Let's explore Chromatin Function and Epigenetics!

Now you should have a good idea of how DNA is wrapped around histone proteins to form nucleosomes and understand that nucleosomes can be tightly packed (heterochromatin) or loosely packed (euchromatin). We will now explore how this basic structure allows for all the necessary functions of DNA and chromatin.

DNA and chromosomes are involved in many important functions of the cell, such as:

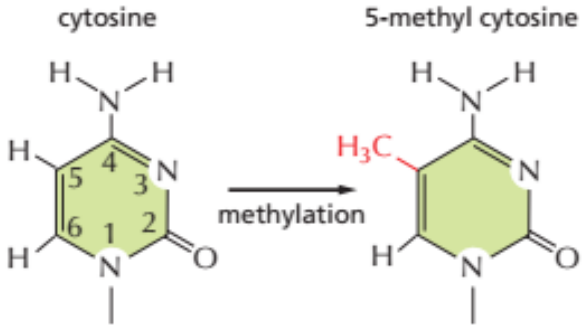
- Transcription of DNA into RNA for gene expression
- Replication of DNA leading to cell division
- DNA repair when a mismatch or DNA break is detected

All these functions and many others require specialized proteins to gain access to the DNA and to be directed to the correct locations to carry out their functions. The way that this is achieved is through markings added to DNA and the histone protein. Collectively these markings are called "epigenetics", meaning "above genetics" because they do not change the nucleotide sequence or base-pairing properties of DNA.

Both DNA and histone proteins can accumulate these markings that direct various structural and functional outcomes. These modifications are reversible allowing the functional changes to be dynamic, depending on input from the environment of the cell. To clarify this complex area, we will model DNA and histone epigenetic modifications separately, but it is important to remember that the modifications work together to carry out a specific function.

DNA Modification: Cytosine methylation

The primary epigenetic modification of DNA is methylation of the 5 carbon of cytosine. Use a cytosine from the Dynamic DNA Kit and identify the 1 through 6 positions of cytosine.



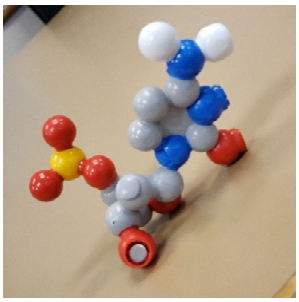
Which positions are involved in base-pairing of cytosine to guanine?

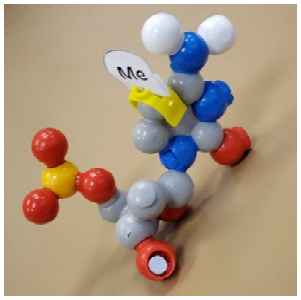
Positions 2, 3, and 4

Which position is bound to the sugar-phosphate backbone?

Position 1

Place a methyl group clip on the 5 position. Notice where it is in relation to other functional groups so that you can easily methylate cytosines within DNA.





Construct this DNA sequence with Dynamic DNA in the 5'→ 3' direction:

5' A T C C G C G T A A T C 3'

Another rule of cytosine methylation as an epigenetic modification is that only cytosines that are followed by guanines (sometimes referred to as CpG dinucleotides) are methylated.

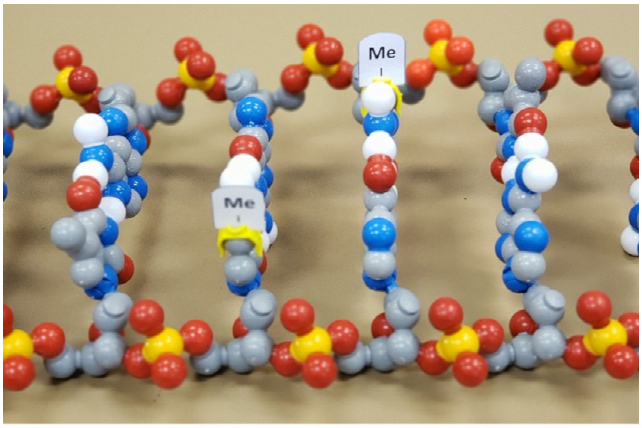
Place a methyl group on each cytosine that is part of a CpG dinucleotide.

5′ A T C C G C G T A A T C 3′

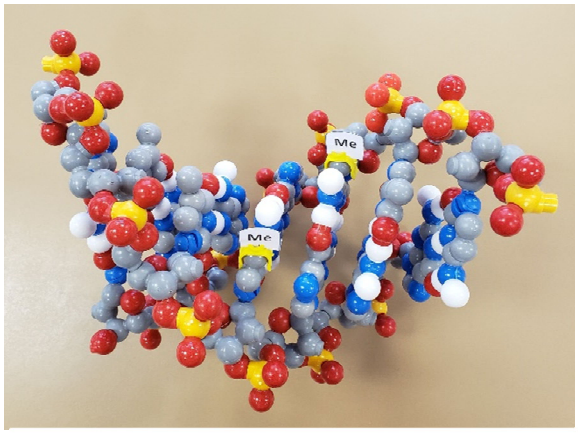
3′ T A G G C G C A T T A G 5′

Notice that every time there is a CpG dinucleotide on one strand, there will be a CpG dinucleotide on the complementary strand as well going in the other direction. Also observe the positions of the methylated cytosines on the two strands. See how close together they are to each other. Position the constructed, methylated Dynamic DNA sequence into a helix.

Are the methyl groups in the major groove or minor groove? Why might that be important?



Position of methyl groups at 5-carbon position in cytosine. Each cytosine in a CpG dinucleotide will always have a paired CpG on the opposite strand.

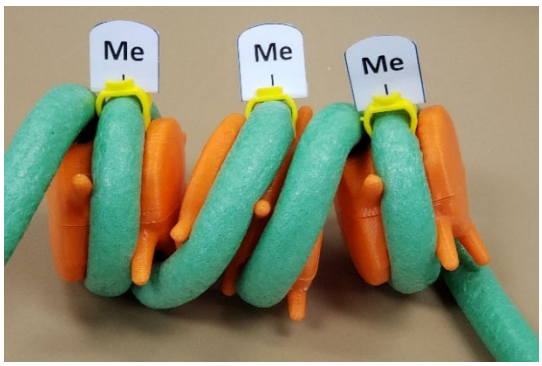


Position of methyl groups within the DNA helix.

What is the effect of cytosine methylation? It is associated with transcriptional silencing. That means that when cytosines are methylated in a gene promoter, it inhibits transcription, thereby reducing the expression of that gene. The exact mechanisms for this are not clear but methylation might make It difficult for necessary proteins like transcription factors to bind to the DNA and begin the process of transcription.

DNA methylation is also known to be associated with tightly packed chromatin or heterochromatin which also inhibits transcription due to the DNA being physically hidden from transcription factors and other DNA binding proteins. This is likely caused by complex interactions between methylated DNA and histone modifications which we will explore in the next section.

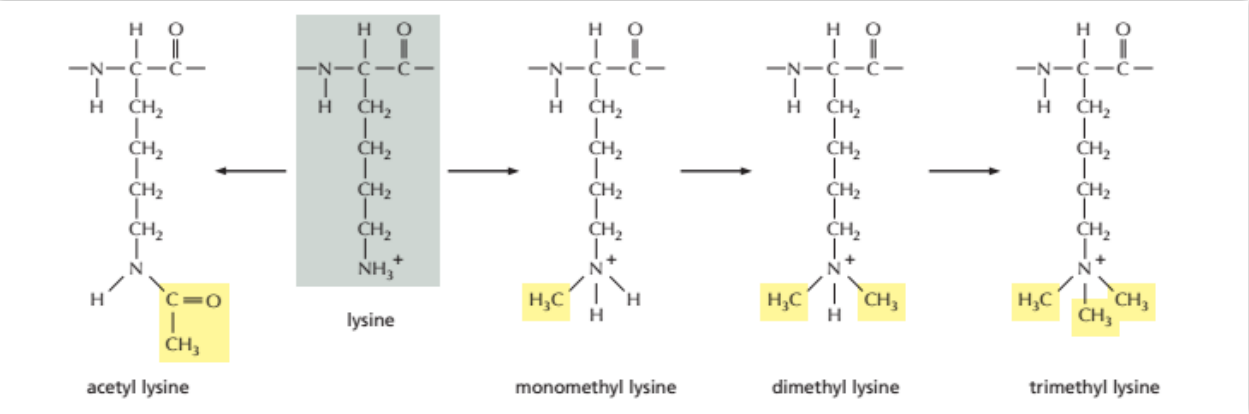
Use the Nucleosome Kit to model methylated DNA and heterochromatin.



Cytosine methylation induces tight packing of nucleosomes, decreasing DNA binding protein access.

Histone modifications – histone tail post-translational modifications

The other type of epigenetic modification involves the histone proteins. Recall that each histone octamer consists of 2 copies of H2A, H2B, H3, and H4 histone proteins, each with a globular portion that makes up the core of the nucleosome, and a long N-terminal tail. These tails can have functional groups added to specific amino acids in the tail. Lysine happens to be an amino acid that is particularly subject to epigenetic modification.



As seen in this diagram, lysine can be acetylated, meaning an acetyl functional group can be added to the end of the lysine sidechain. It can also be methylated with 1, 2, or 3 methyl groups. However, it cannot be *both* acetylated *and* methylated!

Lysine is a basic amino acid with a positive charge. In the Nucleosome lesson, we discussed how positively charged amino acids in the histone proteins can be attracted to the negatively charged DNA backbone and aid in nucleosome packing.

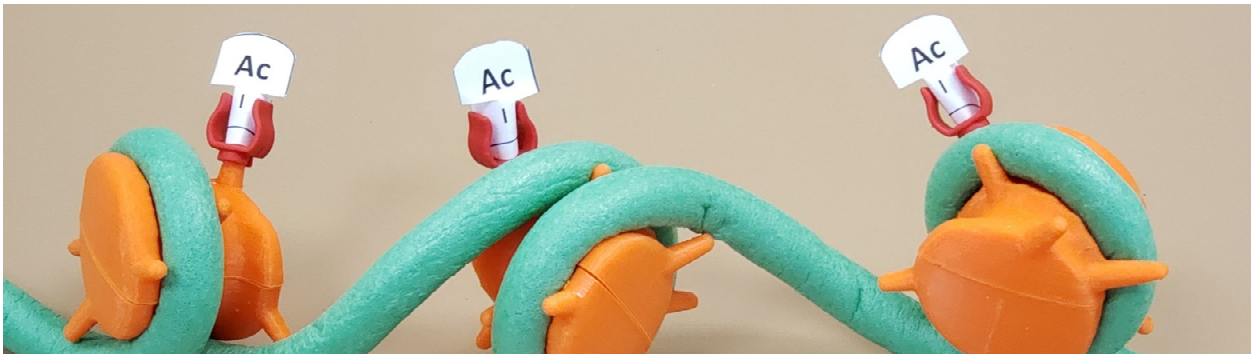
What happens to the charge with the various epigenetic modifications shown in the figure?

Acetylation of lysine removes the positive charge while methylation has no effect.

Which modification changes lysine from positively charged to uncharged? Acetylation

What would you hypothesize this type of modification might do to the association of the DNA with the histone proteins? Reduce the attraction of the histones for DNA and cause unwinding.

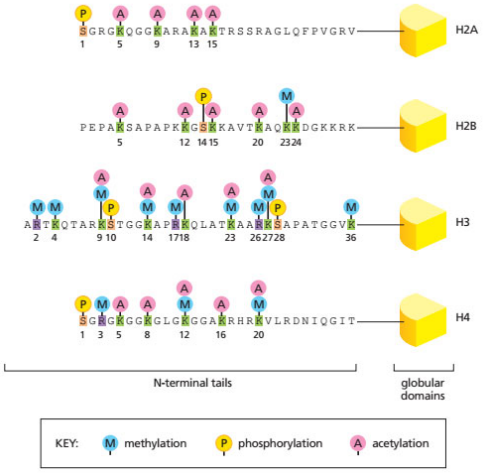
Add acetyl groups to the histone tails of the several nucleosomes and then model how that might affect chromatin packing.



Histone acetylation induces loose packing of nucleosomes and a more open conformation that makes DNA more accessible to DNA binding proteins.

Lysine acetylation of histone tails is almost always associated with areas of chromatin where DNA is loosely wrapped around histone proteins, the DNA is more open and exposed, and where the genes are actively transcribed.

There are lots of different types of histone tail modifications besides acetylation, including methylation, phosphorylation and many others. See the diagram to the right to see a few of the possible modification of various amino acids at specific locations within each histone tail. Unlike acetylation, the functional consequences of other modifications are not as predictable and tend to depend on complex interactions with other neighboring modifications.



Pulling it all together

So how do epigenetic modifications in DNA and histone tails work together to tell the cell which genes to express, determine where replication should begin, or where DNA needs to be repaired, among many other processes? This is where the ***non-histone proteins*** we mentioned in the Nucleosome Structure Activity come in.

There are lots of different proteins that can bind to DNA and/or histones to coordinate and carry out many complex functions. The following are a few classes of proteins and how they work:

Writers and Erasers: These epigenetic modifications to DNA and histones are coordinated and dynamic. Writers are enzymes that add the modifications and erasers remove them. The proteins that add methyl groups to DNA are writers called DNA methyltransferases (DNMTs) and those that remove them are called Ten-Eleven Translocation enzymes (TETs). Writers that add acetyl groups to histones are called histone acetyltransferases (HATs) and the erasers are called histone deacetylases (HDACs) .

Readers and chromatin remodelers: Once the modifications are in place, there are proteins that bind to them in specific places. Some proteins will bind to modifications in both DNA and histone tails. These are known as readers because they "read" the modifications. There are other multiple proteins that bind to the readers and form large complexes that coordinate important processes like transcription, replication, and repair.

Activity: Use the information that you have learned to hypothesize how one of these processes might work and model it. Use the Nucleosome Kit and design potentially important non-histone proteins with cardboard, clay, or other materials.

- What are all the features (structural landmarks locations, etc) that need to be identified before a particular process takes place?
- How might non-histone proteins work together to identify these features and carry out the process?