Introduction to Epigenetics – Life Style Choices and Human Health

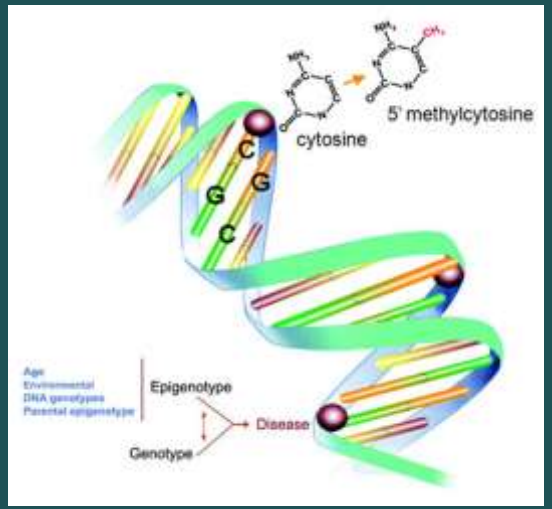
Paul Durham, PhD

Distinguished Professor

of Cell Biology

Director, Center for Biomedical and Life Sciences

Missouri State University



Disclosures

I have received grant support from

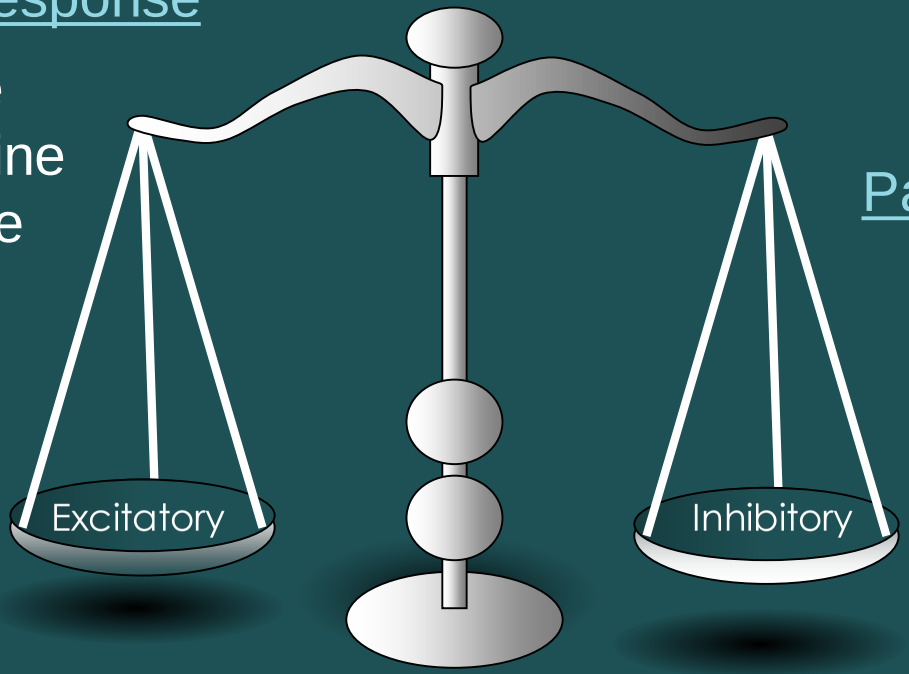
National Institutes of Health
electroCore
International Dehydrated Foods
CBD Life Sciences
Alder Pharmaceutical

Life Is All About Balance

Sympathetic Drive

Fight and Flight Response

Glutamate
Norepinephrine
Epinephrine
Dopamine

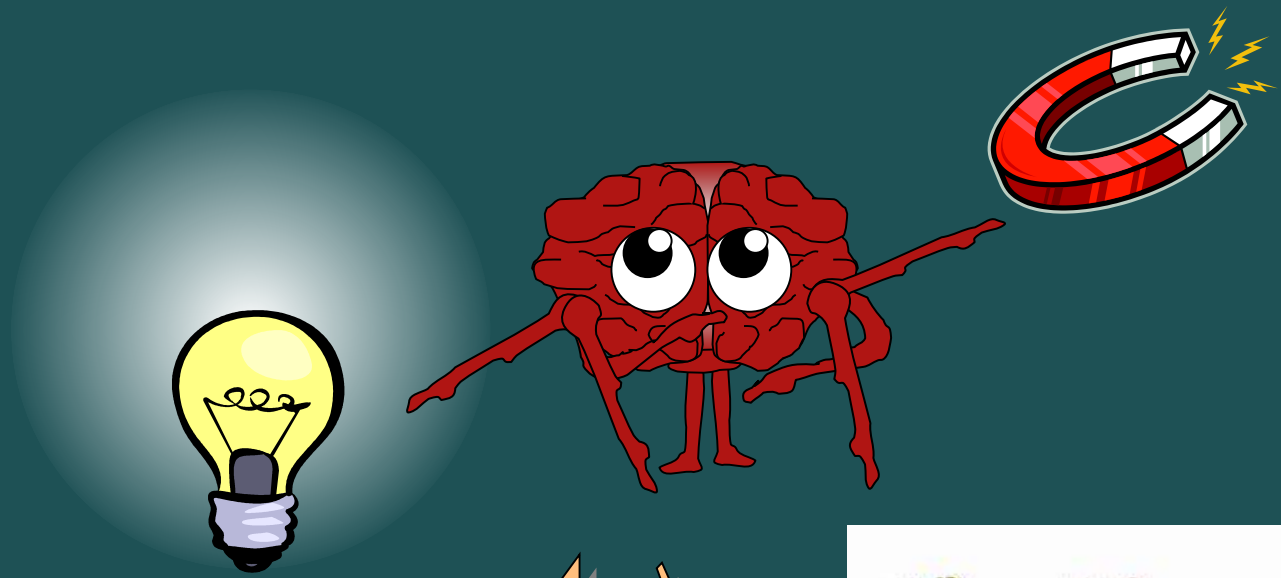


Parasympathetic Drive

Relaxed

Serotonin
GABA

The Hyperexcitable (Hypervigilant) Nervous System



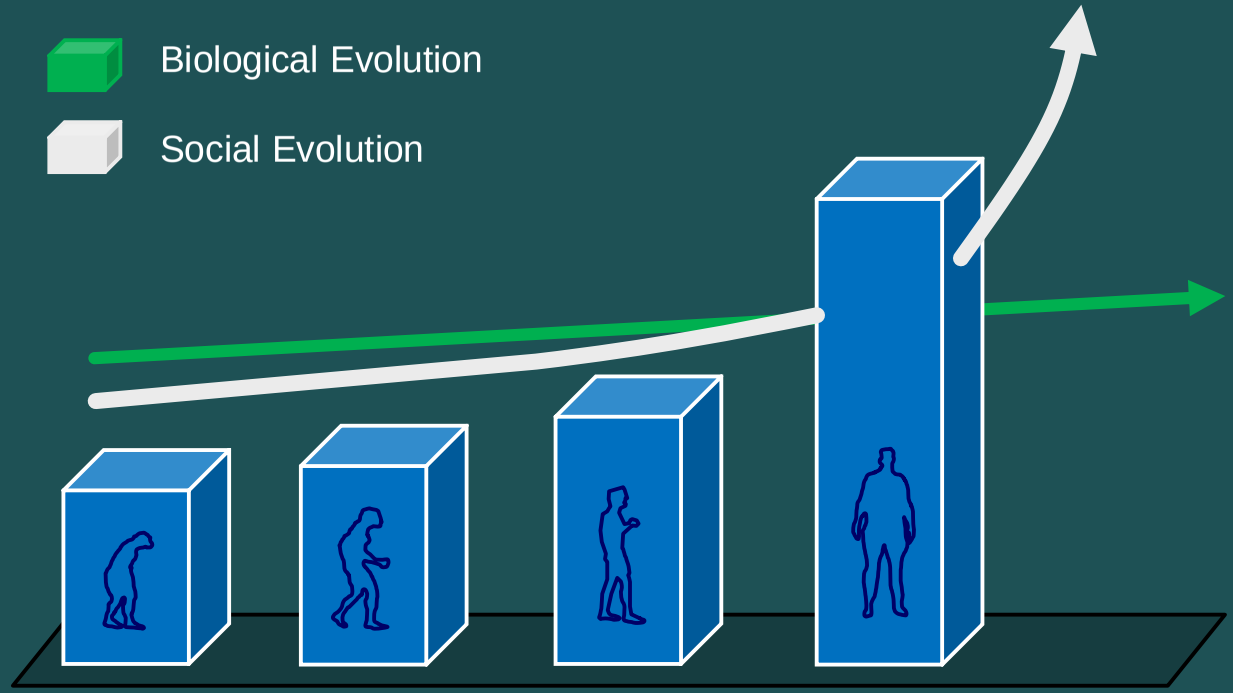
Afra J. *Clin Neurophysiol*. 2000;111:1124-1129

Teleological Explanation of Hypervigilant Nervous System - Advantages

- Some of our great ancestors (200,000 yrs ago) had a brain with increased excitability (gifts) which allowed them to:
  - Find healthy foods
  - Sense and avoid danger
  - Care for children and the tribe

Sentinel

Biological vs Social Evolution - Mismatch



Todays Nervous System Manages Many Things Not in the World of Our Ancestors

Sedentary lifestyle

Food from around the globe

Microwaves

Florescent lights

Artificial environments

Computers

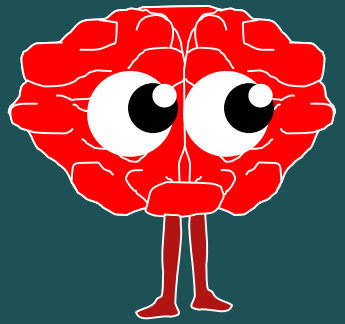
Food additives

Environmental pollution

Pathophysiology of Migraine and Other Craniofacial Diseases

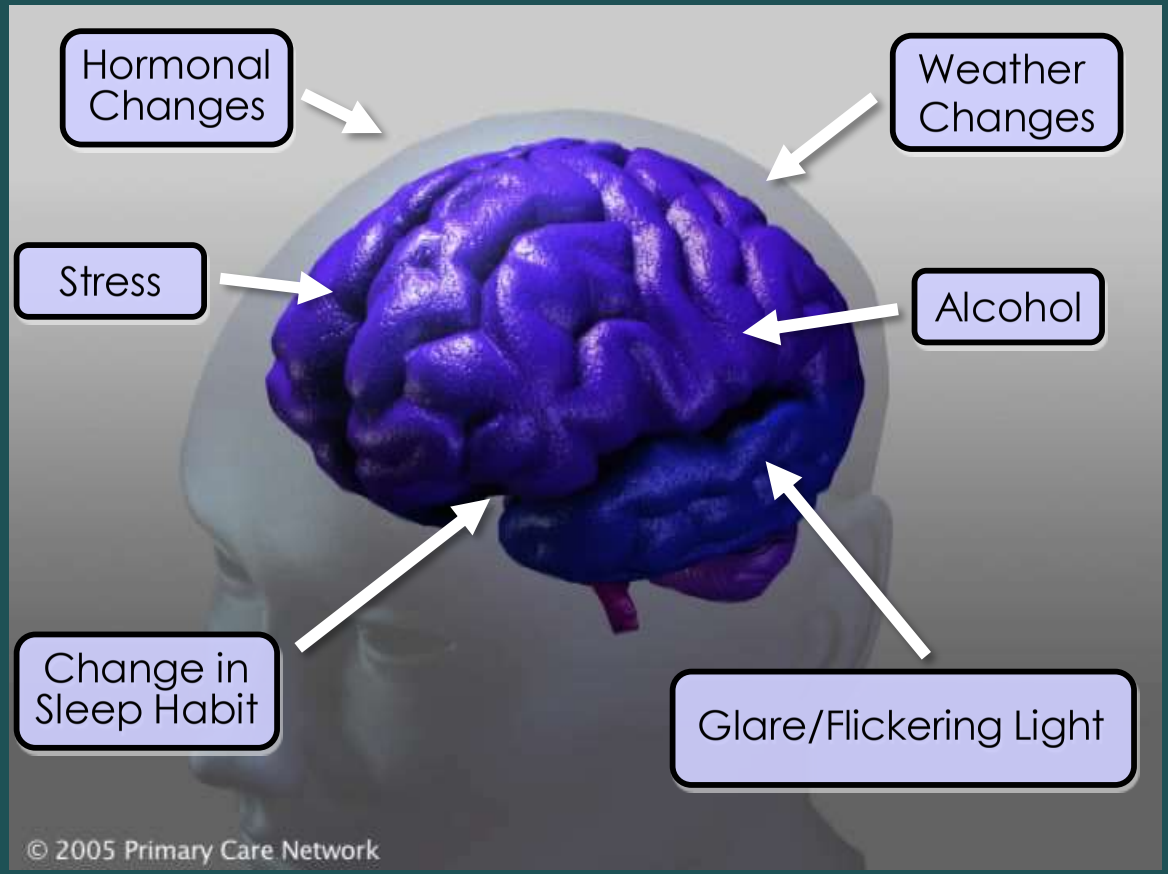
Migraine Brain

(Predisposition)



Environment

Internal Changes External Changes



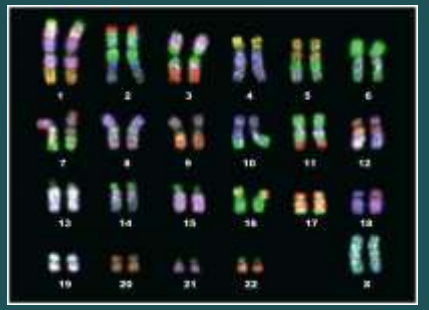
The Migraine Brain – Genetic Predisposition and Environmental Factors

- Genetic hyperexcitability (ion channels, receptors, signaling proteins)
  - Between episodes of migraine
  - During episodes of migraine

- Genetic hyperexcitability also associated with other chronic pain conditions
  - Between episodes of TMD and orofacial pain
  - During episodes of TMD and orofacial pain

Aurora SK, et al. *Cephalalgia.* 2007;27:1442-1453.

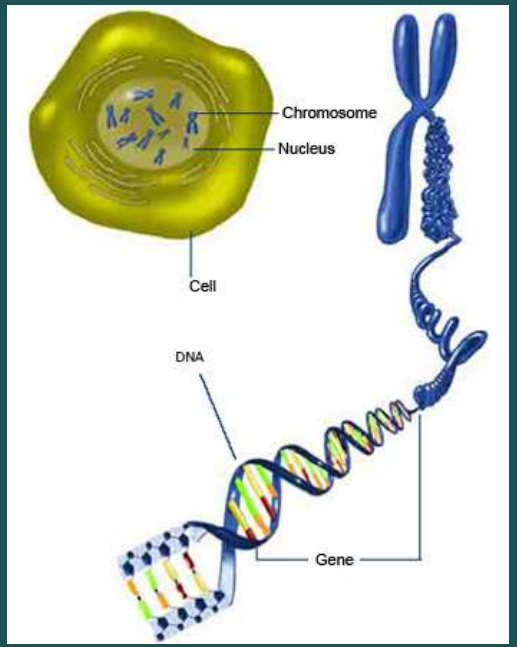
Genes and Your Genome



Each **chromosome** is a single linear DNA molecule associated with proteins

The total DNA in the chromosomes of an organism is its genome

- A gene is the entire nucleic acid sequence that is necessary for the synthesis of a functional polypeptide from mRNA
- Genes also code for tRNA and rRNA
- In humans, genes lie amidst a large expanse of noncoding DNA and genes may contain regions of noncoding DNA

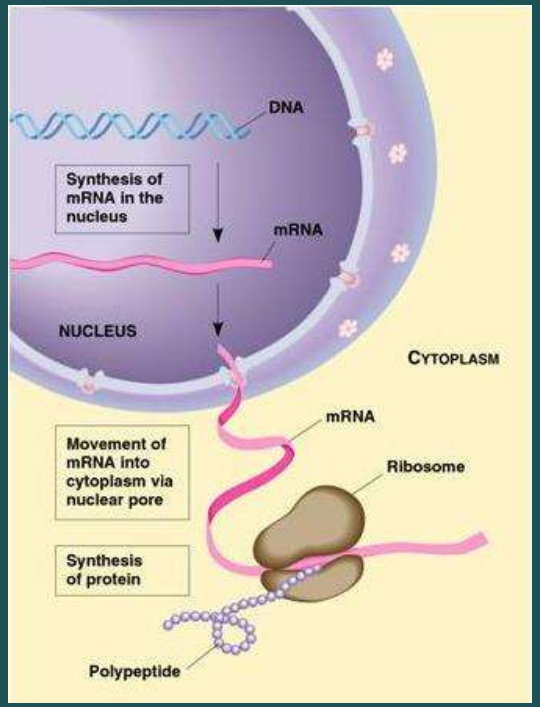


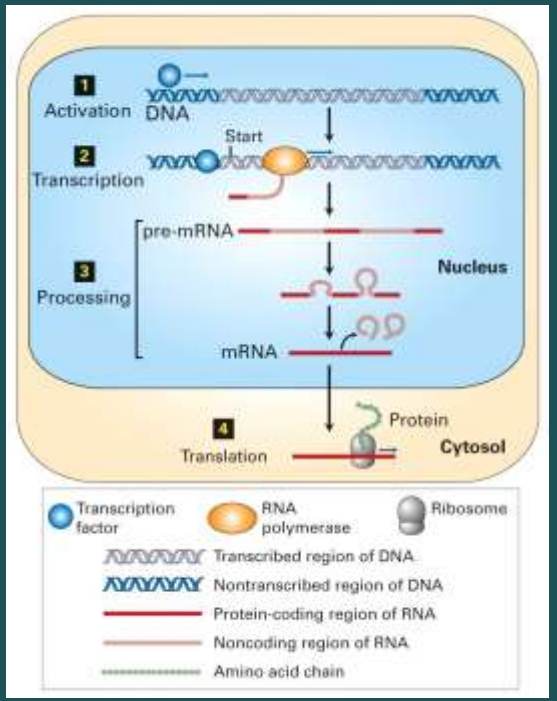
DNA to mRNA to Protein

Protein-coding genes

~ 20,000 copies in human genome (>3 billion base pairs)

~ 1.1% - fraction of human genome (regions containing exons)

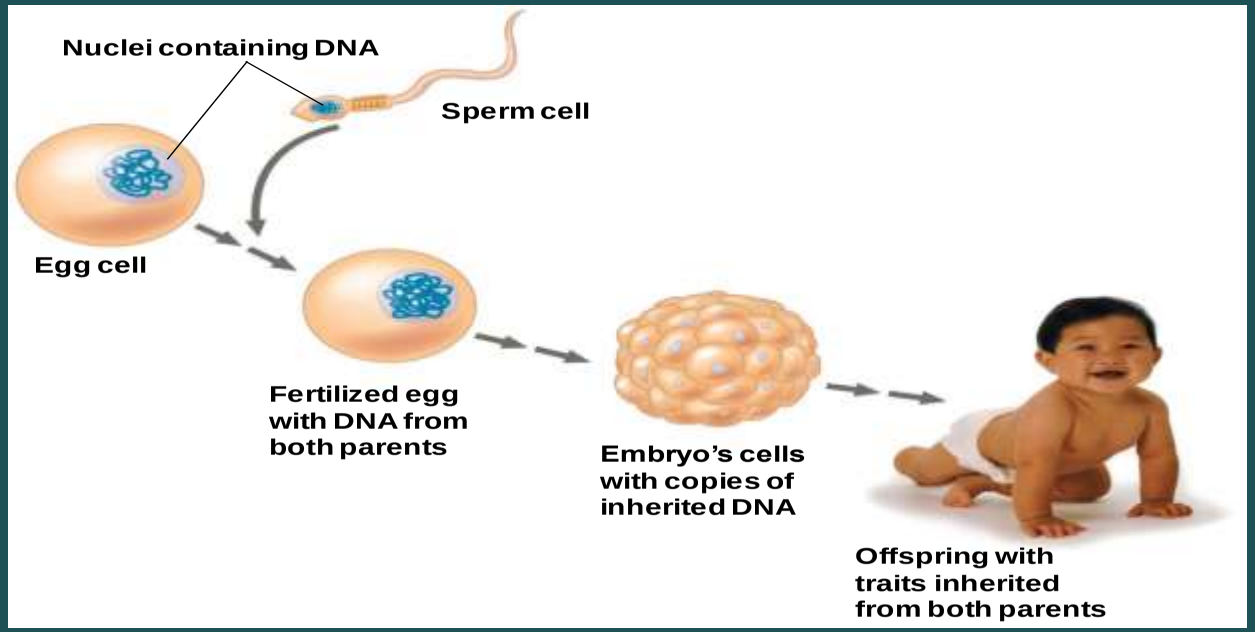


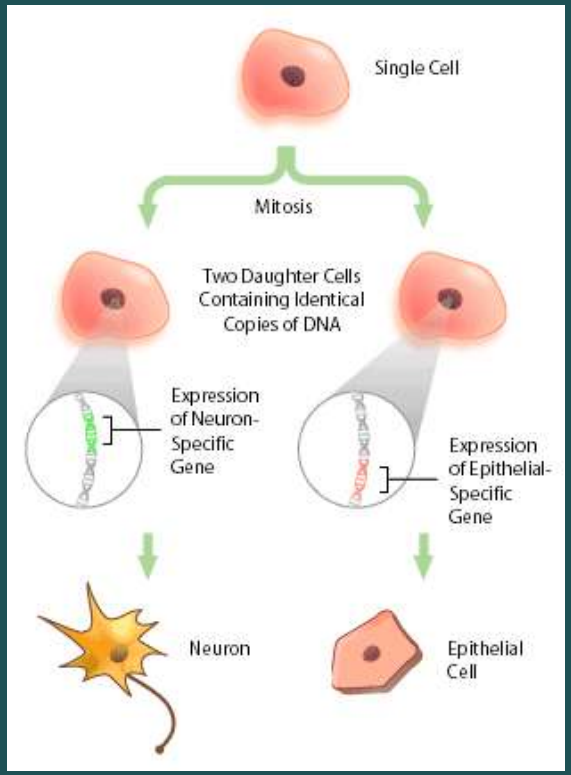


Differential Gene Transcription

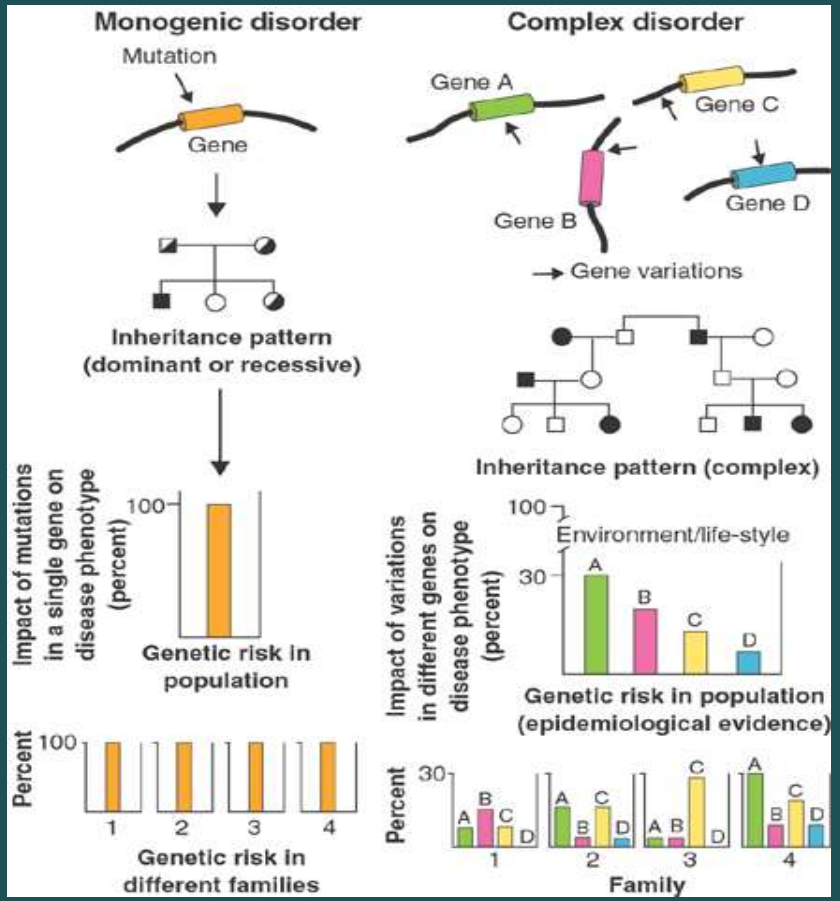
Eukaryotic gene: all DNA sequences required for expression

In any given cell – little more than half of our genes are expressed (10,000-13,000)





Human Disease and Genetic Mutations



Complex disorders are most common –

Chronic Pain
Migraine
TMD
TN

Science
Volume 291, Feb 2001, p. 1224-1229.

Genome-Wide Association Study of Migraine Implicates a Common Susceptibility Variant on Chromosome 8q22.1

Table 3: Association of rs1835740 genotype with gene expression levels

SNP	Gene	Strand	SNP coordinate	Gene start	Distance	SRC *P*
rs1835740	*UQCRB*	–	98,236,089	97,311,911	924,178	0.0013226
rs1835740	*MTDH*	+	98,236,089	98,725,583	489,494	0.0000396[a]
rs1835740	*HRSP12*	–	98,236,089	99,183,743	947,654	0.0028748

"modest support for involvement of MTDH in migraine"

- Function in glutamate pathway

Nature Genetics 42, 869–873 (2010)

Potential Genetic Risk Factors for Chronic TMD: Genetic Associations from the OPPERA Case Control Study

Table 3

Pain. 2011 November ; 12(11 Suppl): T92–101

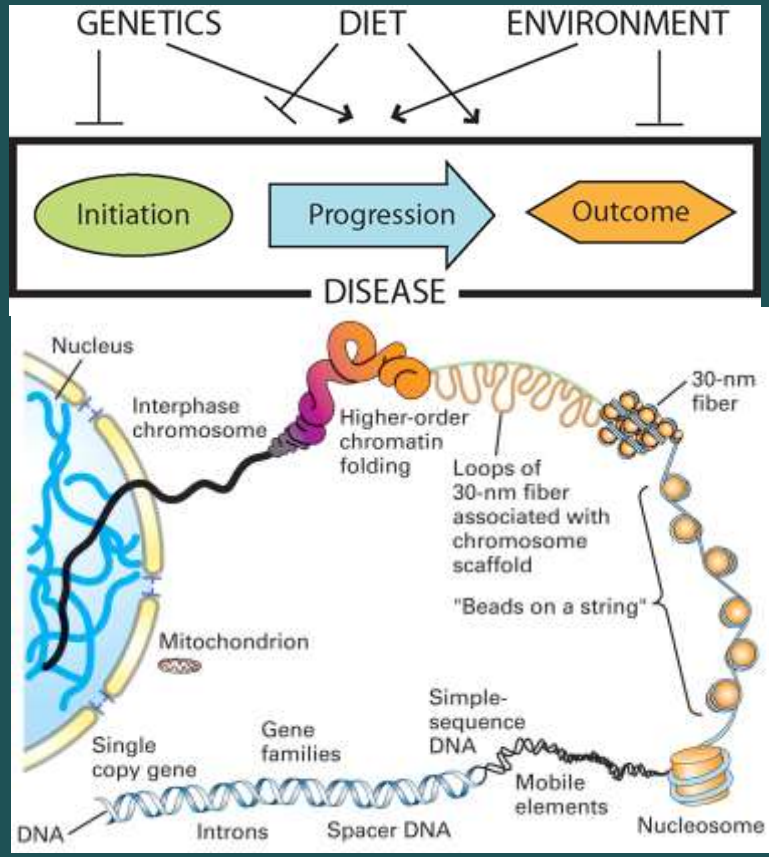
Top Association Results for Combined OPPERA and UNC Cohorts

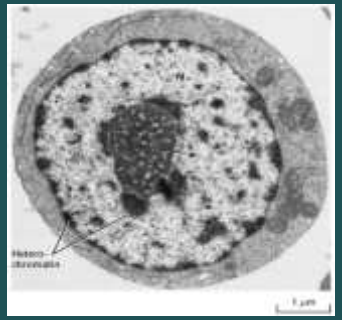
SNP	GENE	CHR	BP	TIER	OR_ADD	P_ADD	OR_DOM	P_DOM	OR_REC	P_REC	MIN	MAJ	MAF
rs2963155	NR3C1	5	142736197	2	.63	6.15E-05	.58	.00012	.47	.016	G	A	.22
rs9324918	NR3C1	5	142747353	2	.56	8.41E-05	.54	.00019	.29	.030	C	T	.14
rs33389	NR3C1	5	142680692	2	.57	.00022	.56	.00049	.30	.033	T	C	.13
rs9316233	HTR2A	13	46331356	2	.64	.00034	.55	4.96E-05	.87	.68	G	C	.25
rs7800170	CHRM2	7	136274860	2	.72	.00062	.53	3.65E-05	.79	.14	A	C	.50
rs3756612	CAMK4	5	110731386	2	1.51	.00064	1.62	.00058	1.61	.19	G	A	.16
rs10491334	CAMK4	5	110800303	2	.63	.00088	.59	.00045	.71	.45	T	C	.15
rs728273	IFRD1	7	111855337	2	1.38	.0012	1.43	.017	1.62	.0037	G	C	.48
rs12415832	GRK5	10	121102317	2	2.40	.0013	2.55	.00098	0	.99	A	C	.03

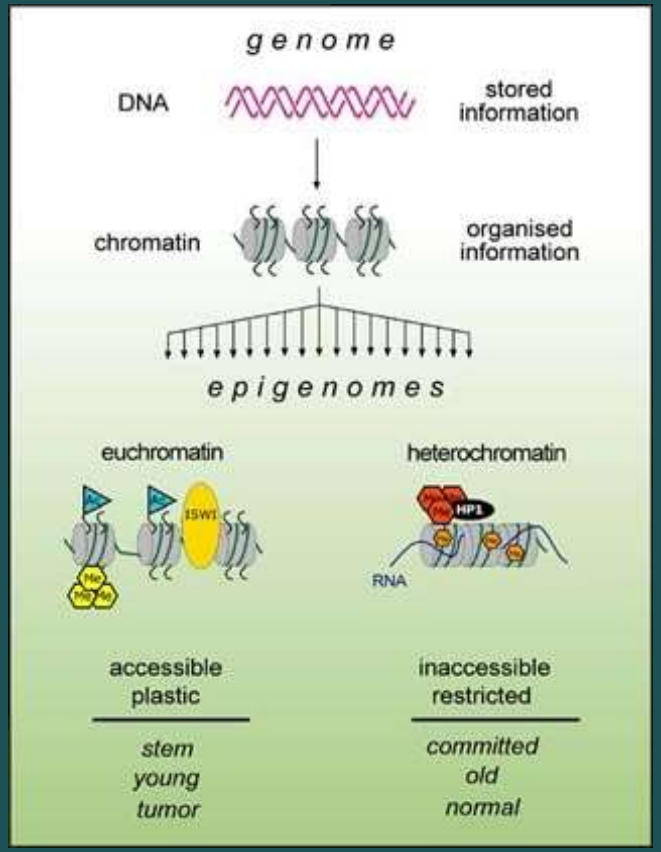
Abbreviations: SNP, single nucleotide polymorphism; CHR, chromosome; BP, genomic location in base pairs; OR_ADD, odds ratio of TMD risk for additive model; P_ADD, *P*-value of TMD risk for additive model; OR_DOM, odds ratio of TMD risk for dominance model; P_DOM, *P*-value of TMD risk for dominance model; OR_REC, odds ratio of TMD risk for recessive model; P_REC, *P*-value of TMD risk for recessive model; Min, minor allele; Maj, major allele; MAF, minor allele frequency.

In summary, the OPPERA case-control study is the first genetic association study of TMD to have investigated a substantial number of pain related candidate genes. While the results require replication, they provide tentative evidence that chronic TMD is influenced by genetic contributions within a number of gene loci, including NR3C1, CAMK4, CHRM2, IFRD1, GRK5, HTR2A and COMT.

Role of Genetics and Epigenetics in Human Disease

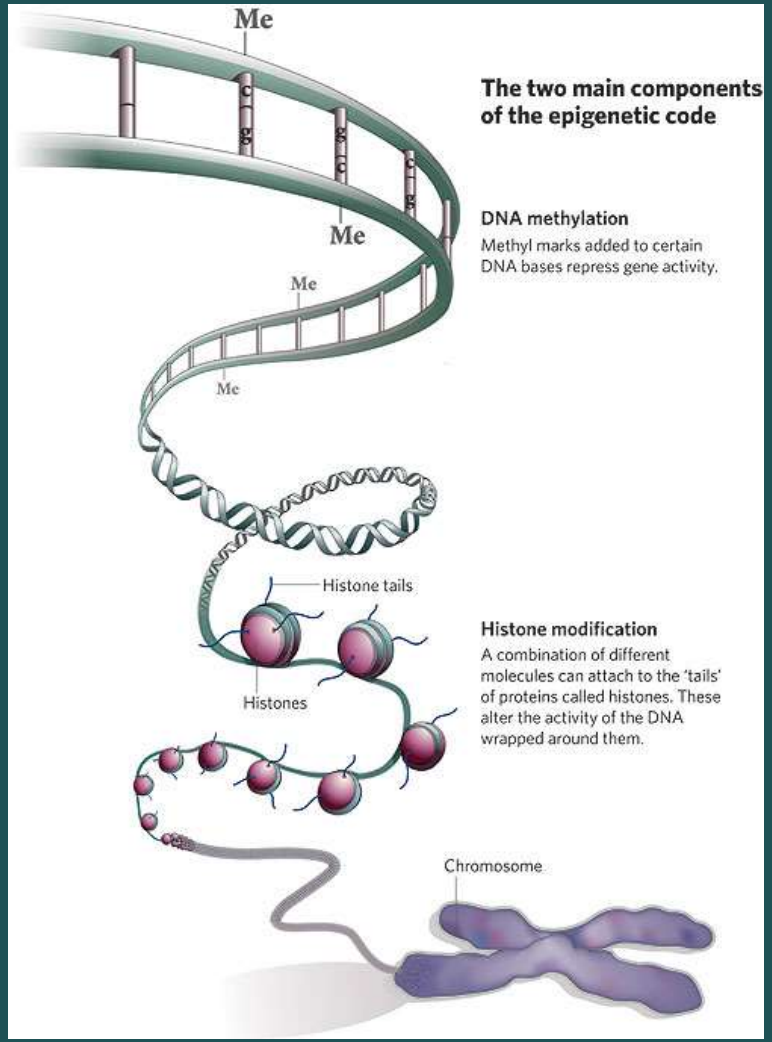






Epigenetics – Basic Mechanisms

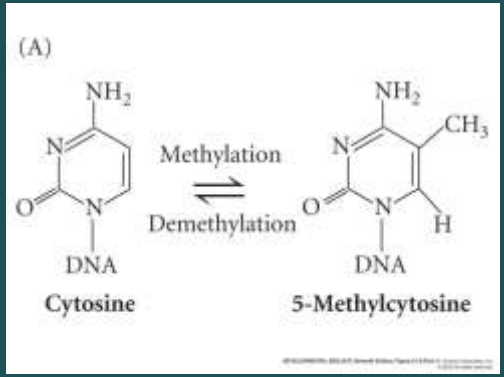
- study of changes in gene activity that do not involve alterations to the genetic code
- patterns of gene expression are governed by the cellular material — the epigenome — that sits on top of the genome, just outside it (hence the prefix *epi-*, which means above)
- epigenetic "marks" that tell your genes to switch on or off, to speak loudly or whisper
- regulation through epigenetic marks by environmental factors like diet, stress and prenatal nutrition can make an imprint on genes



Gene Regulation - Methylation Patterns

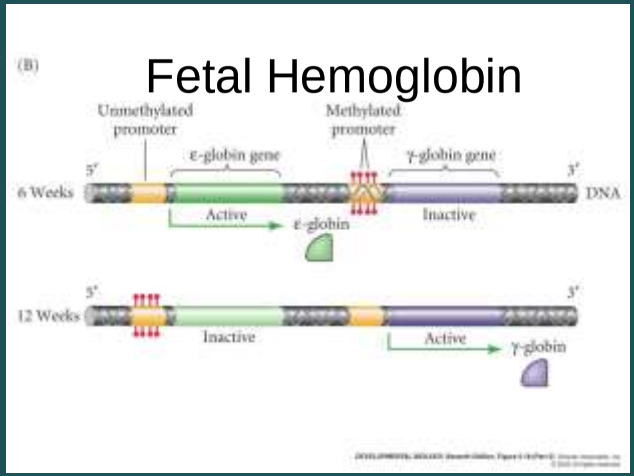
DNA methylation: turns genes off

- control transcription; how cells continue to remain certain cell type even through multiple cell divisions
- promoters get methylated thus preventing genes from being turned on



How are some genes methylated and how does methylation prevent gene expression?

- methyl groups placed on DNA during differentiation is maintained during cell division
- totipotent cells: no methylation pattern
- modification mainly occurs in cytosines that precede guanines, yielding 5-methylcytosine (5-meC) – sites are referred to as CpGs



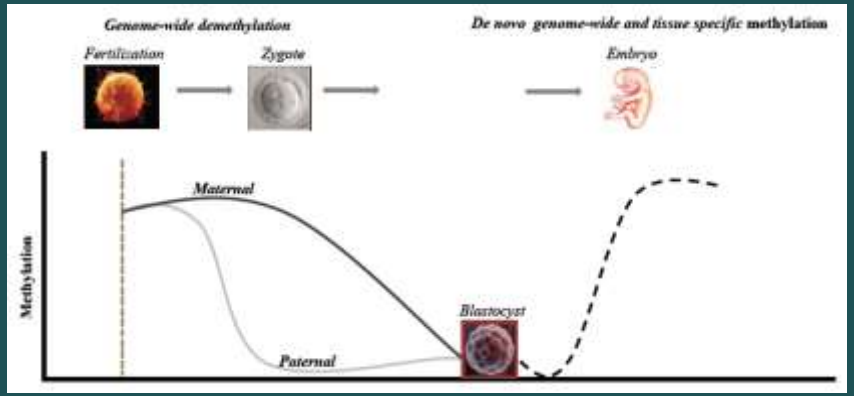
Methylation Control of Gene Expression

- DNA methyl transferases (DNMT):
  add CH_3 group to cytosine

- Methylation patterns similar in identical twins early in development but become altered later – evidence of environmental factors influencing gene expression and affecting health

- Methylation important component in numerous cellular processes, including embryonic development, genomic imprinting, X-chromosome inactivation, and preservation of chromosome stability

- Errors in methylation linked to a variety of devastating consequences, including several human diseases (cancer, lupus, muscular dystrophy, birth defects, neurodegenerative and neurological diseases)



Discovery Medicine, 2010

Gene Regulation: Histone Acetylation

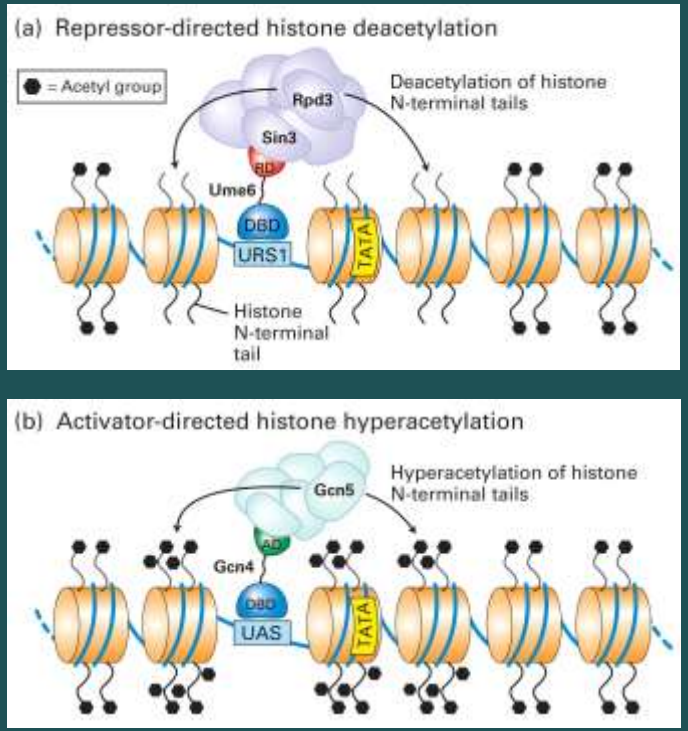
Histone acetylation: clears way for transcription initiation by destabilizing nucleosome structure

Repressors and activators can direct histone deacetylation at specific genes

Key Enzymes:

- histone acetyltransferases (HATs): acetylate lysine residues on histones and loosen up histone-DNA interactions to allow gene expression

- histone deacetylases (HDACs): catalyze removal of acetyl groups from lysine residues and strengthen histone-DNA interactions to prevent gene expression



Epigenetic Inheritance - Effect of Diet, Exercise, and Environmental Conditions

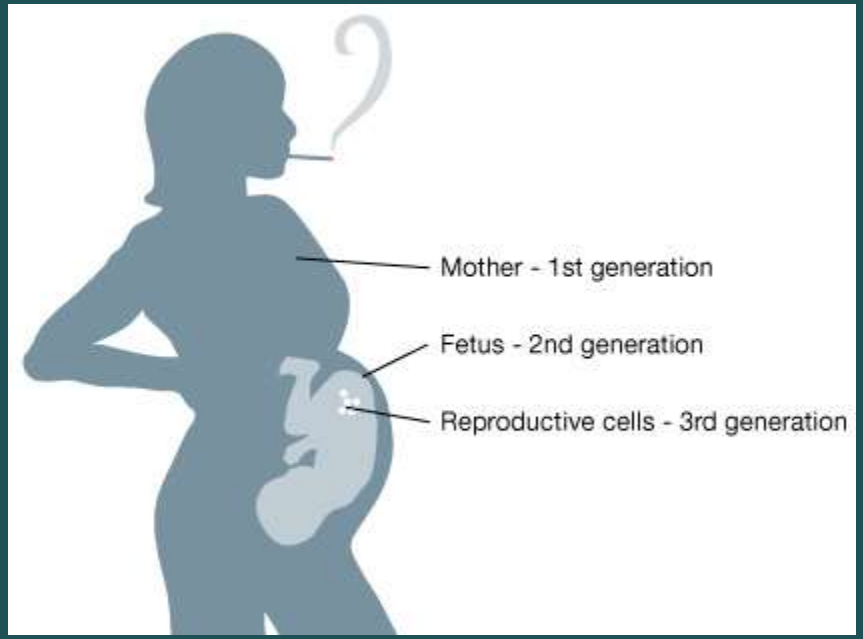
Three generations - exposed to the same environmental conditions (diet, toxins, hormones, etc.)

For epigenetic inheritance - an epigenetic change must be observed in the 4th generation

Epigenetic changes - transient by nature

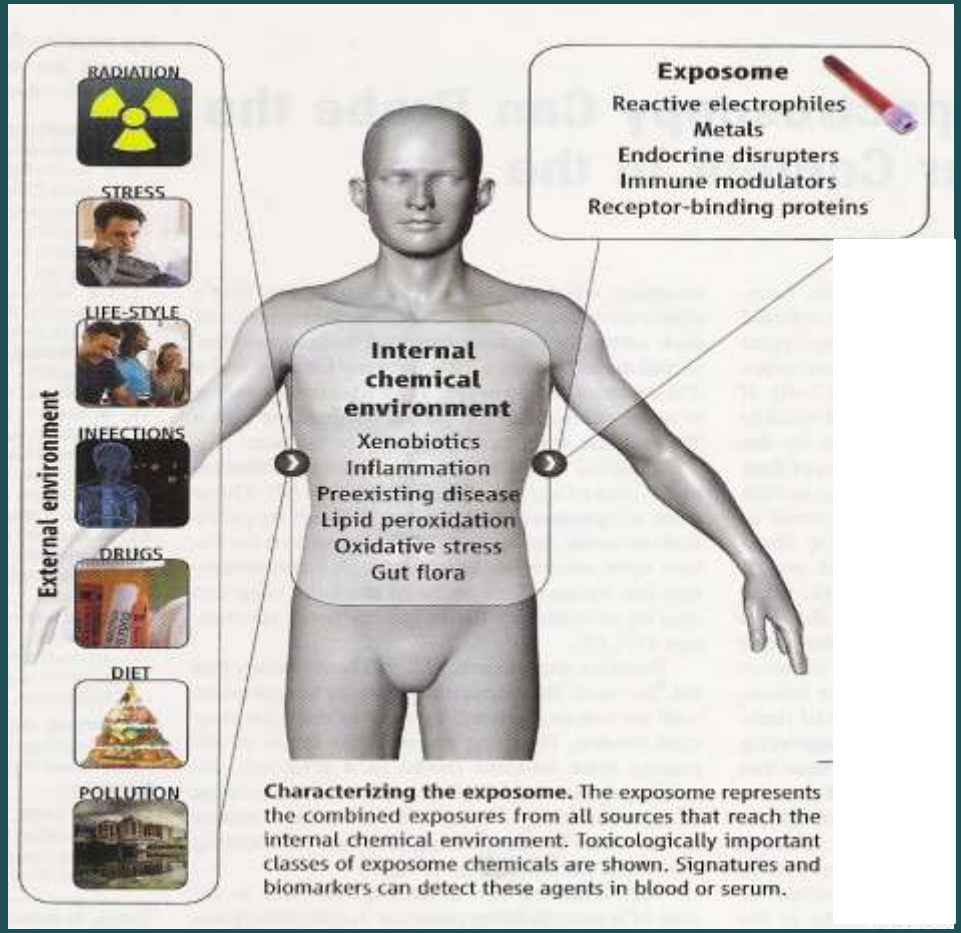
Epigenome changes more rapidly than the relatively fixed DNA code

Epigenetic change triggered by environmental conditions may be reversed when environmental conditions change again



Andrew P. Feinberg *Nature* **447**, 433-440 (24 May 2007)

Epigenetic Signature is Dynamic



Genetics

Maternal factors
In utero nutrition

Epigenotype (birth)

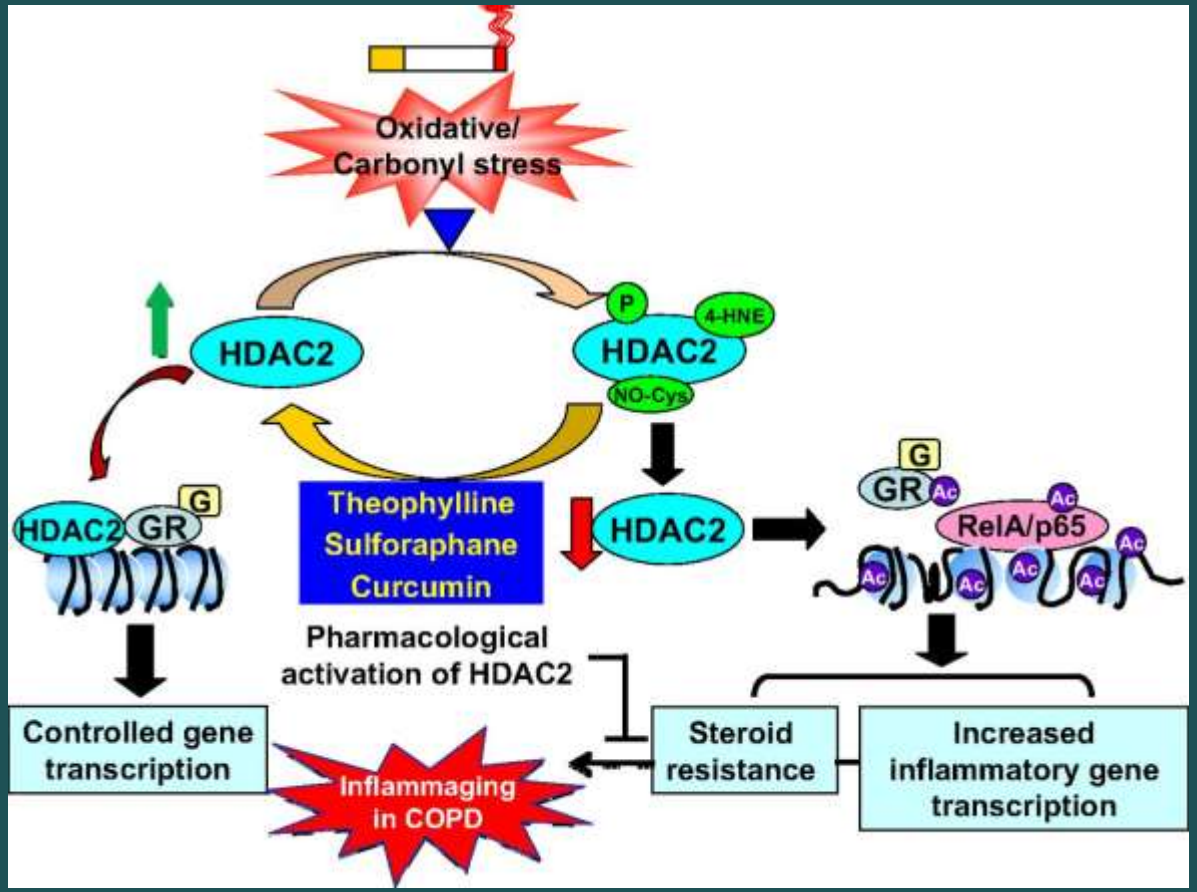
Diet, Sleep
Toxins, Drugs
Smoking
Aging, Illness

Epigenotype (adult)

Negative changes can be suppressed by changing environment – epigenetic modifications are reversible

Science 330:460-461, 2010

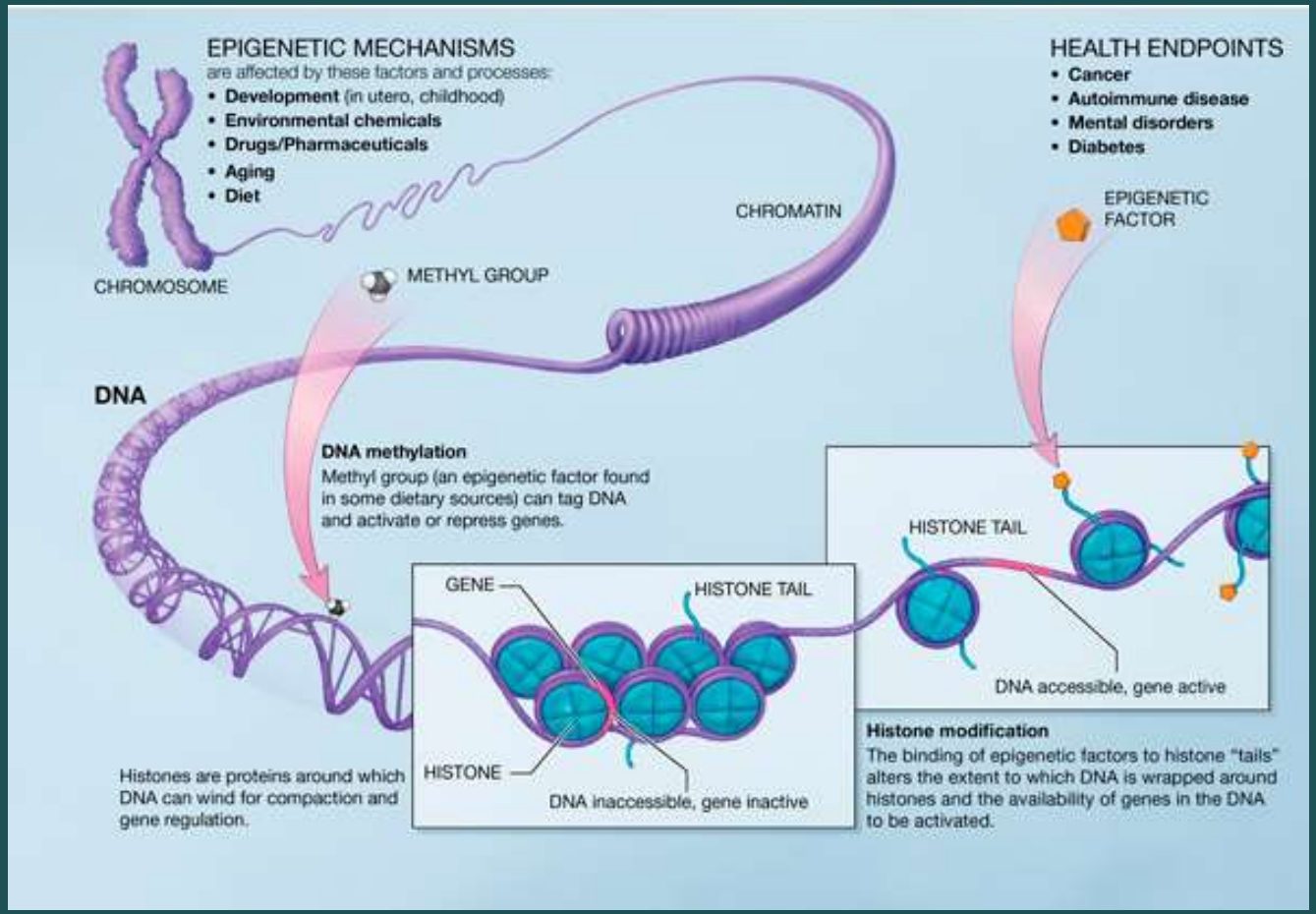
Smoking Induces Epigenetic Changes to Promote Steroid Resistance and COPD



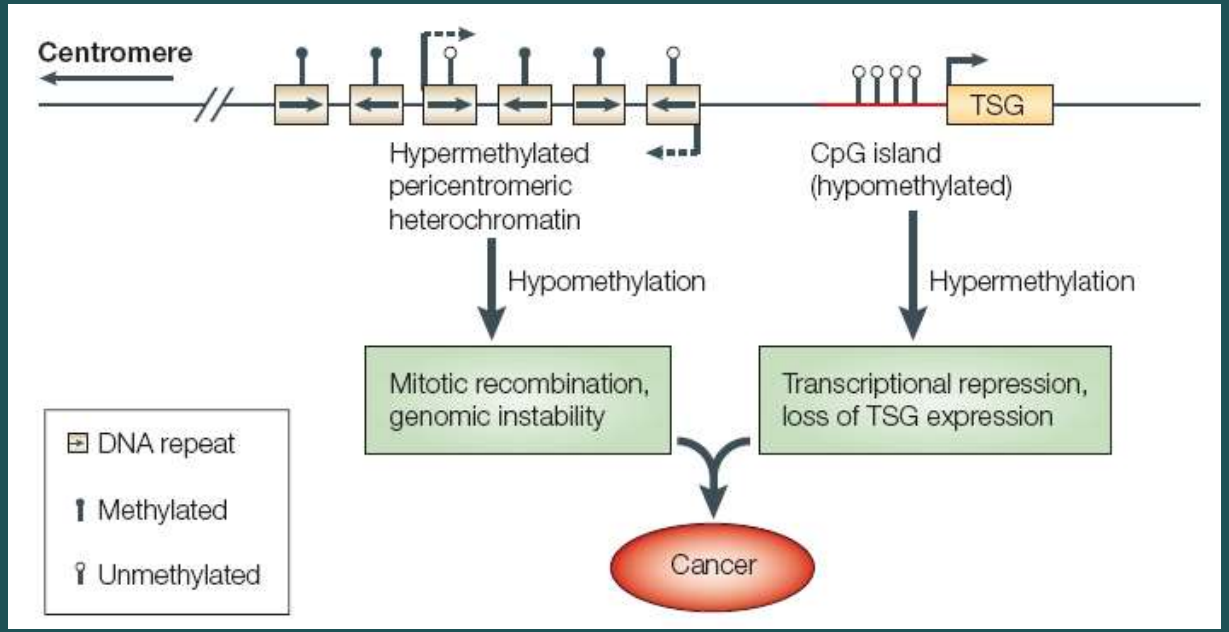
Chest. 2006 129(1):151-5. Reduced histone deacetylase in COPD: clinical implications. Barnes PJ

Respiratory Medicine Volume 106, 2012, Pages 319-328 Asad Tamimia, Dzelal Serdarevica. Nicola A.Hananiab

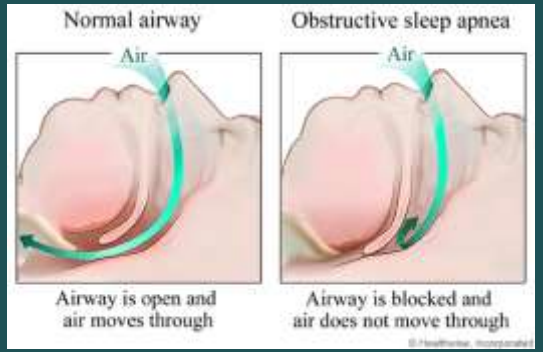
Epigenetic Mechanisms: Disease Implications



Epigenetics - Cancer Incidence and Mortality



- cancer is characterized by global hypomethylation, with hypermethylation of CpG islands
- TSG = tumor suppressor genes (p53, Rb, BRACs)



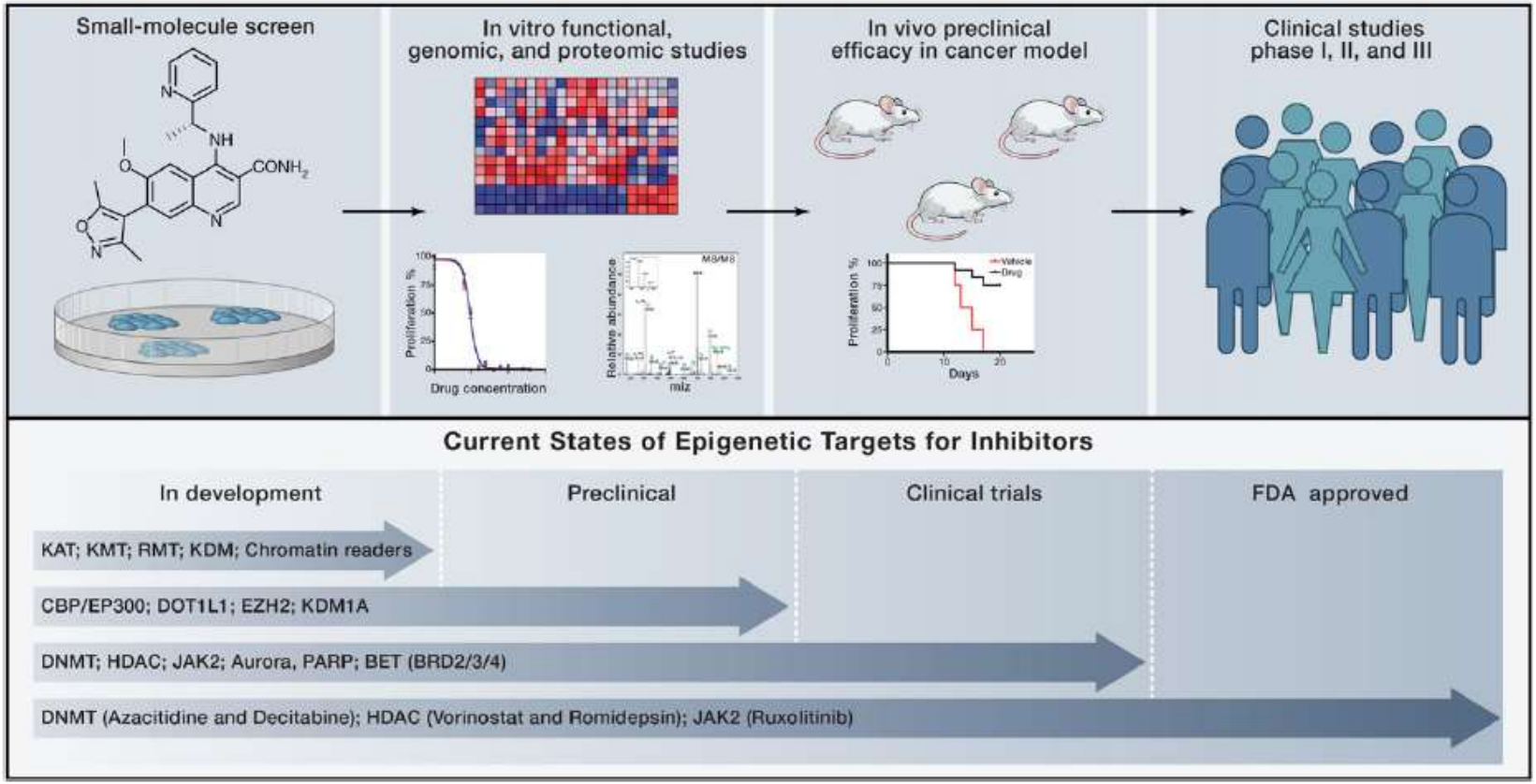
Sleep disordered breathing (SBD) problems linked to increase in cancer mortality.

Increased overnight hypoxia as a surrogate of obstructive sleep apnea severity associated with increased cancer incidence.

Association limited to men and patients <65 years.

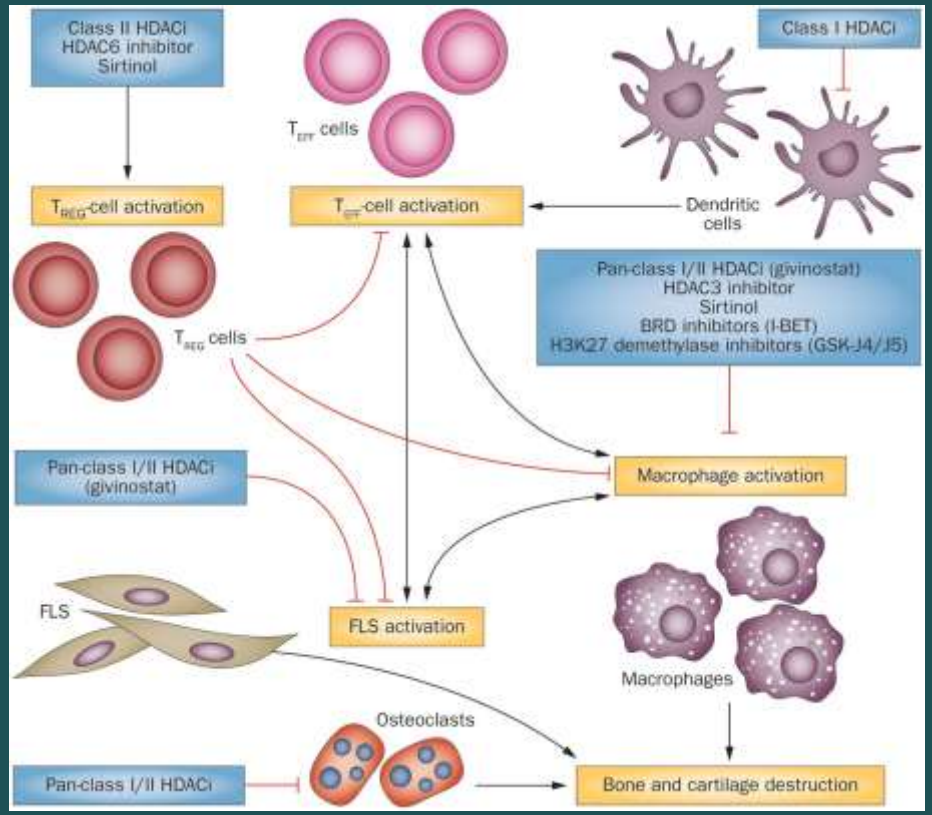
Am J Respir Crit Care Med. 2012

Epigenetic Inhibitors as Cancer Therapy



Cell *150*, July 6, 2012 ©2012

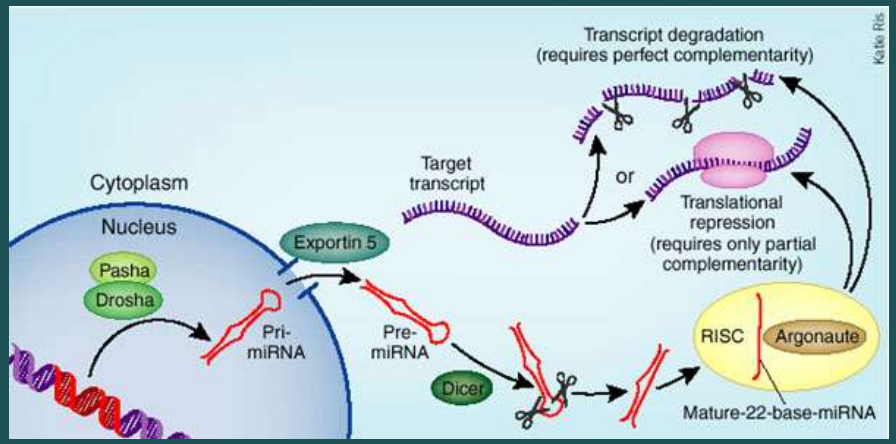
Targeting the Epigenetic Regulatory Machinery – Effects on Rheumatoid Arthritis Cell Populations

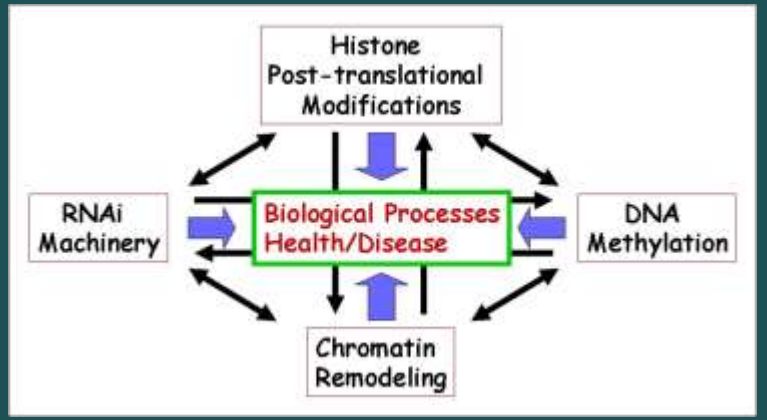


Grabiec, A. M. & Reedquist, K. A. (2013) *Nat. Rev. Rheumatol.*

Role of microRNA in Epigenetics and Implications for Human Disease

- Nonprotein-coding RNAs (~ 1000s in human genome)
- Regulate gene expression by disrupting function of mRNA
- Modulate activity of many cells (immune, sensory neurons, glia) involved in inflammation and nociception
- Estimated that up to 30% of genes regulated by microRNA





George S Mack
Nature Biotechnology **25**, 631 - 638 (2007)

MicroRNA-101 Targets MAPK Phosphatase-1 Blocks Effect of Dexamethasone

Elevated levels of MKP-1 - protective against chronic inflammation

Decrease inflammation and pain following tissue injury

Control effects of TNF and cytokines

The Good Guys!

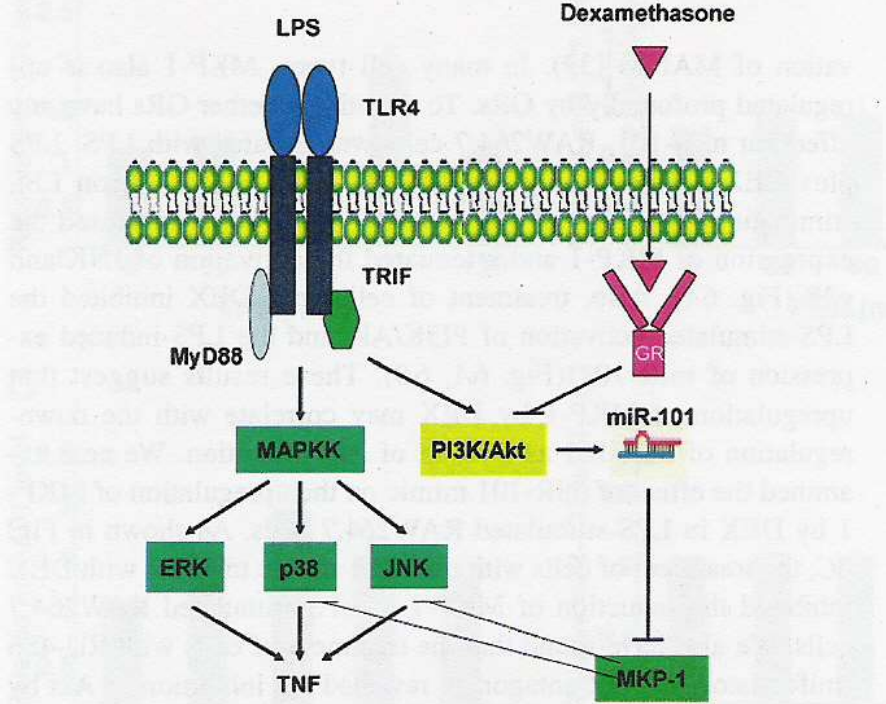


FIGURE 7. Diagram depicting the hypothesized signaling pathways for miR-101 in the regulation of MKP-1.

Zhu et al, J Immunol. 2010 Dec 15;185(12):7435-42

Epigenetically Active Drugs in Development

Table 1 Epigenetically active drugs and their mechanisms

Epigenetics Mechanism	Drug	Action	Clinical Use	Comments
Histone deacetylase inhibitor	Valproic acid	Inhibits classes I and II HDAC	Seizures, pain	Effective for migraine prophylaxis
	Givinostat	Inhibits classes I and II HDAC	Juvenile idiopathic arthritis	Effective in human arthritis trial
	Tricostatin A (TSA)	Inhibits classes I and II HDAC	Laboratory only	Produces analgesia in animal models. Enhances μ-opioid receptor transcription
	Suberoylanilide hydroxamic acid (SAHA)	Inhibits classes I HDAC	Laboratory only	Produces analgesia in animal models
DNA methylation	Glucosamine	Prevents demethylation of IL-1β gene promoter	Arthritis pain	Common clinical use; effect on IL-1β reduces inflammatory cytokine production
	Valproic acid	Induces demethylation of reelin promoter	Seizures, pain	Reelin modulates NMDA function and pain processing
	L-methionine	Induces methylation at glucocorticoid receptor promoter gene	Dietary supplement	Alters experimental stress response; used as dietary supplement for arthritis
RNA interference	siRNA targeted to NMDA receptor subunits	Gene silencing of NR1 and NR2 subunits of NMDA	Experimental	Produces analgesia in animal models
	siRNA to P2X3	Gene silencing of P2X3	Experimental	Produces analgesia in animal models; no observed neurotoxicity with intrathecal use
	siRNA to TNF-α	Gene silencing of TNF-α	Experimental	Produces analgesia in animal models

Buchheit, Van de Ven, Shaw Pain Medicine, 2012

Emerging Therapeutic Strategy: Regulation of the Epigenome

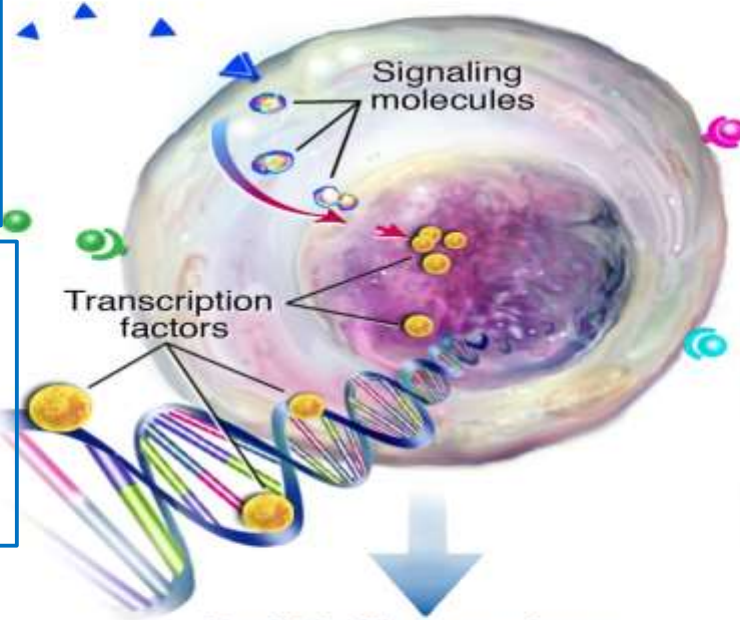
- Epigenetic modifications and enzymes – potential basis for new therapeutics
- Several drugs approved by FDA to treat particular types of hematological cancer
- Many others in development
- Diagnostic tests for diseases or syndromes with epigenetic components
- Utilize genetic and epigenetic information –

Goal of Precision Medicine

Epigenetics and Gene Activation
for Improved Health and Longevity

Excercise
- BDNF

Nutritional Factors
- Calorie Restriction
- Mediterranean Diet
- Polyphenols



Environment
- Clean air, water and soil
- No smoking

Emotional Health
- Religion
- Meditation
- Spirituality

Anti-Inflammatory
Anti-oxidant, Anti-mutation

Summary

1. Epigenetics
   - changes in gene expression caused by environmental factors (diet, sleep, exercise, exposure to drugs/toxins)

   - implicated in pathology of inflammatory and non-inflammatory diseases

2. Epigenetic modifications are dynamic and reversible

3. Importance of epigenome in underlying pathology of diseases of the head and face likely as significant as mutations in genome

4. While one's predisposition to a particular disease is largely heritable, disease progression is strongly influenced by life style and environmental factors