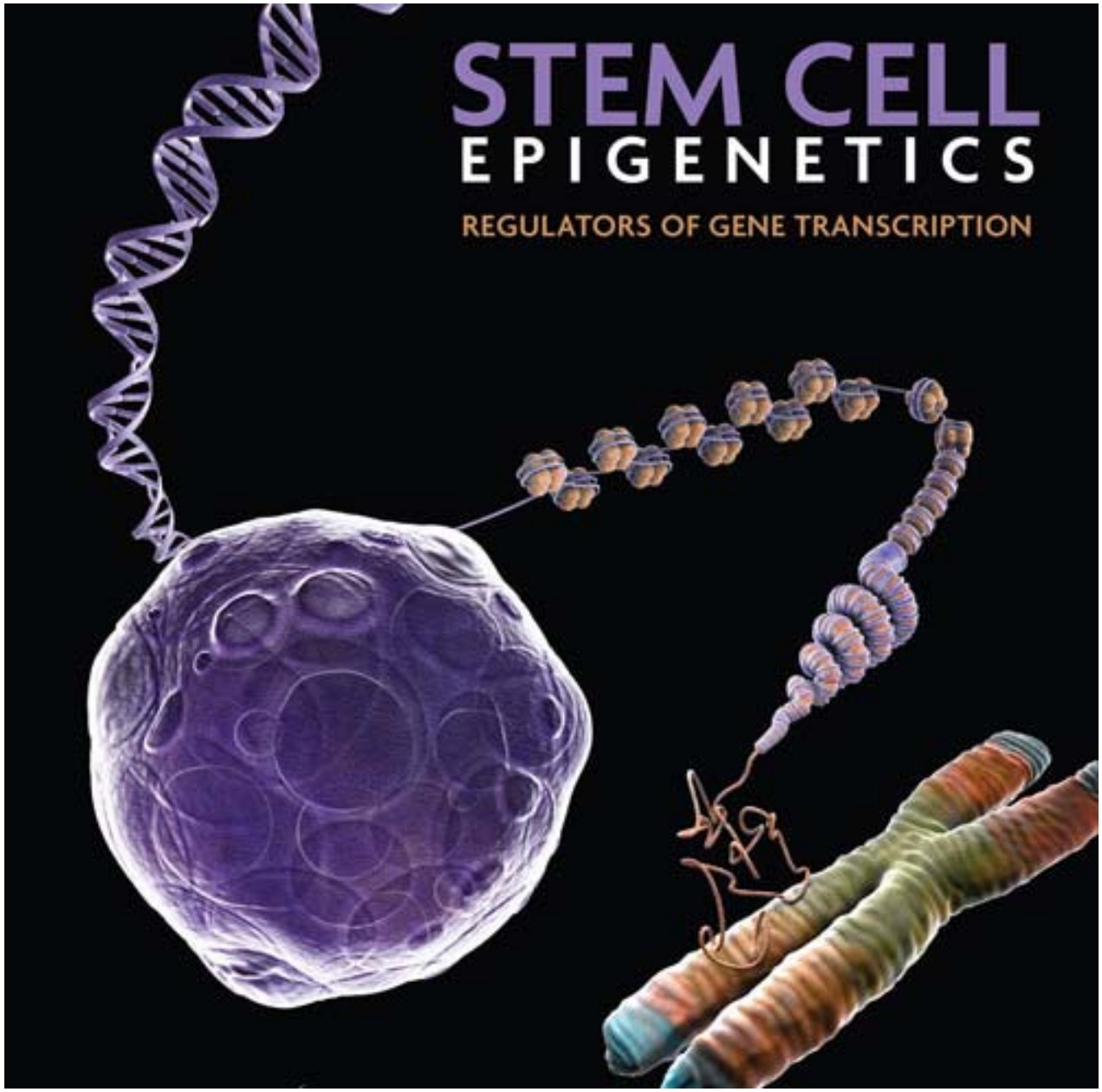


Stem cell epigenetics

Assistant Professor

Ahmed Al-Shammari, PhD

ICCMGR

Experimental Therapy Department

Stem Cells Class

For

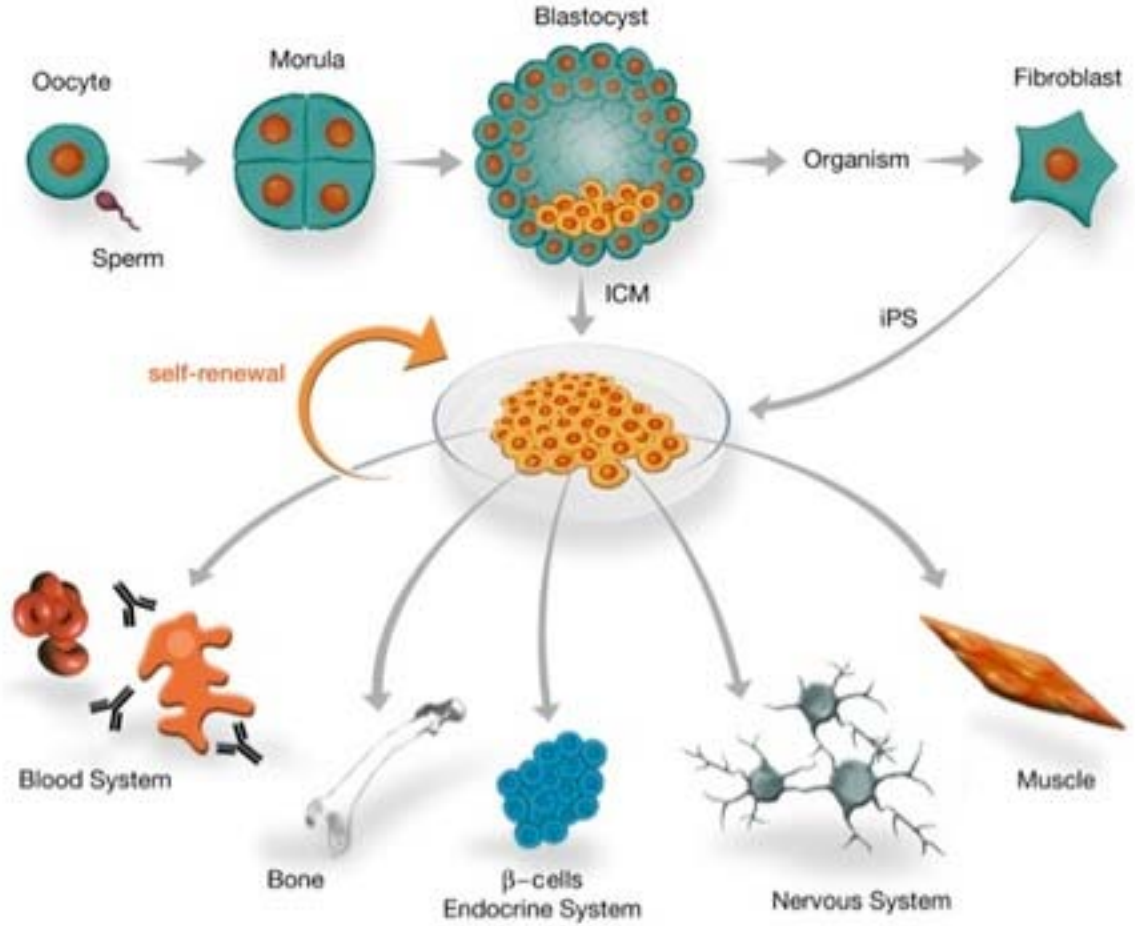
Postgraduate Students

What is Epigenetics?

- **epigenetics** is the study of biological mechanisms that will switch genes on and off.
- **Epigenetics Controls Genes**. Certain circumstances in life can cause genes to be silenced or expressed over time. In other words, they can be turned off (becoming dormant) or turned on (becoming active).
- Epigenetics Is Everywhere. What you eat, where you live, who you interact with, when you sleep, how you exercise, even aging – all of these can eventually cause chemical modifications around the genes that will turn those genes on or off over time. Additionally, in certain diseases such as cancer or Alzheimer's, various genes will be switched into the opposite state, away from the normal/healthy state.
- Epigenetics Makes Us Unique. Even though we are all human, why do some of us have blonde hair or darker skin? Why do some of us hate the taste of mushrooms or eggplants? Why are some of us more sociable than others? The different combinations of genes that are turned on or off are what makes each one of us unique. Furthermore, these epigenetic changes can be inherited.
- Epigenetics Is Reversible. With 20,000+ genes, what will be the result of the different combinations of genes being turned on or off? The possible permutations are enormous! But if we could map every single cause and effect of the different combinations, and if we could reverse the gene's state to keep the good while eliminating the bad… then we could theoretically cure cancer, slow aging, stop obesity, and so much more.

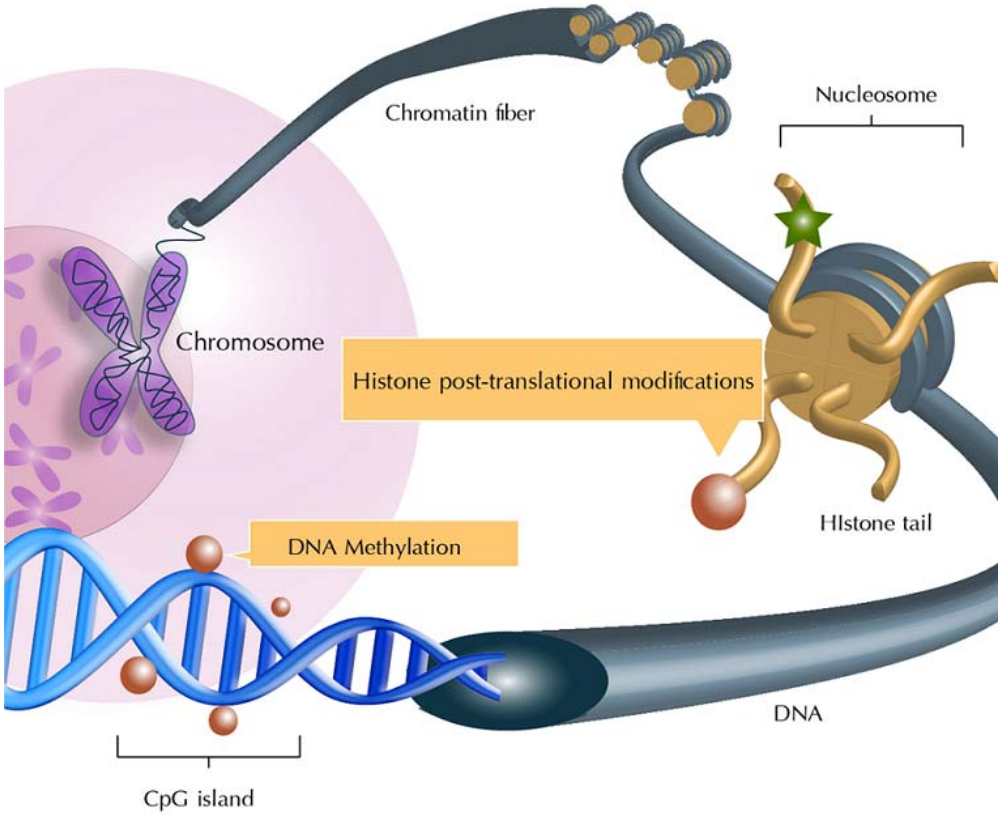
What is Epigenetics?

- epigenetics refers to heritable changes in gene expression (active versus inactive genes) that does not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. Epigenetic modifications can manifest as commonly as the manner in which cells terminally differentiate to end up as skin cells, liver cells, brain cells, etc. Or, epigenetic change can have more damaging effects that can result in diseases like cancer. At least three systems including DNA methylation, histone modification and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change

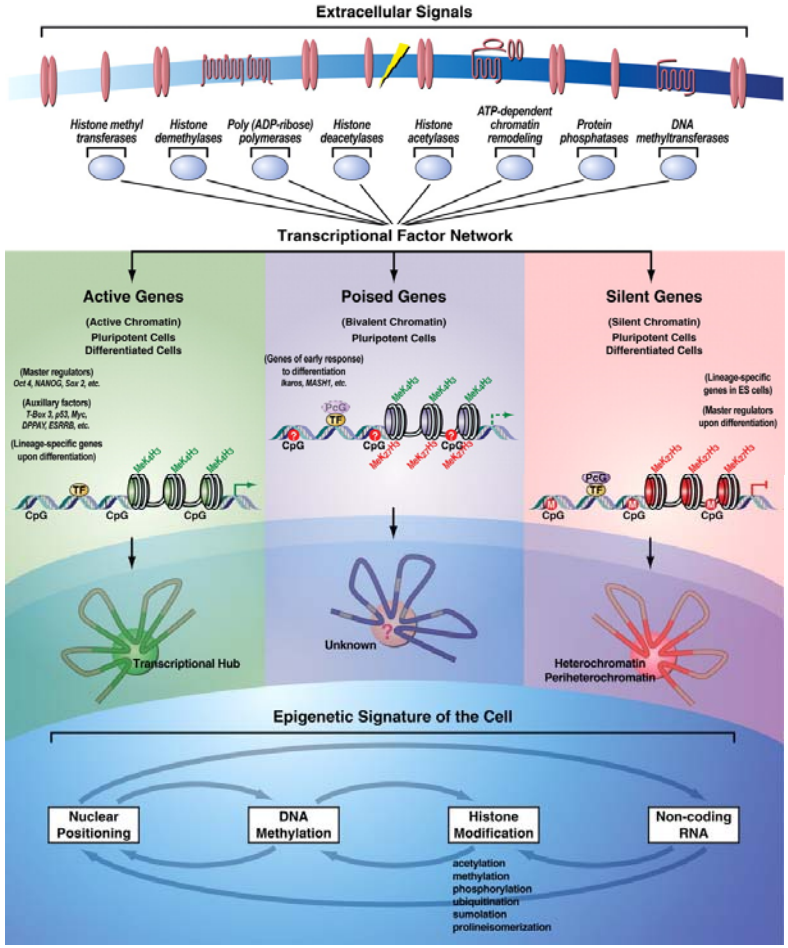

Blastocyst
Morula
Oocyte
Fibroblast
Organism
Sperm
ICM
iPS
self-renewal
Blood System
Bone
β-cells
Endocrine System
Nervous System
Muscle

Modern directions in the field of epigenetic research aimed to decipher the epigenetic signals that give stem cells their unique ability to self-renew and differentiate into different cell types

- Epigenetic mechanisms include: modifications of the histones and incorporation of histone variants; changes in DNA methylation and Adenosine-5′-triphosphate-dependent chromatin remodeling; implementation of RNAi pathways and non-protein-coding RNAs (ncRNA). These mechanisms represent the final outcome in the transcriptional hierarchy mediated by transcriptional factors and are designated to alter the gene function without changes to the DNA sequence.


Chromatin fiber
Nucleosome
Chromosome
Histone post-translational modifications
HIstone tail
DNA Methylation
DNA
CpG island

Epigenetic response to extrinsic signals occurs through the transcriptional factors network.



Victoria V. Lunyak, and Michael G. Rosenfeld Hum. Mol. Genet. 2008;17:R28-R36

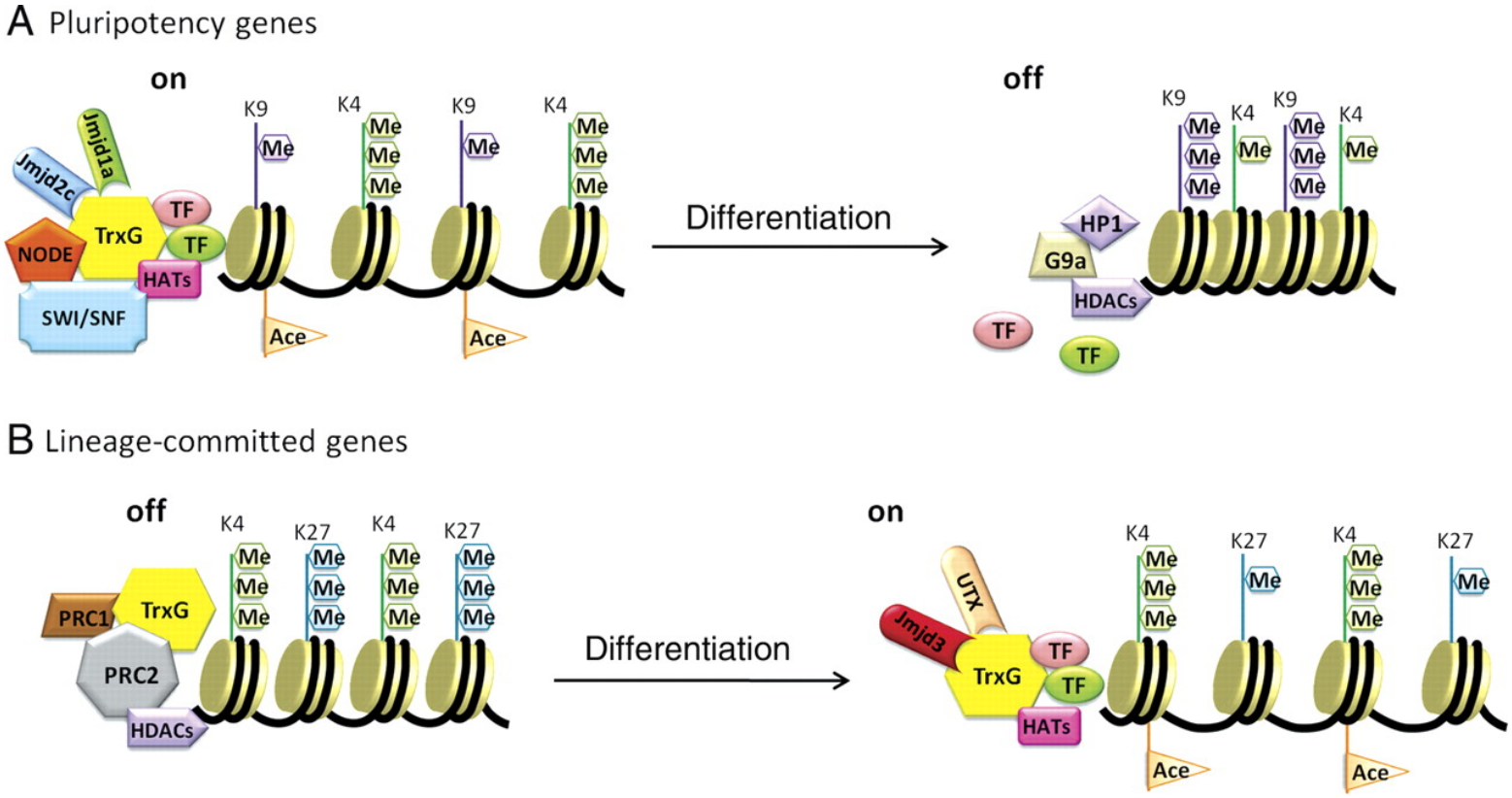
Human Molecular Genetics

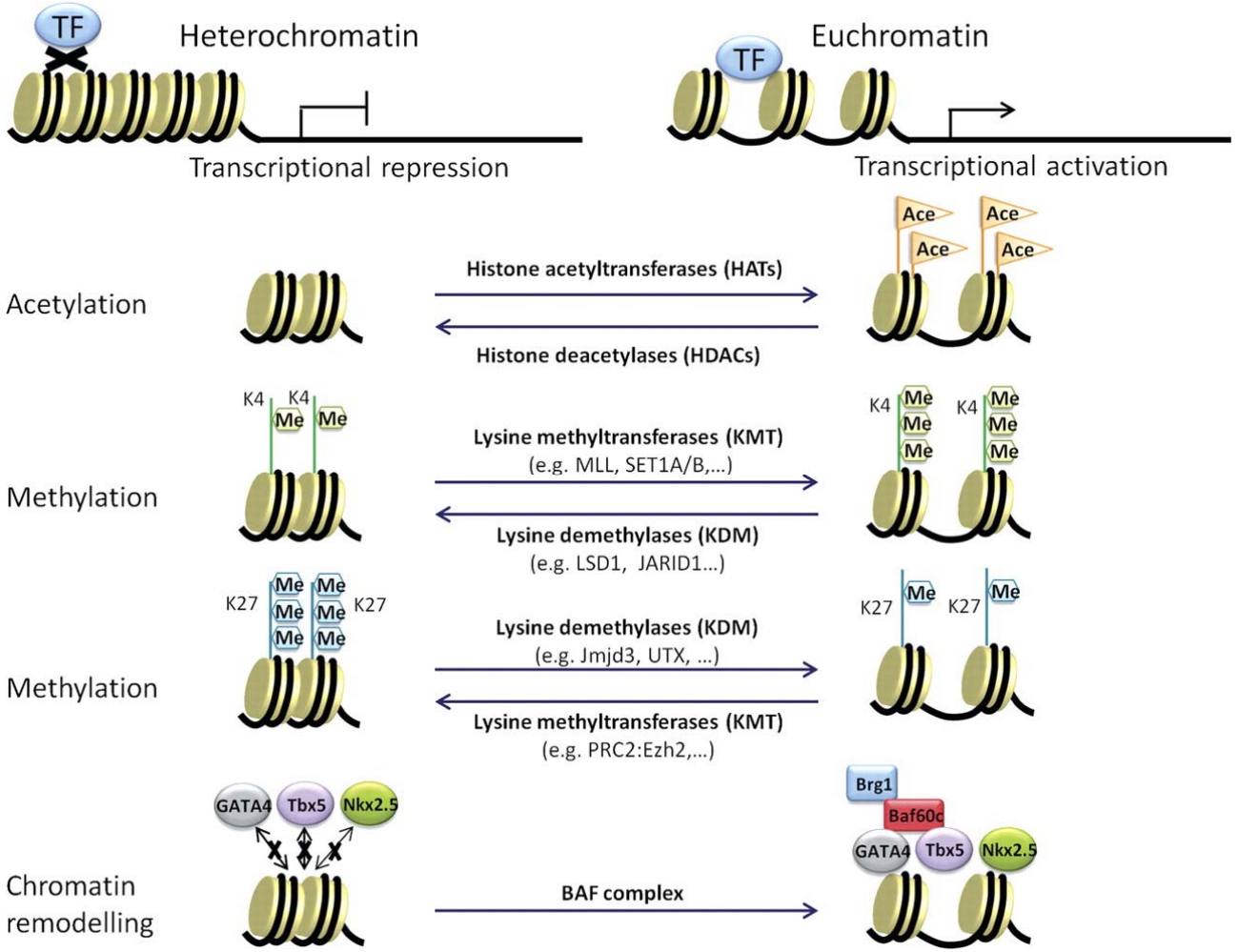
STEM CELLS AND EPIGENETICS

- The multipotency of stem cells is reduced over time due to progressive gene silencing.
- Genes active in earlier progenitors are gradually silenced at developmentally later stages, and subsets of cell type-specific genes are turned on.
- This progression is the result of selective expression of transcription factors (TFs) in concert with classis ‘corner stones’ of epigenetics – chromatin remodeling and chromatin modifications, DNA methylation.
- As a result of these events compactization of the chromatin, its accessibility and positioning within specialized nuclear domains undergo dynamic changes.
- For example, it has been shown that heterochromatic markers, such as HP1 proteins, as well as heterochromatic histone modifications change their localization from dispersed and very dynamic in ESC to more concentrated distinct loci during cellular differentiation (6,7).
- This suggests that differentiation leads to the restructuring of the chromatin accompanied by the change in the global nuclear architecture, thus allowing the pluripotent nature of ESC genome to become more condensed, and, therefore, more transcriptionally restrained with maturation of the heterochromatin.

TRANSCRIPTIONAL FACTORS NETWORKS AND EPIGENETICS

- Batteries of TFs have been proposed to control stem cell self-renewal and lineage progression and are also a powerful mechanism for generating cell diversity (10). Progression along the lineage from stem cell to differentiated cell is characterized by striking morphological and functional changes at each stage of the lineage commitment. The sequential expression of TFs and other signaling molecules, which elicit cascades of gene expression, regulate each other and interact with epigenetic control factors to form large gene regulatory networks. The identification of the common target sites by chromatin immuno-precipitation (ChIP) assay together with genome-wide location analysis has suggested that OCT3/4, SOX2 and NANOG might form a regulatory network that maintains pluripotency and self-renewal in mouse and human ESC


A Pluripotency genes
on
Jmjd1a
Jmjd2c
TrxG
TF
TF
NODE
HATs
SWI/SNF
K9
K4
K9
K4
Me
Ace
Differentiation
off
HP1
G9a
HDACs
TF
TF
B Lineage-committed genes
off
PRC1
TrxG
PRC2
HDACs
K4
K27
K4
K27
Differentiation
on
UTX
Jmjd3
TF
TrxG
TF
HATs
Ace


TF
Heterochromatin
Transcriptional repression
TF
Euchromatin
Transcriptional activation
Acetylation
Histone acetyltransferases (HATs)
Histone deacetylases (HDACs)
Ace
Methylation
Lysine methyltransferases (KMT)
(e.g. MLL, SET1A/B,…)
Lysine demethylases (KDM)
(e.g. LSD1, JARID1…)
K4
Me
Methylation
Lysine demethylases (KDM)
(e.g. Jmjd3, UTX, …)
Lysine methyltransferases (KMT)
(e.g. PRC2:Ezh2,…)
K27
Chromatin remodelling
BAF complex
GATA4
Tbx5
Nkx2.5
Brg1
Baf60c

Epigenetic memory and histone variants

- In order to maintain the stable self-renewal of ESCs, the mechanisms that prevent their differentiation and promote their proliferation must be transmitted to their daughter cells, which imply the significance of the ‘epigenetic memory’ mechanisms in this process. This ‘epigenetic memory’ allows cells to maintain their identity, even when exposed to extracellular environments that induce formation of other cell fates. This is also important for maintaining stem cells over time and in preventing tumor formation. Historically cellular inheritance was explained by methylation of a promoter DNA, but recently published data argues in favor that DNA methylation is not the only mechanism elaborated by the cell to provide for epigenetic memory. Histone variants deposition into chromatin of actively transcribed genes (for example H3.3) can contribute to the cellular memory phenomena. Experiments performed by Ng and Gurdon in Xenopus laevis provide for the first documented evidence of the persistence of epigenetic memory of a transcriptionally active state and propose the role of histone variant H3.3 in this process

An epigenetic modifier–the Polycomb-group complex

- A series of recent studies have revealed that in order to maintain the pluripotency, mouse and human embryonic cells elaborate epigenetic mechanisms for a dynamic repression of genes regulating developmental pathways in such a way, that this repression can be maintained through cell division (20).

EPIGENETICS IN CELL-FATE CONVERSION

- The responsiveness of stem cells to extrinsic signals changes over time, and their developmental potential becomes more restricted due to changes in their internal state (60–62). There is growing evidence that epigenetic modifications are required for nuclear reprogramming and cell-fate conversion (63,64). Recent advances in epigenetics suggest that cell fates can be reset by the alteration of epigenetic marks on histones or DNA methylation, and that such converted cells are functional when transplanted in vivo.
- Experimental data suggests that epigenetic modifications might permit the generation of new sources of neuronal SCs (stem cells) and neurons from non-neuronal SCs. It was demonstrated that the addition of either valproic acid, an histone deacetylases inhibitor, or 5-azacytidine (5-AzaC), a DNA methylation inhibitor, can convert bone marrow stromal cells to NSCs

Experiment: Effect of TSA on stem cell differentiation

- Leukemia inhibitory factor (LIF) was removed from all the cell lines. LIF inhibits cell differentiation, and its removal allows the cell lines to go through cell differentiation. The cell lines were treated with Trichostatin A (TSA) - a histone deacetylase inhibitor for 6 days. One group of cell lines was treated with 10nM of TSA. The western analysis showed the lack of initial deacetylation on Day-1 which, was observed in the control for the embryonic stem cell differentiation. The lack of histone deacetylase activity allowed the acetylation of H3K9 and histone H4. Embryonic stem cells were also analyzed morphologically to observe the formation of embryoid body formation as one of the measures of cell differentiation. The 10nM TSA treated cells failed to form the embryoid body by Day-6 as observed in the control cell line. This implies that the ES cells treated with TSA lacked the deacetylation on Day-1 and failed to differentiate after the removal of LIF. Second group,'-TSA Day4' was treated with TSA for 3days. As soon as the TSA treatment was stopped, on day 4 the deacetylation was observed and the acetylation recovered on Day-5. The morphological examination showed the formation of embryoid body formation by Day-6. In addition, "Interestingly" the embryoid body formation was faster than the control cell line. This suggests that the '-TSA Day4' lines were responding to the removal of LIF but, were unable to acquire any differentiation phenotype. They were able to acquire the differentiation phenotype after the cessation of TSA treatment and at rapid rate. The morphological examination of the third group,' 5 nM TSA' showed the intermediate effect between the control and 10nM-TSA group. The lower dose of TSA allowed the formation of some embryoid body formation. This experiment shows that TSA inhibits histone deacetylase and the activity of histone deacetylase is required for the embryonic stem cell differentiation. Without the initial deacetylation on Day-1, the ES cells cannot go through the differentiation

- Lunyak VV, Rosenfeld MG. Epigenetic regulation of stem cell fate. Human molecular genetics. 2008 Apr 15;17(R1):R28-36.
- NIH Stem Cell Information Home Page. In Stem Cell Information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2016 [cited February 1, 2019] Available at < //stemcells.nih.gov/info.htm>