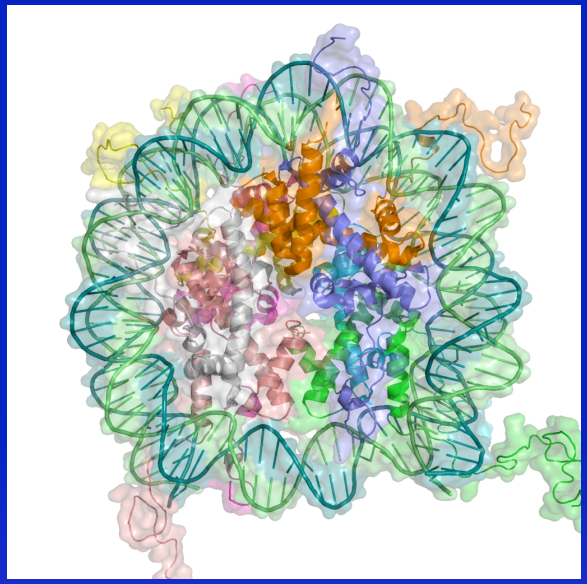
Regulation of Cell Identity by a Changing Epigenetic Landscape



Brian P. O'Connor
Center for Genes, Environment & Health
National Jewish Health

Epigenetic regulation of Cellular Identity

Epi- (*greek: "over, above")* genetic*:* heritable changes in gene expression via mechanisms that are separate from the DNA sequence and regulated by environmental signals.

<u>The processes that controls Chromatin architecture:</u>

DNA Methylation

- CpG islands: Repression of gene transcription
- Other areas: More Complicated

Histone Modifications

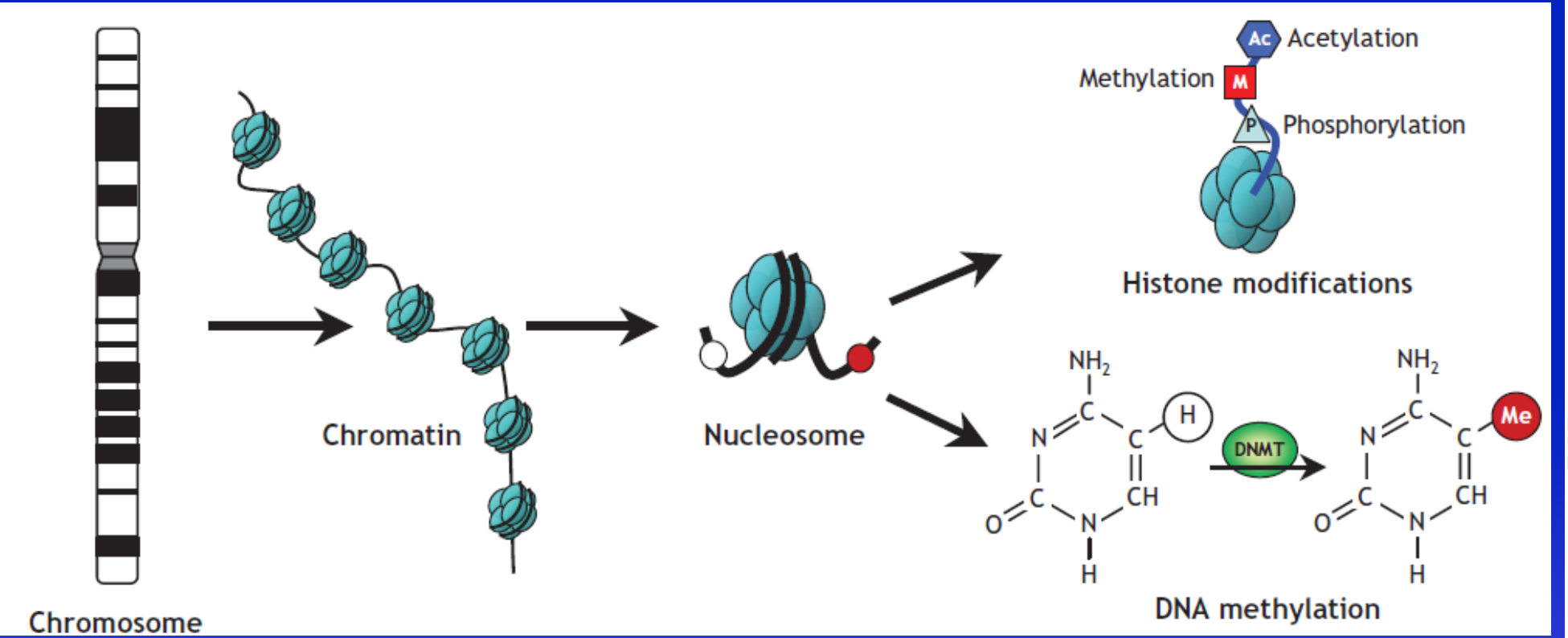
- Multiple types of modifications
- Multiple residues at the N-terminal tails
- Combinations of modifications either support or repress transcription

Epigenetic regulation of Cellular Identity

Epi- (*greek: "over, above")* genetic*:* heritable changes in gene expression via mechanisms that are separate from the DNA sequence and regulated by environmental signals.

	Environment	*Heritable*
Organism level:	Nutrition, Behavior, Pollution, Sun light, Toxins, Circadian rhythms, Viruses, Bacteria	Parent to child to grandchild?
Cellular-level:	The tissue microenvironment (Hypoxia)	Reproduction of epigenetic marks from parent cell to daughter cell during mitosis

Nucleosomes are the basic building block of Chromatin



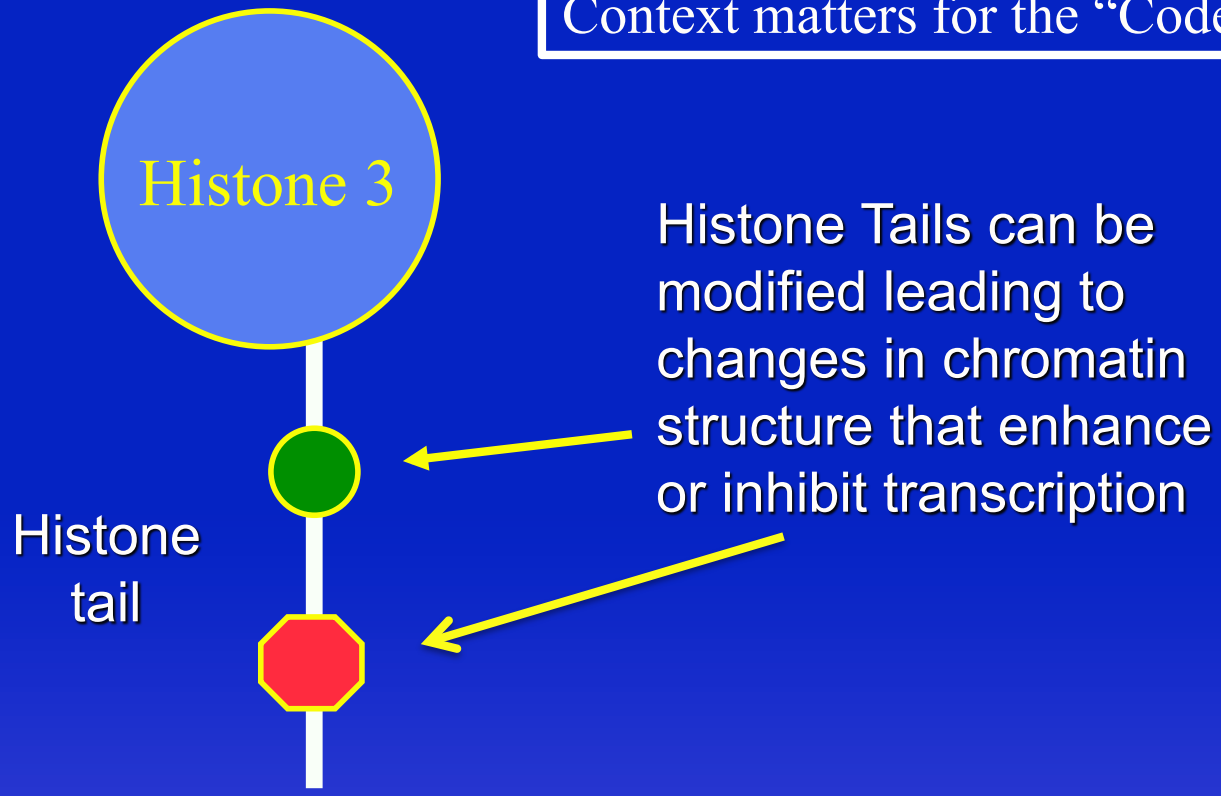
Rodenhiser& Mann CMAJ 2006 174(3):341

Post-translation Modifications to the Histone Tails

Luger, K. et al., Nature, Vol.389:251-260. 1997

Histone Posttranslational Modifications:
Defining the Epigenetic Code

Context matters for the "Code"



Histone Tails can be modified leading to changes in chromatin structure that enhance or inhibit transcription

Go

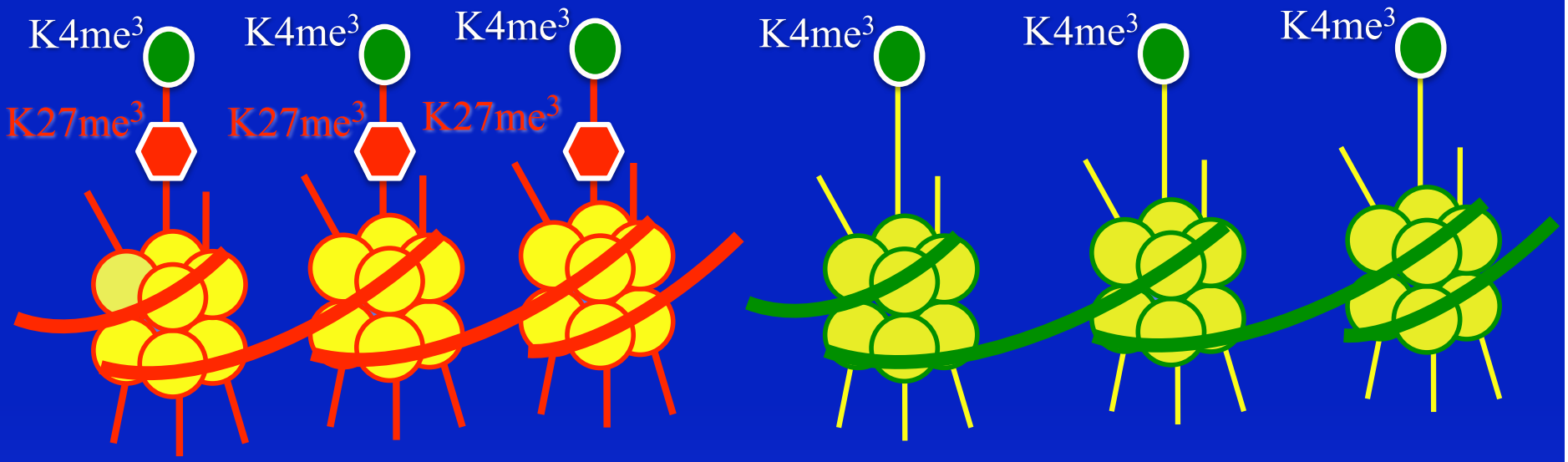
Acetylation

Methylation

Stop

Methylation

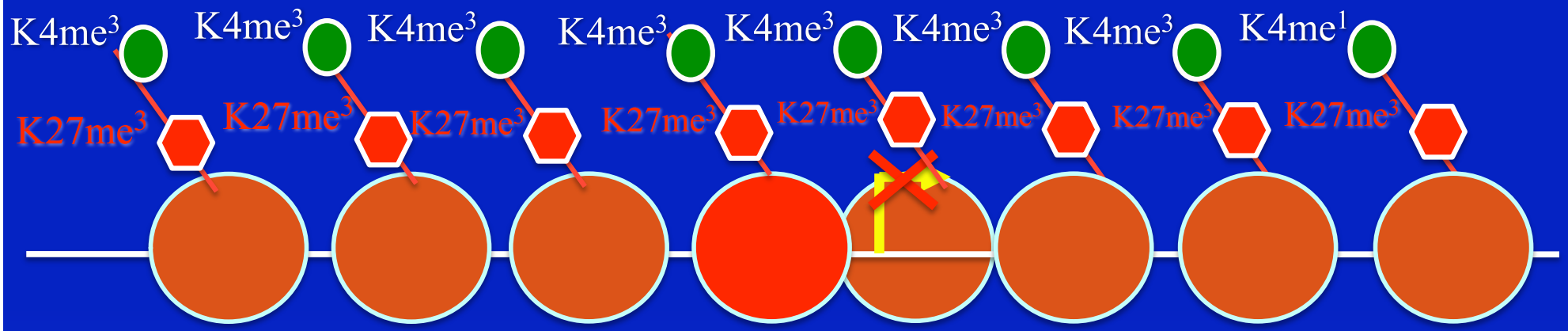
The Epigenetic Code:
Combinations of Histone Modifications regulate Transcription



Histone Mark:	Repressive Bivalent, Poised	Monovalent, Permissive, Active
Transcription:	Off or Low	On
Gene Identity:	Non-lineage specific gene Lineage specific Transcription Factors	Lineage specific gene; Enhancer; Regulatory regions

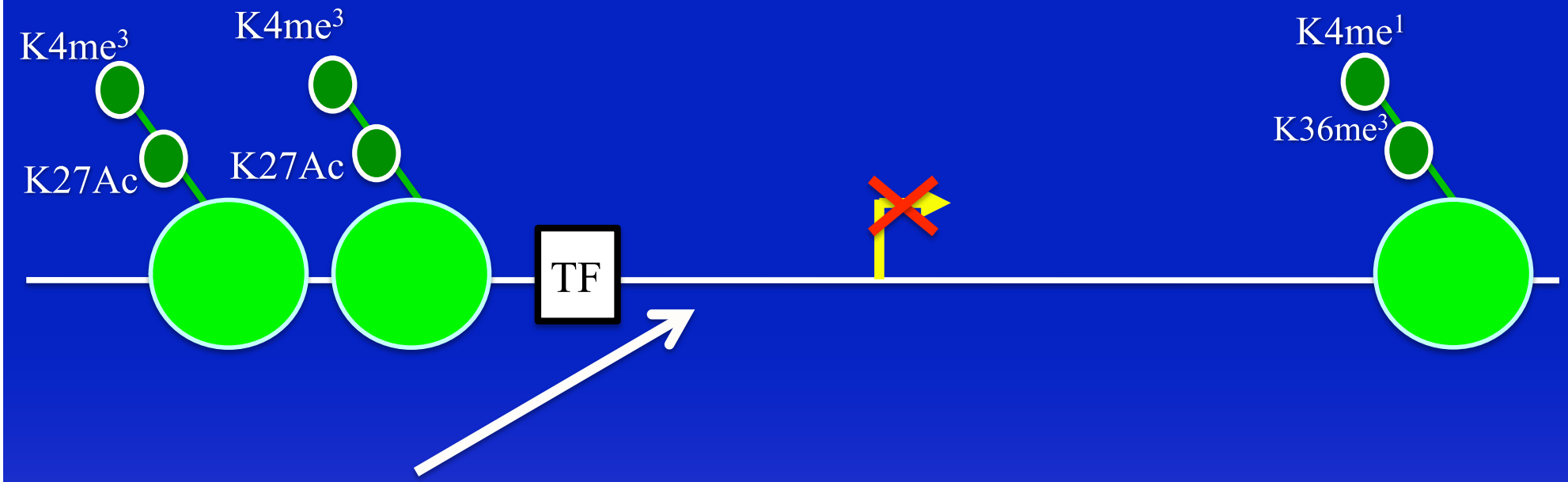
The Epigenetic Code regulates Chromatin Structure and Gene Transcription

Bivalent



The Epigenetic Code regulates Chromatin Structure and Gene Transcription

Open



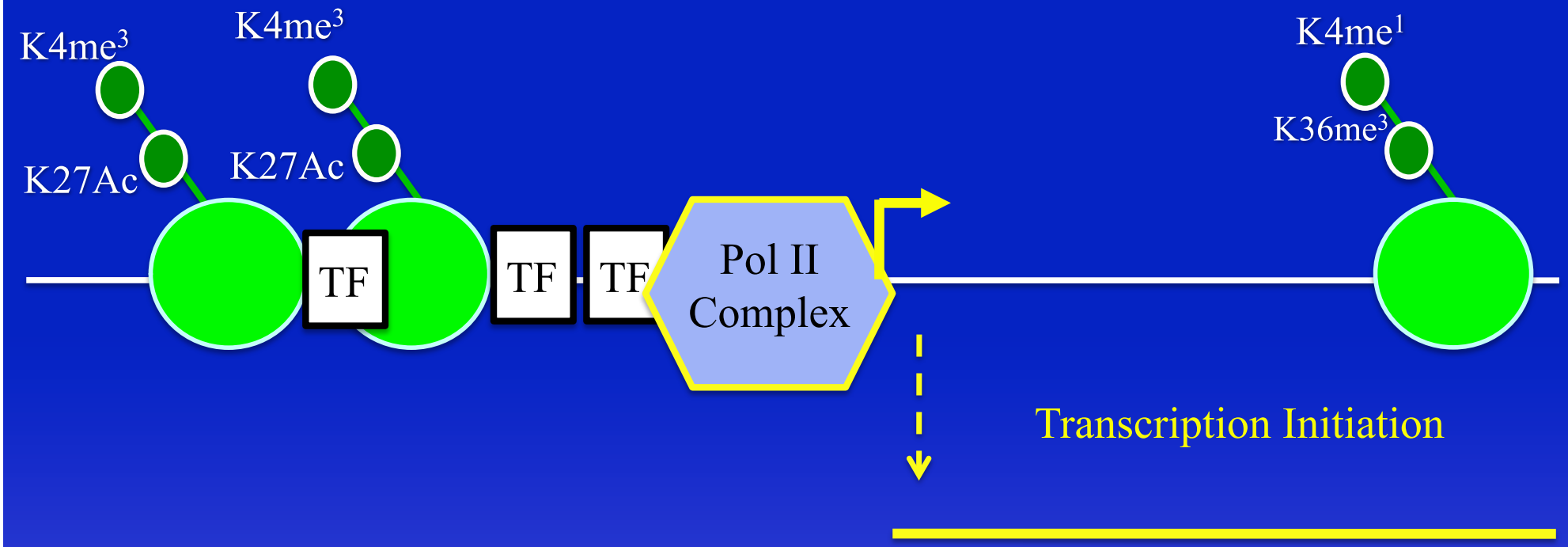
NFR: Nucleosome Free Region

TF

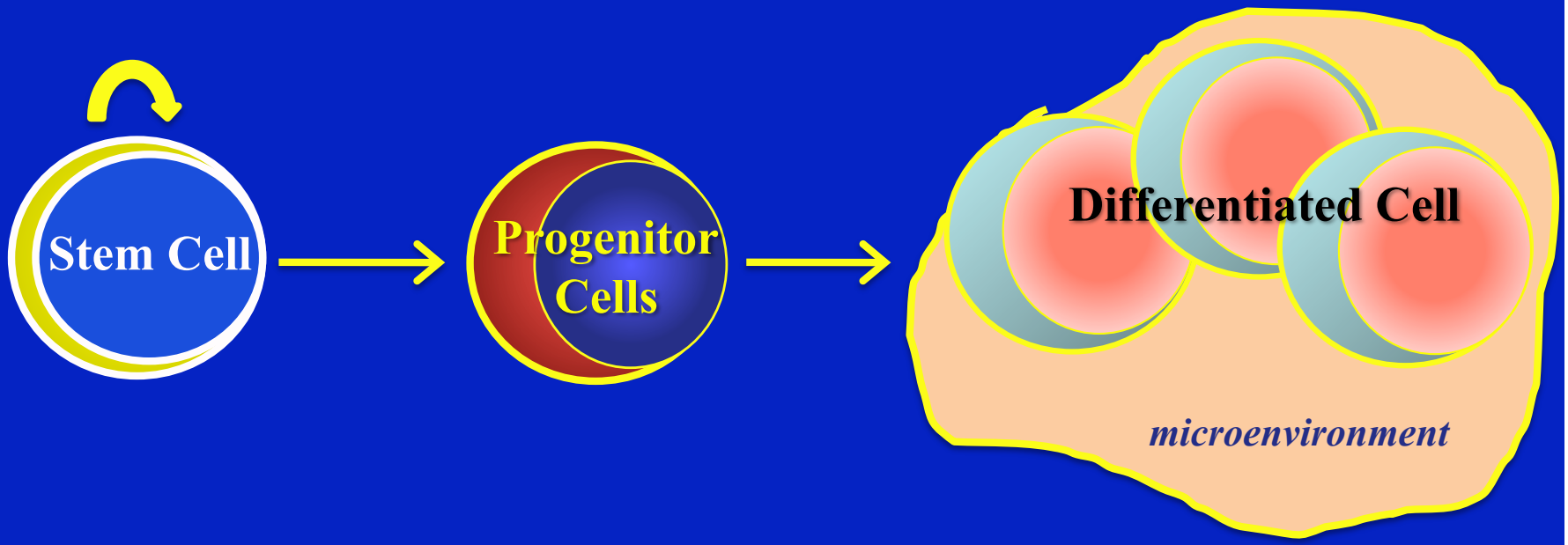
Pol II Complex

The Epigenetic Code regulates Chromatin Structure and Gene Transcription

Active



Epigenetic Regulation of Cell Identity



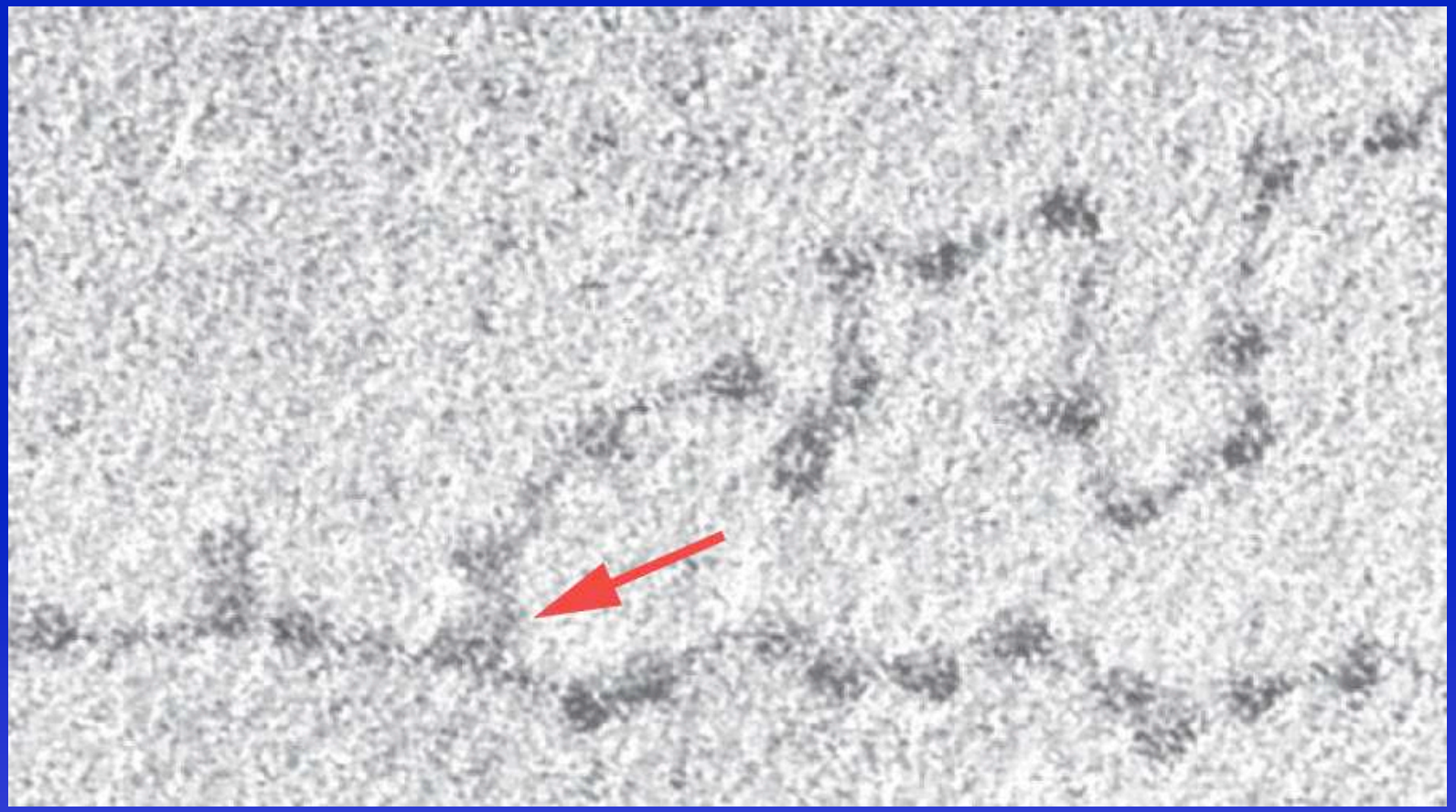
Bivalent Histone Signature

Monovalent Histone Signature

Malleable Identity

Fixed Identity

Nucleosome "replication" during S Phase: Inheritance of Histone Marks through Mitosis



X-ChIP: Chromatin ImmunoPrecipitation Assay

Assay to examine in vivo DNA-protein interaction dynamics:

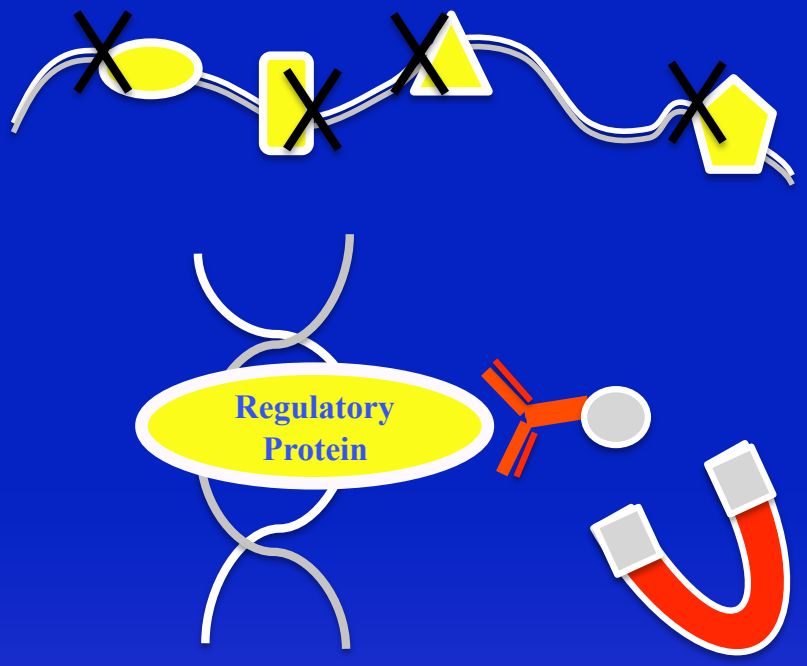
Step 1: Cross-link the DNA and protein

Step 2: Shear the DNA with Sonication

Step 3: Use Specific Antibody to target specific Protein-DNA domains: Regulatory proteins, Transcription Factors, Histone Modifications, etc.

Step 4: Use Magnetic Beads to isolate Ab-Protein-DNA fragments

Step 5: Elute, Reverse the Cross-links and purify the DNA, which is ready for analysis



Analyzing ChIP isolated DNA

ChIP-qPCR

ABI 7900: qPCR analysis

Targeted analysis of
specific genomic loci

ChIP-seq

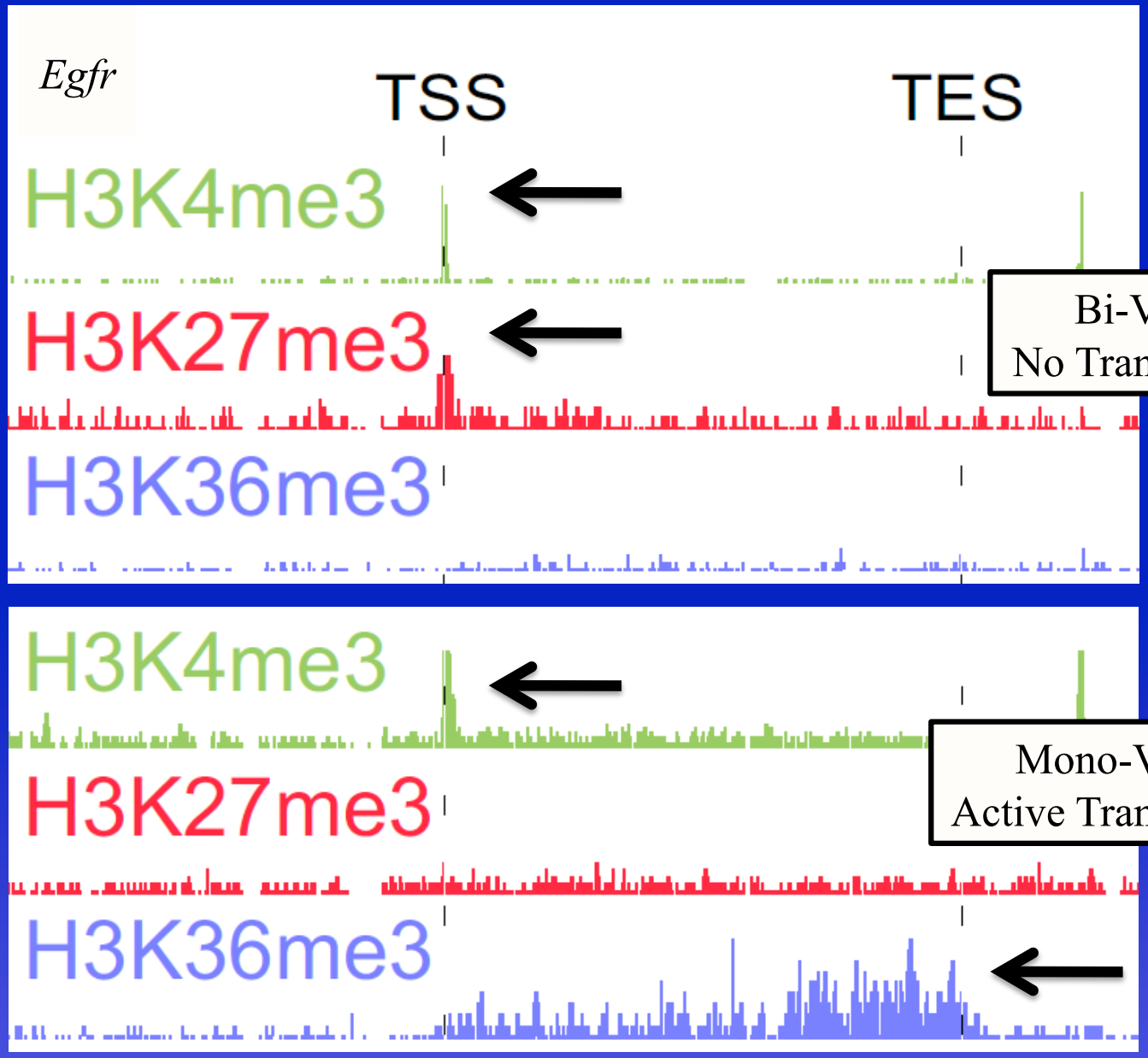
ABI Solid4: Next-Gen Sequencing

Genome-Wide analysis

ChIP-Seq analysis: Histones regulate cell differentiation

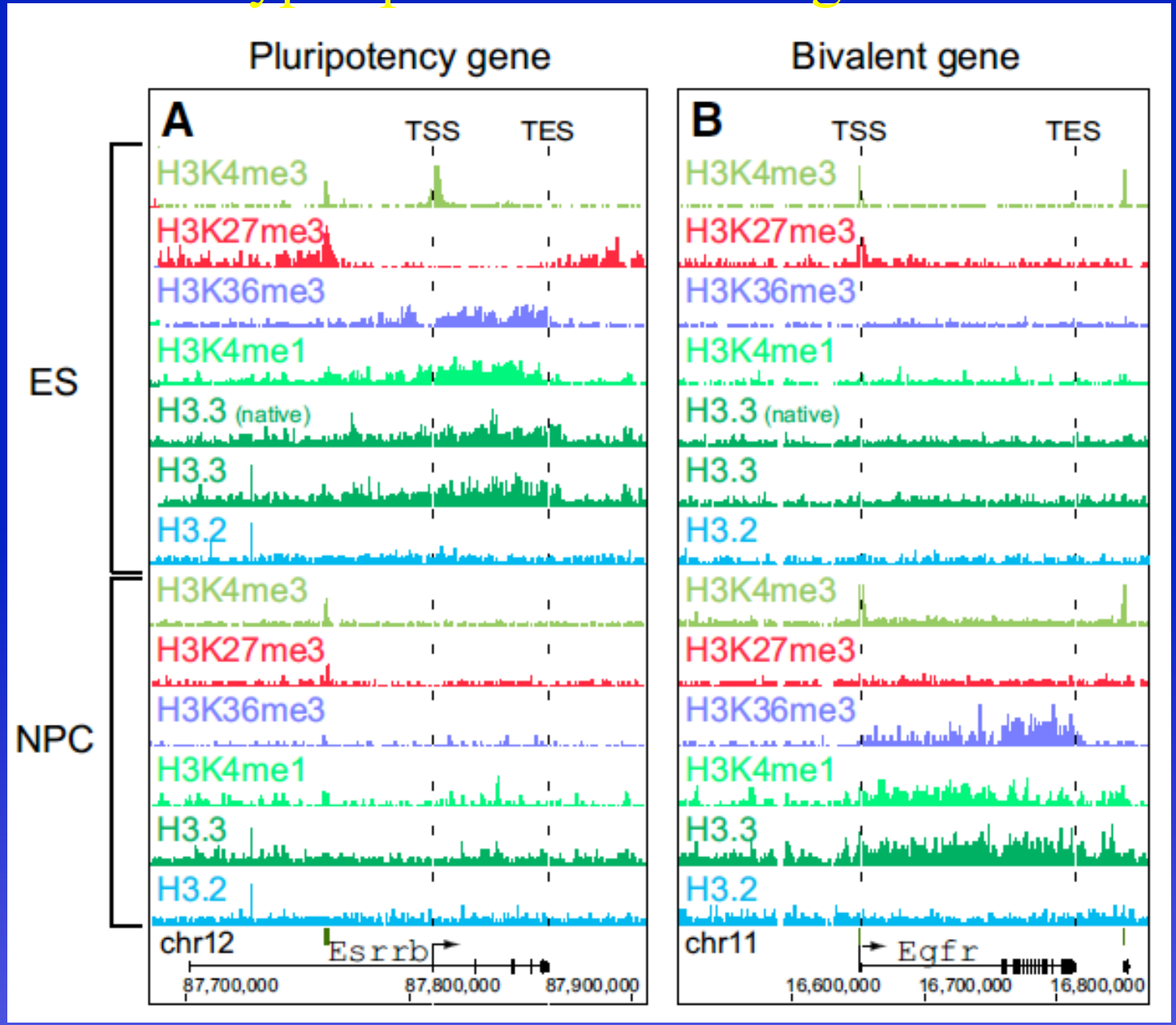
Stem Cell:
Non-Expressed
Gene

Neural Progenitor:
Expressed
Gene



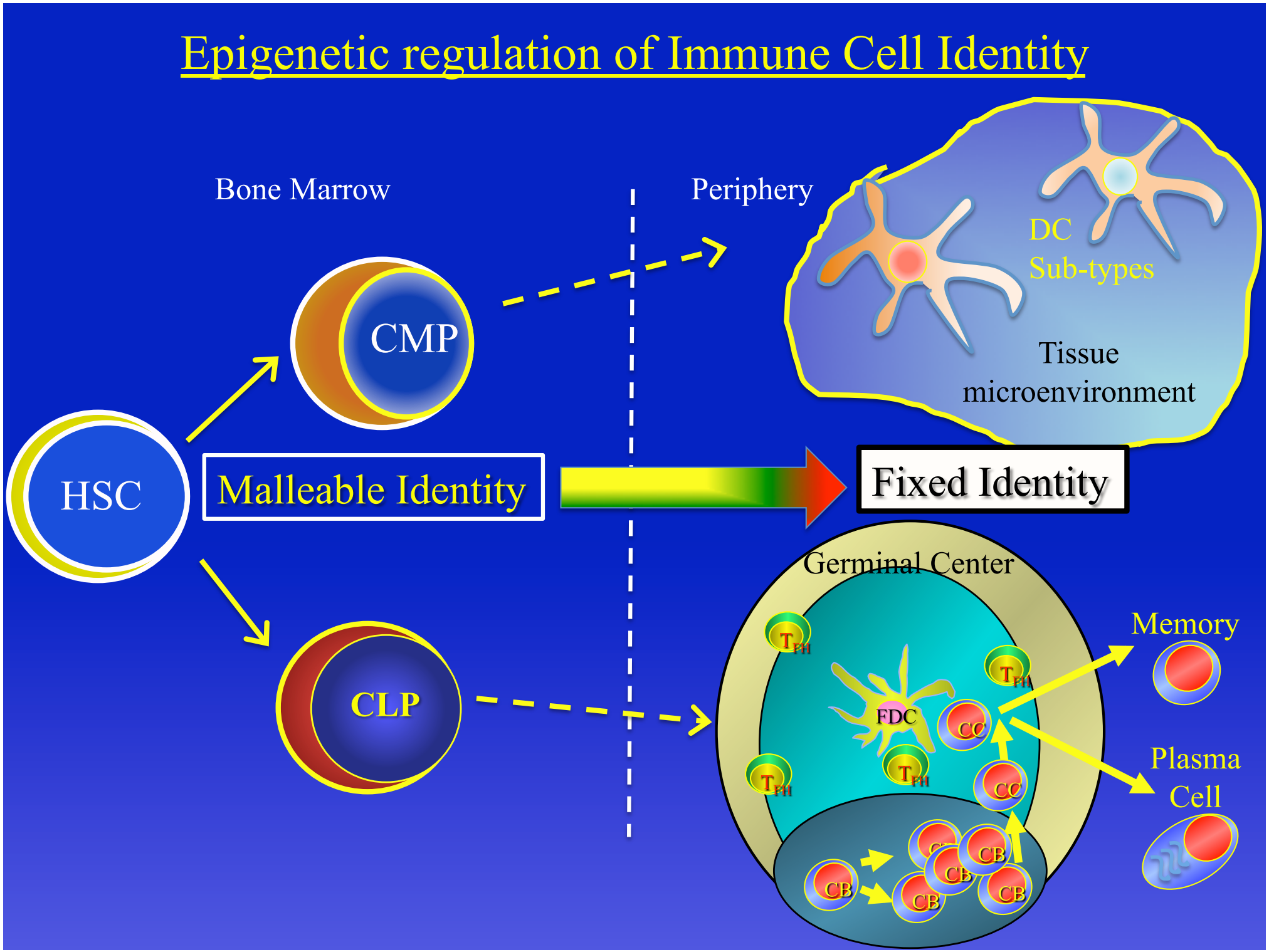
Goldberg et al., Cell, 140: 678-691. 2010

Mapping the Epigenetic Landscape: Cell-type specific histone signatures

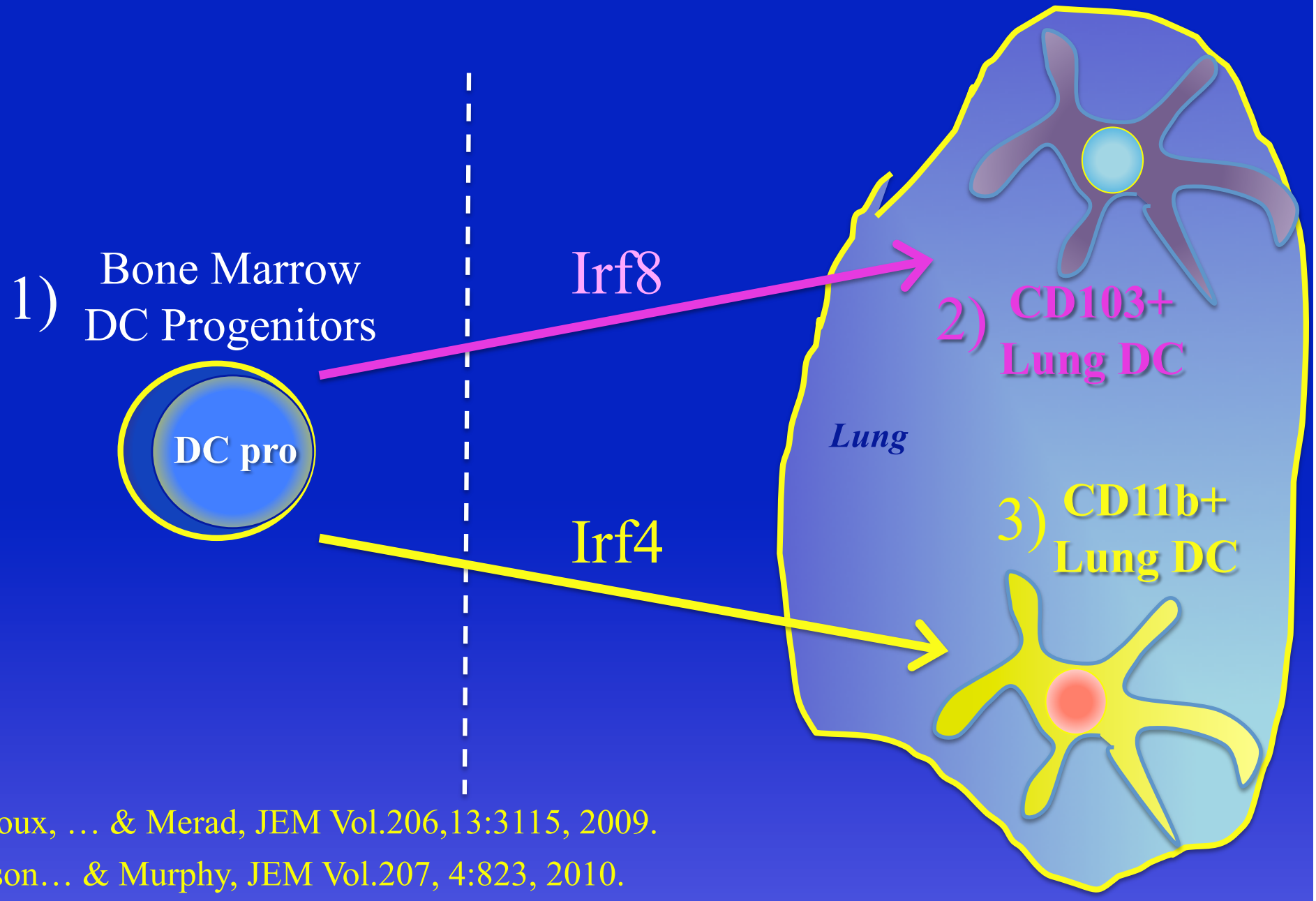


Goldberg et al., Cell, 140: 678-691. 2010

Epigenetic regulation of Immune Cell Identity



Epigenetic Regulation of Dendritic Cell Development

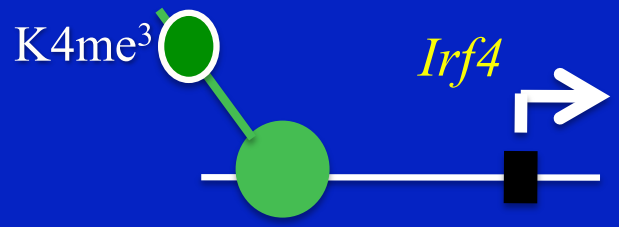


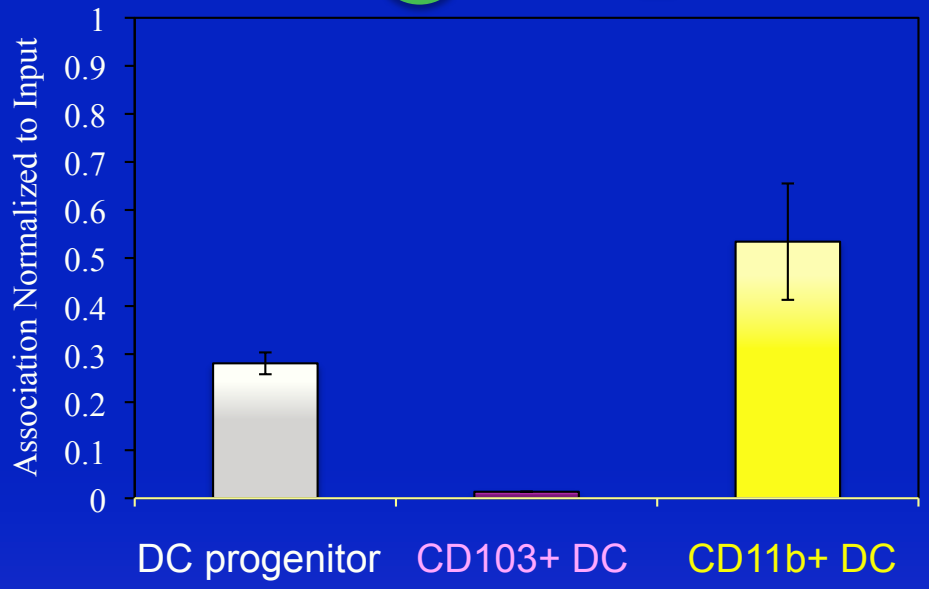
Ginhoux, … & Merad, JEM Vol.206,13:3115, 2009.

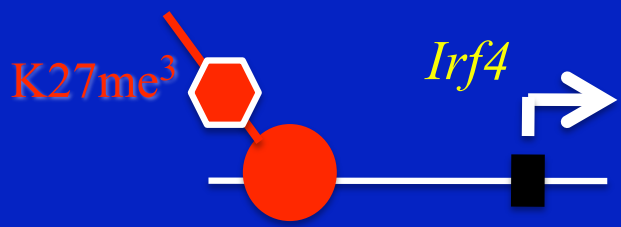
Edelson… & Murphy, JEM Vol.207, 4:823, 2010.

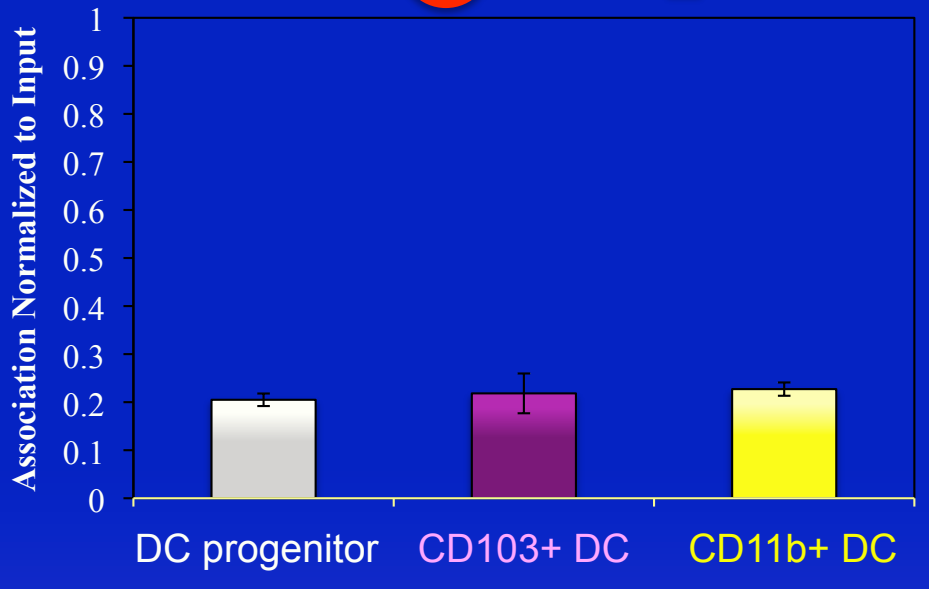
Randolph; Rudensky; Nussenzweig: Science, Vol324, 17April2009.

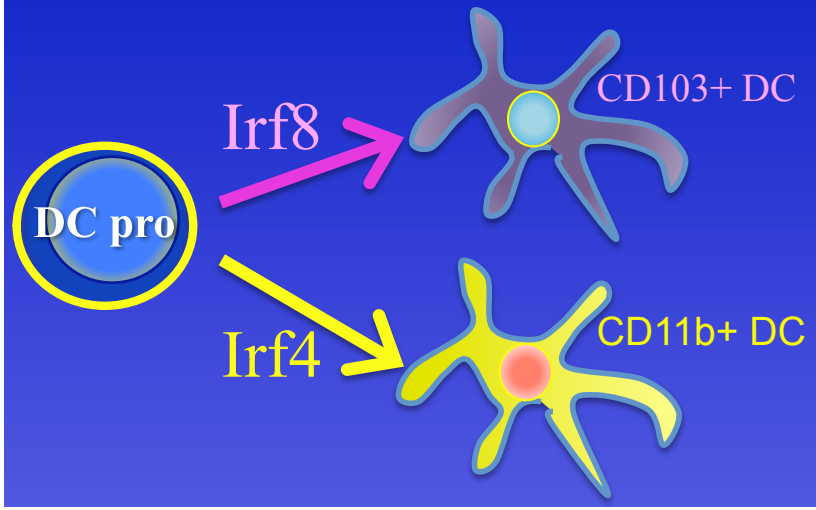
ChIP analysis of Irf4 Core Promoter Region









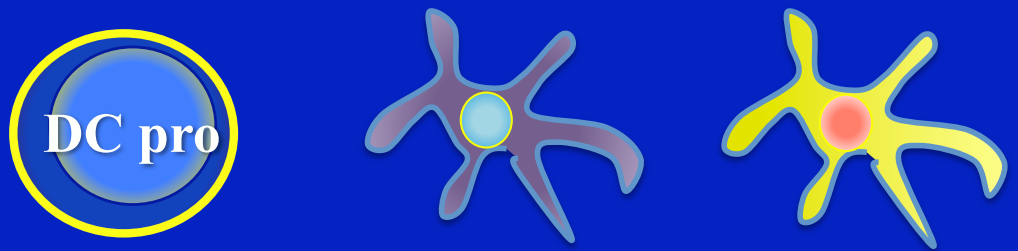


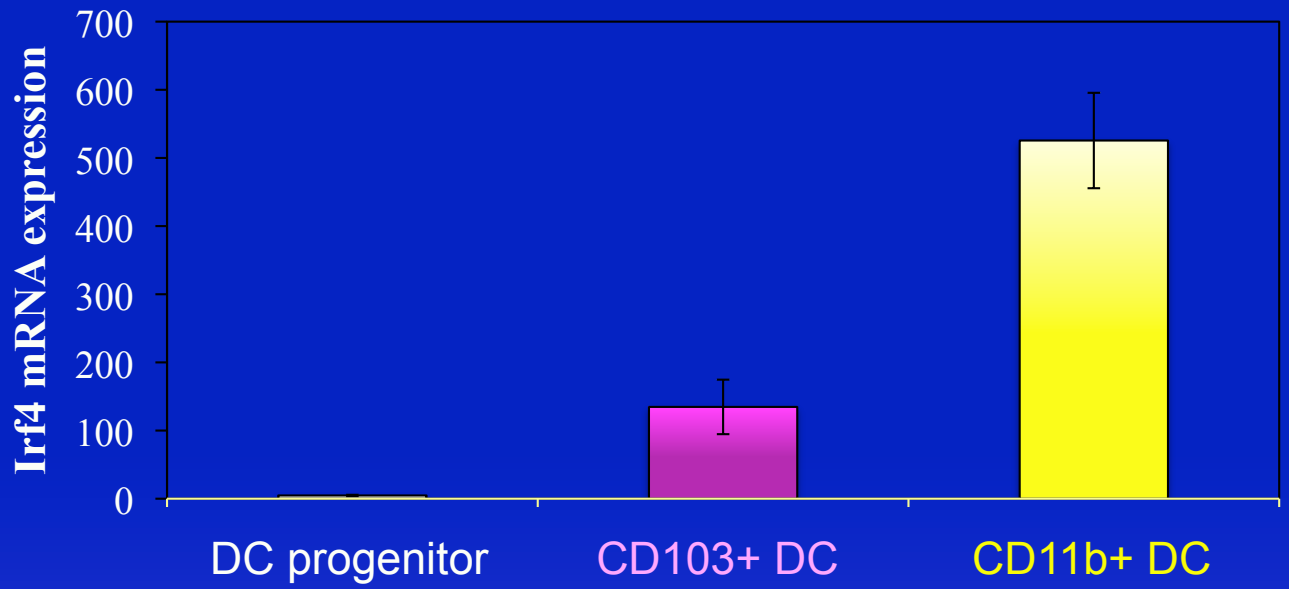
BM DC progenitor Irf4 promoter is bi-valent/ poised

CD103+ DC Irf4 promoter is repressive

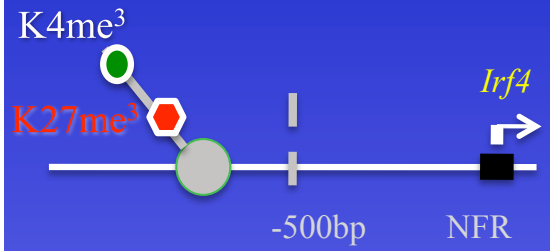
CD11b+ DC Irf4 promoter is active

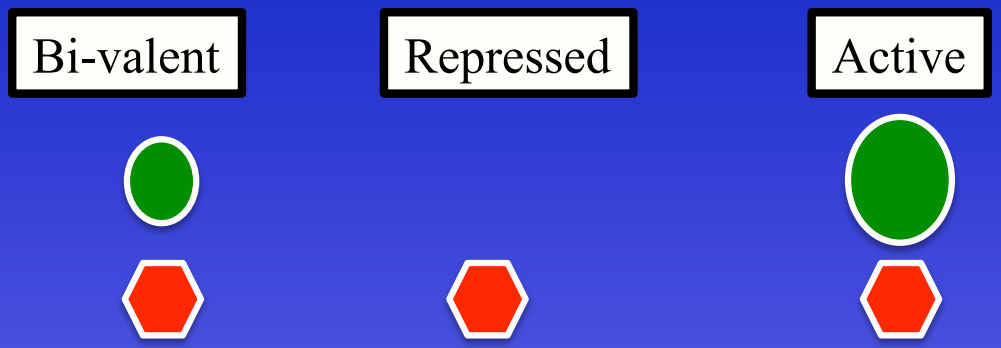
Irf4 Gene Expression Changes during DC development



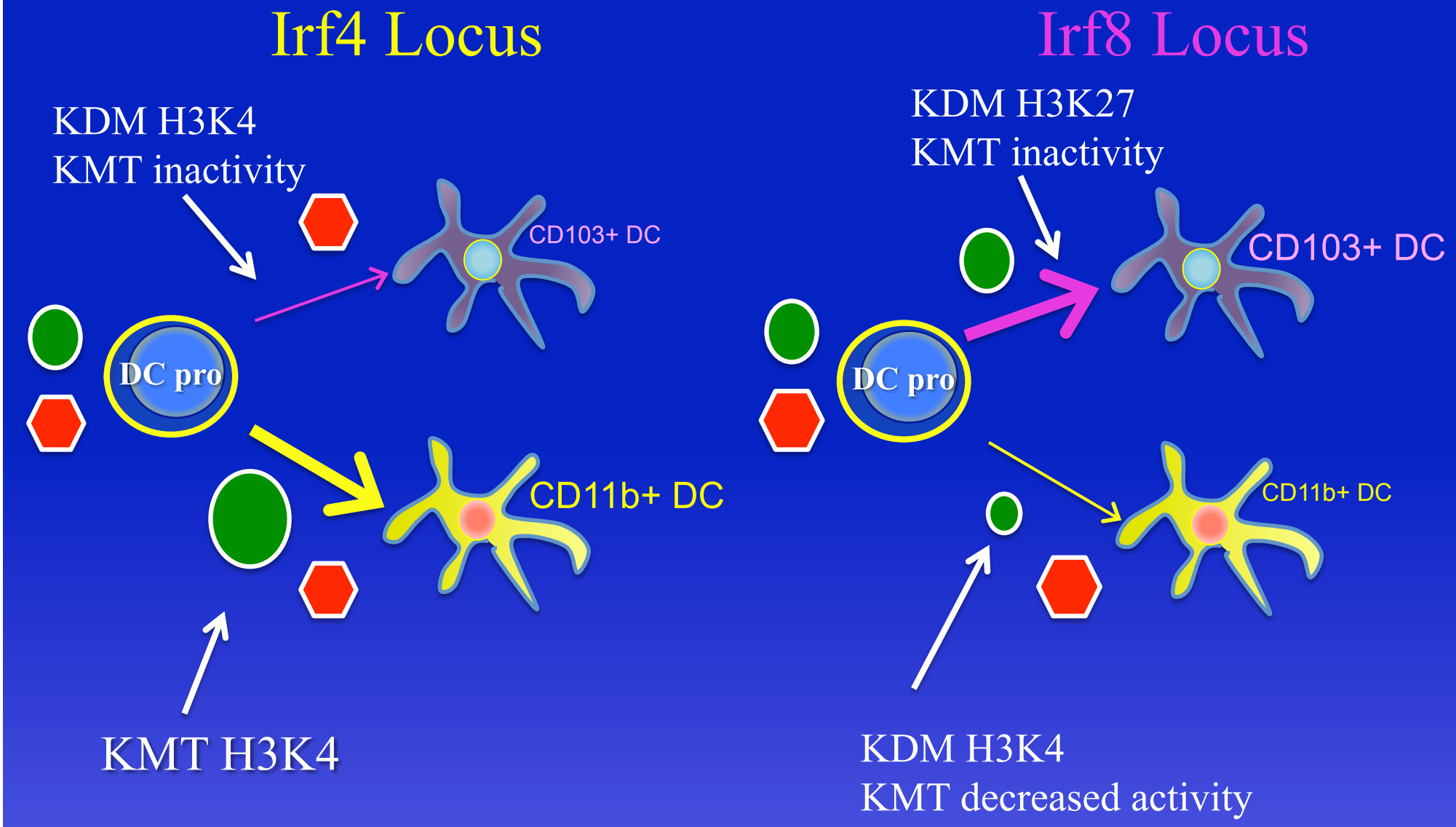


ChIP Analysis of Irf4 Promoter:



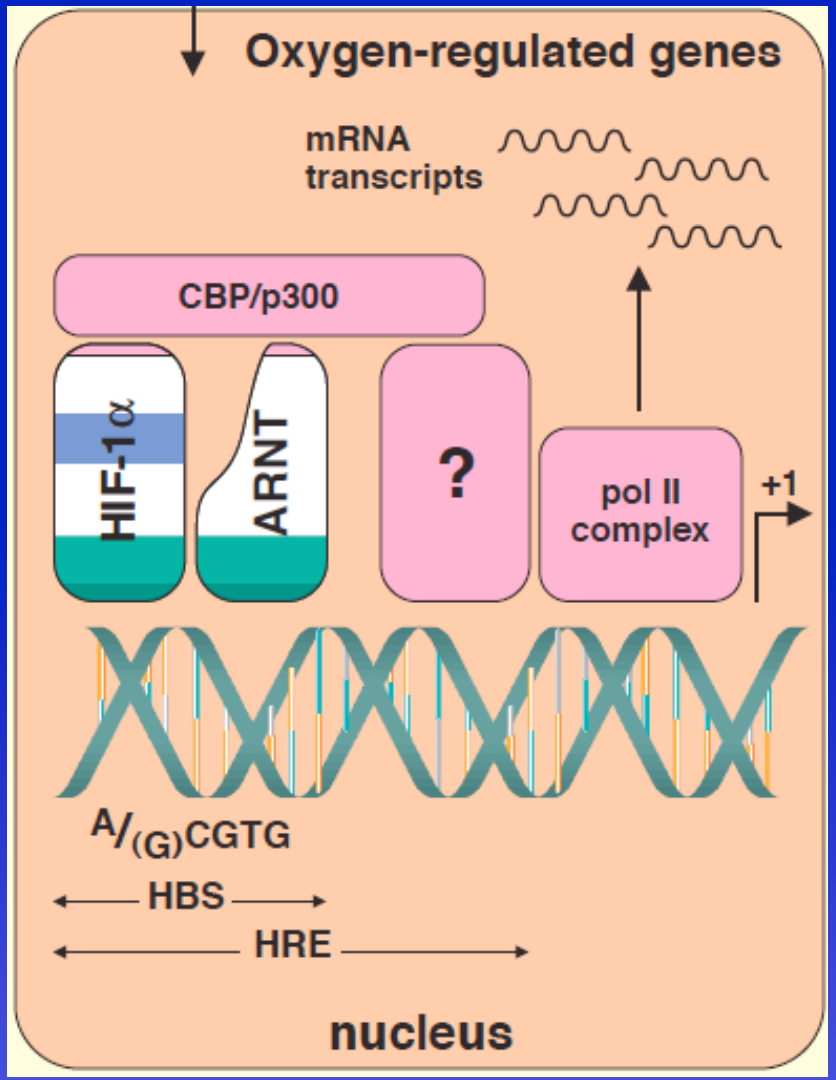


Summary: Unique Histone Signatures Characterize Specific Cell Lineages



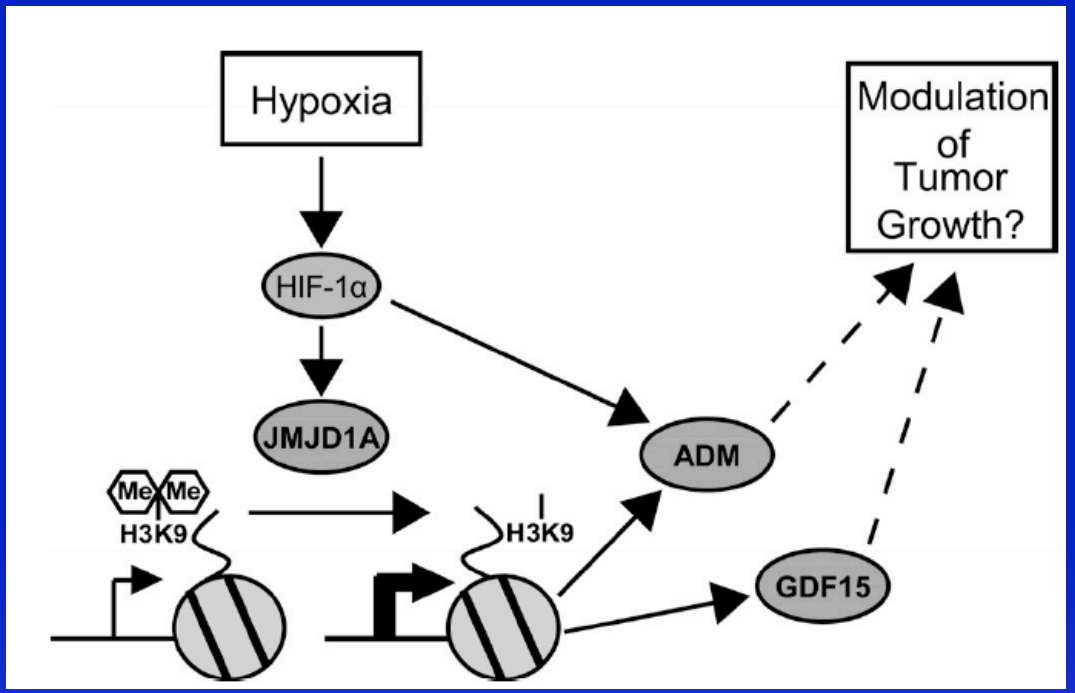
Hypoxia controls Histone Modifications at Oxygen-Regulated Genes

Direct Model



Wenger RH, Journal of Exp Bio; 203:1253-1263, 2000.

In-Direct Model



Krieg et al., Mol and Cell Bio; 30,1:344-353, 2010.

What have we learned ?

- Post-translational histone modifications alter chromatin structure and create “landing pads” for Chromatin binding proteins/DNA-binding proteins/transcription factor binding at specific loci throughout the genome. Coding & Non-Coding.

- The local chromatin structure of genes are unique to each cell lineage.

- The cell differentiation process can utilize opposing types of histone modifying enzymes.

- The epigenetic regulation of differentiation is affected by local microenvironments.

- The tissue microenvironment (Inflammation, Hypoxia) can affect histone modifying enzyme activity, and thus regulates the malleable process of progenitor cell differentiation to terminally differentiated cells.