

Overview of epigenetics section

- *Review of Plant Meristematic Cell Suspension Culture Technology - a source of unique methylation patterns*
- *Benefits of Meristematic Cell Suspension Cultures*
- *Examination of Epigenetic Methylation via Human Epigenomic Arrays*
- *Summary*

Uses of plant tissue culture

Screening of cells for beneficial characteristics

- Plant breeders may look for a high content of an active

Meristem tip culture

- Produces plant material free from viruses, often for plants propagated vegetatively

Forestry and floriculture

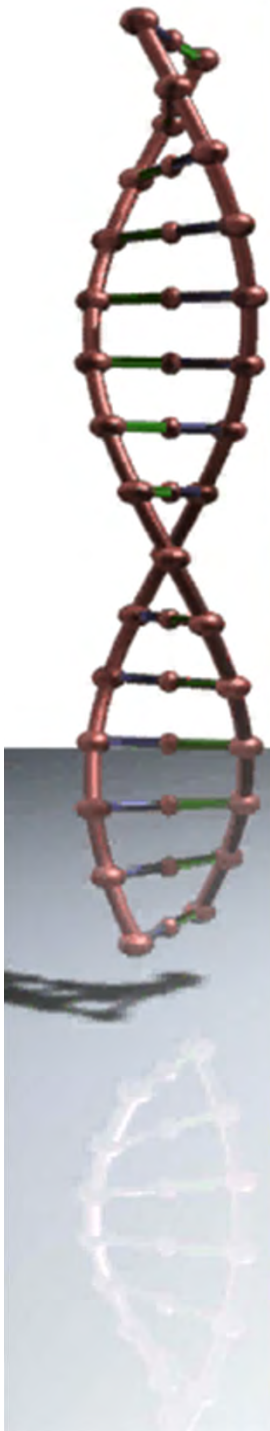
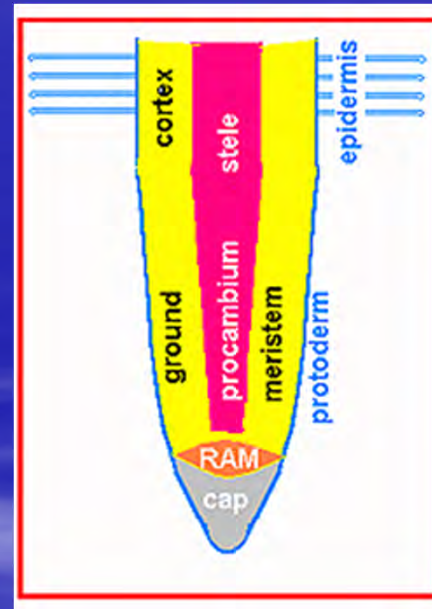
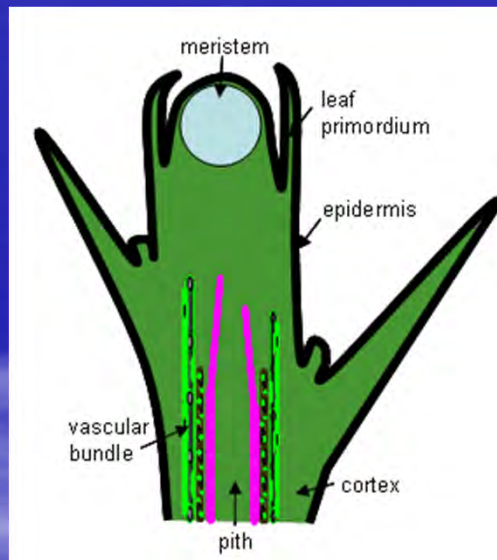
- For conservation of rare and endangered plant species

Large-scale growth of plant cells as a source of secondary metabolites



Review of Meristematic Cell Culture Technology

- Meristem – tissue in plants that contain undifferentiated cells, occurs at the shoot and root apex
- Callus – Mass of undifferentiated cells



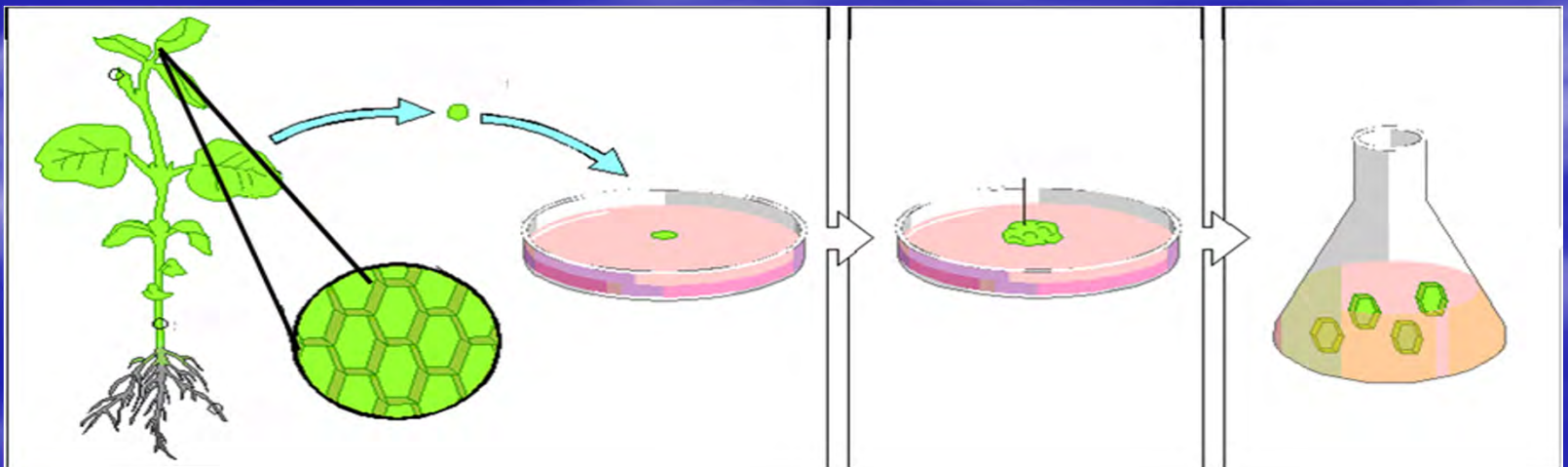
Review of Meristematic Cell Culture Technology

- Meristem – tissue in plants that contain undifferentiated cells, occurs at the shoot and root apex
- Callus – Mass of undifferentiated cells
- Totipotent – ability of a cell to produce all of the differentiated cells in an organism
- Suspension culture – liquid media in which the plant cells grow

Tissue sample from adult plant is cultured

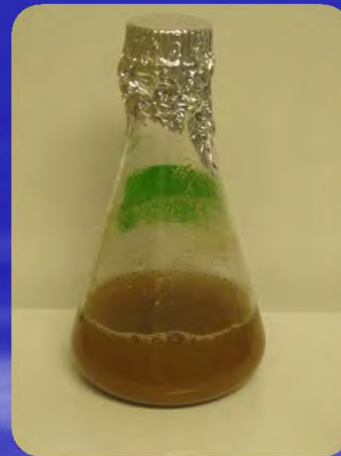
Undifferentiated callus forms

Callus separated / single cells cultured



Plant Meristematic Suspension Culture Scale-Up

Overview of Product Development



Plant callus → ***Shaker Flask*** → ***Bioreactor***

Plant Meristematic Cell Cultures Combine Two Current Technologies

Biotech-derived
compounds
(fermentation)

Natural Bioactives
(Plant extracts)

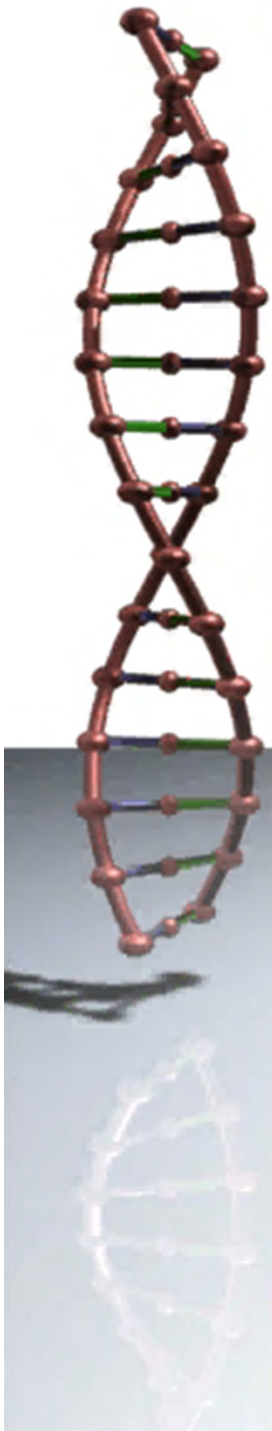
Growing organisms
in bioreactors



Botanicals and their
natural bioactives



Plant Meristematic Cell Cultures



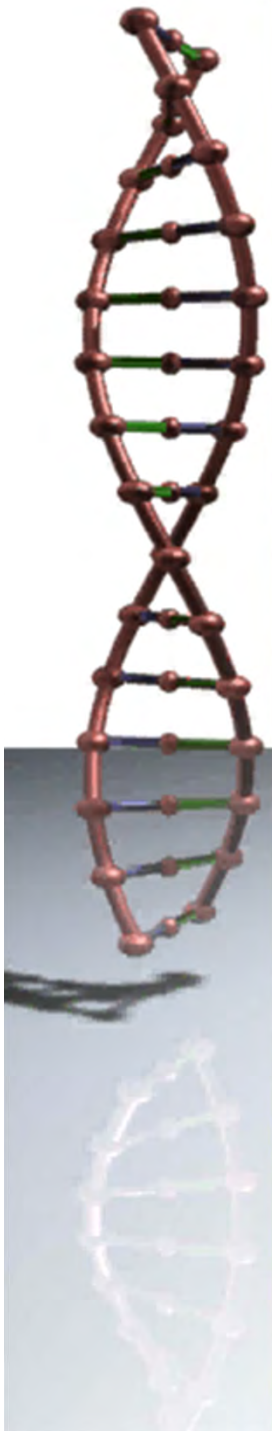
Rice meristem culture: the concept

Plant tissue culture:
Undifferentiated cells

Elicitation:
Increases secondary metabolites and actives

Rice culture

Epigenetic DNA modification:
Rejuvenation and renewal of cells



Benefits of Meristematic Suspension Cultures

Access to rare and hard to obtain plants

- Opens frontier to new actives

Easier way to procure uniform botanicals

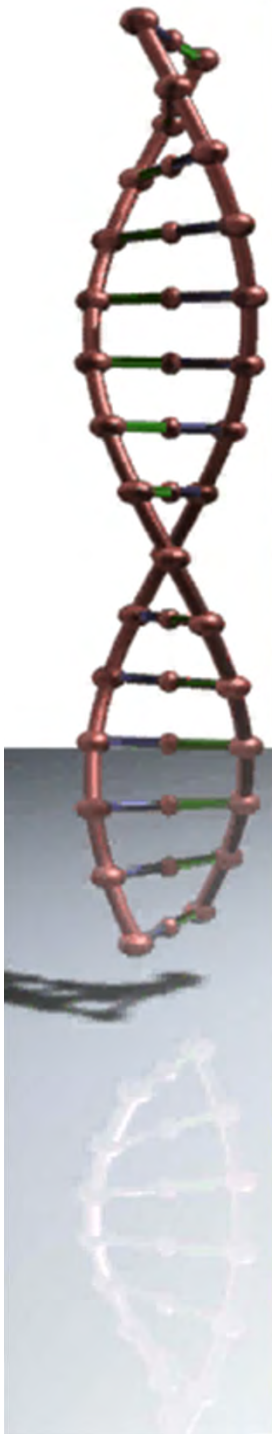
- No environmental variation in weather, sunlight, soil and water

Very reproducible biomass and concentration of actives

- Allantoin
- Tea & EGCG



Tacca chantrieri

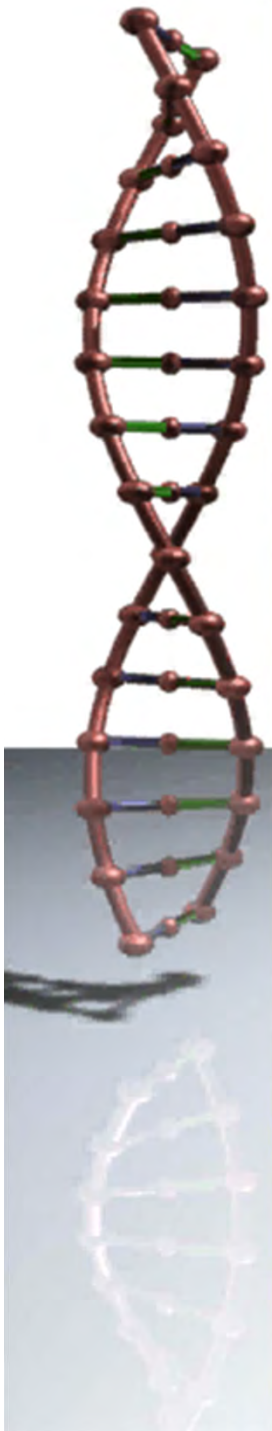


Benefits of Meristematic Suspension Cultures

- More environmentally responsible - Green technology
 - Prevents depletion of wild-grown plants that may be scarce
- Enables growth of plants under conditions otherwise unattainable in a field
 - Defensive stress
 - Higher concentration of actives
- A natural product
 - Just as yeast fermentation is considered natural, so are plant suspension cultures



Trillium



Benefits of Meristematic Suspension Cultures

- Ability to harvest epigenetic and transcription factors and novel plant compounds not produced or produced in minute quantities in whole mature plants
- Meristematic cultures enable harvest of proteins and other compounds that would degrade too quickly from traditional harvest plant



Welwitschia mirabilis



Cryptocereus anthonyanus



Wollemia nobilis

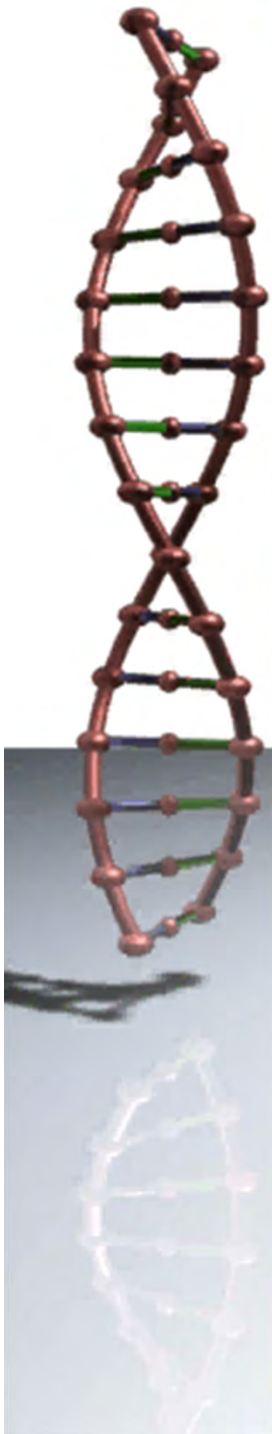
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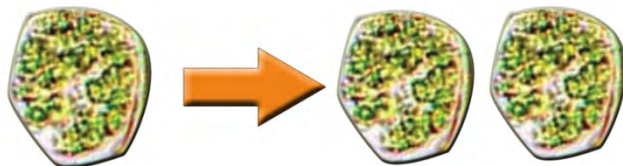


Efficient production of actives needs elicitation

- Undifferentiated cells primarily grow, not produce actives
- In order to increase secondary metabolite production, elicitation is needed.
- Elicitors can include ozone and specific chemicals
- Cells containing no actives will have little benefit in topical application

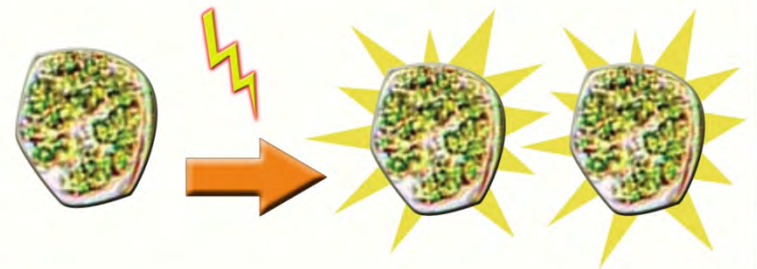
Untreated cells

Cells primarily grow and divide

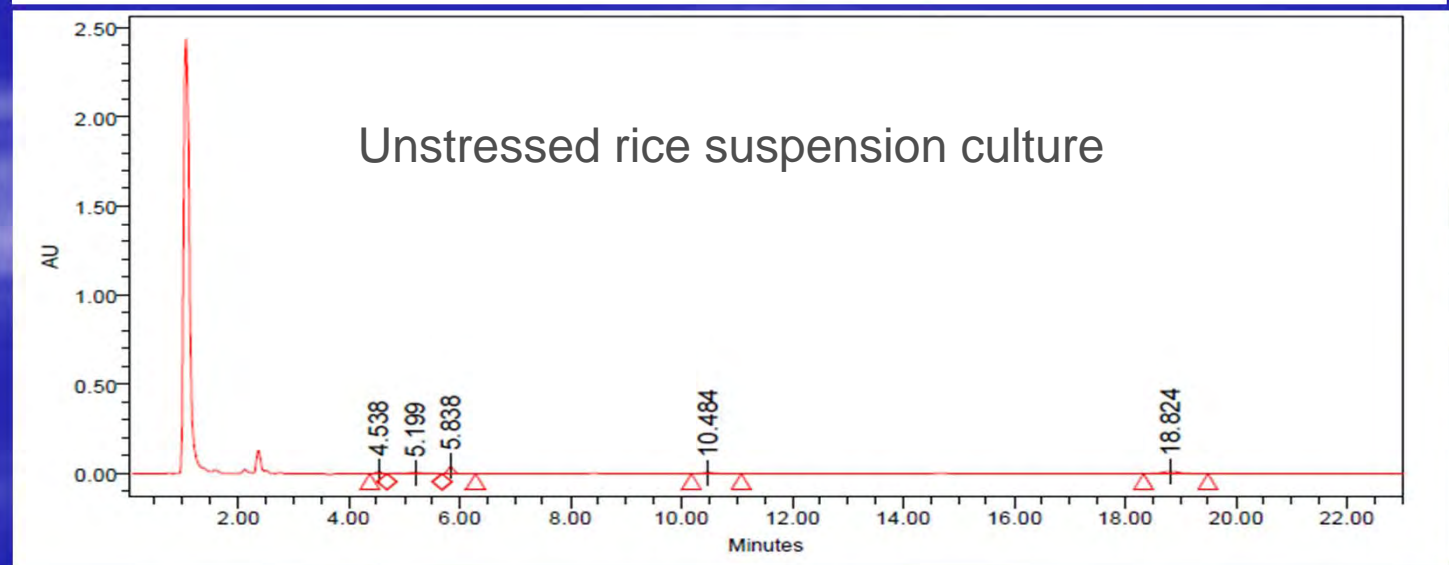
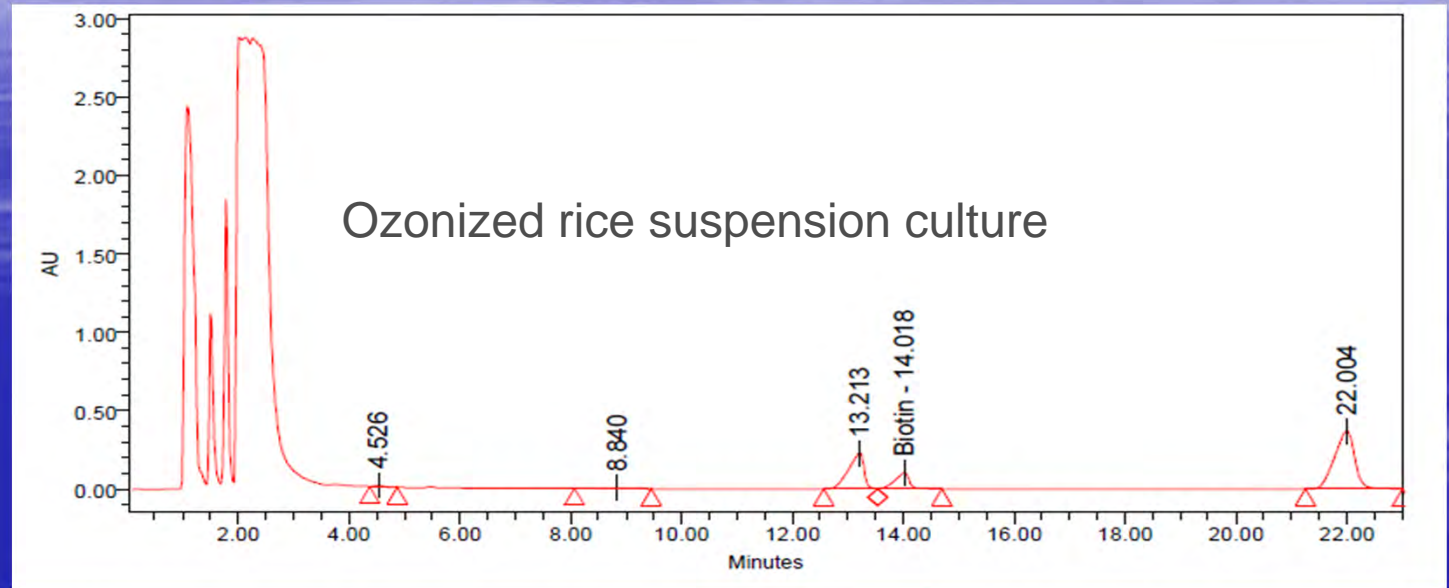
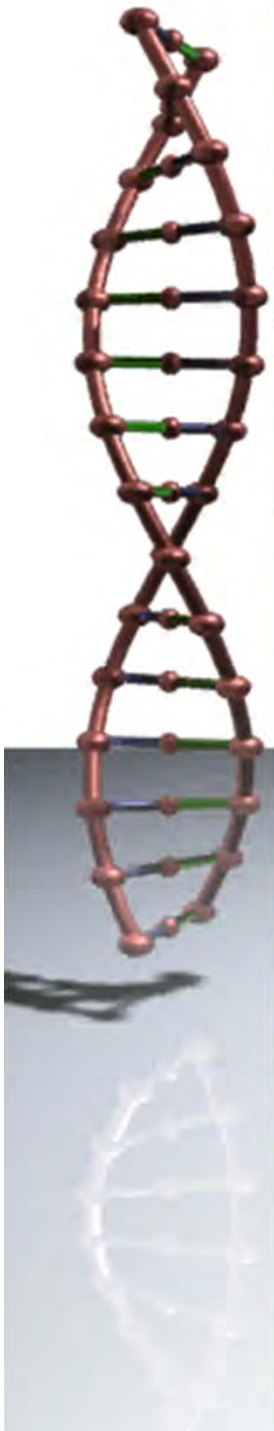


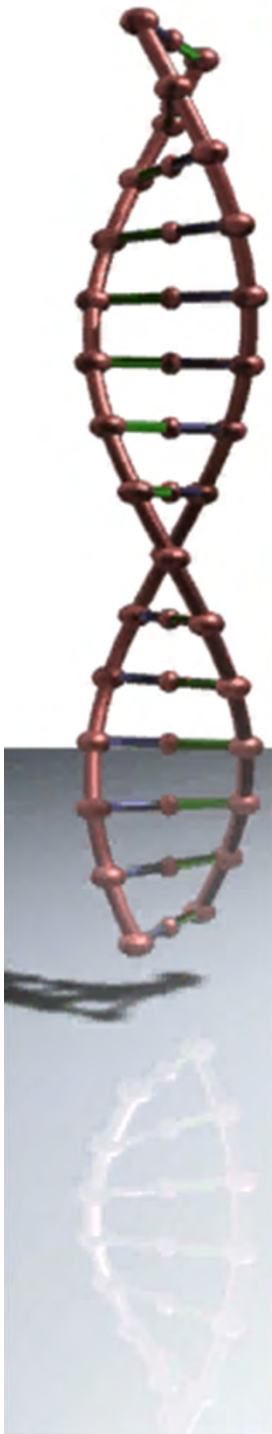
Elicited cells

Cells produce secondary metabolites



Elicitation of actives through use of ozone





Overview of epigenetics section

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- Benefits of Meristematic Cell Suspension Cultures
- Examination of Epigenetic Methylation via Human Epigenomic Arrays
- Summary



The Emerging Evidence of the Influence of Epigenetics on Aging

PLoS Genet. 2010 May 27;6(5):e1000971.

Aging and chronic sun exposure cause distinct epigenetic changes in human skin.

Grönniger E, Weber B, Heil O, Peters N, Stäb F, Wenck H, Korn B, Winnefeld M, Lyko F.

Antioxid Redox Signal. 2011 Jan 15;14(2):241-59. Epub 2010 Nov 22.

Epigenetic control of aging.

Muñoz-Najar U, Sedivy JM.

Exp Gerontol. 2010 Apr;45(4):255-68. Epub 2010 Jan 10.

Experimental approaches to the study of epigenomic dysregulation in ageing.

Thompson RF, Fazzari MJ, Greally JM.

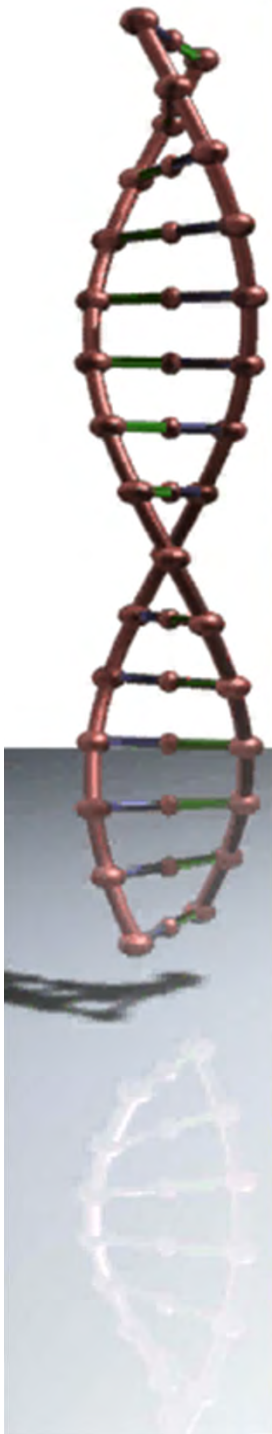
Pflugers Arch. 2010 Jan;459(2):247-58. Epub 2009 Sep 19.

Epigenetic factors in aging and longevity.

Gravina S, Vijg J.

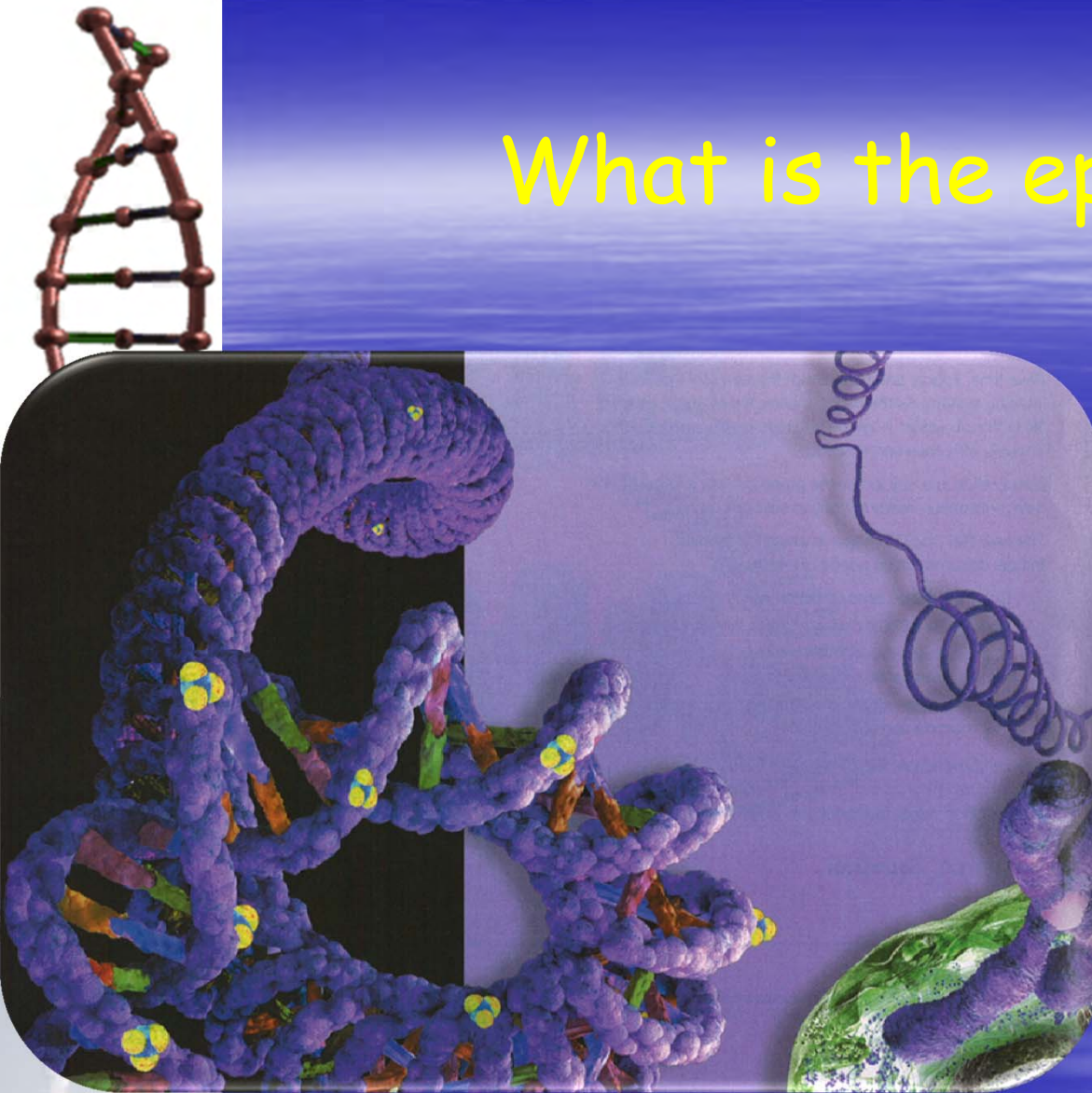
What is epigenetics?

- Definition: heritable changes in gene expression that occur by a mechanism other than changes to the DNA sequence
- The mechanism by which cells “remember”
- How does a cell and its progeny remember that they are skin cells and not nerve cells? Through epigenetics
- Epigenetics plays a part in:
 - Cellular differentiation
 - Development
 - Aging
 - Disease
 - Differences between identical twins
- The “youth switch”



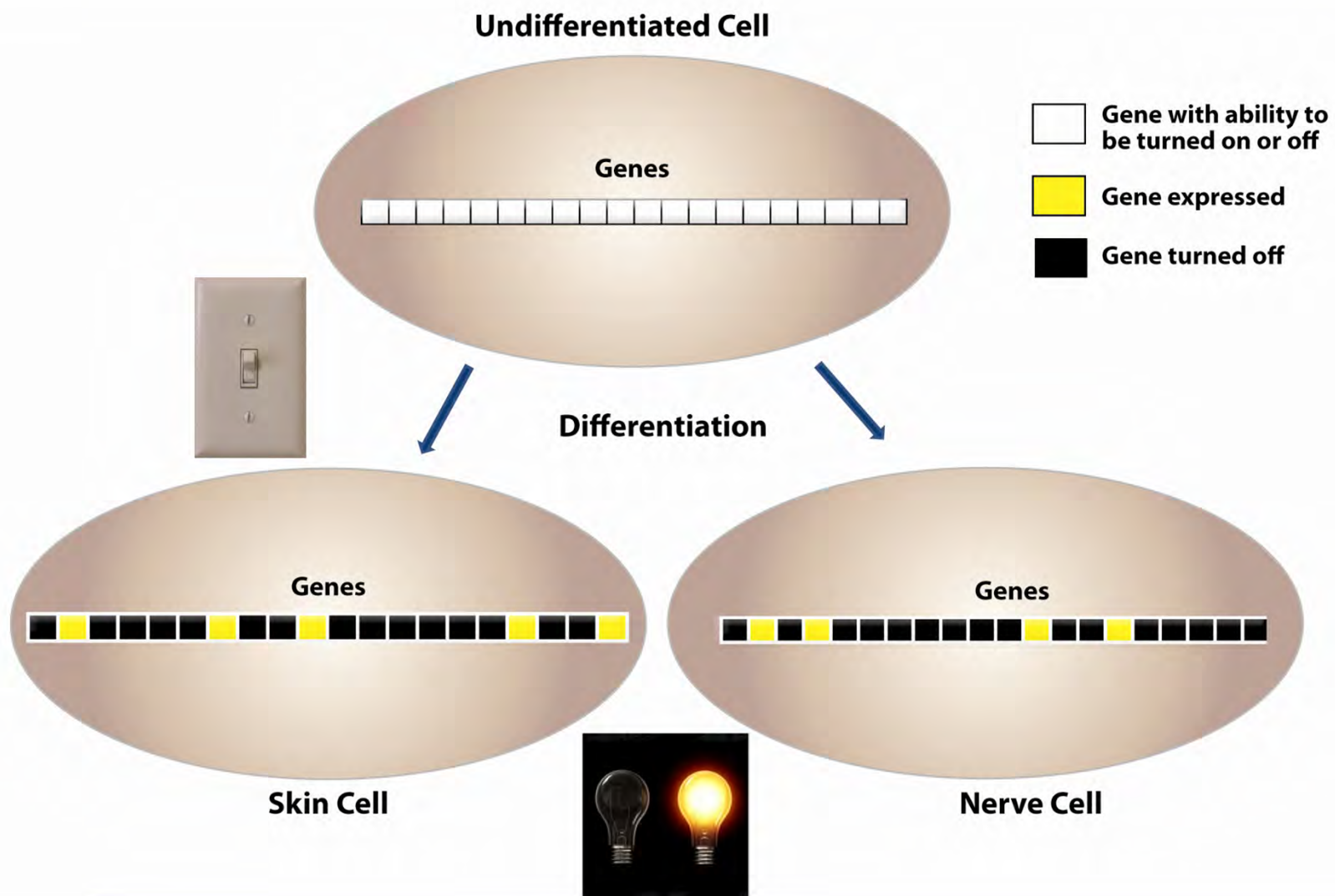
What is the epigenome?

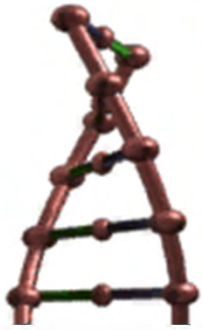
- Epigenetics is a heritable “switch” that controls how well a gene is able to pass its messages via RNA synthesis.
- This controlling switch simply confers a mechanism by which the DNA wraps around its histones and so “packs” into the nucleus.



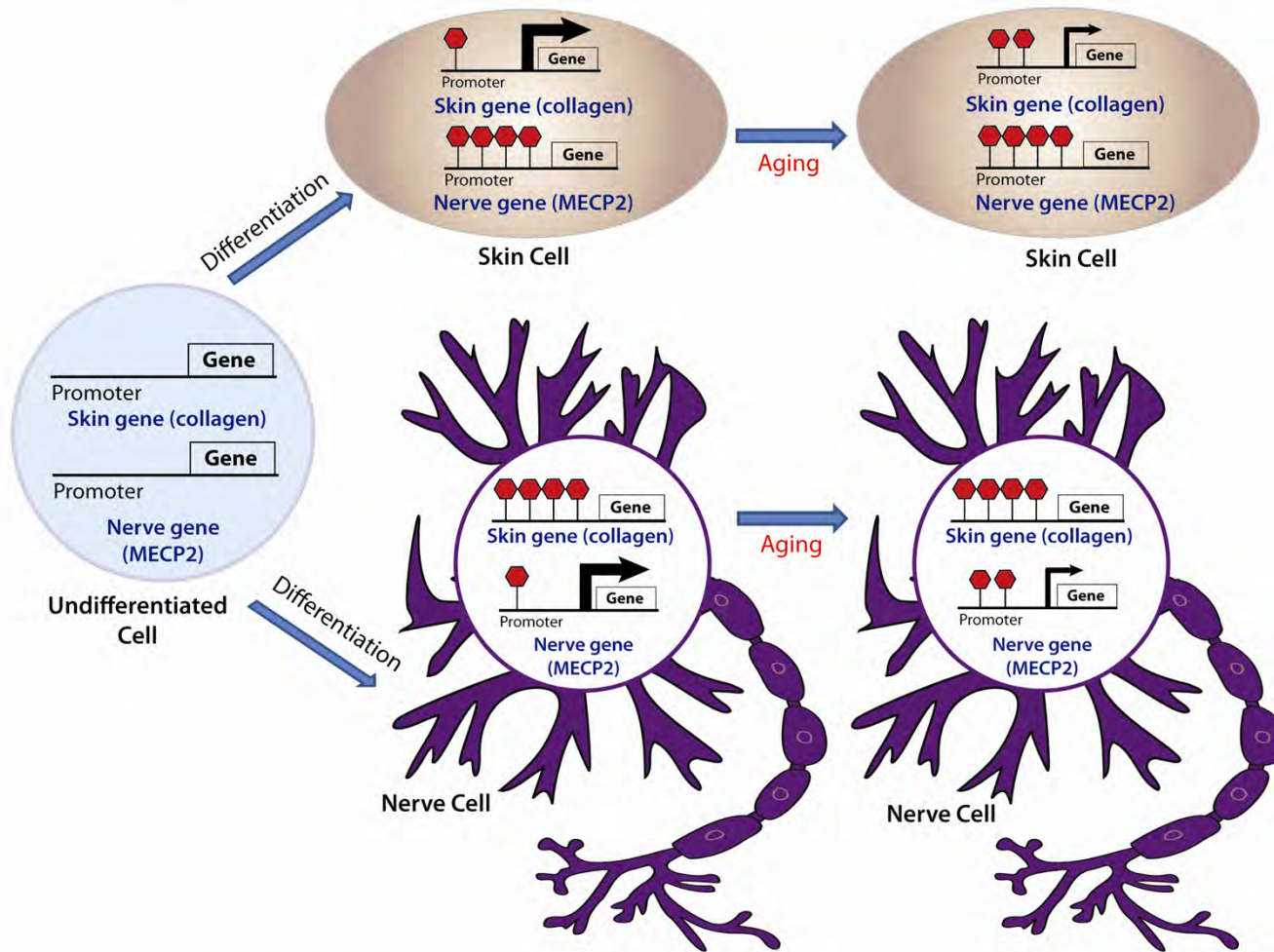


Gene expression and differentiation





Methylation, Differentiation, and Aging

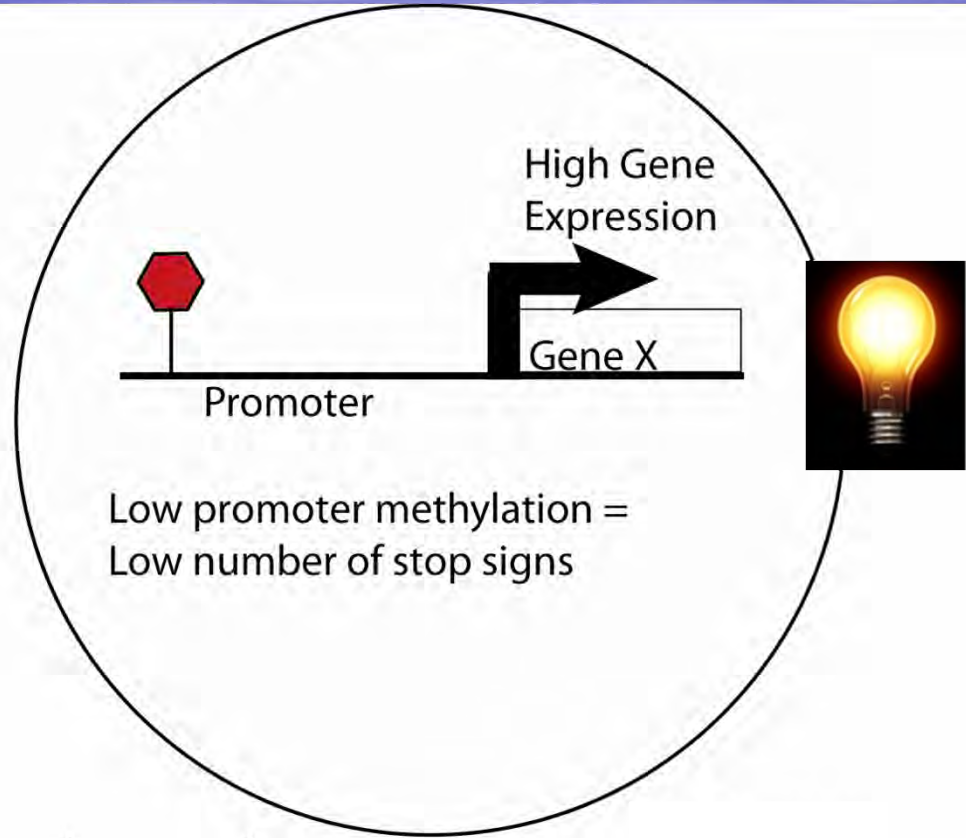


Cells from different types of tissue have different genes expressed. As aging occurs, methylation of the gene promoters increases, deregulating the cell's initial gene expression patterns.

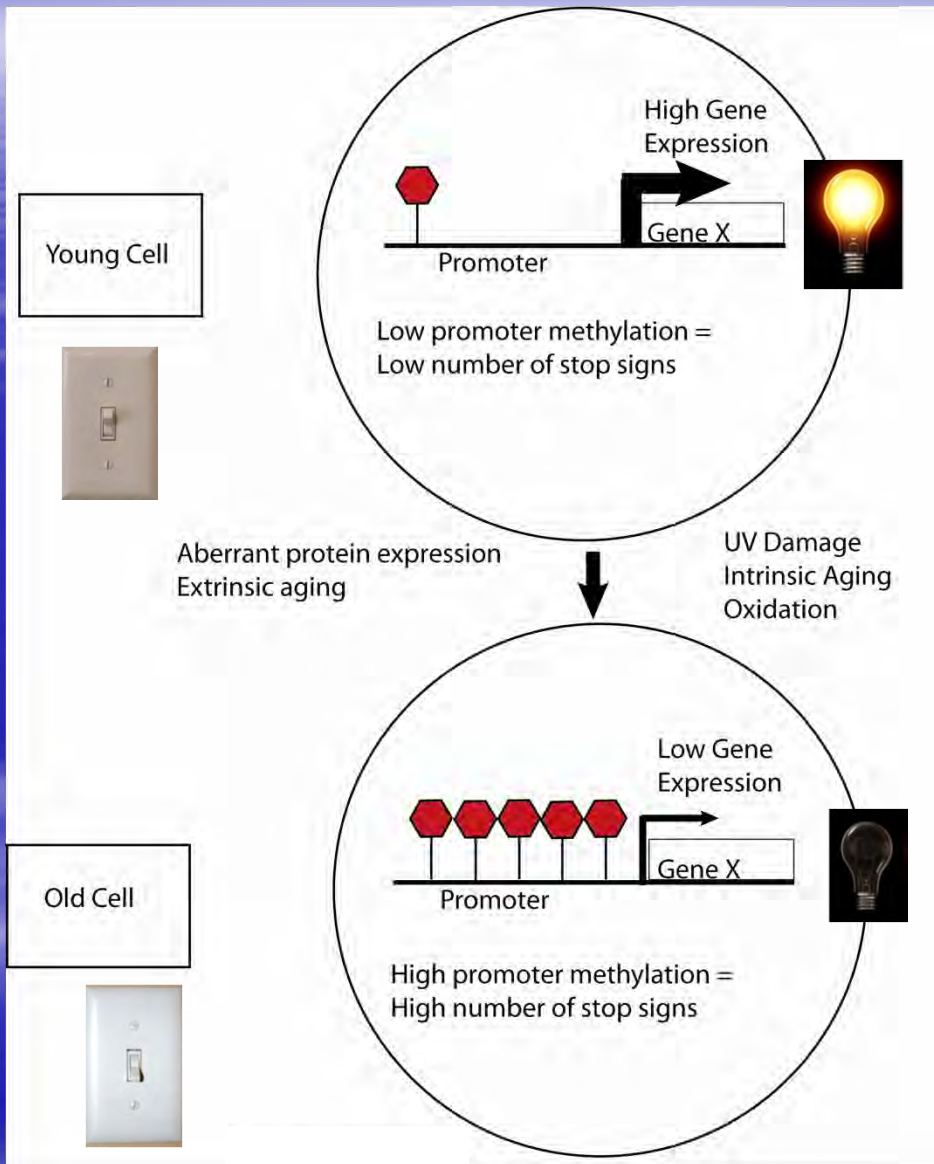
Aging and epigenetic changes



Young Cell



Aging and Epigenetic Changes



As cells age, the regions of the genome known as promoters become progressively more methylated resulting in diminishment of gene transcription (the ability of the gene to transfer its protein assembling message to the RNA).



Aging and Epigenetic Changes Literature References

Aging and Environmental Exposures Alter Tissue-Specific DNA Methylation Dependent upon CpG Island Context

Brock C. Christensen^{1,2}, E. Andres Houseman^{2,3}, Carmen J. Marsit¹, Shichun Zheng⁴, Margaret R. Wrensch⁴, Joseph L. Wiemels⁵, Heather H. Nelson⁶, Margaret R. Karagas⁷, James F. Padbury⁸, Raphael Bueno⁹, David J. Sugarbaker⁹, Ru-Fang Yeh⁵, John K. Wiencke⁴, Karl T. Kelsey^{1,2*}

PLoS Genetics August 2009 | Volume 5 | Issue 8 |

Dynamic changes in the human methylome during differentiation

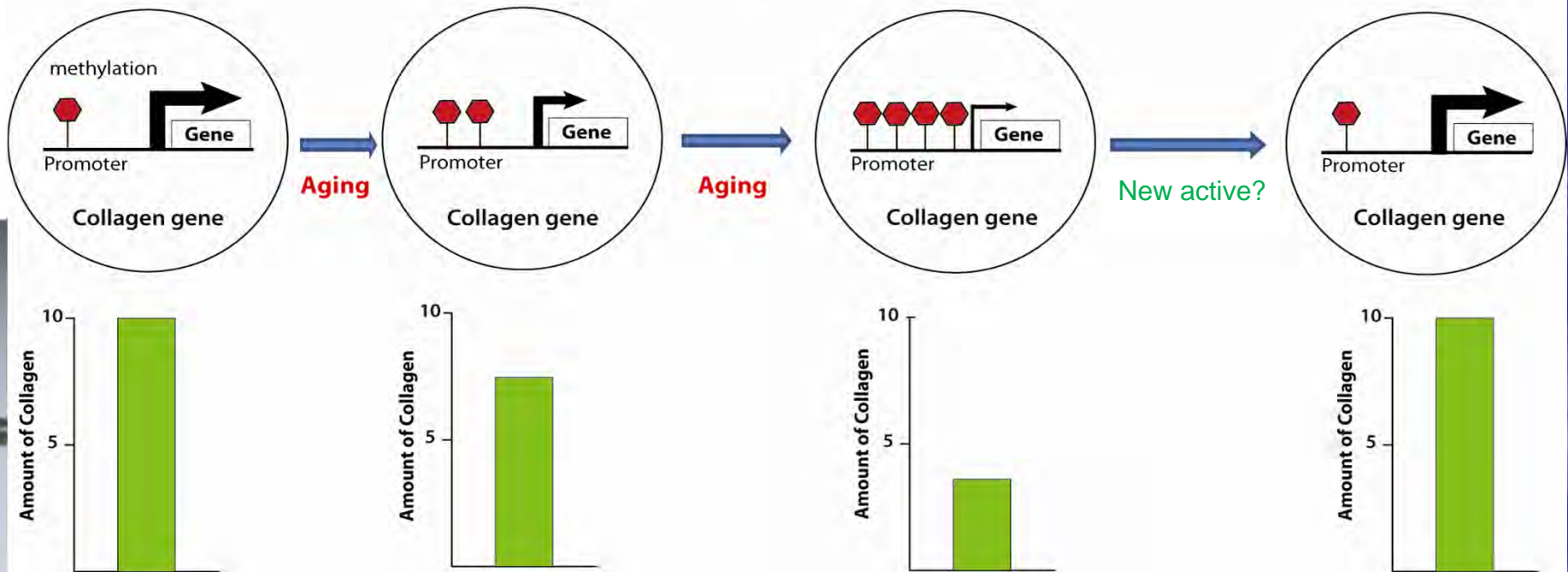
Louise Laurent, Eleanor Wong, Guoliang Li, et al.

Genome Res. 2010 20: 320-331 originally published online February 4, 2010



Correlating methylation to protein production

AGING OF SKIN CELLS



As a gene promoter becomes methylated, the gene is expressed less, leading to a decrease in protein production. A new active was desired to be able to modulate the methylation patterns and decrease methylation, hence increasing protein production.

Testing Epigenetic Changes



Cells

DNA Extraction
Methylated DNA
Isolation

Fluorescent labeled
DNA applied onto
microarray chip



Fluorescent Data
Measured and
Analyzed

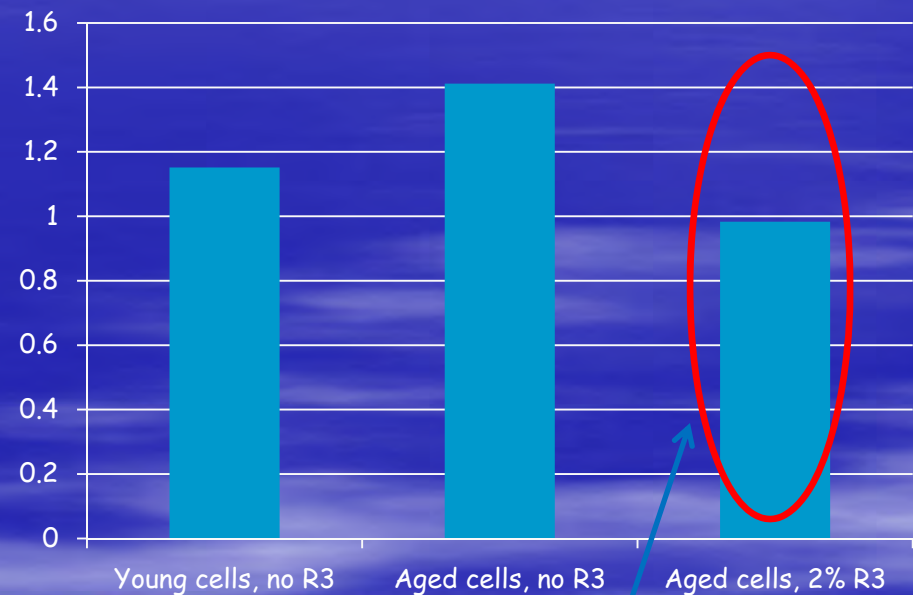
- Fibroblasts were aged both intrinsically (8 population doubling) and extrinsically (UVB). Some cells were treated with 2% of a meristematic rice extract (R3).
- DNA was extracted and examined for CpG methylation at the promoter regions of the genome and at specific genes

in vitro CpG methylation assay: genome wide promoters

Treatment Regime:

- 1. Non-Aged Cells (harvested after a few days in culture)
- 2. Intrinsic Aging + Extrinsic Aging
 - Fibroblasts taken through period of 8 cell culture passages with repeated UVB exposure to produce intrinsic and extrinsic aging
- 3. Intrinsic Aging + Extrinsic Aging + 2% Red Rice culture.

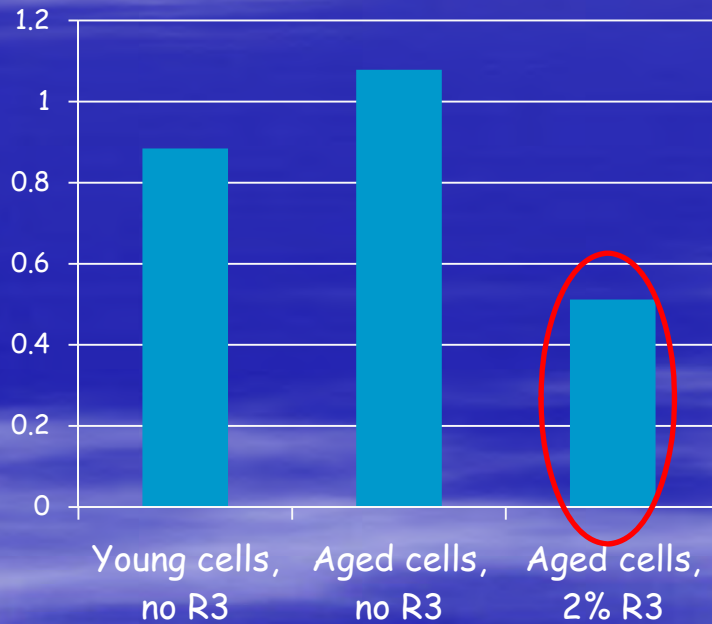
Average CpG methylation at all gene promoters genome wide



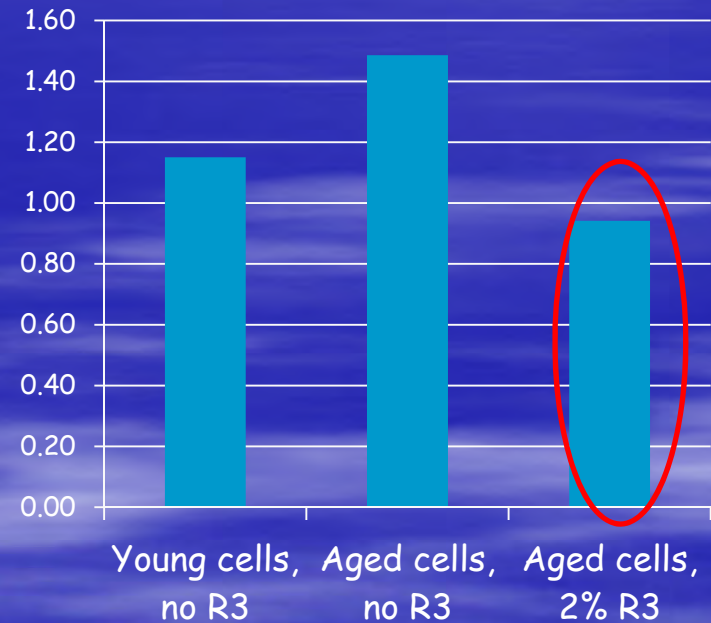
Red Rice culture was able to decrease age related CpG promoter methylation genome wide, rejuvenating the cells. Reducing methylation increases the gene's ability to express transcribe proteins. Shown as ratio to average CpG promoter methylation of GAPDH.

in vitro CpG methylation assay: Collagen1A promoters

Average CpG methylation
at Collagen1A1 gene
promoter



Average CpG methylation
at Collagen1A2 gene
promoter

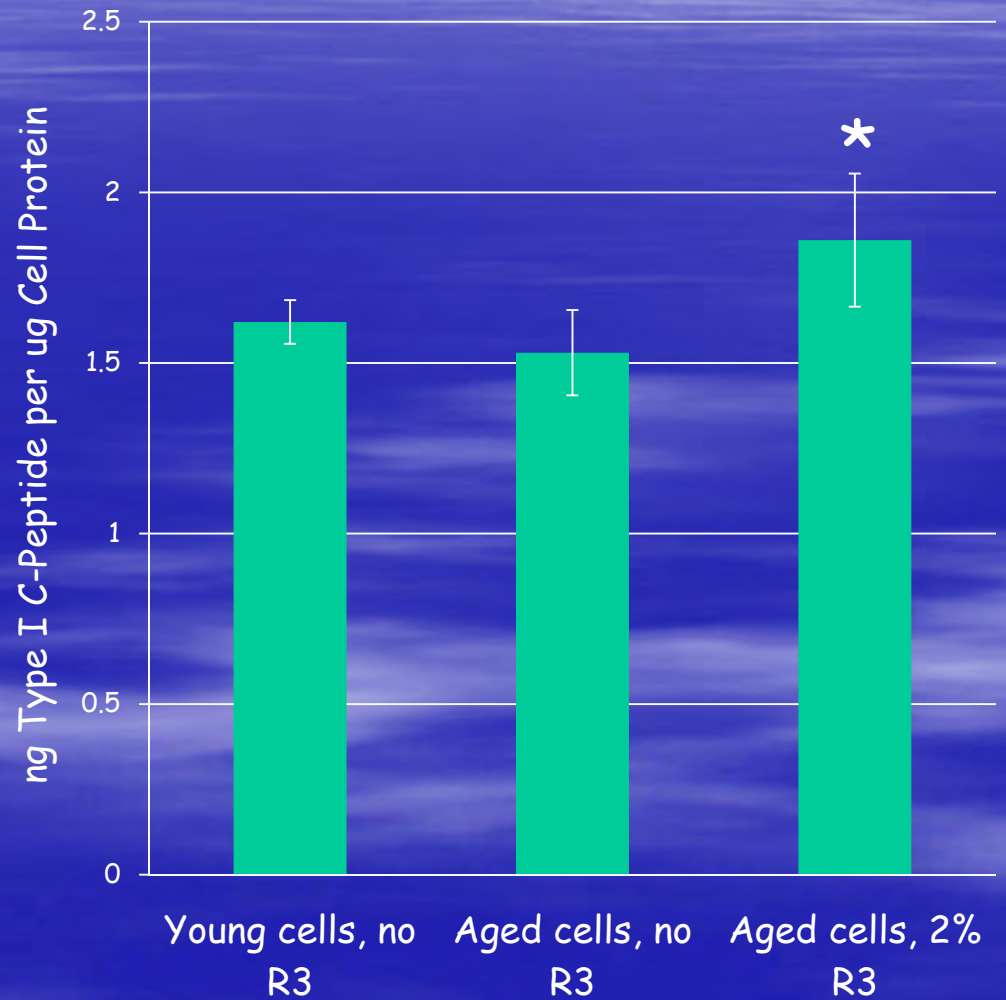


R3 meristematic rice extract was able to decrease age related CpG promoter methylation in the Collagen 1A1 and 1A2 gene promoters. Shown as ratio to average CpG methylation of GAPDH gene promoter.

in vitro collagen Protein assay

Expression of Collagen Type 1A is markedly increased in the cells treated with meristematic rice extract. This increase could translate into skin that appears more firm and with less wrinkles.

Collagen 1A protein levels





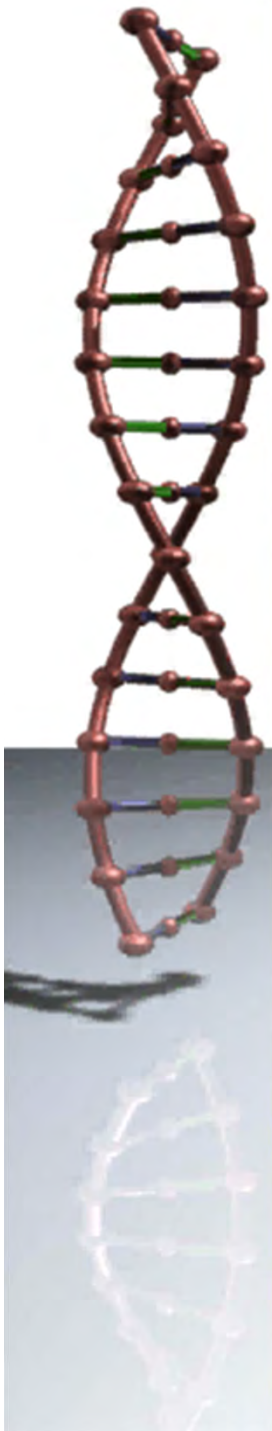
Collagen gene methylation and aging; Literature support

Age-dependent alterations in mRNA level and promoter methylation of collagen $\alpha 1(I)$ gene in human periodontal ligament

Masaki Takatsu ^a, Shinji Uyeno ^{b,1}, Jun-ichiro Komura ^b,
Makoto Watanabe ^a, Tetsuya Ono ^{b,*}

Mechanisms of Ageing and Development
110 (1999) 37–48

The data suggest the possible importance of alterations in collagen $\alpha 1(I)$ gene expression and its DNA methylation in promoter region in age-dependent degeneration of periodontal ligament. © 1999 Elsevier Science Ireland Ltd. All rights reserved.



Summary of epigenetic results

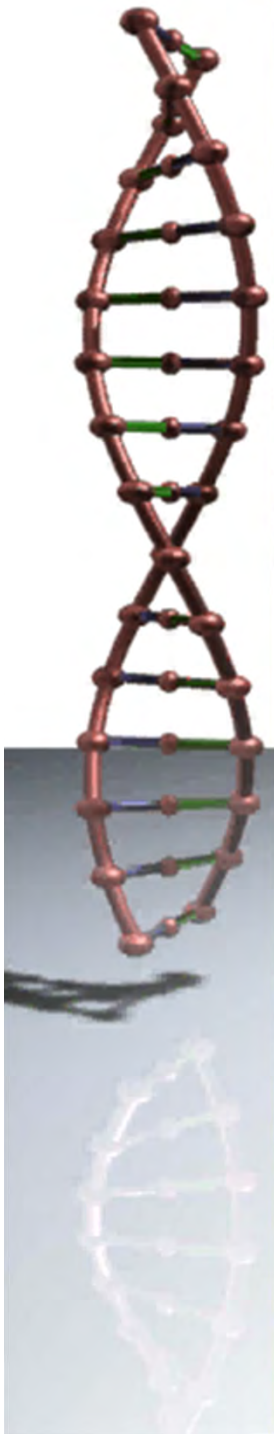
- The emerging science related to epigenetics is rapidly demonstrating that how we live our lives can actually influence how our skin cells age.

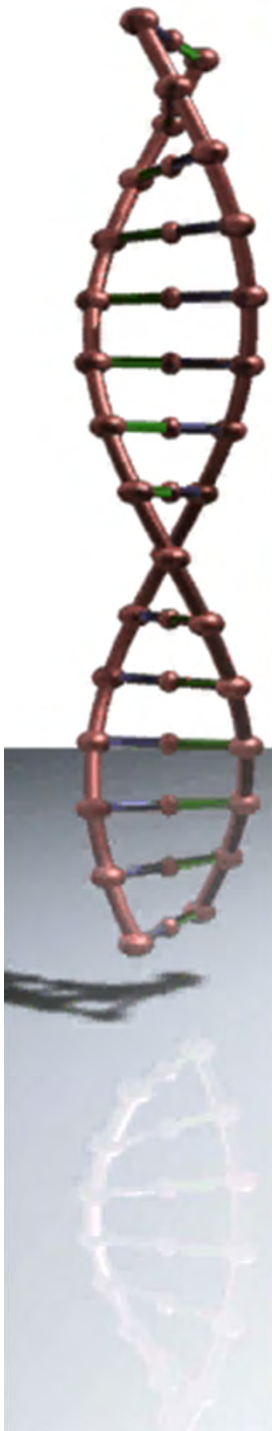
The epigenome is like a switch that can turn a gene on or turn it off. It appears that as we age, the switches within our skin cells tend to be more frequently turned "off". However, these changes can be moderated with ingredients that can diminish promoter methylation.

As more knowledge of epigenetic effects on skin aging become known, this will become a target of more intensive research and product development.

Overall Conclusions

- Human microarrays can help guide research into the effects of skin ingredients on skin cells
- While not always directly matching, gene expression and protein expression usually correlate (Mother Nature doesn't waste time)
- There may be common pathways that certain well known ingredients influence to improve skin health
- Sometimes, results can be surprising and somewhat unexpected such as the skin lighteners influencing tyrosinase and ferritin gene and protein expression
- The epigenome is like a switch that can turn a gene on or turn it off. As we age, the switches within our skin cells tend to be more frequently turned "off". However, these changes can be moderated with ingredients that can diminish promoter methylation.
- New findings can lead to new directions for ingredient developments



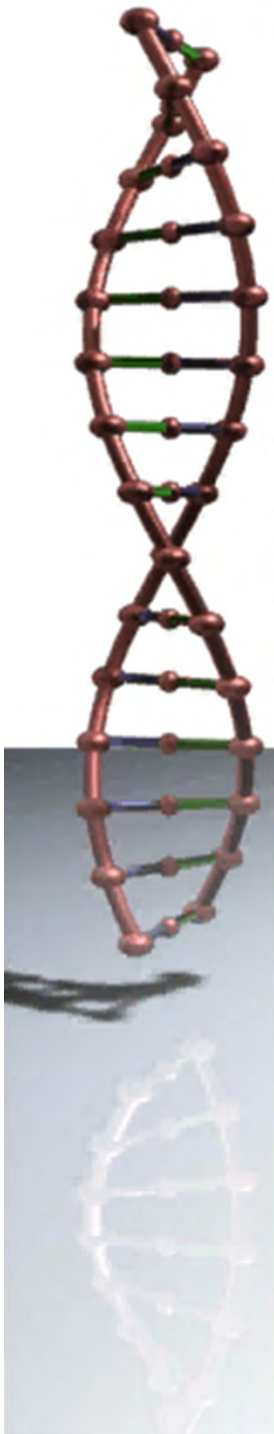


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SOCIETY OF COSMETIC CHEMISTS

SCC



THANK YOU