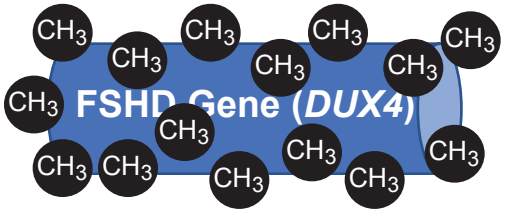
What is DNA methylation? (Pt 1)

You have heard us refer to FSHD as being an epigenetic disease, and you have heard that DNA methylation is an epigenetic mark, and our FSHD research test is an epigenetic test, and your reports show your DNA methylation levels. But, what is DNA methylation?

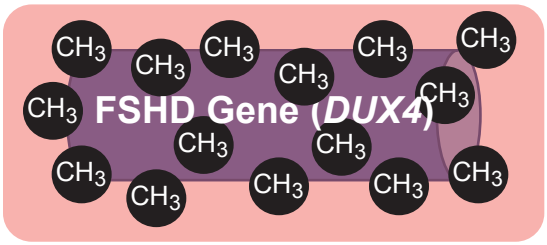
DNA methylation is a post-replicative addition of a methyl group (CH3) to the DNA. It does not change the DNA sequence.

Healthy

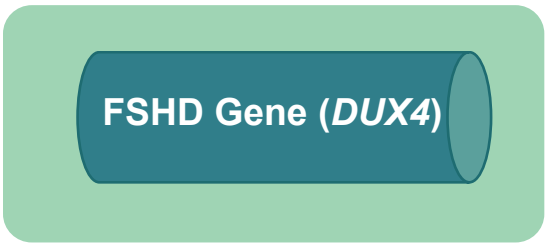


FSHD

DUX4 is OFF

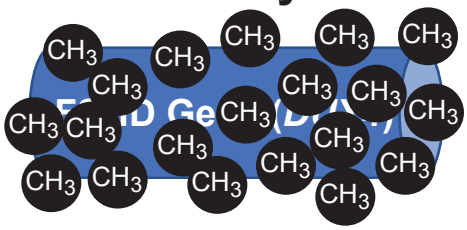


DUX4 is ON

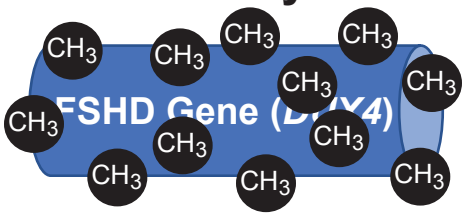


DNA methylation levels vary between individuals

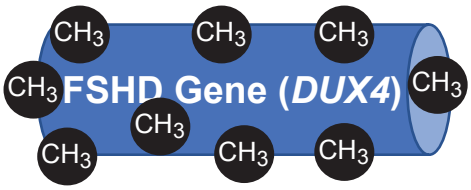
Healthy



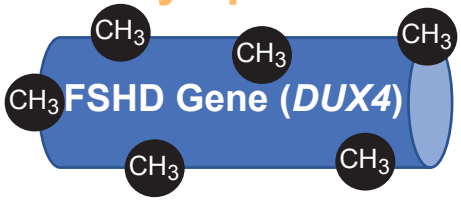
Healthy



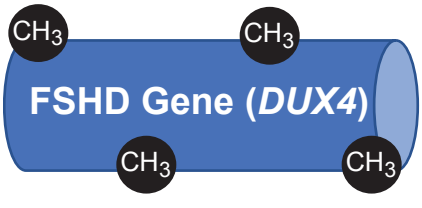
Borderline



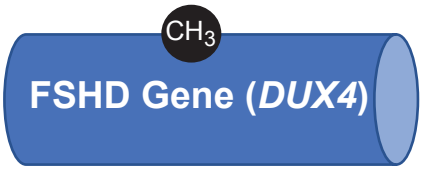
Mild FSHD or Asymptomatic



Typical FSHD



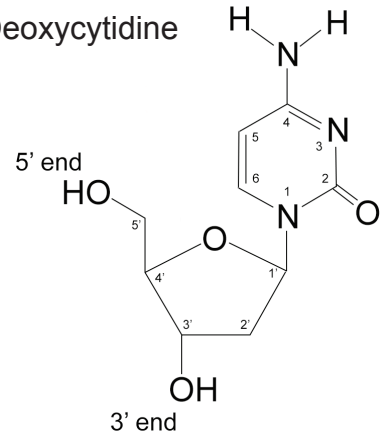
Severe FSHD



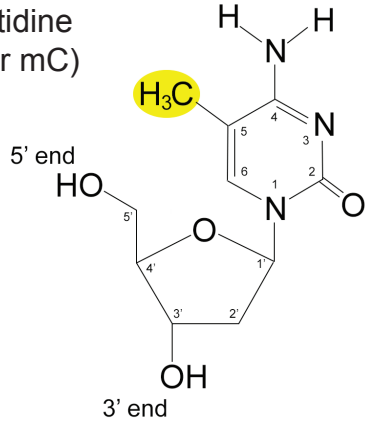
What is DNA methylation? (Pt 2)

DNA methylation is a post-replicative addition of a methyl group (CH3) to the DNA. It does not change the DNA sequence.

Deoxycytidine

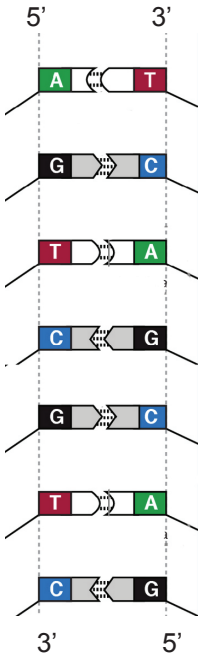


5-methyl-deoxycytidine
(aka 5-methyl-C or mC)

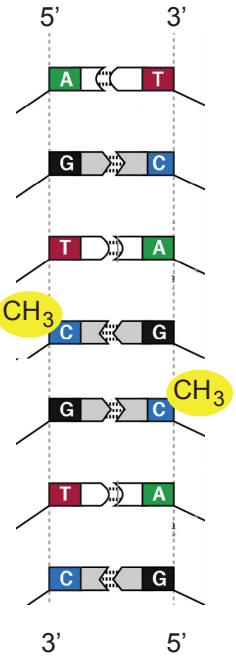


In mammals (such as people), deoxycytidine (C) is the only DNA base that is methylated (mC) and only when paired with a deoxyguanosine (G). Thus, CG is the only one of the 16 possible DNA base pairs that can be methylated (CG or mCG).

Unmethylated DNA sequence



Same DNA sequences, different methylation; they are epigenetically different.



Methylated DNA sequence

National Human Genome Research Institute

DNA sequences that only differ by DNA methylation status are called epialleles. For example, the sequence above left is the same as the one on the right, but the right sequence is methylated. These are epialleles of the same gene.

What is DNA methylation? (Pt 3)

In mammals (such as people), deoxycytidine (C) is the only DNA base that is methylated (mC) and only when paired with a deoxyguanosine (G). Thus, CG is the only one of the 16 possible DNA base pairs that can be methylated (CG or mCG).

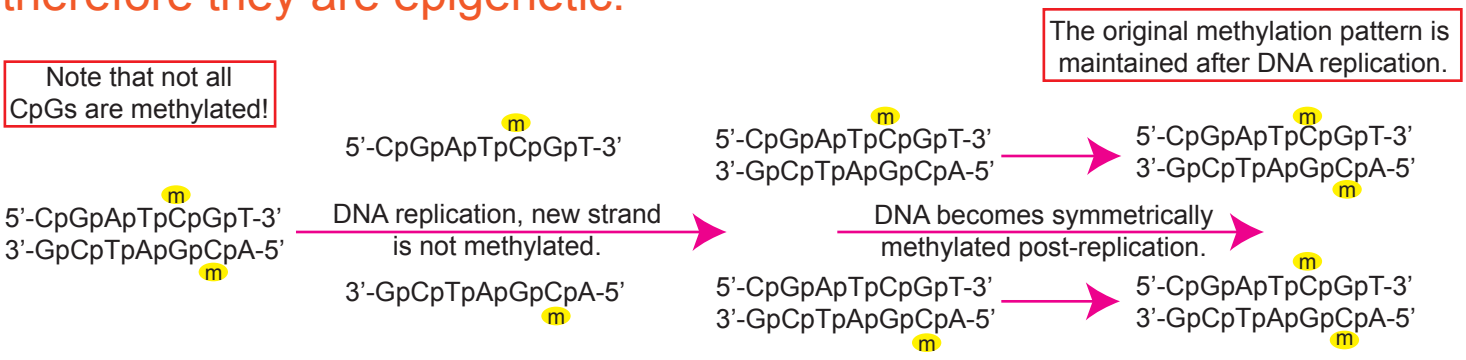
DNA ribonucleotides are connected by a phosphodiester backbone, signified by a "p". DNA also has direction, 5' to 3', based on carbon positions in the nucleotide sugar-ring. Thus DNA convention is CpG dinucleotide oriented 5'-CpG-3'.

The complementary DNA strand runs the other direction.

CpG is the only dinucleotide that is the same in both strands!

Methylation of C in a CpG dinucleotide is always symmetrical. Thus, the methylation mark is maintained after replication. This is what makes CpG DNA methylation "epigenetic"; its pattern is heritable without changing the DNA sequence!

DNA methylation patterns are heritable after replication; therefore they are epigenetic.



DNA methylation is a mechanism for regions of the genome (or genes) to exist in two different states (methylated or unmethylated) without altering the DNA sequence.

In FSHD, the *DUX4* gene is unmethylated (active or ON state); whereas in healthy individuals the *DUX4* gene is methylated (silenced or OFF state).

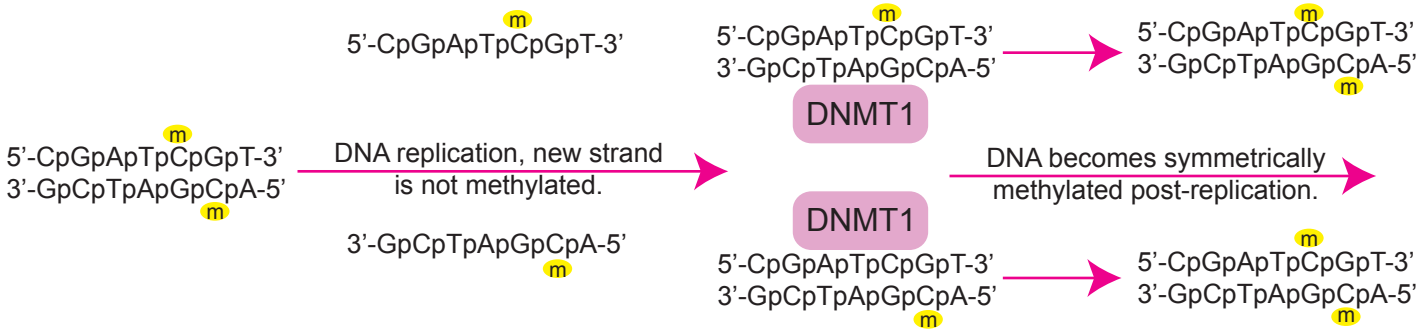
What is DNA methylation? (Pt 4)

How does the DNA methylation mark get there?

There are two types of DNA methylation, maintenance methylation and de novo methylation. These are carried out by different enzymes.

Maintenance of DNA methylation patterns after replication is carried out by DNA methyltransferase 1 (DNMT1).

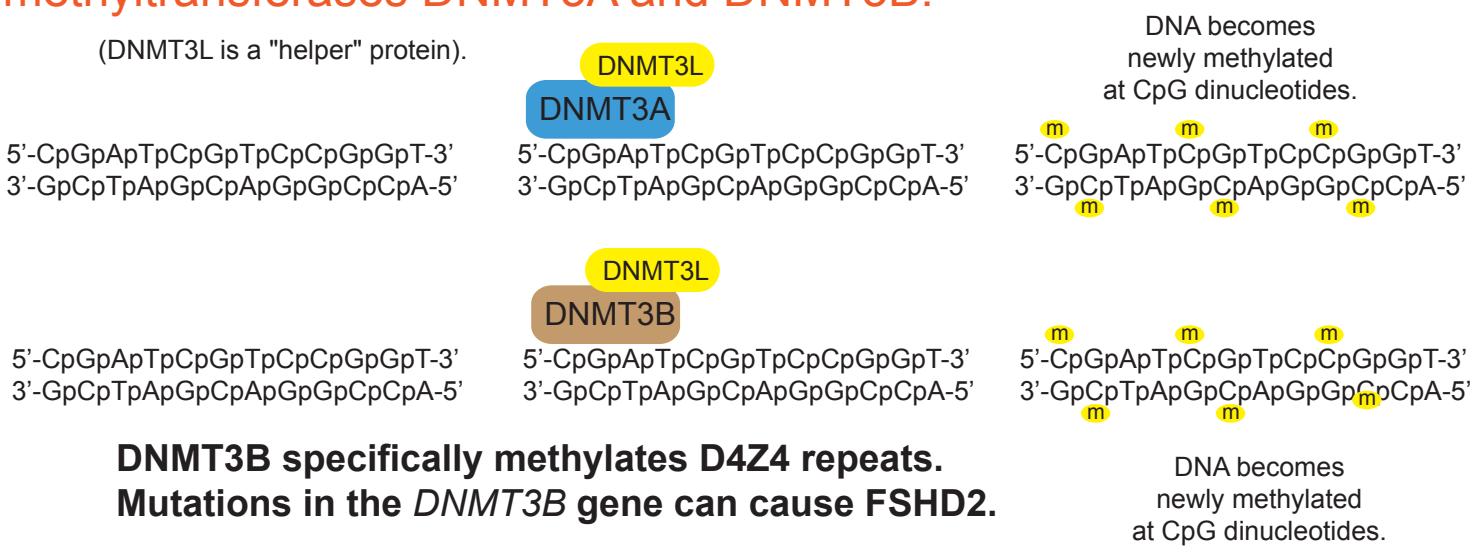
The original methylation pattern is maintained after DNA replication.



DNMT1 recognizes hemi-methylated DNA and adds methylation to the newly synthesized strand of DNA after replication.

New DNA methylation patterns are created by the *de novo* DNA methyltransferases DNMT3A and DNMT3B.

(DNMT3L is a "helper" protein).



DNMT3B specifically methylates D4Z4 repeats.
Mutations in the *DNMT3B* gene can cause FSHD2.

That genetic engineering 2.0 "On-Off switch for gene editing" uses CRISPR/dCas9 to recruit the DNMT3A+DNMT3L to methylate, and thus turn OFF specific genes.
What is the catch? It is too large to therapeutically deliver systemically to people.