α / ARNT
  - O_2
  - Fe^{+2}
  - α-ketoglutarate
  - Ascorbate
- **PAS** (Per/Arnt/Sim) Domain Proteins (NADPH & NADH sensitive)

AP-1 (activator protein-1) activity is controlled by reversible cysteine oxidation



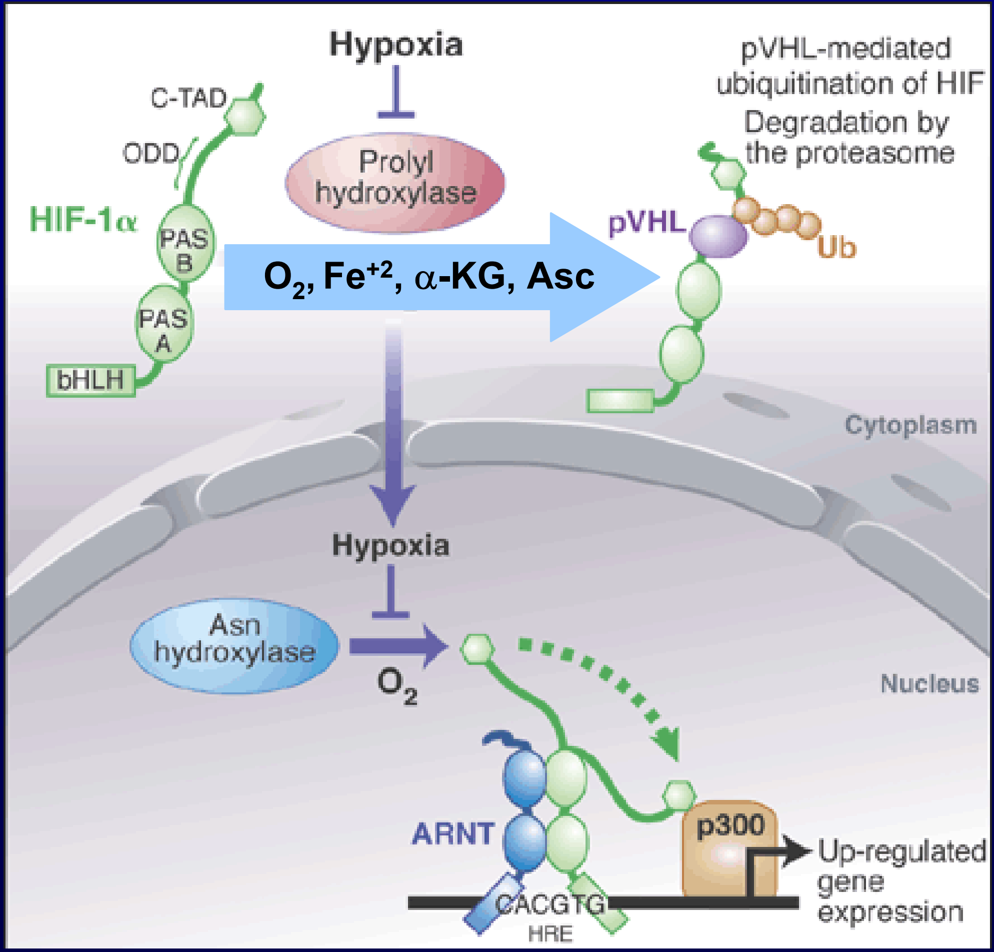
Evans, AR, et al., Mutat. Res. *461*, 83-108, 2000

Zinc Fingers are a common redox sensitive DNA binding motif

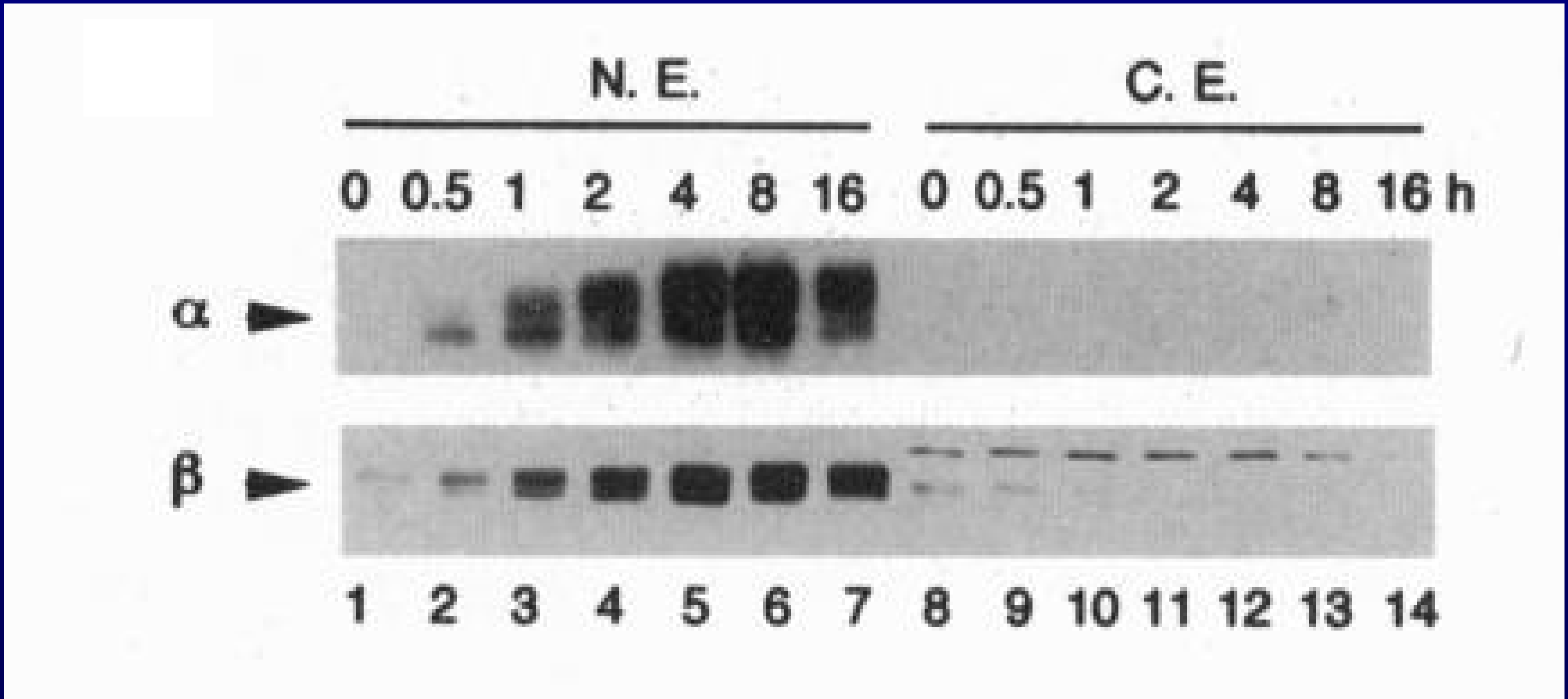


Alberts et al., Molecular Biology of the Cell, 4th Edition

HIF-1α is Post-Translationally Regulated

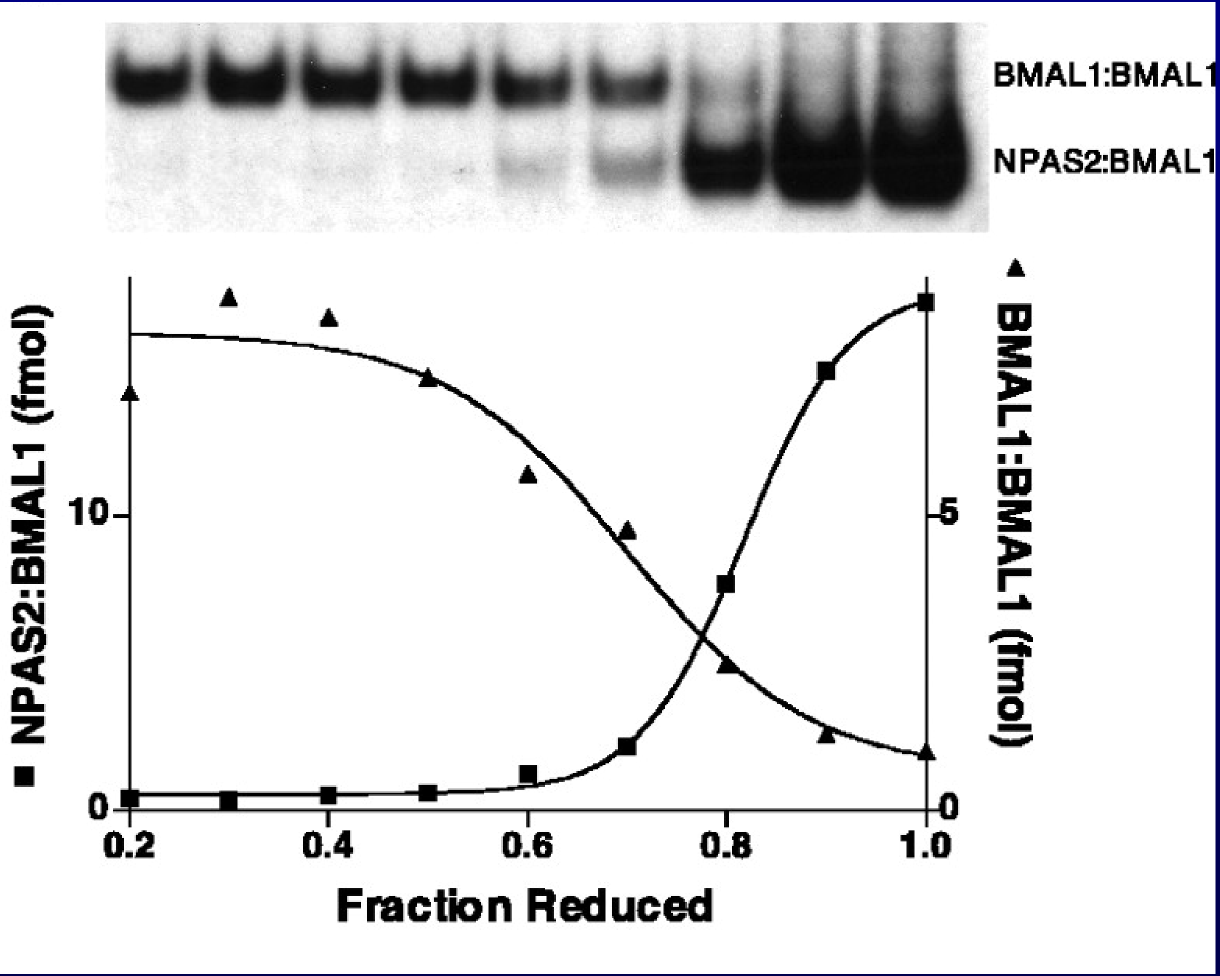


HIF-1α is O_2 sensitive



Wang GL, et al., Proc Natl Acad Sci 92(12): 5510, 1995

PAS Domain Proteins are Sensitive to Reduced NADPH



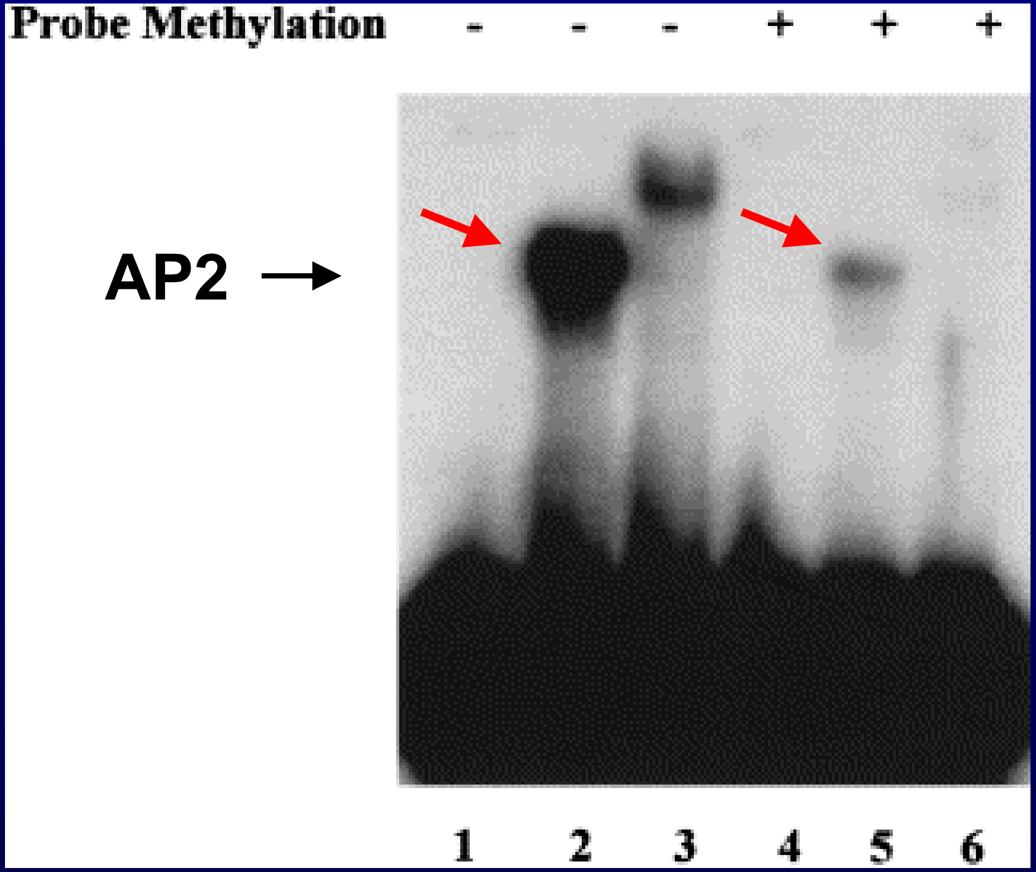
Rutter, J et al., Science 293:510, 2001

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

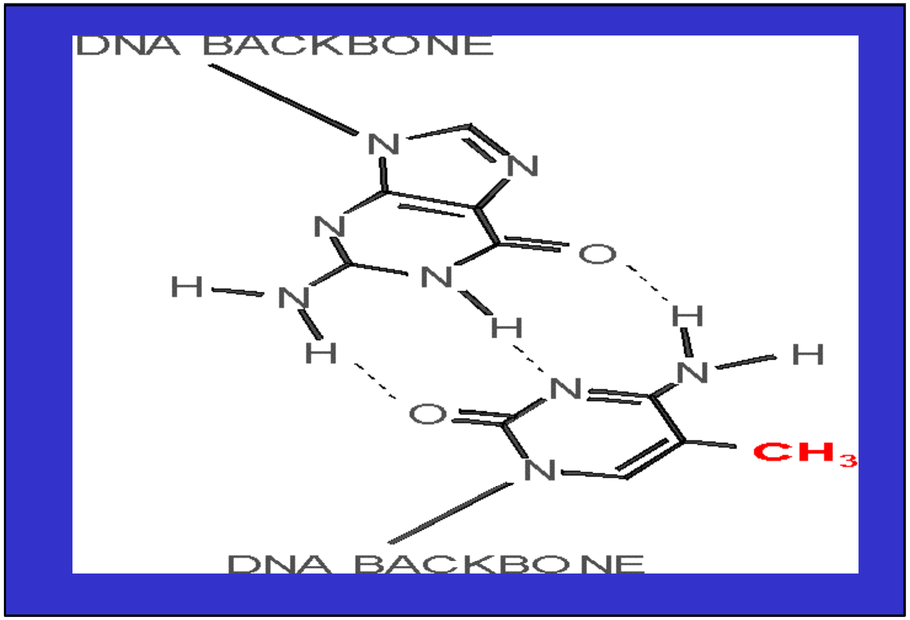


Huang Y, et al, Free Radic Biol Med.23:314, 1997

Overview of Cytosine Methylation

- 5-methyl cytosine – the 5th base
- CpG dinucleotides
- Distribution of CpG in the genome
- Cytosine methylation patterns
- DNA Methyltransferases (DNMTs)

5-Methylcytosine



- 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

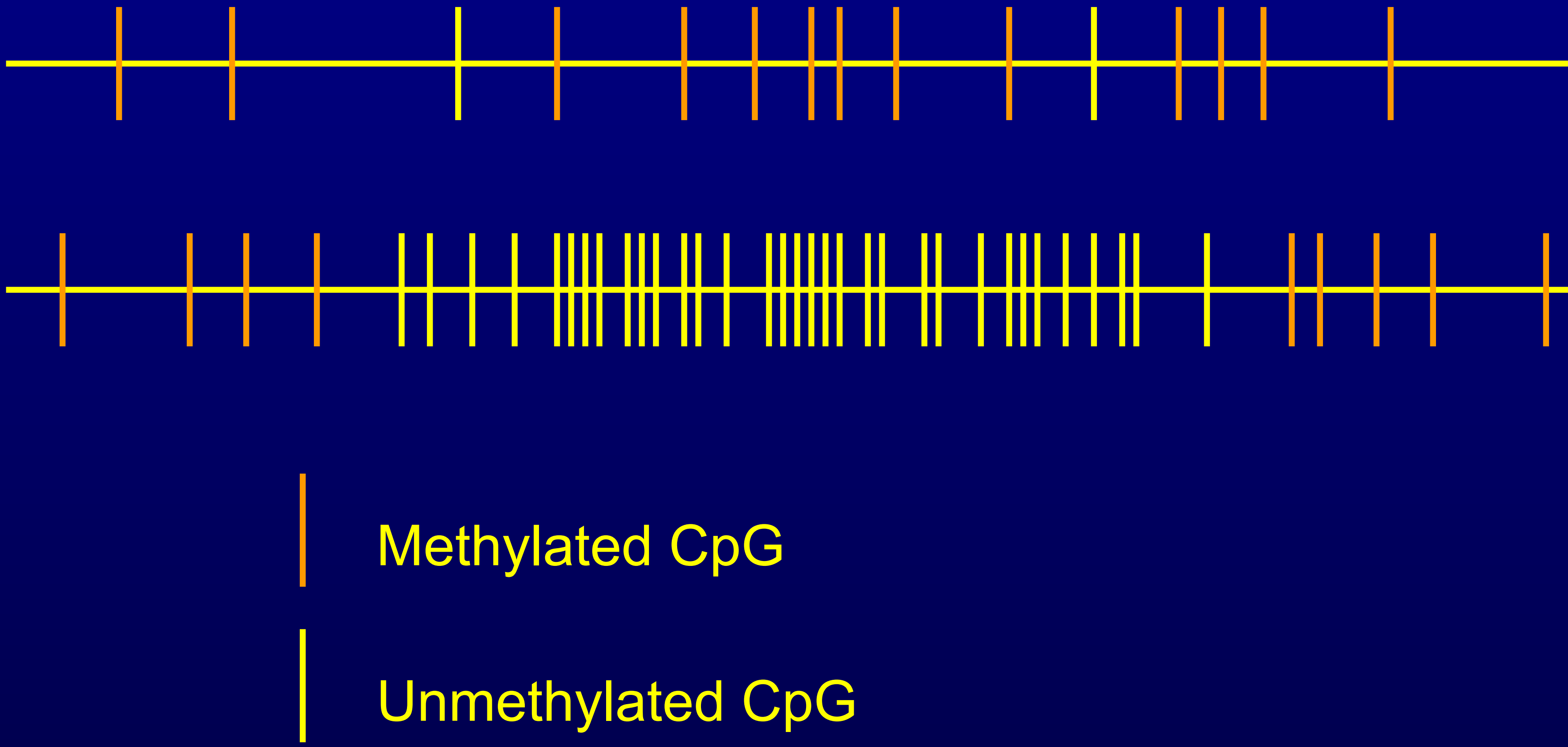
Aberrant CpG Methylation Leads to Tumor Suppressor Gene Silencing in Human Cancers

Gene	Tumors with methylation
RB	Retinoblastoma
p16/INK4A	Most common solid tumors
p27/KIP	Pituitary cell line
E-cadherin	Bladder, breast, colon, liver tumors
WT-1	Wilms tumors

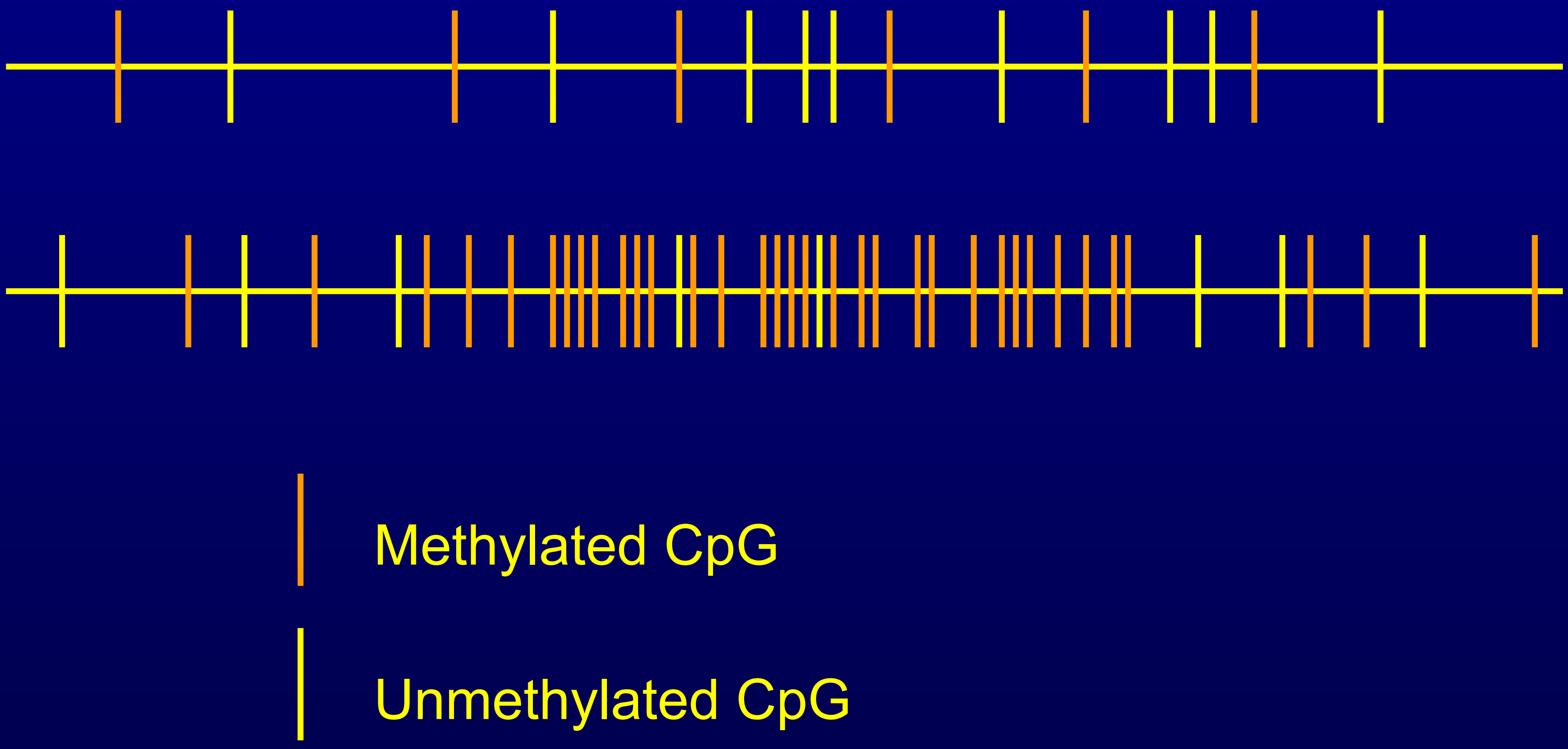
Gene	Tumors with methylation
VHL	Renal carcinoma
p15/INK4B	Acute leukemia, Burkitt lymphoma
h-MLH1	Colon
BrCA1/2	Breast Cancer
maspin	Breast Cancer

Baylin SB *et al*., (1998) Adv Cancer Res. **72**:141-96. Herman JG *et al*., (1997) Cancer Res. **57**:837-41. Domann FE *et al*., (2000) Int J Cancer. **85**:805-810.

Distribution of methylated CpG in Normal Cells



Distribution of methylated CpG in Cancer Cells



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

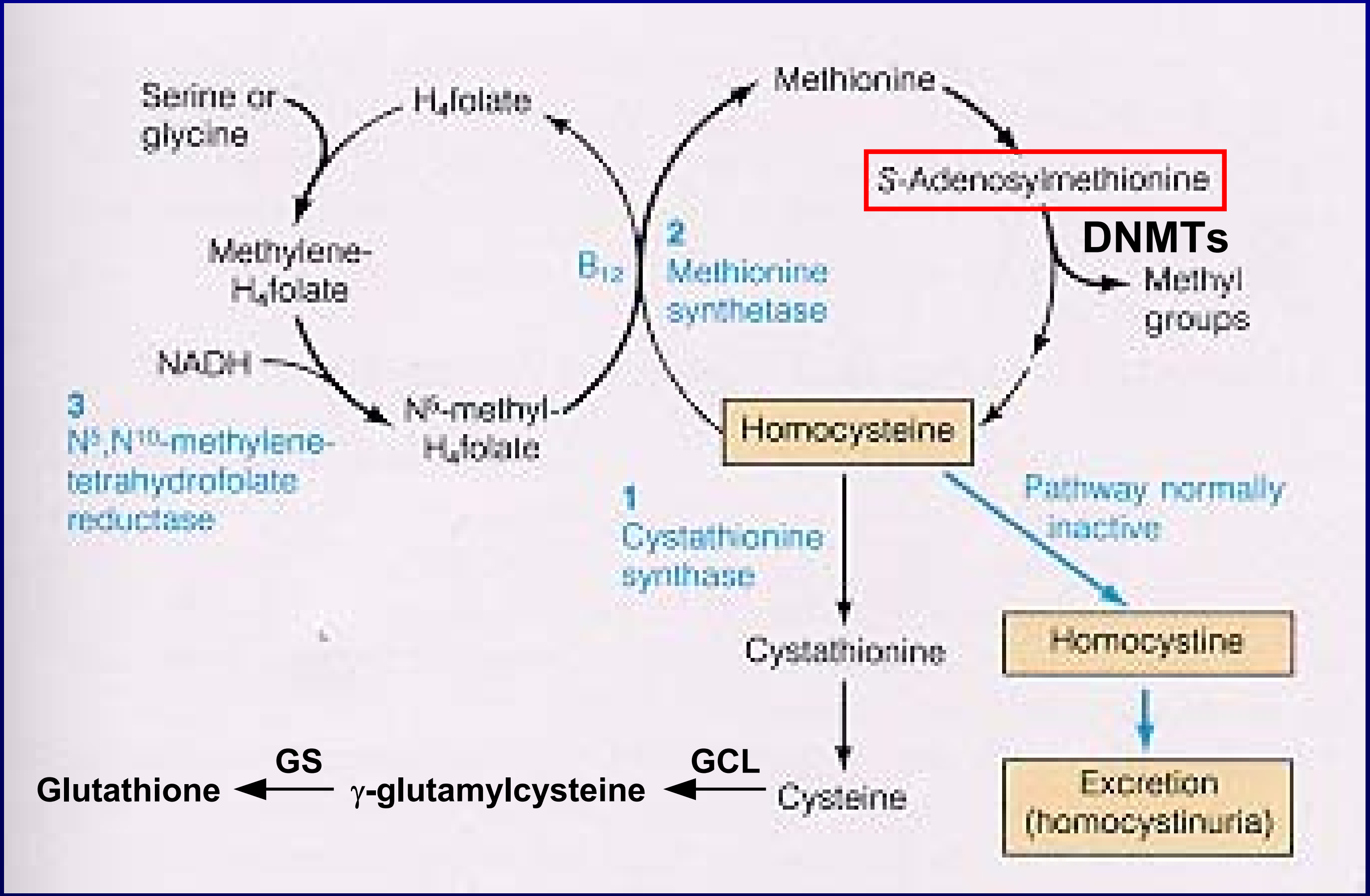
Is DNA Methylation Redox Sensitive?

Biological Response

GSH

Compensatory increase

Overview of one carbon metabolism featuring the SAM cycle

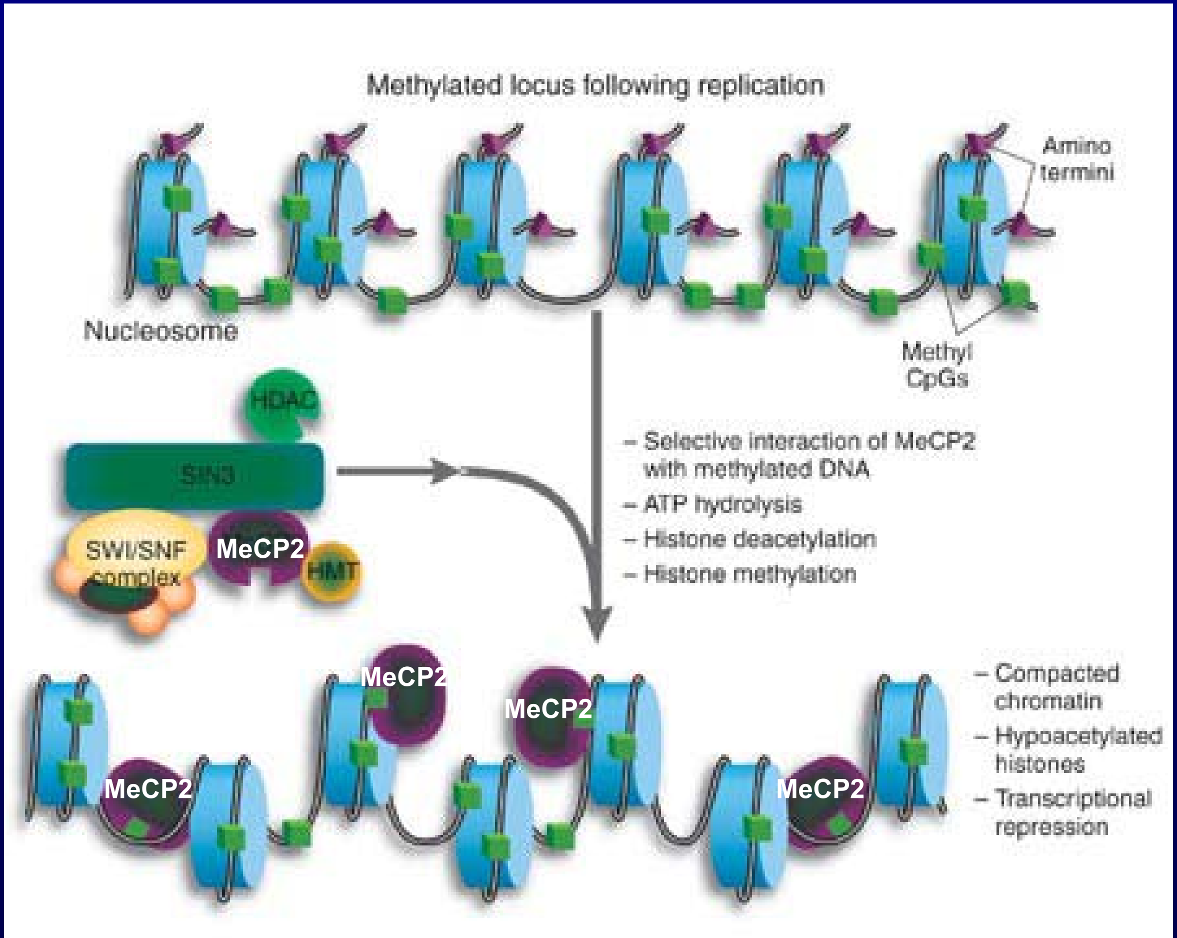


*** Note the metabolic link to cysteine and thus glutathione (GSH) synthesis**

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



Wade P, *Nature Genetics* **37**, 212 - 213 (2005)

What’s Chromatin?

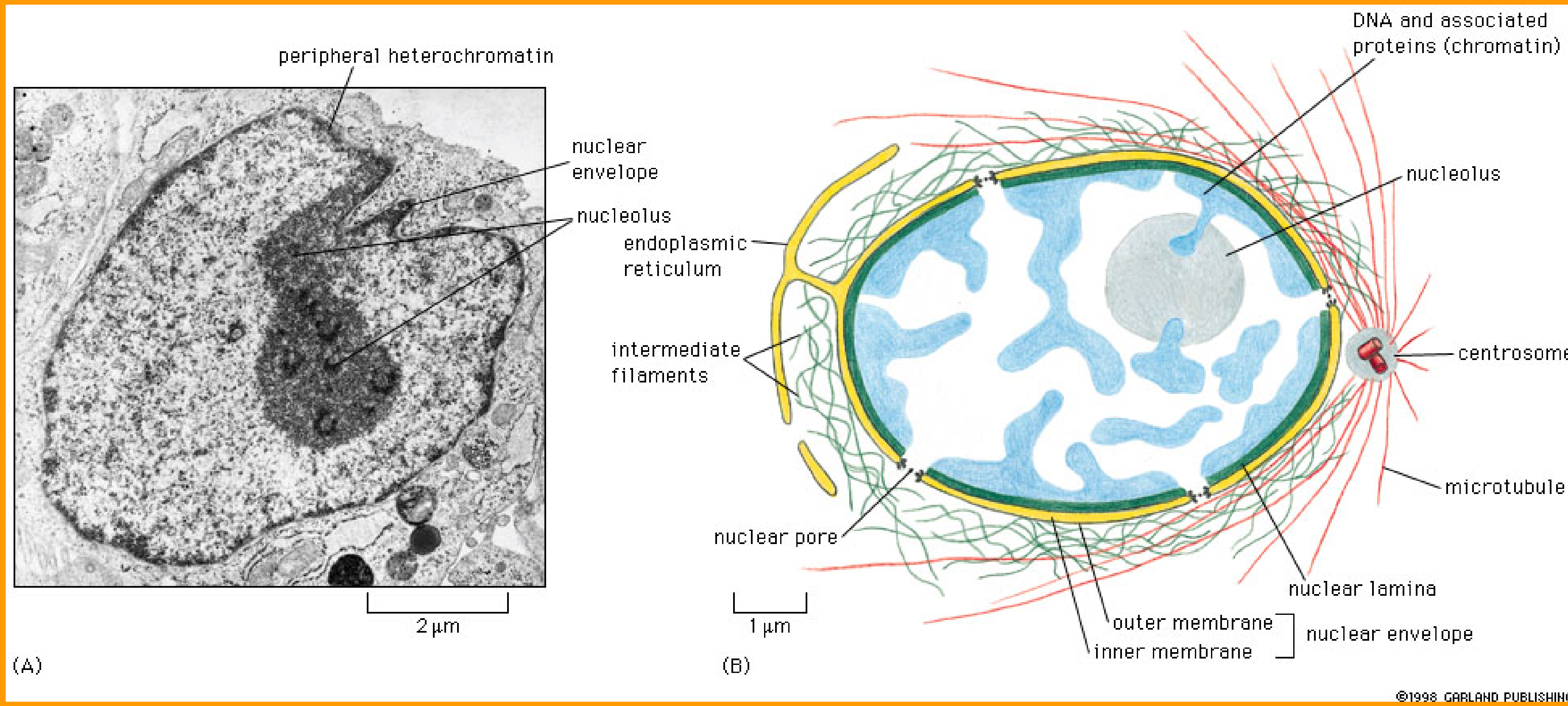
Located in cell nucleus

DNA and its associated proteins

DNA exists on nucleosomes composed of histone proteins

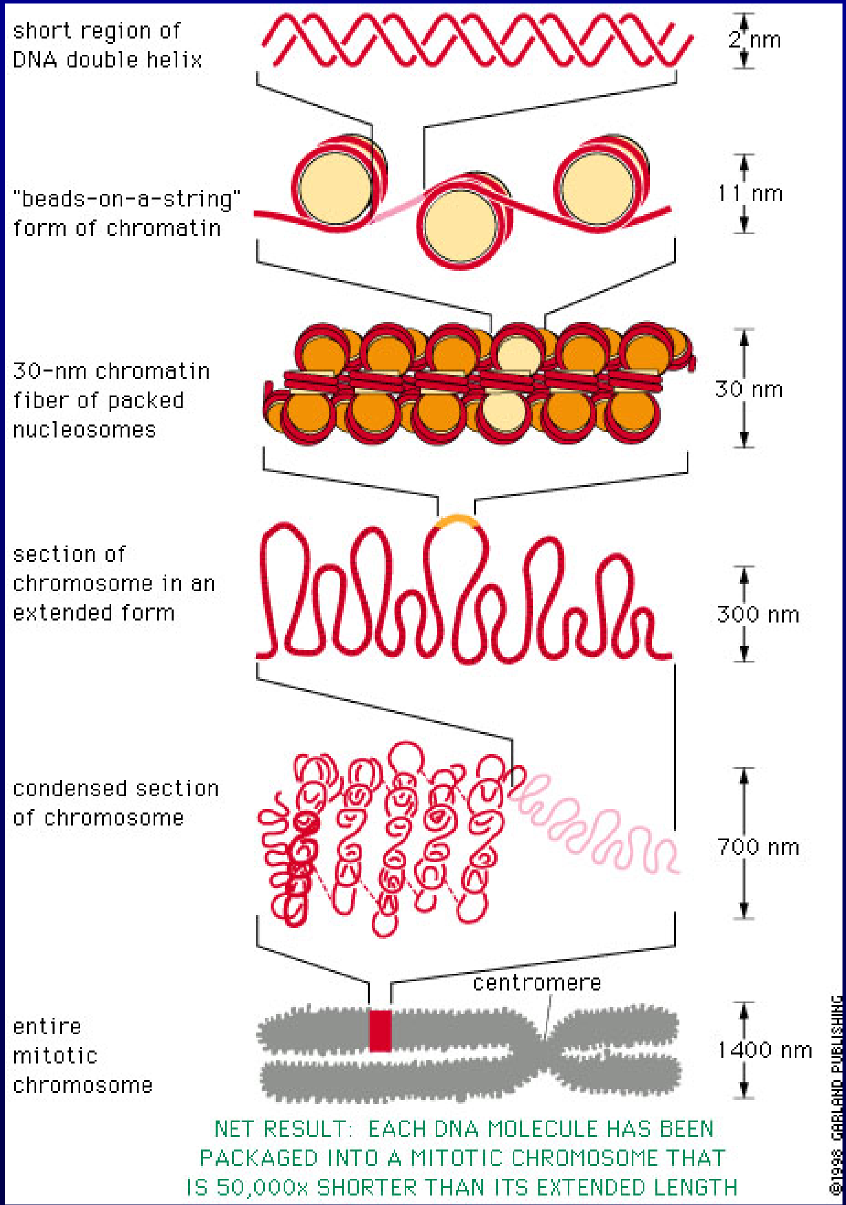
One histone octamer contains 2 subunits each of H2A, H2B, H3, H4

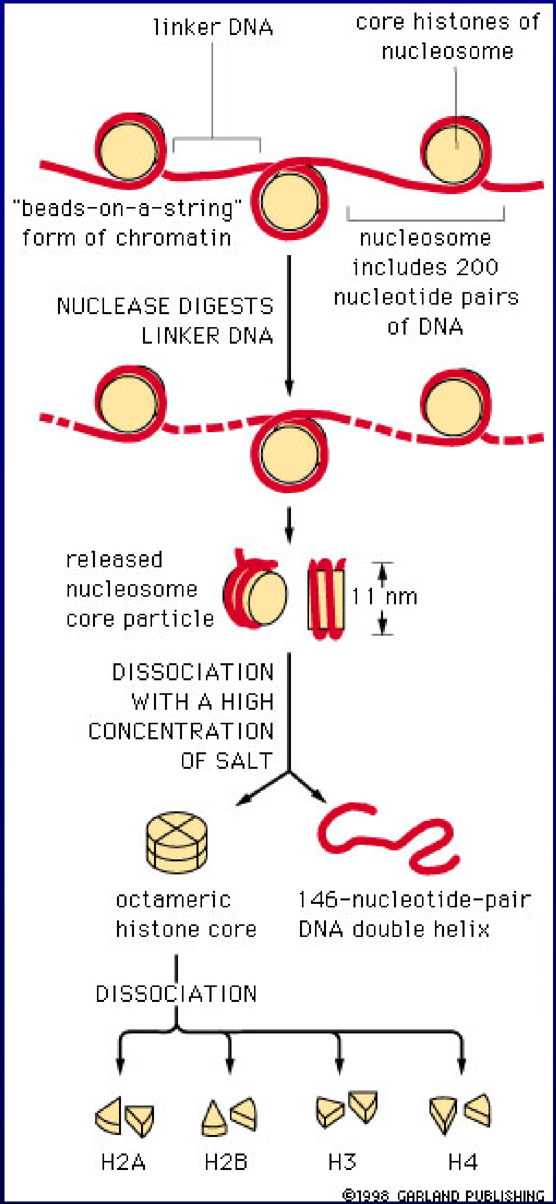
Nuclear Organization



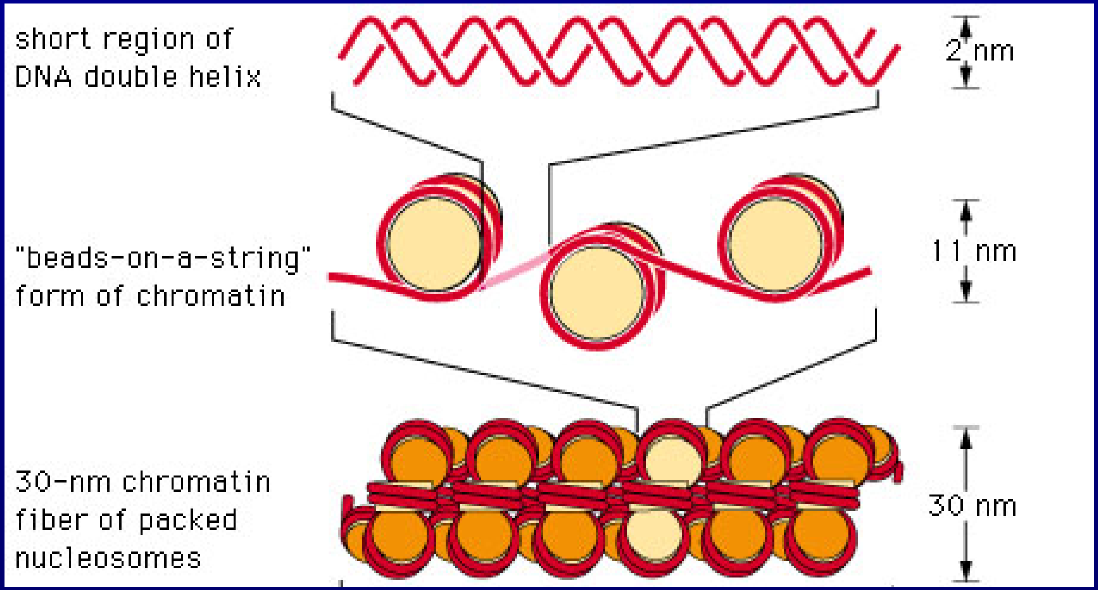
Essential Cell Biology, by Alberts et al., 1998, Garland Publishing Inc

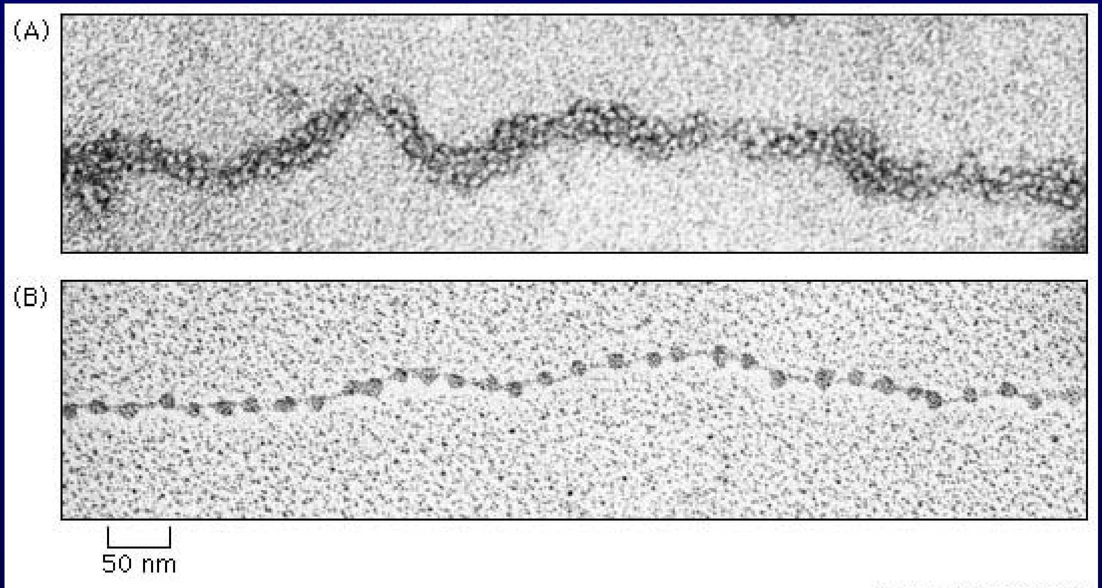
Chromatin Structure and Organization



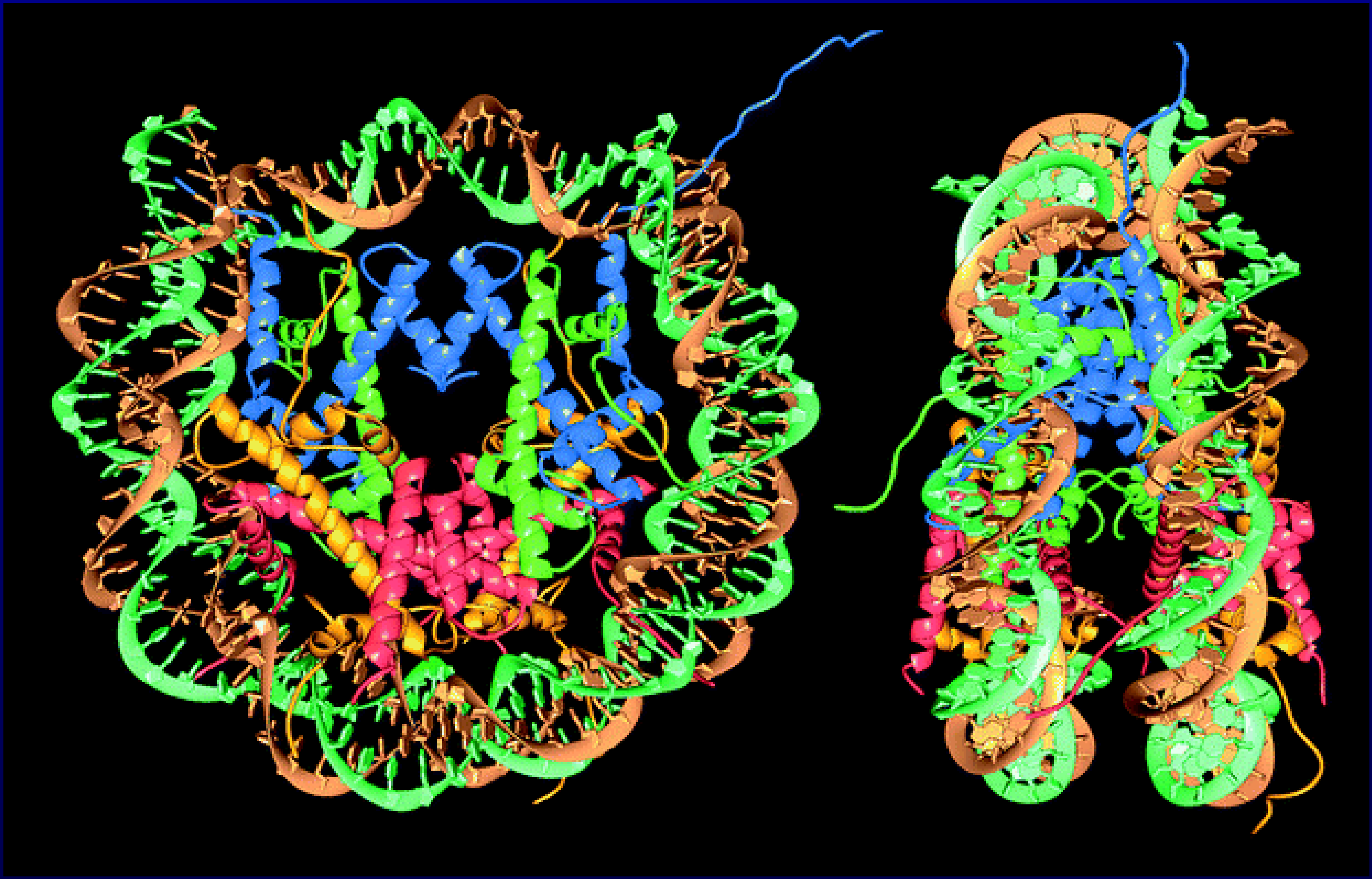


Essential Cell Biology, by Alberts et al., 1998, Garland Publishing Inc


short region of
DNA double helix
2 nm
"beads-on-a-string"
form of chromatin
11 nm
30-nm chromatin
fiber of packed
nucleosomes
30 nm

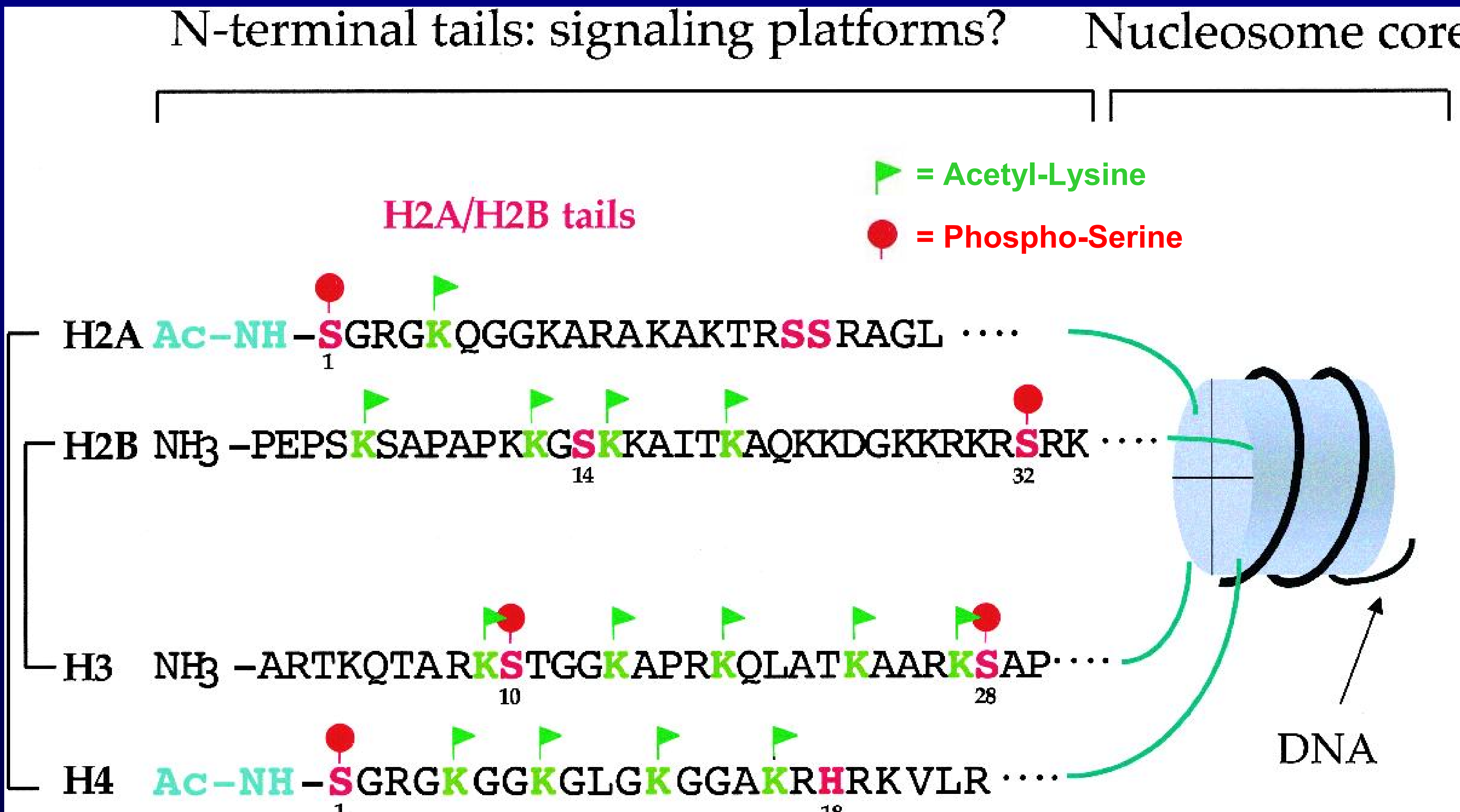

(A)
(B)
50 nm

Nucleosome Structure



K. Luger, et al., *Nature* **389**, 251 – 260, 1997

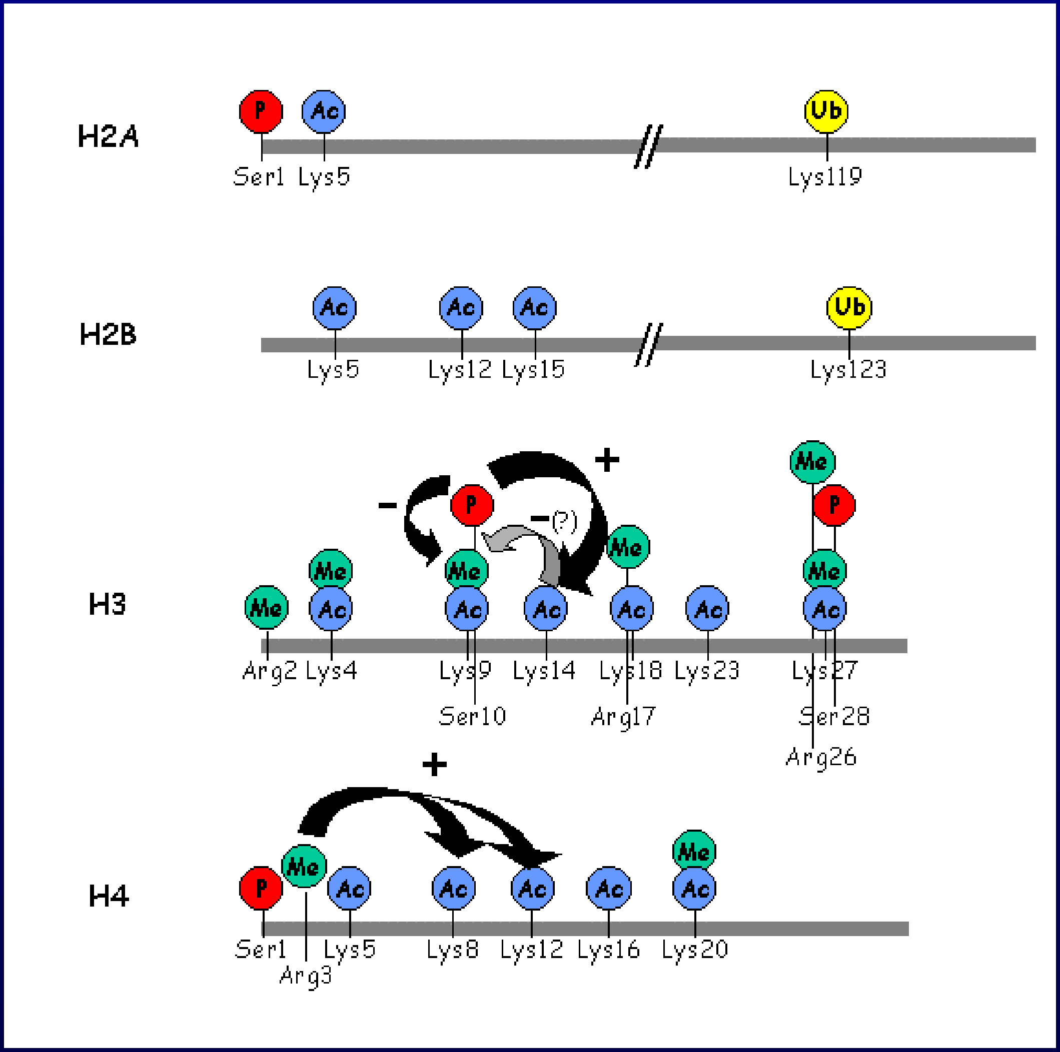
Nucleosome Tails are Post-Translationally Regulated



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

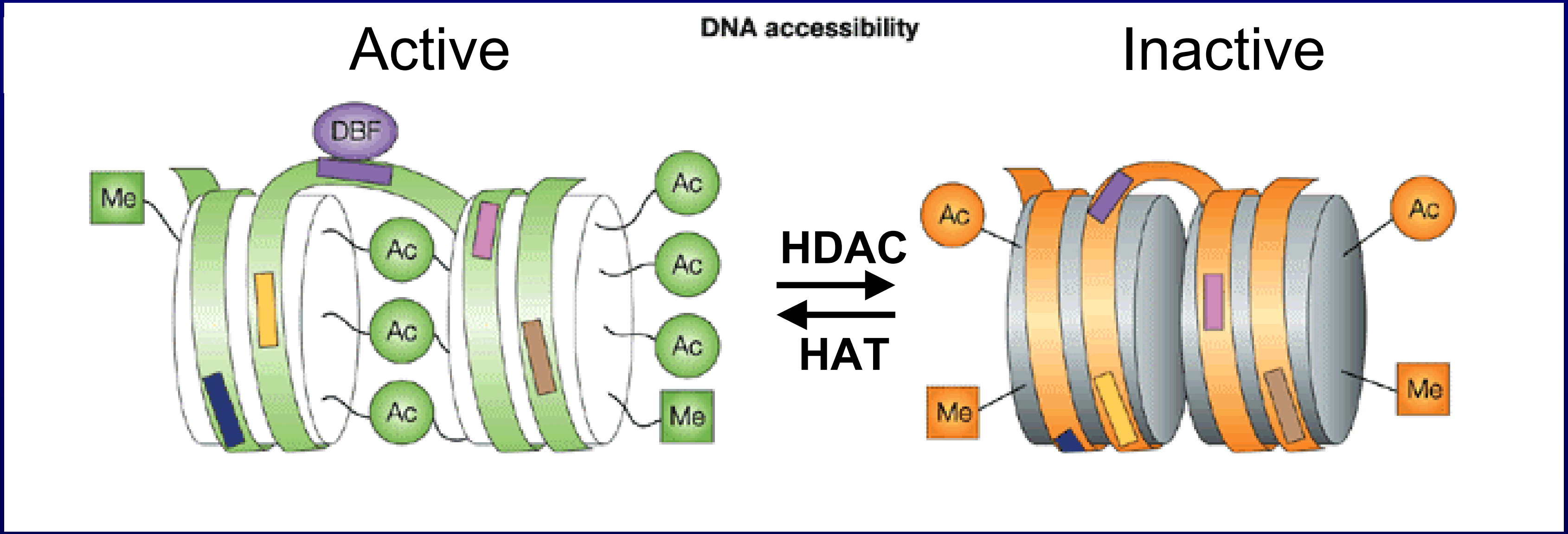
A heritable change in phenotype that is independent of a change of genotype.

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

Holliday Hypothesis- ca. 1975

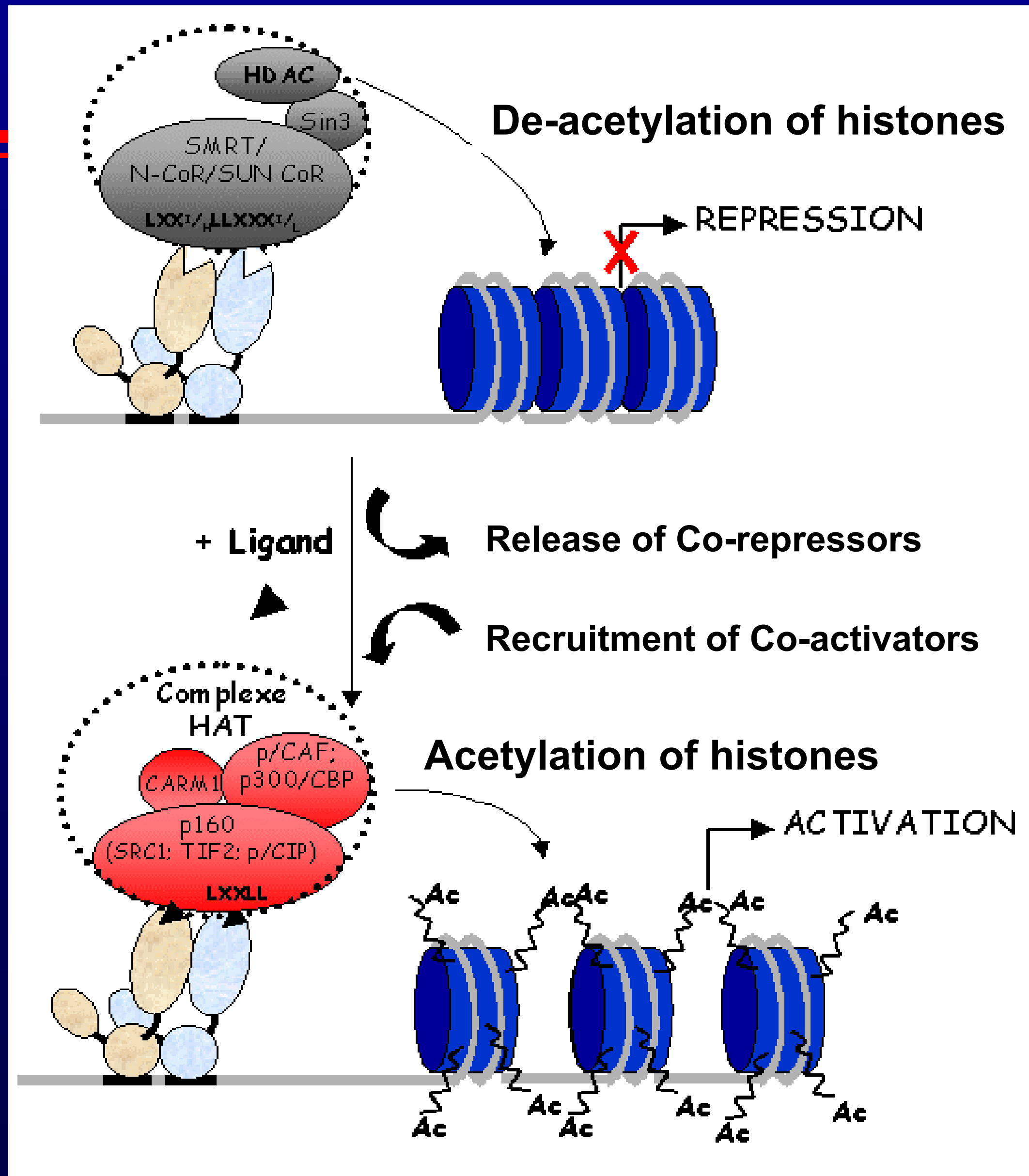
Chromatin Structure Governs DNA Accessibility

Plasticity of the epigenetic state

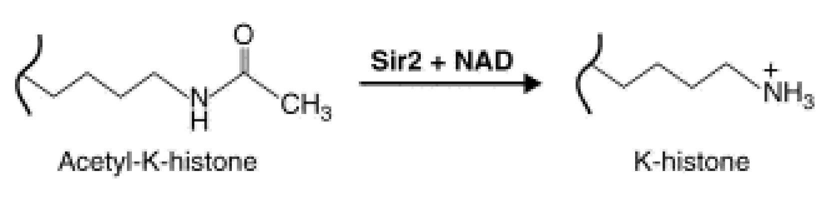


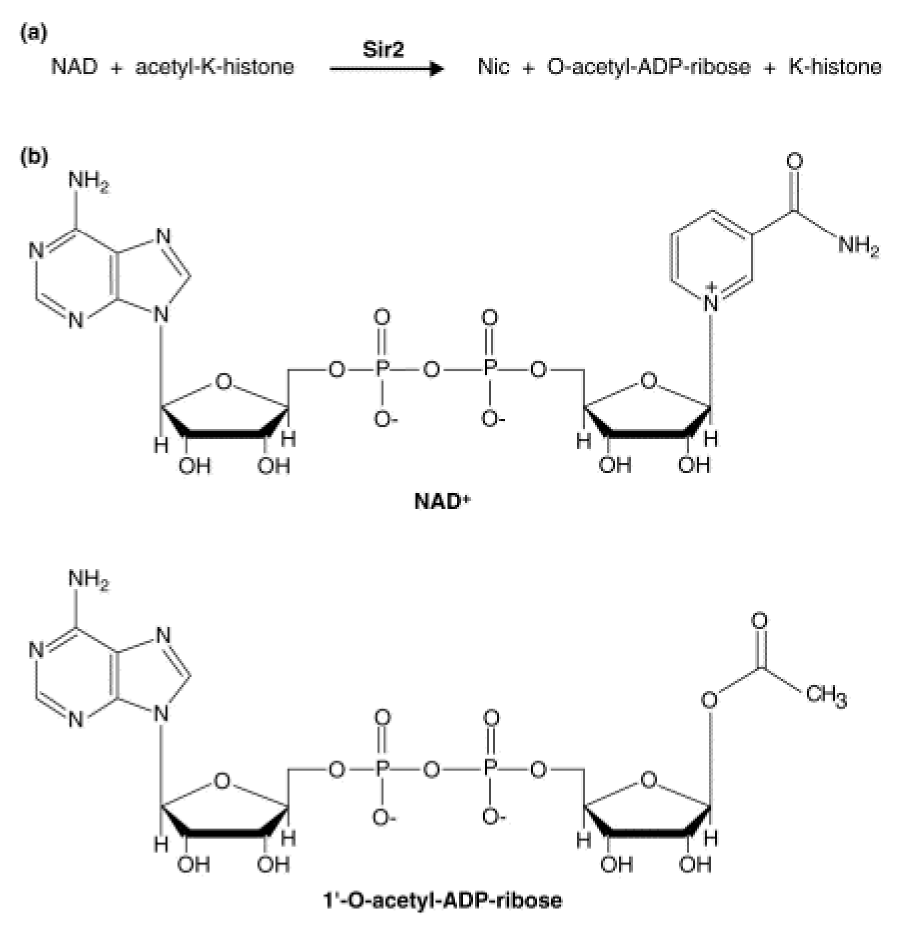
Georgopouos K, *Nature Reviews Immunology* **2**, 162, 2002

Some transcription factors function through chromatin remodeling



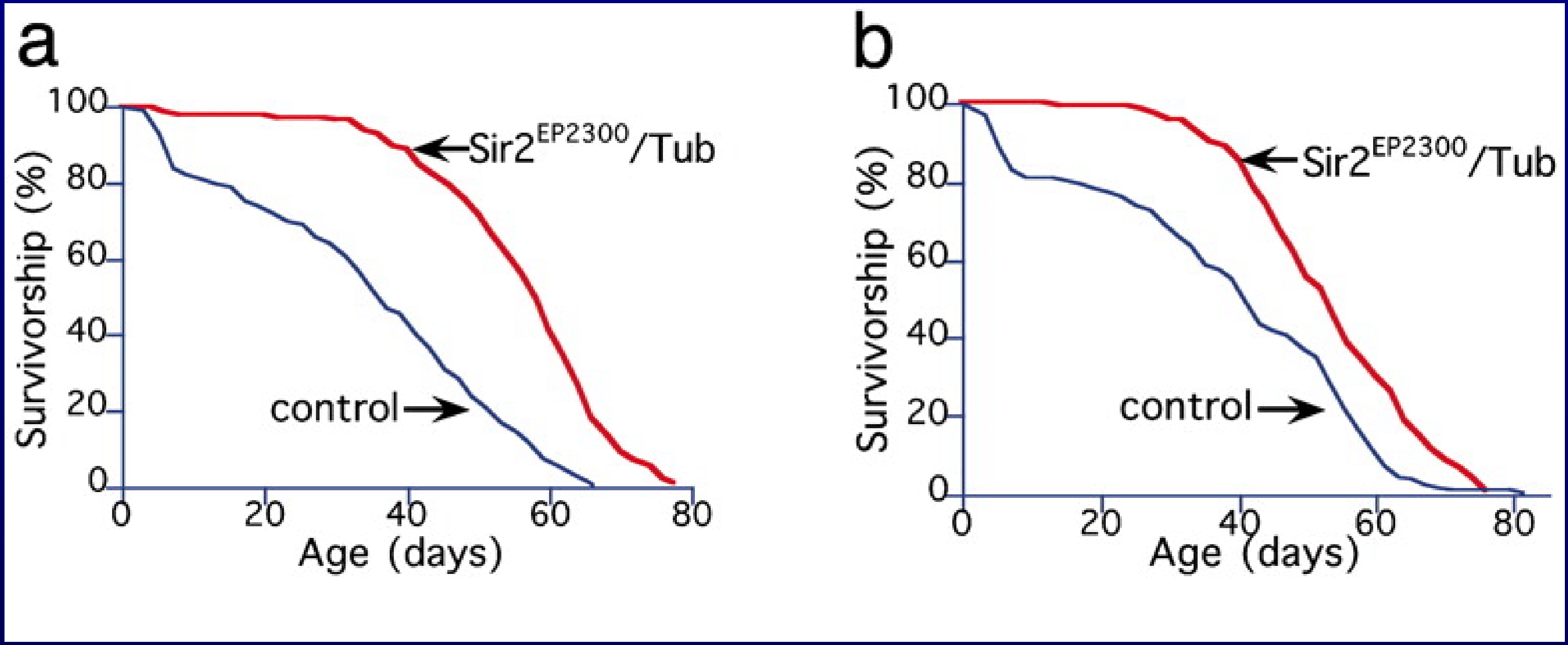
One Type of Histone Deacetylase, Sir2, Yields a Unique Product





Moazed D, Curr Opin Cell Biol. 13(2): 232, 2001

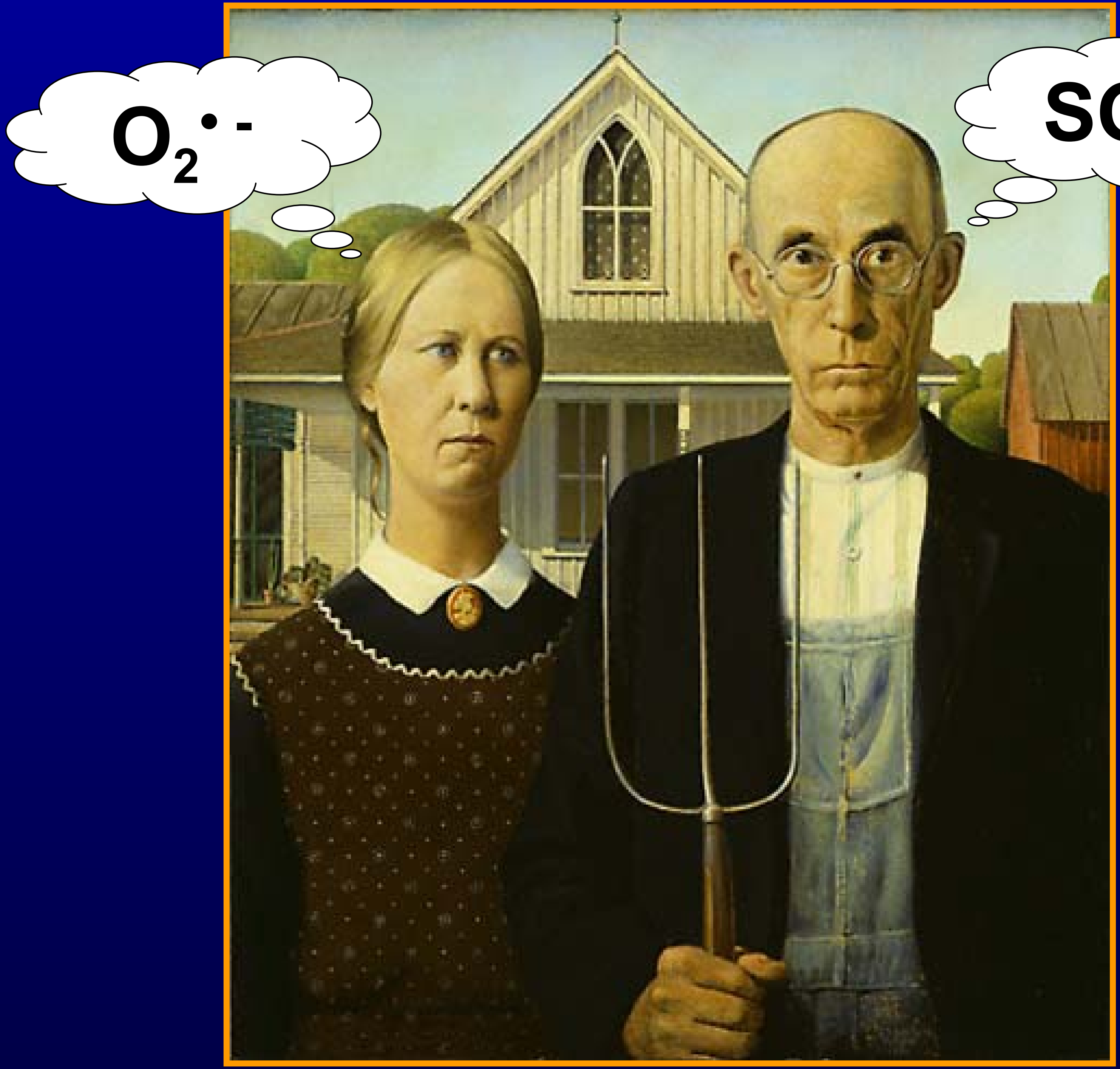
An Increase in Sir2 Extends Lifespan



Rogina B, Proc Natl Acad Sci: 101:15998, 2004

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!!!!



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Grant Wood

Free Radicals,
an
Iowa Tradition