**SUPPLEMENTAL MATERIAL: Epigenetic regulation of gene expression**

***Part I***

***Differential regulation of gene expression via DNA methylation***

In this activity, the students will be working with seven genes (both monoallelic and biallelic) that have tissue-specific differential expression. The goal is to identify and compare the promoter methylation pattern of these genes in somatic cells during different developmental stages and in male and female gametes.

Students should use the information provided in the table and determine when the CpG islands for each gene promoter would be methylated or unmethylated.

The genes are represented on three different chromosomes. The table below indicates the expression pattern of these genes in two different tissues and shows if the gene is monoallelic or biallelic. The table also shows if the monoallelic gene is maternally (m) or paternally (P) imprinted.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Type of gene | Expressed in liver | Expressed in muscle | Maternally (m) imprinted | Paternally (p) imprinted\* |
| A | Biallelic | yes | No | No | No |
| B | Biallelic | No | yes | No | No |
| C | Biallelic | yes | No | No | No |
| D | Biallelic | No | yes | No | No |
| E | Biallelic | No | yes | No | No |
| F | Biallelic | yes | yes | No | No |
| G | Monoallelic | yes | yes | yes | No |

**The following symbol represents the CpG dinucleotide where the promotor can be methylated. For each cell, fill in the circles for the genes that would have a methylated promoter.**

In adult liver and muscle somatic cells, which genes will have methylated promoters?



***Part II***

***In the following section, each question presents an embryonic cell at different stage of development.***

**The following symbol represents the CpG dinucleotide where the promotor can be methylated. For each cell, fill in the circles for the genes that would have a methylated promoter.**

1. What would be the predicted methylation state of the promoters of the seven genes in early developmental stages (morula stage) during the restoration of totipotency?



1. What is the predicted methylation state of the genes in the Primordial Germ Cells (PGC) in the zygote from question 1 after reprogramming?



1. What is the predicted methylation state of the genes in the gametes produced by the zygote from question 1, if the zygote develops into either a male embryo producing sperms or a female embryo producing eggs (oocyte).



**Instruction notes:**

**Description**

A simple and engaging in-class group activity that helps students to reflect and demonstrate their understanding of the dynamic nature of epigenetic modifications during development and how each cell type will have its unique pattern of DNA methylation.

**Classroom presentation**

The instructor can present this activity after teaching the basic concepts of epigenetics and before the effect of the environment on Epigenetic modifications. The instructor should summarize the activity and ask students to work in groups.

*Part I*

Give the students a few minutes to read the first part of the activity and then start a class discussion. The instructor can start the discussion with the following questions; How many genes involved in this activity, what type of genes, and what is their pattern of expression?

* The activity includes seven genes, six are biallelic and one is monoallelic.
* The pattern of expression:
* Biallelic genes: two genes are expressed in the liver (A, and C), and three genes are expressed in Muscles (B, D, and E). One gene (F) is expressed in both cell types.
* The monoallelic gene (G) is maternally imprinted and is expressed from the paternal allele in both liver and muscle.

Before answering the question in part-I of the activity, the instructor is encouraged to briefly review and remind the students of the effect of Promoter methylation on gene expression. Then allow them to fill in the circles in the question.

The Answer:

* The liver cell: the circles on the promoter of genes A, C, and F remain unfilled (not methylated), whereas the circles for genes B, D, E, are filled (methylated).
* The muscle cell: the circles on the promoter of genes B, D, E, and F remain unfilled (not methylated), whereas the circles for genes A, C are filled (methylated).
* The promoter that regulates gene F is not methylated in both cells because it is expressed in both cell types.
* The case for gene G is slightly different. Gene G is maternally imprinted, and for simplicity, it is expressed in both cell types. Therefore, the circles on the maternal chromosome are filled, and the maternal allele is silenced in both cell types, whereas the paternal allele is expressed in both cell types, and the circles remain unfilled.

*Part II*

The instructor can use fig. 2 from the article to review the stages of zygote cellular development. The instructor should briefly remind the students of the dynamic nature of epigenetic modification during embryonic development and epigenetic reprogramming, then allow the students to work on question 1,2 and 3.

Answer:

Question 1: The morula stage occurs before implantation. During the morula stage, the first phase of epigenetic reprogramming occurs to establish totipotency. Therefore, all the circles should be unfilled except for gene G promoter on the maternal chromosome, because imprinted genes do not undergo reprogramming until the stage of gametogenesis.

Question 2: All the circles for genes A, B, C, D, E, and F will be filled because these genes are not expressed in PGCs. However, both maternal and paternal gene G promoters will be unmethylated (unfilled circle) because of the reprogramming step to establish parent-of-origin imprint.

Question 3: These are haploid cells and have only one set of chromosomes. Again, all the circles for genes A, B, C, D, E, and F will be filled in both gametes because these genes are not expressed in gamete cells. However, since gene G is maternally imprinted, its promoter will be unmethylated (unfilled circles) in the sperm (paternal gamete) and methylated (filled circles) in the egg (maternal gamete).