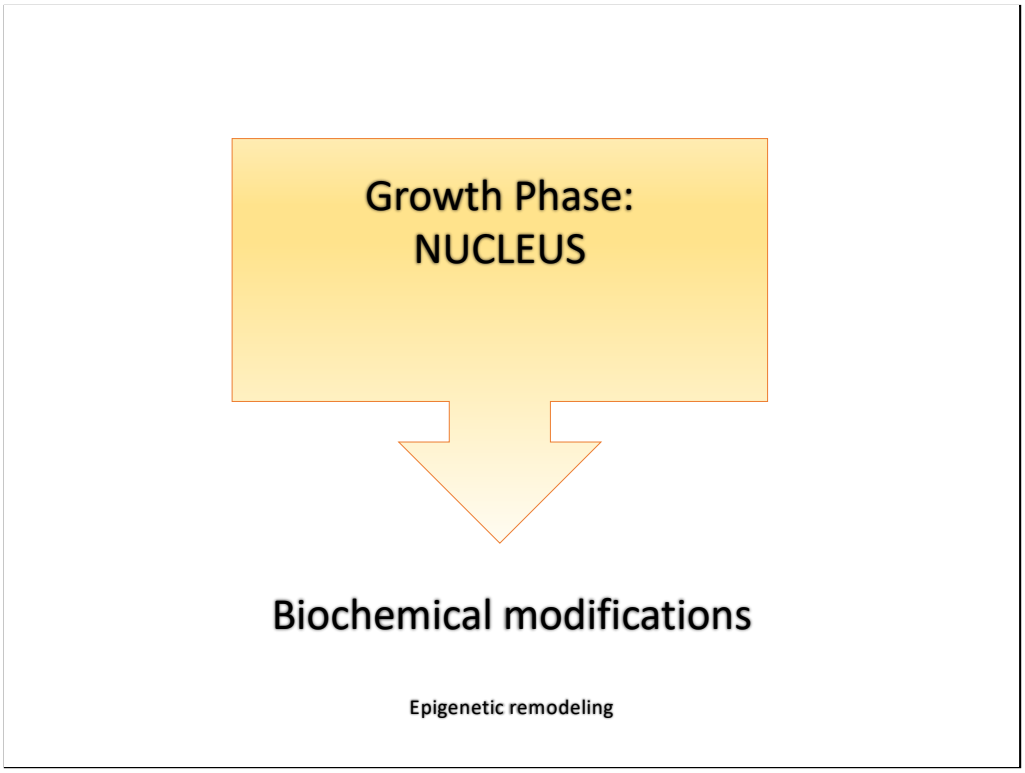
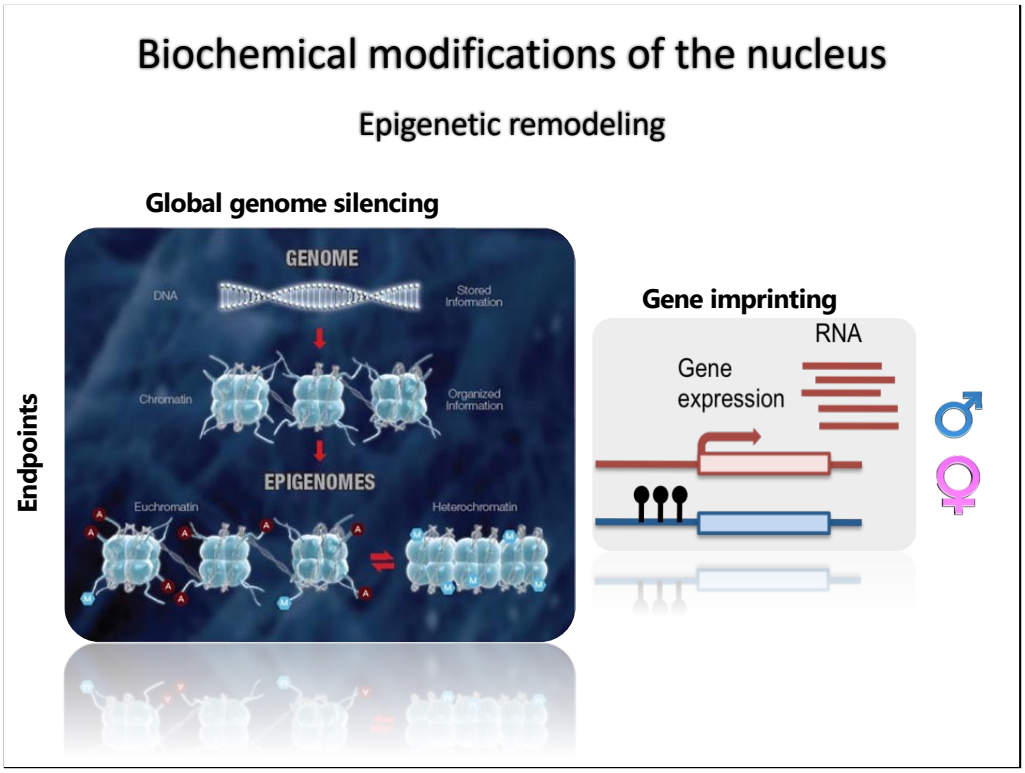


Biochemical modifications of the oocyte nucleus



An important biochemical change involving the nucleus of the oocyte during the growth phase is chromatin epigenetic remodeling. This process allow to close (heterochromatin) or open (Euchromatin) specific target regions of the DNA.

Epigenetic remodeling aims to two mainly functional endpoints:

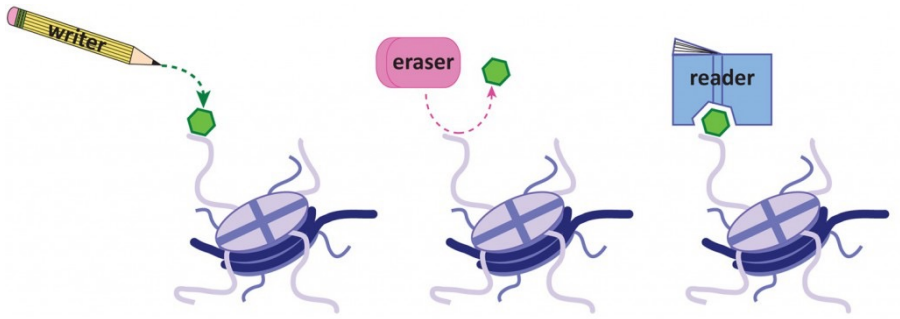
1. The first one is to silence at global level the maternal genome transcription, a phenomenum that must occur before fertilization

2. The second process involves the permanent suppression of gene expression on a specific class of genes. This gene category is referred to as primary imprinted genes, and their inhibition initiates during gametogenesis, persisting from fertilization throughout adult life.

Which epigenetic mark could be involved in genome silencing?

Macromolecular complexes work together to remodel chromatin.

- **DNA MODIFYERS**
- **HISTONE MODIFYERS**



Some example:

Acetiltransferases	Deacetiltransferases	Bromodomanins
Methyltransferases	Demethyltransferases	Cromodomains
Kinases	Phosphatases	Etc…
Ubiquitinases	Deubiquitinases	

In epigenetics, "writers," "erasers," and "readers" refer to proteins and enzymes involved in the modification, maintenance, and interpretation of epigenetic marks on chromatin. Here's a brief definition of each:

Writers:

Definition: Writers are enzymes responsible for adding or depositing specific chemical modifications onto chromatin, such as DNA methylation or histone modifications.

Example: DNA methyltransferases (DNMTs) are writers responsible for adding methyl groups to DNA, contributing to DNA methylation patterns

Erasers:

Definition: Erasers are enzymes that remove or reverse the epigenetic modifications present on chromatin, restoring the chromatin to its original state.

Example: Histone deacetylases (HDACs) are erasers that remove acetyl groups from histone proteins, leading to chromatin condensation.

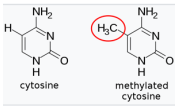
Readers:*Definition:* Readers are proteins that recognize and bind to specific epigenetic marks on chromatin. They interpret these marks, translating them into functional responses, such as gene expression or repression.

Example: Bromodomain-containing proteins are readers that specifically recognize acetylated lysines on histones, influencing chromatin structure and gene regulation.

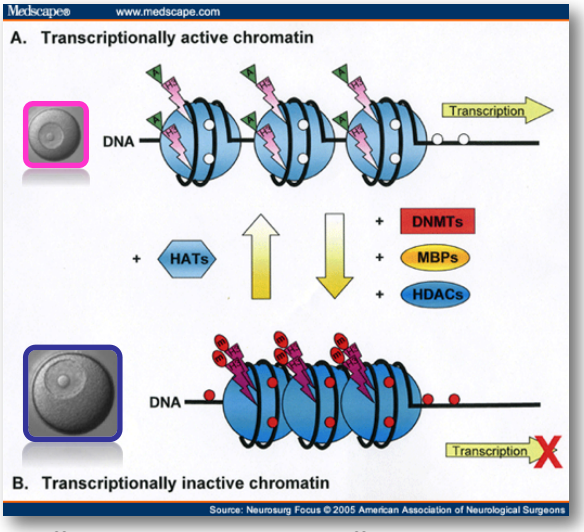
In summary, writers add epigenetic marks, erasers remove or modify these marks, and readers interpret the marks to orchestrate various cellular processes based on the epigenetic landscape of the chromatin. These processes play a crucial role in regulating gene expression, cellular differentiation, and other biological functions.

Epigenetic remodeling

DNA Methylation



DNMTs



This efficient mechanism globally affects maternal DNA, resulting in the inhibition of DNA transcription.

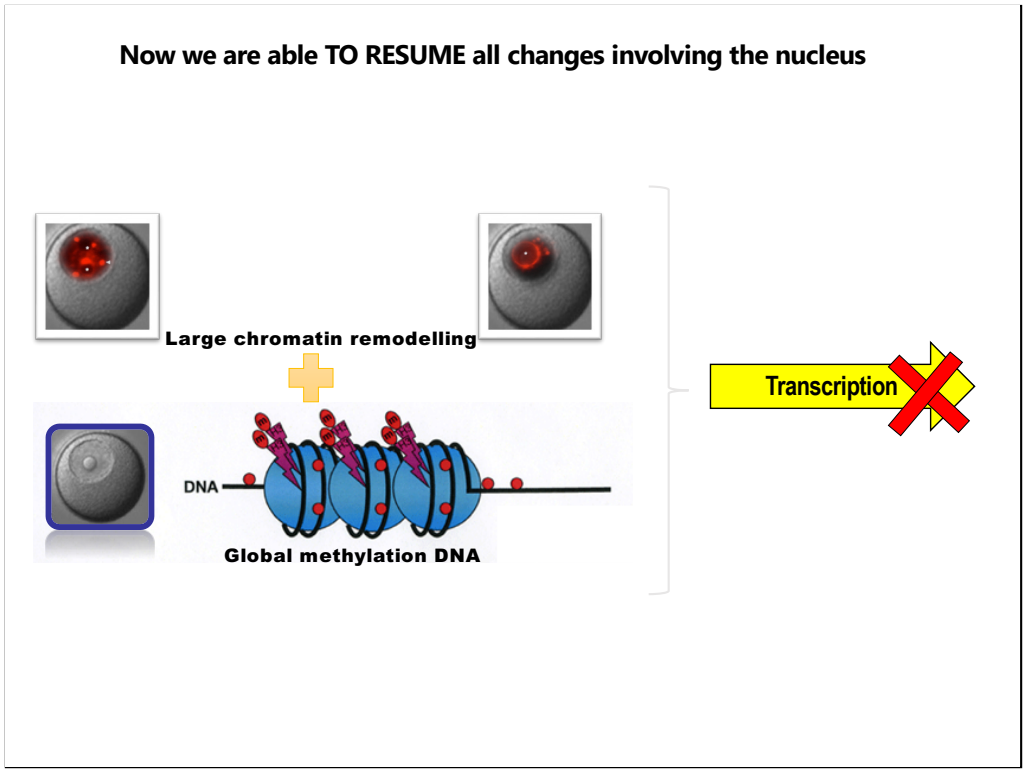
The most significant epigenetic process that involves the oocyte during the growth phase is DNA methylation.

DNA methylation is an heritable epigenetic mark that entails the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases (DNMTs), forming 5-methylcytosine.

DNA methylation is not the only epigenetic modification contributing to gene regulation. Other epigenetic processes involving histones are finely regulated simultaneously with DNA methylation, such as histone acetylation. Macromolecular complexes work together to remodel chromatin. For instance, before DNA is methylated, acetyl groups on histones are removed.

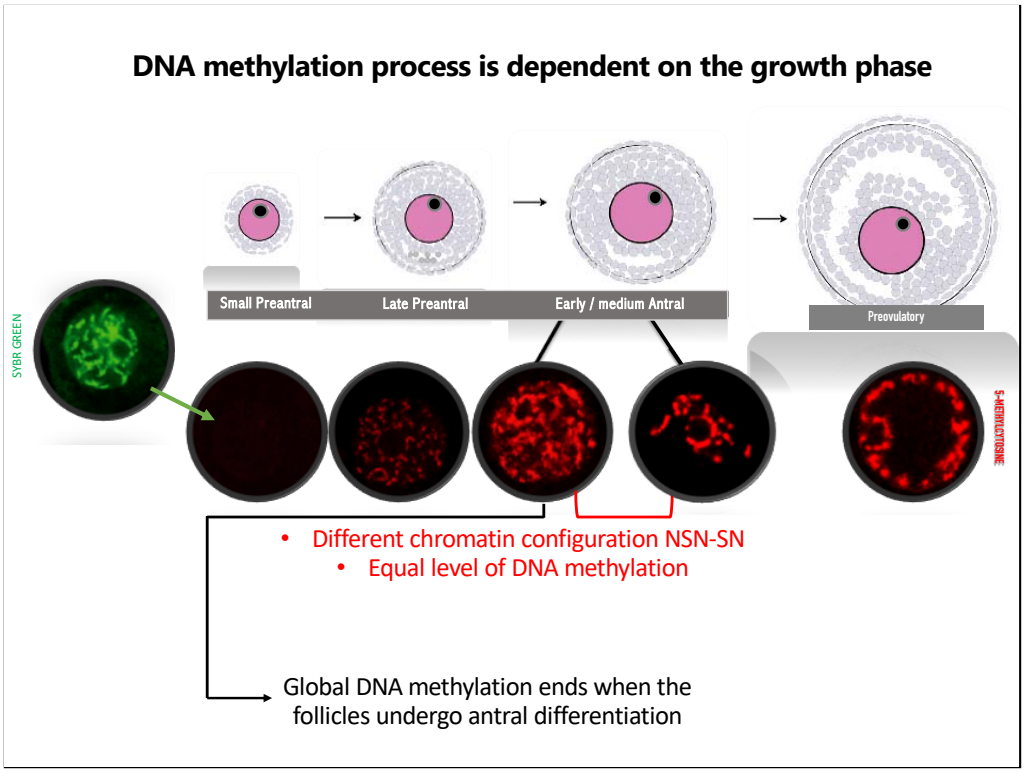
As a consequence of the insertion of methyl groups, chromatin progressively compacts, acquiring a more condensed structure that is no longer accessible to transcriptional enzymes.

This efficient mechanism globally affects maternal DNA, resulting in the inhibition of DNA transcription.



Now we are able to resume all changes involving the nucleus of the oocyte during the growing phase, which are crucial in the transformation from a transcriptionally active genome to a transcriptionally inactive one. These changes include:
Extensive chromating configuration changes (this is about localization into the nucleus)

Epigenetic remodeling (this is about DNA and histone marks)



This global DNA methylation process is strictly dependent on the growth phase, as illustrated in this slide.

As seen, we can assess the DNA methylation status through a straightforward immunofluorescent procedure, allowing the analysis of global methyl status on individual oocytes.

Materials and Methods: In detail, the degree of global DNA methylation is analyzed using a specific anti-methyl 5 cytosine antibody. This commercial antibody allows us to stain the methyl groups of DNA, visible through a secondary antibody conjugated with a red fluorescent dye (CY3). The red fluorescence in these images indicates the degree of DNA methylation within the nuclei of ovine oocytes collected from follicles at different developmental stages. A higher fluorescence signal corresponds to a higher concentration of methyl groups inside the chromatin.

Conclusions: These images demonstrate:

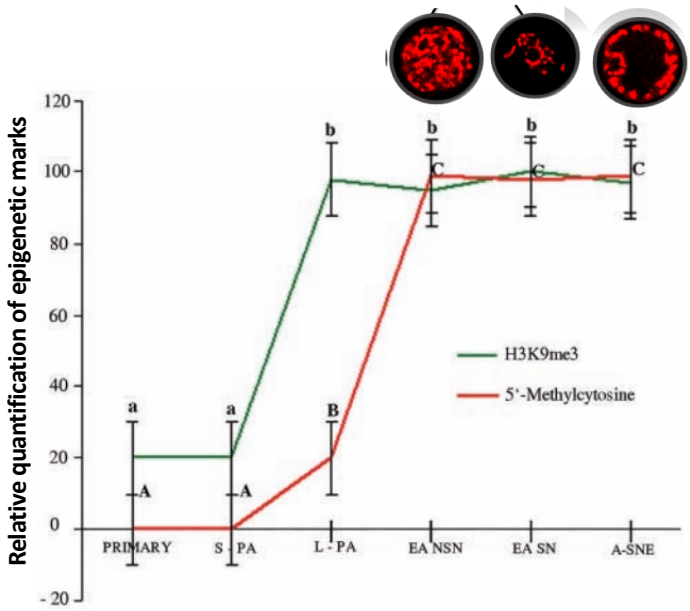
•Oocytes in the early stage of the growth phase (from primordial to small preantral follicles) are completely demethylated. No methyl groups are present in the DNA, visible exclusively with SYBR green dye. This open chromatin is freely accessible to transcriptional enzymes.

•The process of DNA methylation occurs later in oogenesis, at least in sheep. Specifically, the degree of chromatin methylation increases in nuclei of oocytes isolated from late preantral follicles, displaying low levels of fluorescence.

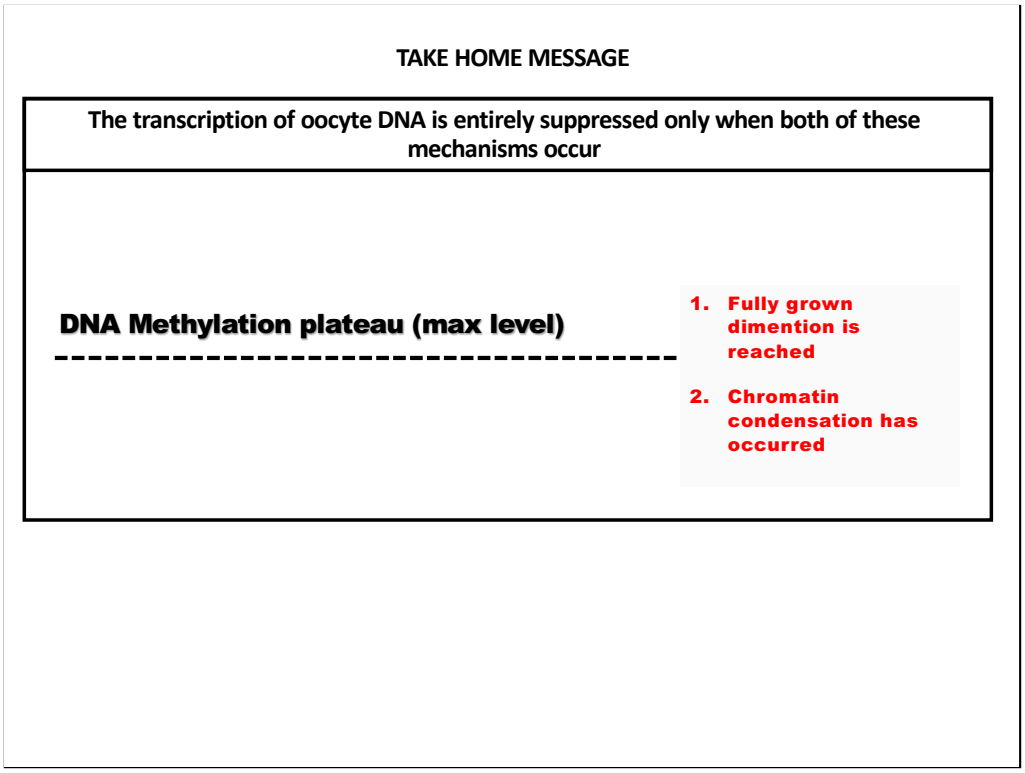
•Global DNA methylation increases and reaches a plateau of fluorescence in oocytes collected from early antral to antral follicles, independent of oocyte diameter, which increases further during the transition from early antral to medium antral follicle.

•The fluorescent signal increases in oocytes collected from early antral follicles, showing a high degree of methylation. This is not related to chromatin configuration changes from NSN to SN; both NSN and SN oocytes display a high and similar degree of methylation that does not increase further.

•Similar fluorescence intensity was recorded in oocytes from early antral follicles up to preovulatory follicles, indicating that the process of global DNA methylation ends when the follicles undergo antral differentiation. This process precedes the oocyte growth, concluding later, and occurs before the extensive chromatin remodeling, reaching the SNE configuration in medium antral follicles in sheep.



This graph provides a visual perspective on the rate of global DNA methylation accumulation, as well as the recruitment of acetylation on histone H3 at lysine 9 (H3K9me3). These marks reach a peak during antrum differentiation. There is no further increase recorded afterward, even though chromatin configuration continues to change until the preovulatory stage of the follicle.



Based on these evidence, we can summarize that the process of DNA methylation reaches its maximum level when:

- chromatin condensation is established (early antral follicles), and

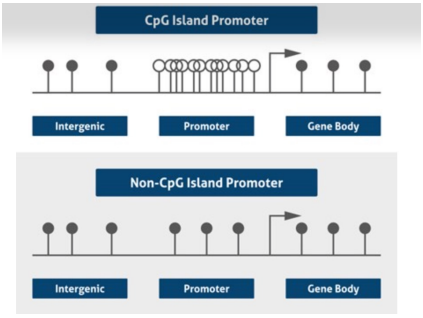
- fully-grown dimension (in medium large antral follicles) is reached.

The transcription of oocyte DNA is entirely suppressed only when both of these mechanisms occur precisely at the conclusion of the growth phase.

Methylation of CpG islands

CpG Islands Definition
1,000 base pairs long and are characterized by their elevated G+C base composition and little to no methylation under normal conditions

CpG Islands Location
Often located at or near the transcription start sites of genes.



Role of CpG Island methylation

1. Serves for the establishment of Primary Gene Imprinting
2. Is responsible for allele-specific expression based on parental origin
3. Involves a small number of genes

≠ **Global DNA methylation**

Timing of CpG Island methylation

Occurs during oocyte growth where creates germline **Differentially Methylated Regions** (gDMRs) in specific genes

Next to global DNA methylation, we find the methylation of CpG islands.

CpG islands are regions of DNA that have a high frequency of CpG sites, where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases. These islands are typically 1,000 base pairs long and are characterized by their elevated G+C base composition and little to no methylation under normal conditions. CpG islands are often located at or near the transcription start sites of genes.

CpG methylation is responsible for allele-specific expression based on parental origin. This specific methylation involves a relatively small number of genes. Methylation of CpG islands occurring during oocyte growth creates germline differentially methylated regions (gDMRs) in specific genes. Failure to establish methylation at these gDMRs can result in severe developmental abnormalities. gDMRs are also referred to as Imprinting Control Regions (ICRs).

Conversely, Global DNA methylation is responsible for the widespread methylation across the genome affecting a large number of cytosines in CpG contexts.

ROLE: gene silencing, regulating plasticity of the chromatin structure.

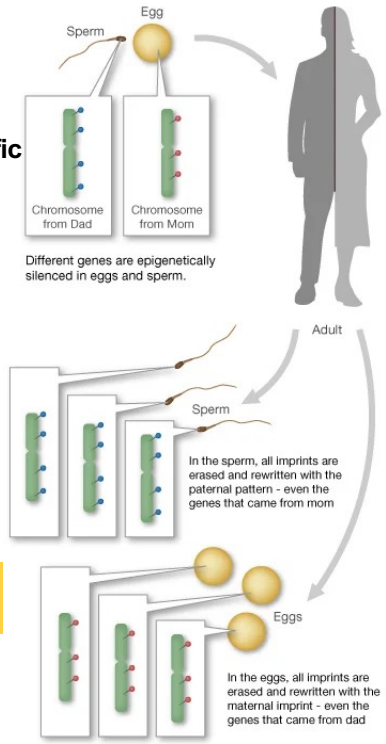
Primary imprinting

The process of parental-dependent allele-specific methylation.

In human…

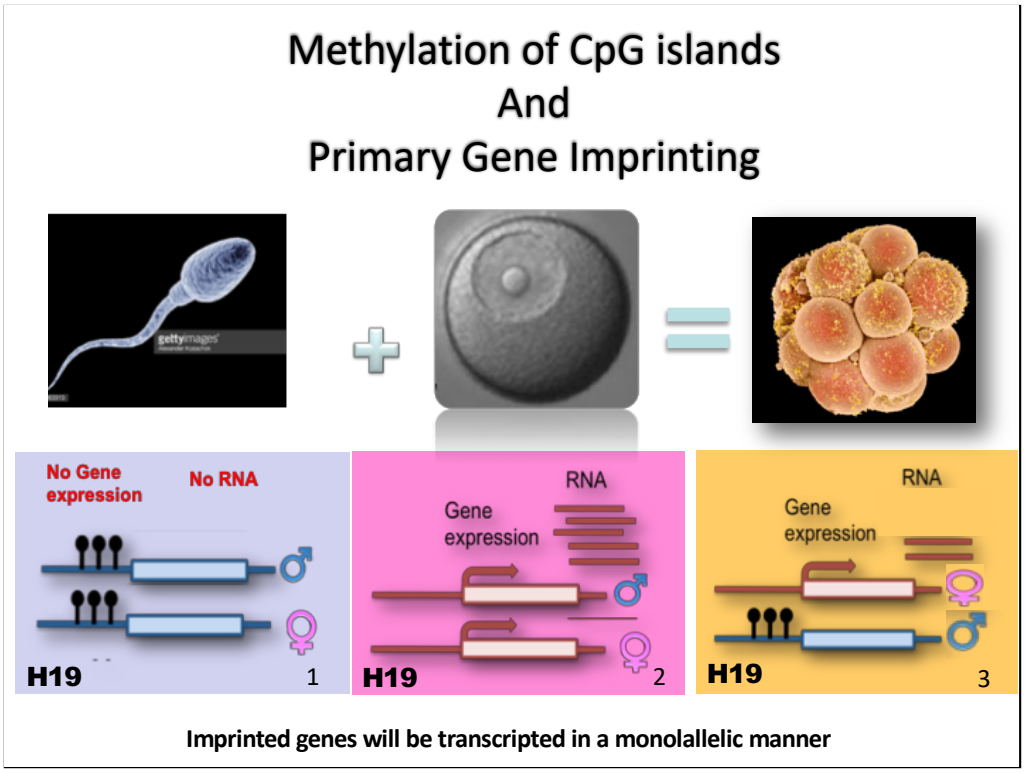
70 or so from the male parent

30 or so from the female parent



This process of parental-dependent allele-specific methylation is known as genomic imprinting. In humans, it involves around 100 different genes, with approximately 70 being imprinted from the male parent and 30 from the female parent.

Methylation of CpG islands	*vs.*	Global Methylation
Methylation occurring in C residues of CpG islands of specific genes (approx 100 genes in humans).		Widespread methylation randomically affecting several C into CpG islands.
Genome imprinting, responsible for allele-specific expression based.		Gene silencing, regulation of chromatin architecture (hetero or eu chromatin)



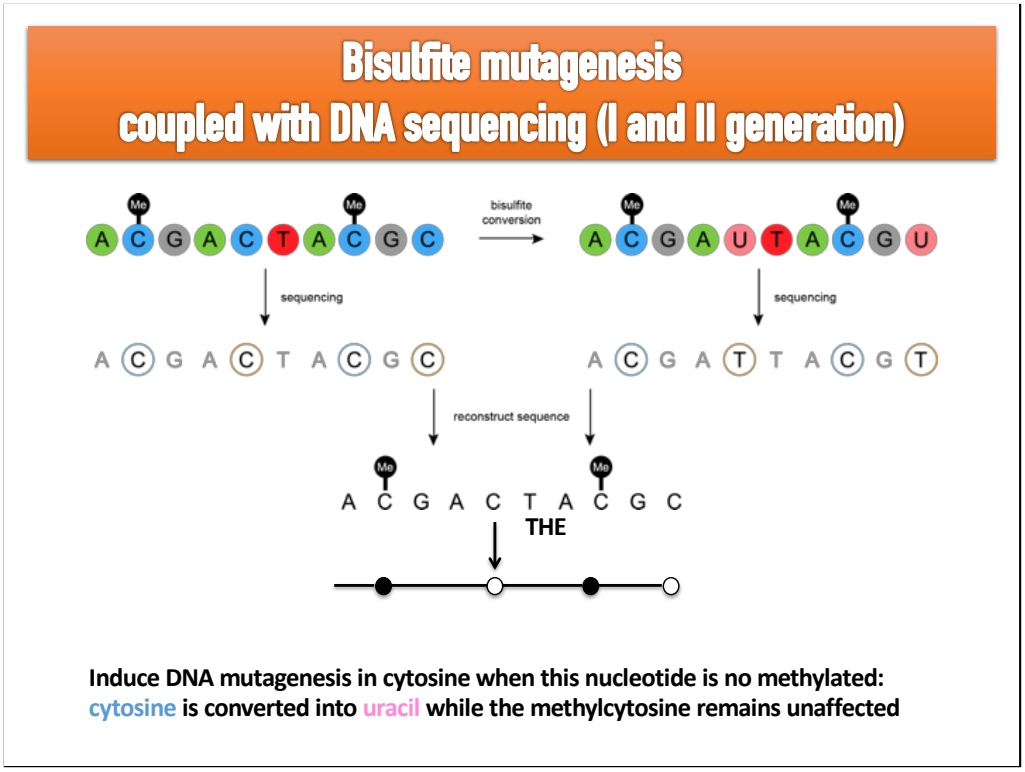
An example of imprinted maternal gene is H19 gene which encoded for a long non-coding RNA (it works as a tumor suppressor).

It is methylated in the spermatozoon. It means that is not expressed into the male gamete (see panel 1).

It isn't methylated into the oocyte, so it means that this gene is expressed in the female gamete (see panel 2).

The imprinting process will, of course, impact on the transcription of such category of genes in the new organism since these imprinted marks persist and so these imprinted genes will be transcripted in a monolallelic manner (see panel 3).

An example of paternally imprinted gene in IGF2 (insulin like growth factor 2); it is only expressed from the maternallly-inherited chromosome.



The bisulfite mutagenesis technique can be used with success to detect the presence of methyl groups on cytosine of GpC islands

- using single oocyte and by sequencing single genes.

Material and Methods

DNA extraction

Bisulfite treatment: It is able to induce DNA mutagenesis in cytosine when this nucleotide is no methylated

In this case the unmethylated cytosine is converted into uracil while the methylcytosine ones remains unaffected

Thus, bisulfite introduces specific changes into DNA in relation of the status of methylation of cytosine that then can be identified by using DNA sequencing.

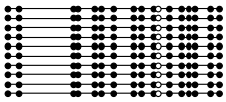
Indeed, by sequencing specific segment of DNA and yielding single nucleotide information we can assess the presence of methyl group on each cytosine base.

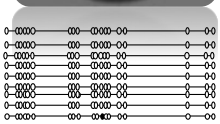
The first-generation sequencing (known as Sanger) and second-generation sequencing (known as Next Generation Sequencing-NGS) use a similar process but differ in the amount of sample that can be sequenced. While the Sanger method allows sequencing a fragment of DNA of modest size (700 – 1000 base pairs), through the use of NGS technology, it is possible to sequence multiple DNA fragments simultaneously.

How will be expressed these imprinted genes in the new organism?

Small Nuclear Ribonucleo protein Polypeptide N

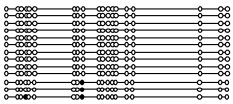
SNRPN

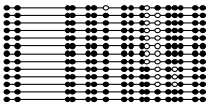




Maternal expressed gene

IGF2R





Paternal expressed gene

- Methylated gene regions
- No Methylated gene regions

Third generation sequencing

Research Article

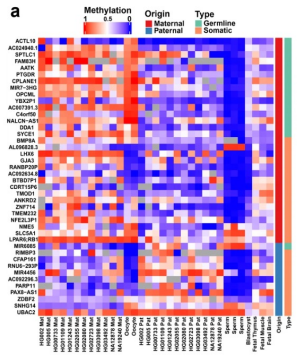
Computational and Systems Biology, Genetics and Genomics

Genome-wide detection of imprinted differentially methylated regions using nanopore sequencing

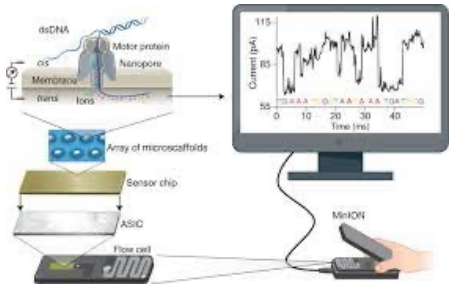
Vahid Akbari, Jean-Michel Garant, Kieran O'Neill, Pawan Pandoh, Richard Moore, Marco A Marra, Martin Hirst, Steven JM Jones

Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Canada; Department of Medical Genetics, University of British Columbia, Canada; Department of Microbiology and Immunology, Michael Smith Laboratories, University of British Columbia, Canada

Jul 5, 2022 · https://doi.org/10.7554/eLife.77898



Heatmap displaying average methylation of the 42 nanopore-detected DMRs



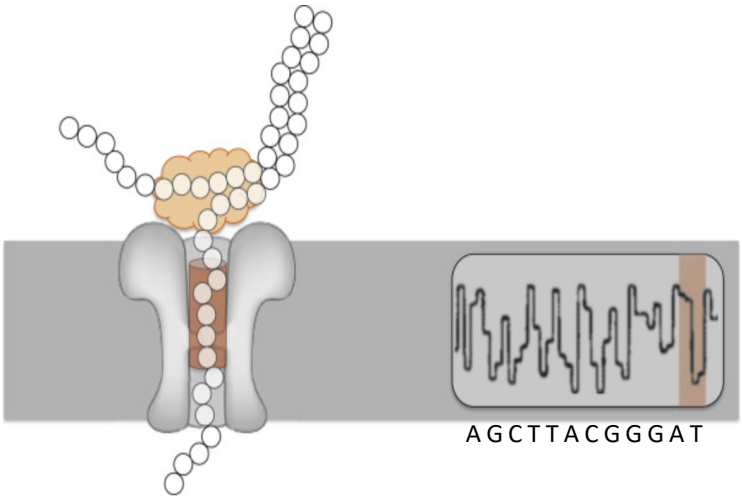
Nanopore sequencing works on the principle of minute changes in electric current across the nanopore immersed in a conducting fluid with voltage applied when a moving nucleotide (or DNA strand) passes through it.

And then there are more innovative and recent methods that do not require bisulfite treatment, such as third-generation sequencing.

Nanopore sequencing technology is an example:

Nanopore sequencing works on the principle of minute changes in electric current across the nanopore immersed in a conducting fluid with voltage applied when a moving nucleotide (or DNA strand) passes through it.

Certainly, these technologies still need validation using older techniques like whole-genome bisulfite sequencing (WGBS), a second generation technique.



Principle of nanopore sequencing. A biological nanopore is embedded in a membrane with high electric resistance immersed in an electrolyte solution. Ions may only pass through the nanopore if a suitable potential is applied across the membrane generating an ionic current.
If an **analyte** enters the pore and passes the "sensing zone," a disruption of the current can be detected.