A Taste of Epigenetics

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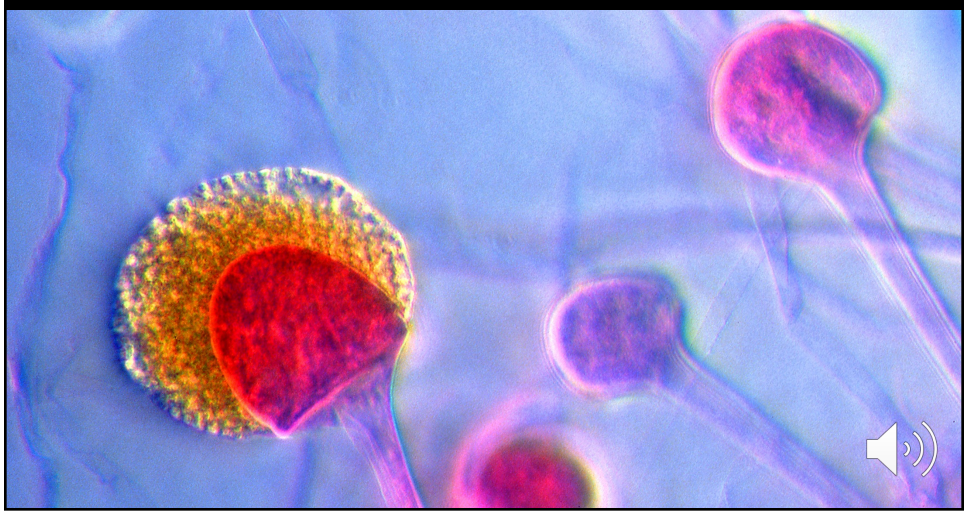

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Disclosures

James Ryan is a principal in Progene DX, a company that sells the transcriptomic test called GENIE

Gene ExpressioN: Inflammation Explained

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Lab Update

1. One year after submission of our application, we have our CLIA inspection scheduled for October 20
2. Supply chain disruptions and scarcity of supplies is nearly over
3. Results taking 2-3 weeks

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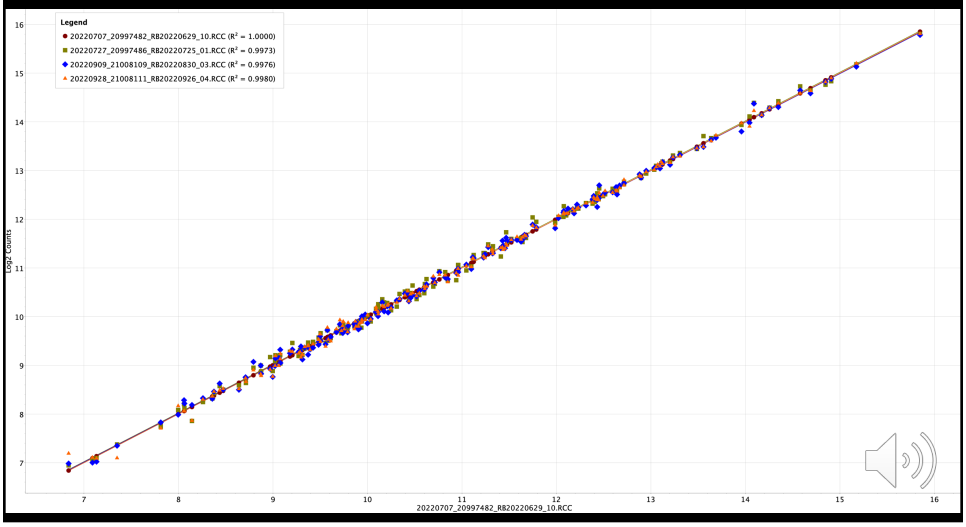
Replicate Blood Tube Controls



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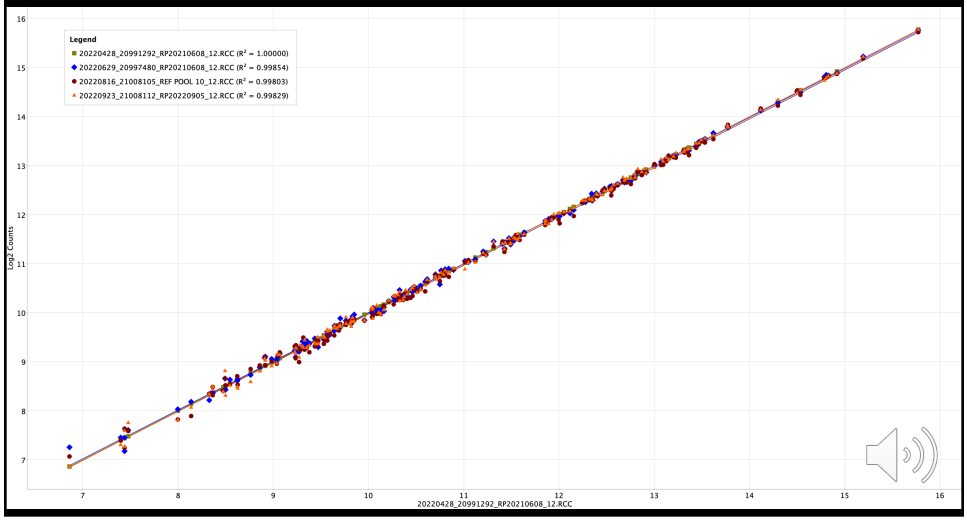
RNA Controls



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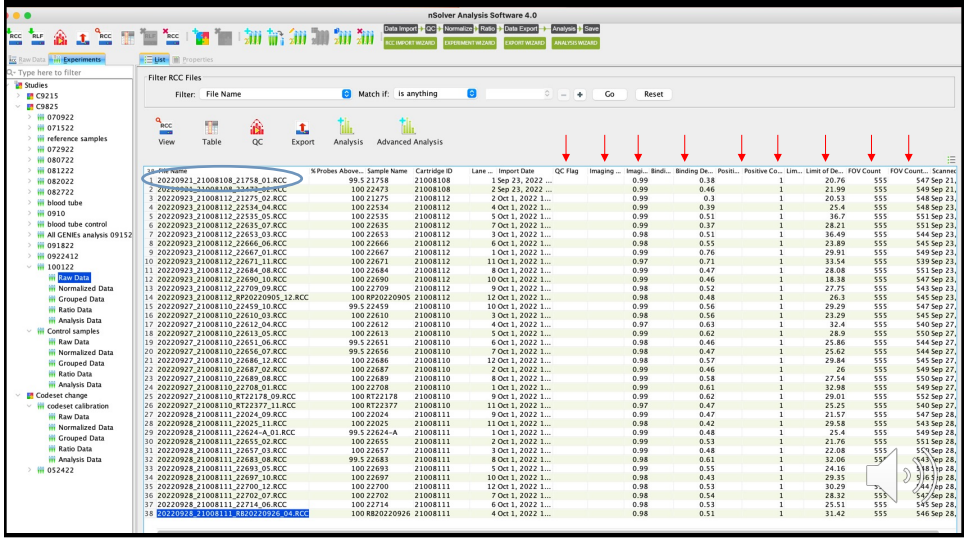
Run Control Metrics



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Basics – Why are cells different

1. Our bodies are made of tissues, cells organized for a common function
2. Cells derive different functions by differential gene expression from the same genome
3. Although some cells are highly specialized, e.g., neuron vs epithelial cell, they all use the same basic machinery, e.g., ribosomes, mitochondria. However, how do they become different to begin with?

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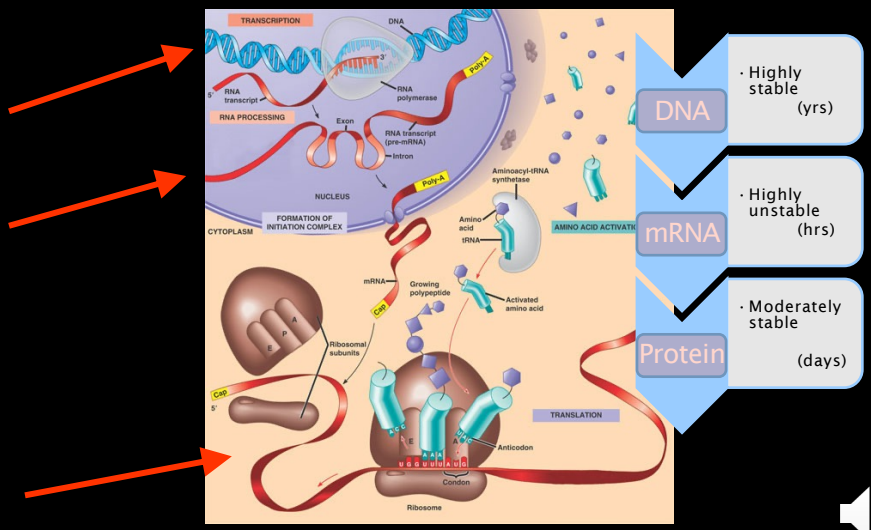
Central Dogma of Molecular Biology



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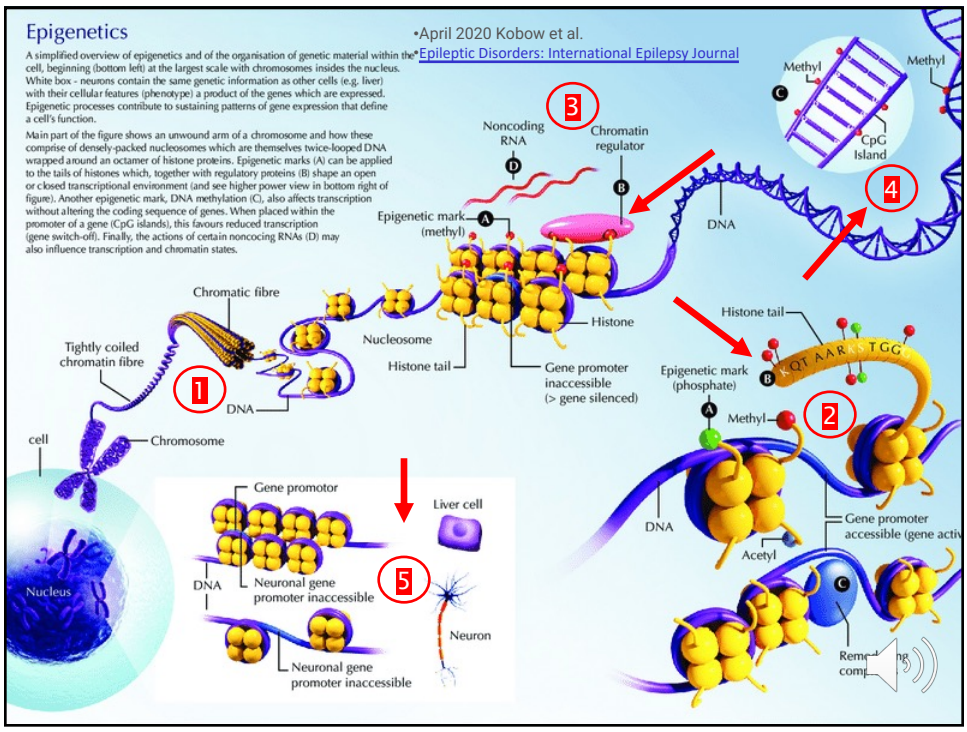
Epigenetics
•April 2020 Kobow et al.
•Epileptic Disorders: International Epilepsy Journal
A simplified overview of epigenetics and of the organisation of genetic material within the cell, beginning (bottom left) at the largest scale with chromosomes insides the nucleus. White box - neurons contain the same genetic information as other cells (e.g. liver) with their cellular features (phenotype) a product of the genes which are expressed. Epigenetic processes contribute to sustaining patterns of gene expression that define a cell's function.
Main part of the figure shows an unwound arm of a chromosome and how these comprise of densely-packed nucleosomes which are themselves twice-looped DNA wrapped around an octamer of histone proteins. Epigenetic marks (A) can be applied to the tails of histones which, together with regulatory proteins (B) shape an open or closed transcriptional environment (and see higher power view in bottom right of figure). Another epigenetic mark, DNA methylation (C), also affects transcription without altering the coding sequence of genes. When placed within the promoter of a gene (CpG islands), this favours reduced transcription (gene switch-off). Finally, the actions of certain noncoding RNAs (D) may also influence transcription and chromatin states.
Methyl
Methyl
CpG Island
Noncoding RNA
Chromatin regulator
Epigenetic mark (methyl)
DNA
Chromatic fibre
Tightly coiled chromatin fibre
Nucleosome
Histone
Histone tail
Gene promoter inaccessible (> gene silenced)
Histone tail
Epigenetic mark (phosphate)
Methyl
Acetyl
DNA
Gene promoter accessible (gene activ
cell
Chromosome
Nucleus
Gene promotor
Liver cell
DNA
Neuronal gene promoter inaccessible
Neuron
Neuronal gene promoter inaccessible
1
2
3
4
5

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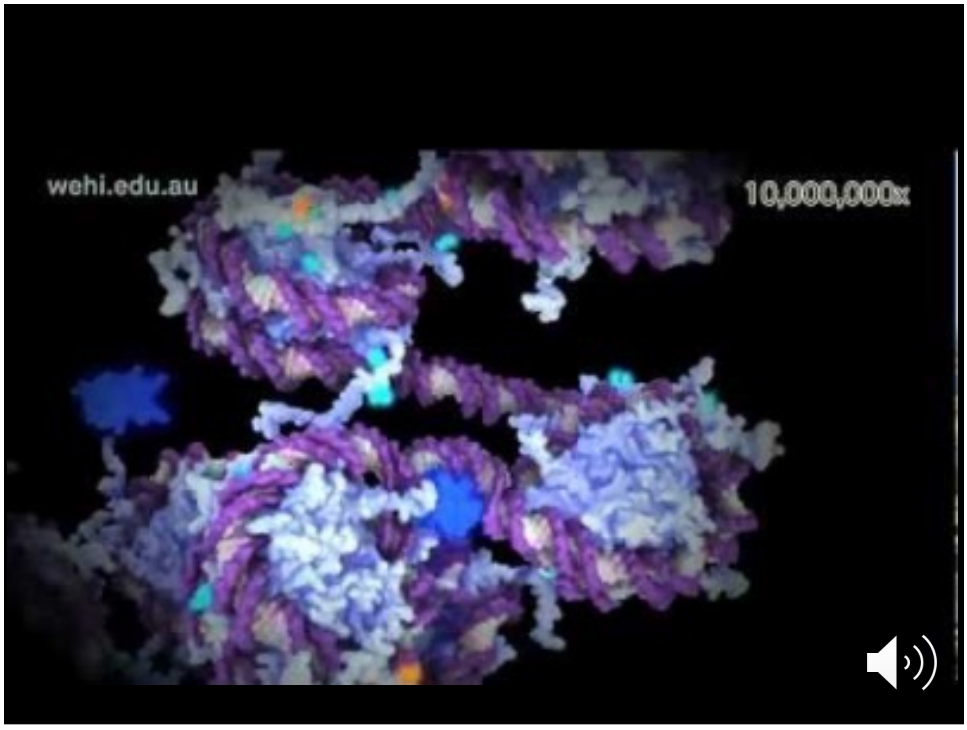
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CD3D Sorted	
CD40LG	1.4E-22
CD52	2.8E-30
CD127	2.8E-26
HIF1a	1.2E-22
IQSEC1	2.3E-25
MAP3K5	5.8E-27
MAPK9	1.4E-33
STAT3	3.1E-33

KDM4C Sorted	
CD40LG	1.3E-29
CD3	1.0E-28
CD127	9.9E-32
YLPM1	1.8E-37
IQSEC1	3.2E-34
MAP3K5	1.9E-23
MAPK9	1.2E-35
STAT3	1.7E-32

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KDM4C

KDM4C – Lysine demethylase that regulates gene expression and chromosome segregation. Chromosomal aberrations and changes in expression of this gene may be found in tumor cells.

Diseases associated with KDM4C include Primary Mediastinal B-Cell Lymphoma and Lung Sarcomatoid Carcinoma. Among its related pathways are Coregulation of Androgen Receptor activity and Signal Transduction.

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Histone Methylation on GENIE

1. KMT2D – Lysine Methyl Transferase 2D is a histone methyltransferase that catalyzes methyl group transfer from S-adenosyl-L-methionine to the epsilon-amino group of 'Lys-4' of histone H3
2. KDM4C – Lysine DE-Methylase 4C is a histone demethylase that specifically demethylates 'Lys-9' and 'Lys-36' residues of histone H3

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KMT2D

- **Lysine Methyltransferase 2D** [2][3][5]
- MLL4 [2][3][4][5]
- ALR [2][3][4][5]
- Histone-Lysine N-Methyltransferase 2D [2][3][4]
- CAGL114 [2][3][5]
- MLL2 [3][4][5]
- Myeloid/Lymphoid Or Mixed-Lineage Leukemia 2 [2][3]
- Lysine (K)-Specific Methyltransferase 2D [2][3]
- Trinucleotide Repeat Containing 21 [2][3]
- Lysine N-Methyltransferase 2D [3][4]
- ALL1-Related Protein [3][4]
- TNRC21 [3][5]
- Myeloid/Lymphoid Or Mixed-Lineage Leukemia Protein 2 [4]
- Histone-Lysine N-Methyltransferase MLL2 [3]
- Kabuki Make-Up Syndrome [3]
- ALL1-Related [2]
- EC 2.1.1.364 [4]
- KABUK1 [3]
- AAD10 [3]
- KMS [3]

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Rewiring the Epigenetic Networks in *MLL*-Rearranged Leukemias

Chan et al, Front. Cell Dev. Biol., 15 May 2019

"Specific modifications or epigenetic histone marks have differential effects on gene expression. For example, acetylated histone marks (e.g., H3K9ac and H3K27ac) are usually associated with gene activation Krejci et al., 2009; Creyghton et al., 2010; Hawkins et al., 2011; Hezroni et al., 2011). In contrast, methylated modifications are context-dependent: for instance, methylation on H3K4 or H3K79 is associated with gene activation (Schubeler et al., 2004), whereas methylation on H3K9 or H3K27 is associated with gene silencing Musselman et al., 2012)"

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ME Literature

Histone H3 lysine 9 di-methylation as an epigenetic signature of the interferon response, Fang et al

- The key role of H3K9me2 in the IFN response raises a question of the role of H3K9me2 methylating and demethylating enzymes in inflammatory and autoimmune diseases that are rooted in increased IFN production.

Epigenetic Regulation: A Link between Inflammation and Carcinogenesis, Vezzani et al

- Histone lactylation is a recently studied posttranslational modification. Zhang et al. found that lactic acid, already known to promote gene expression and histone acetylation, can directly tag lysine residues on H3, H4, H2A, and H2B histones. Histone lactylation also occurs in lung tumors and melanoma cells. Moreover, exogenous lactate decreases the HDAC content in the nucleus, HDAC activity, and chromatin methylation

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ME Literature 2

Epigenetic regulation of innate immune memory in microglia, Zhang et al.

- Increased transcription of inflammatory genes, and transcription inducing H3K4me3 and H3K27Ac modifications were found in microglia of mice treated with LPS during the acute response. In case of tolerance, abundance of these marks is decreased after LPS re-exposure, which, at least partially, explains compromised induction of gene expression after secondary LPS challenge. Possibly, there is a second layer of gene expression repression by inhibitory histone marks. Previous data suggest a role for the inhibitory histone marks H3K9me2/3 in this context. The TF RELB has a recruiting role for H3K9me2/3 at the *Il1β* locus after LPS challenge, which leads to transcriptional repression of *Il1β* in response to a secondary LPS challenge

Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma, Morin et al

- 32% of diffuse large B-cell lymphoma and 89% of Follicular lymphoma cases had somatic mutations in *MLL2 (KMT2D)*, which encodes a histone methyltransferase

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Epigenetic Modification: A Key Tool for Secondary Metabolite Production in Microorganisms

Bind et al, Front. Microbiol., 13 April 2022

- Secondary metabolites are products made by an organism that are not necessary for growth but give the organism an advantage in particular environments
- Fungal-derived secondary include antibacterials, antifungals, immunosuppressants, mycotoxins.
- SM gene clusters remain in transcriptionally inactive or repressive heterochromatin state. Due to different environmental cues (light, temperature, pH, carbon, nitrogen, and iron), chromatin changes from heterochromatin state to euchromatin (transcriptionally active) state, with the help of epigenetic modificaitons, thus activating the SM biosynthetic pathway.

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Conclusions

- Epigenetic controls include adding or removing chemical tags on either DNA or on DNA histones, making gene promoters more or less available to transcription factors.
- Although epigenetic mechanisms are needed for normal physiology, they can also be the root of pathology. Several drugs are already in clinical trials to control epigenome modifying enzymes.
- Without knowing all the epigenetic modifications of a patient, GENIE still measures whats important, the gene transcripts produced from the epigenome. However, maybe particularly stubborn cases of CIRS can be explained by epigenome intransigence. Still lots of dots to be connected.

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