Epigenetics.

Significance of epigenetic regulation of gene expression. Mechanisms of epigenetic regulation: DNA methylation, RNA interference. Mechanisms of epigenetic regulation: histone modifications, histone variants.

1. Explain the importance of epigenetic regulation and its role in heritability of cellular traits.
2. Explain the role of DNA methylation in regulation of gene expression.
3. Explain the mechanism of RNAi.
4. Describe chromatin structure at the levels of organization: nucleosome, 30-nm fiber, chromosome.
5. Explain the effects of histones on transcription.
6. Explain how transcription is affected by: nucleosome positioning, histone acetylation and methylation, chromatin remodeling.
7. Describe the mechanisms and major players of above mentioned processes.

Since the early days of genetics research, scientists have noted certain heritable phenotypic differences that are not due to differences in the nucleotide sequence of DNA. Current evidence suggests that these "epigenetic" phenomena might be controlled by a number of mechanisms, including the modification of DNA cytosine bases with methyl groups, the addition of various chemical groups to histone proteins, and the recruitment of protein factors to specific DNA sites via interactions with non-protein-coding RNAs.

Epigenetics is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype — which in turn affects how cells read the genes. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. Epigenetic modifications can manifest as commonly as the manner in which cells terminally differentiate to end up as skin cells, liver cells, brain cells, etc. Or, epigenetic change can have more damaging effects that can result in diseases like cancer.

At least three systems including DNA methylation, histone modification and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change.**1** New and ongoing research is continuously uncovering the role of epigenetics in a variety of human disorders and fatal diseases.

DNA Methylation

DNA methylation works by adding a chemical group to DNA. Typically, this group is added to specific places on the DNA, where it blocks the proteins that attach to DNA to "read" the gene. This chemical group can be removed through a process called demethylation. Typically, methylation turns genes "off" and demethylation turns genes "on."

Histone modification

DNA wraps around proteins called histones. DNA wrapped tightly around histones cannot be accessed by proteins that "read" the gene. Some genes are wrapped around histones and are turned "off" while some genes are not wrapped around histones and are turned "on." Chemical groups can be added or removed from histones and change whether a gene is unwrapped or wrapped ("on" or "off").

Non-coding RNA

Your DNA is used as instructions for making coding and non-coding RNA. Coding RNA is used to make proteins. Non-coding RNA helps control gene expression by attaching to coding RNA, along with certain proteins, to break down the coding RNA so that it cannot be used to make proteins. Non-coding RNA may also recruit proteins to modify histones to turn genes "on" or "off."

While epigenetic changes are required for normal development and health, they can also be responsible for some disease states. Disrupting any of the three systems that contribute to

epigenetic alterations can cause abnormal activation or silencing of genes. Such disruptions have been associated with cancer, syndromes involving chromosomal instabilities, and mental retardation .

Epigenetics and Cancer

The first human disease to be linked to epigenetics was cancer, in 1983. Researchers found that diseased tissue from patients with colorectal cancer had less DNA methylation than normal tissue from the same patients (Feinberg & Vogelstein, 1983). Because methylated genes are typically turned off, loss of DNA methylation can cause abnormally high gene activation by altering the arrangement of chromatin. On the other hand, too much methylation can undo the work of protective tumor suppressor genes.

As previously mentioned, DNA methylation occurs at CpG sites, and a majority of CpG cytosines are methylated in mammals. However, there are stretches of DNA near promoter regions that have higher concentrations of CpG sites (known as CpG islands) that are free of methylation in normal cells. These CpG islands become excessively methylated in cancer cells, thereby causing genes that should not be silenced to turn off. This abnormality is the trademark epigenetic change that occurs in tumors and happens early in the development of cancer. Hypermethylation of CpG islands can cause tumors by shutting off tumor-suppressor genes. In fact, these types of changes may be more common in human cancer than DNA sequence mutations/

Furthermore, although epigenetic changes do not alter the sequence of DNA, they can cause mutations. About half of the genes that cause familial or inherited forms of cancer are turned off by methylation. Most of these genes normally suppress tumor formation and help repair DNA, including O^6-methylguanine-DNA methyltransferase (*MGMT*), MLH1 cyclin-dependent kinase inhibitor 2B (*CDKN2B*), and *RASSF1A*. For example, hypermethylation of the promoter of *MGMT* causes the number of G-to-A mutations to increase

Epigenetics and Mental Retardation

Fragile X syndrome is the most frequently inherited mental disability, particularly in males. Both sexes can be affected by this condition, but because males only have one X chromosome, one fragile X will impact them more severely. Indeed, fragile X syndrome occurs in approximately 1 in 4,000 males and 1 in 8,000 females. People with this syndrome have severe intellectual disabilities, delayed verbal development, and "autistic-like" behavior .

Fragile X syndrome gets its name from the way the part of the X chromosome that contains the gene abnormality looks under a microscope; it usually appears as if it is hanging by a thread and easily breakable (Figure 3). The syndrome is caused by an abnormality in the *FMR1* (fragile X mental retardation 1) gene. People who do not have fragile X syndrome have 6 to 50 repeats of the trinucleotide CGG in their *FMR1* gene. However, individuals with over 200 repeats have a full mutation, and they usually show symptoms of the syndrome. Too many CGGs cause the CpG islands at the promoter region of the *FMR1* gene to become methylated; normally, they are not. This methylation turns the gene off, stopping the *FMR1* gene from producing an important protein called fragile X mental retardation protein. Loss of this specific protein causes fragile X syndrome. Although a lot of attention has been given to the CGG expansion mutation as the cause of fragile X, the epigenetic change associated with *FMR1* methylation is the real syndrome culprit.

Fragile X syndrome is not the only disorder associated with mental retardation that involves epigenetic changes. Other such conditions include Rubenstein-Taybi, Coffin-Lowry, Prader-Willi, Angelman, Beckwith-Wiedemann, ATR-X, and Rett syndromes .

Combating Diseases with Epigenetic Therapy

Because so many diseases, such as cancer, involve epigenetic changes, it seems reasonable to try to counteract these modifications with epigenetic treatments. These changes seem an ideal target because they are by nature reversible, unlike DNA sequence mutations. The most popular of these treatments aim to alter either DNA methylation or histone acetylation.

Inhibitors of DNA methylation can reactivate genes that have been silenced. Two examples of these types of drugs are 5-azacytidine and 5-aza-2′-deoxycytidine (Egger *et al*., 2004). These medications work by acting like the nucleotide cytosine and incorporating themselves into DNA while it is replicating. After they are incorporated into DNA, the drugs block DNMT enzymes from acting, which inhibits DNA methylation.

Drugs aimed at histone modifications are called histone deacetylase (HDAC) inhibitors. HDACs are enzymes that remove the acetyl groups from DNA, which condenses chromatin and stops transcription. Blocking this process with HDAC inhibitors turns on gene expression. The most common HDAC inhibitors include phenylbutyric acid, SAHA, depsipeptide, and valproic acid (Egger *et al*., 2004).

Caution in using epigenetic therapy is necessary because epigenetic processes and changes are so widespread. To be successful, epigenetic treatments must be selective to irregular cells; otherwise, activating gene transcription in normal cells could make them cancerous, so the treatments could cause the very disorders they are trying to counteract. Despite this possible drawback, researchers are finding ways to specifically target abnormal cells with minimal damage to normal cells, and epigenetic therapy is beginning to look increasingly promising.

Literature:

1. Alberts et al., pp. 194-207; Alberts et al., pp. 404-413; Alberts et al., pp. 429-436;
2. Weaver, pp. 355-387; Weaver, pp. 488-507.