Environmental Health: The Lasting Effects of Pesticides

Critical Discussion of an Environmental Health Research Paper

CEHS workshop for Life Sciences Teachers

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Presented at an MIT COEC Teacher Workshop 2007

The Paper…

Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility

MD Anway, AS Cupp, M Uzumcu, and MK Skinner, *Science*, June 3, 2005

Our Goals:

- Realize the importance of critical reading in ANY area of science research…consider many possibilities, not just the author's conclusions.
- Understand the connection between the environment, our health, and their interactions.
- Understand the main points of the paper and critique the paper as a whole.

One way the environment effects our health…

- Pesticides are chemicals used to kills “pests,” often insects or fungi that feed on our food sources or plants.
- People and animals are often exposed to small doses of pesticides in the foods they eat and the air they breathe.
- Pesticide exposure has been repeatedly and strongly linked to a large variety of cancers, as well asthma and other health problems, especially among children.
- BUT, can the damage caused by pesticides be passed down from generation to generation???

Rev Environ Health. 2005 Jan-Mar;20(1):15-38.
Breast Cancer Res Treat. 2005 Mar;90(1):55-64.

Introduction: Meet the pesticides…

Vinclozolin:

- A pesticide used to kill fungi in vines (such as grapes), strawberries, vegetables, fruit and ornamentals. It may also be used on turf grass. This fungicide works by inhibiting spore germination.
- It is not acutely toxic, but over time it has been shown to cause cancer in a variety of animal studies.

Methoxychlor:

- An insecticide against flies, mosquitoes, cockroaches, chiggers, and a wide variety of other insects. It is used on agricultural crops and livestock, and in animal feed, barns, grain storage bins, home garden, and on pets.
- Methoxychlor is also not acutely toxic, but high doses can cause depression of the central nervous system, progressive weakness, trembling, convulsions, diarrhea, and death in animal studies.

Both pesticides are chemically similar to human sex hormones.

http://pmep.cce.cornell.edu/profiles/extoxnet/pyrethrins-ziram/vinclozolin-ext.html
http://www.epa.gov/safewater/contaminants/dw_contamfs/methoxyc.html

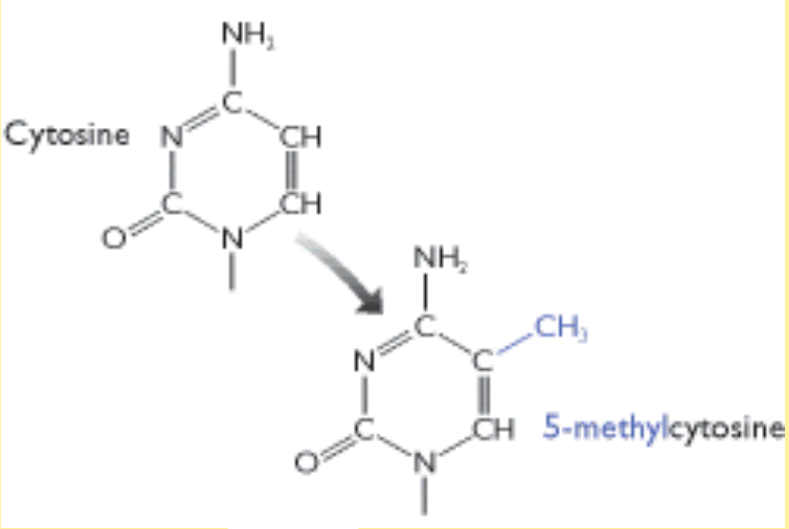
A brief introduction to EPIGENETICS…

What is **epigenetics**?

- The study of *heritable* changes in gene function that occur without a change in the sequence of nuclear DNA.
- This includes the study of how environmental factors affecting a parent can result in changes in the way genes are expressed in the offspring.
- The idea has been around since 1942, but only in the last few years has it been observed directly…

Where are heritable changes stored if not in DNA?!?

DNA methylation… (one type of epigenetic change)



The Cytosine (C) base of DNA can be specifically modified by the addition of a methyl group ($-CH_3$). This is called **DNA methylation.**

Like DNA itself, the DNA methylation pattern seen in a parent is passed along to their offspring. (Though it's different between males and females).

When cells divide, C's that have this modification in the parent cell are modified in the same way in the new cell's DNA.

What effect does methylation have on DNA? Genes that have been marked by DNA methylation are **inactivated**, meaning the gene is not transcribed into RNA or expressed as protein.

Several human diseases have been tied to mutations in the genes that regulate DNA methylation.

Some definitions…

Apoptosis: a type of cell death - a **cell suicide** mechanism that controls cell number and eliminates cells that threaten the animal's survival.

F0-F4: Generation of rats, from F0 = parental, to F4 = 4th generation.

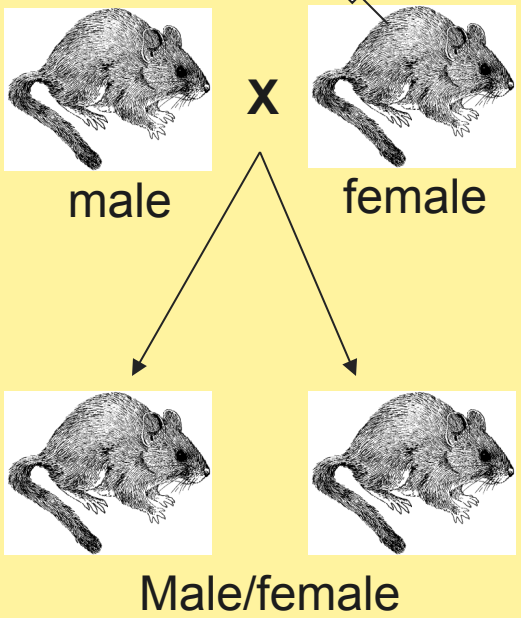
PCR: Polymerase Chain Reaction, a method of making many copies of a region of DNA.

What the scientists say they showed…

- Exposure of a pregnant rat to pesticides can have a bad effect not only on the children in her womb, but on every generation born in that genetic line thereafter (i.e. her grandchildren, great-grandchildren, etc.).
- The bad effects observed in this study are passed from father to son for all four generations tested, and include: low sperm count, fewer viable sperm, and a higher incidence of male infertility.

Do we agree that they showed this? Let's look at their evidence…

How did the scientists approach this issue…



Pregnant female rats were treated with vinclozolin or methoxychlor between embryonic days 8 and 15.

The offspring of these treated females were mated to each other (but not inbred), and their offspring were mated, and so on, for 4 generations.

Generations of rats…


male

X


female

F_0


male

X


female

F_0



Male/female

F_1 X



Female/male

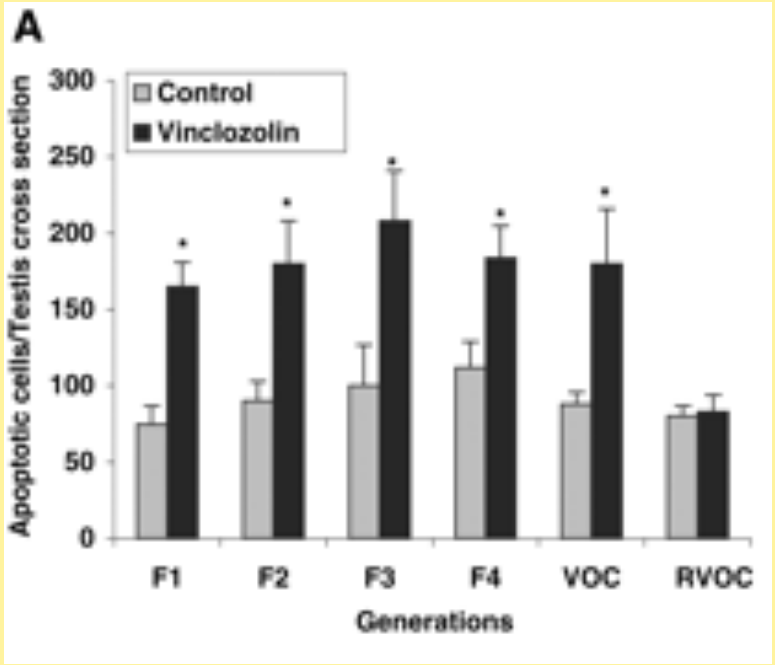
F_1



Male/female

F_2

Figure 1A: Problems with the offspring…



By several measures, the male offspring had sexual problems, including:

A) The number of cells that died by apoptosis

F1-F4 are all bred from both male and female offspring of the treated F0,

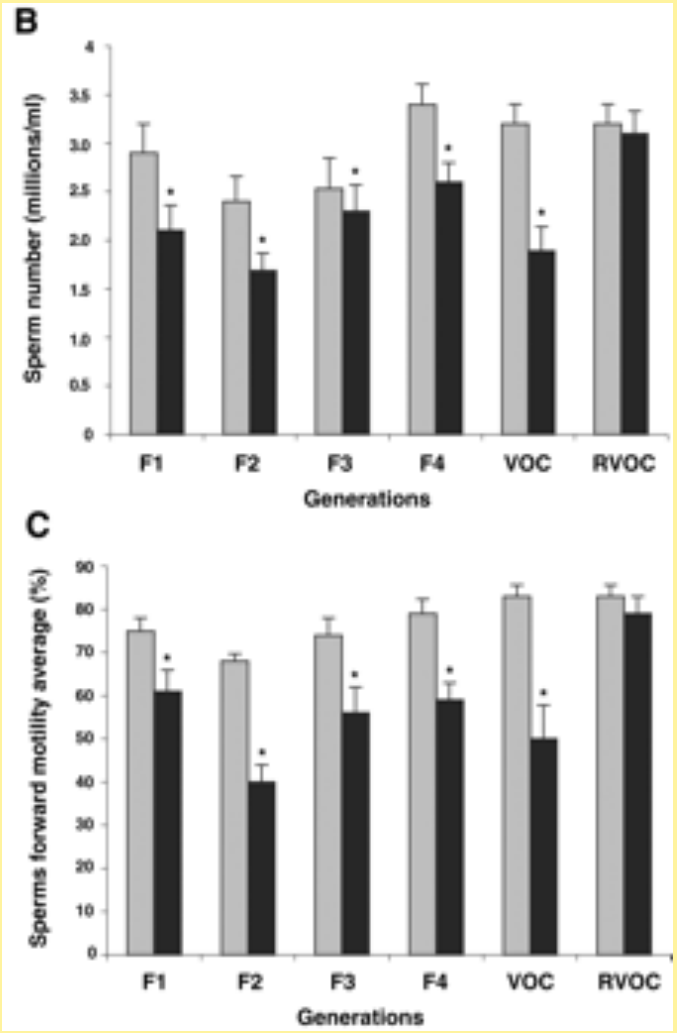
VOC are F2 males bred with untreated females and

RVOC are F2 females bred with untreated males.

What does this show?

That 4 generations of males bred from vinclozolin-treated females continue to show a higher level of testis cell death regardless of whether they are mated with “treated” or untreated females.

Figure 1b, c: More problems with the offspring…



The authors also measured:

B) Total sperm number and

C) The percentage of sperm that move normally.

F1-F4 are all bred from both male and female offspring of the treated F0,

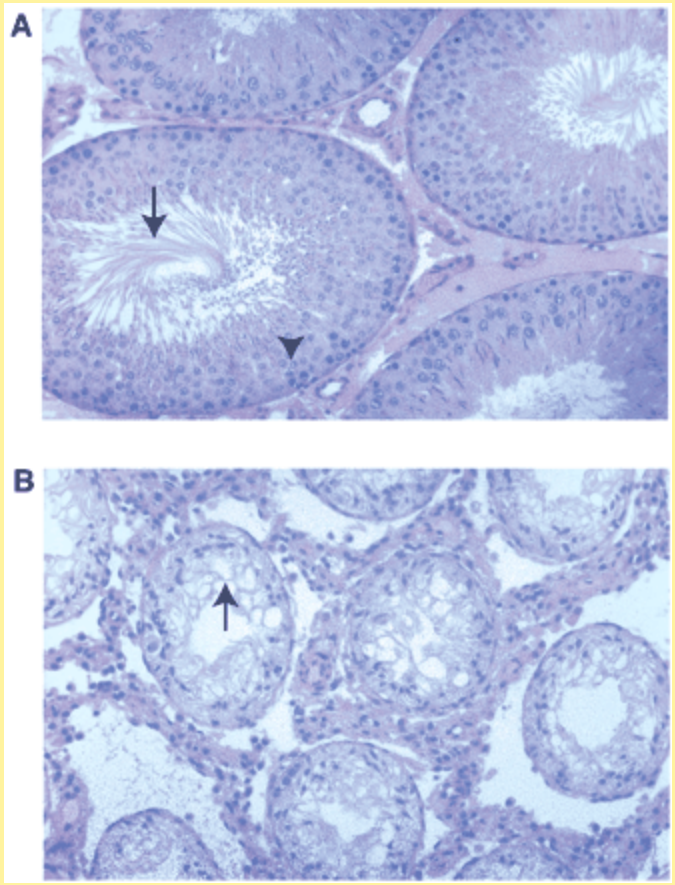
VOC are F2 males bred with untreated females and

RVOC are F2 females bred with untreated males.

Again, the problems seem to be passed exclusively through the males. These effects were observed in >90% of the vinclozolin males.

Also, 8% of males were infertile, compared to 0% of the controls.

Figure 2: What's happening microscopically…



A close look at the testes of

A) Untreated and

B) Vinclozolin-treated males

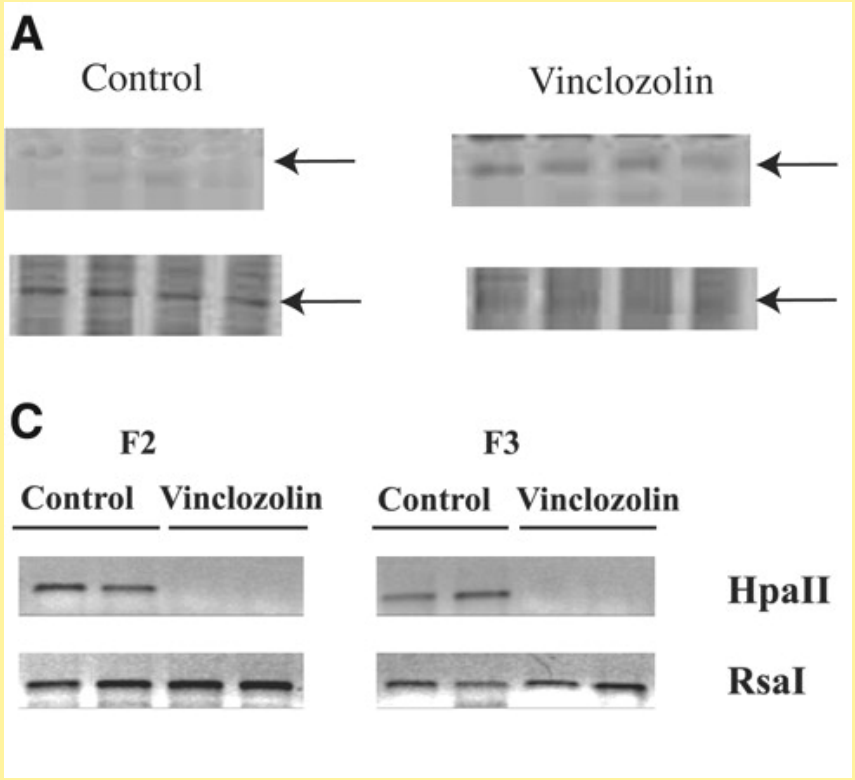
The arrow in A) point to the tails of elongate spermatozoa and the arrowhead points to spermatocytes

In B) the arrow indicates the lack of germ cells at their expected location.

This shows a loss of normal sperm development in the vinclozolin males.

As the animals aged (i.e. older than 90 days) many males showed problems with sperm production (~20%).

Figure 3: A look at DNA methylation patterns…



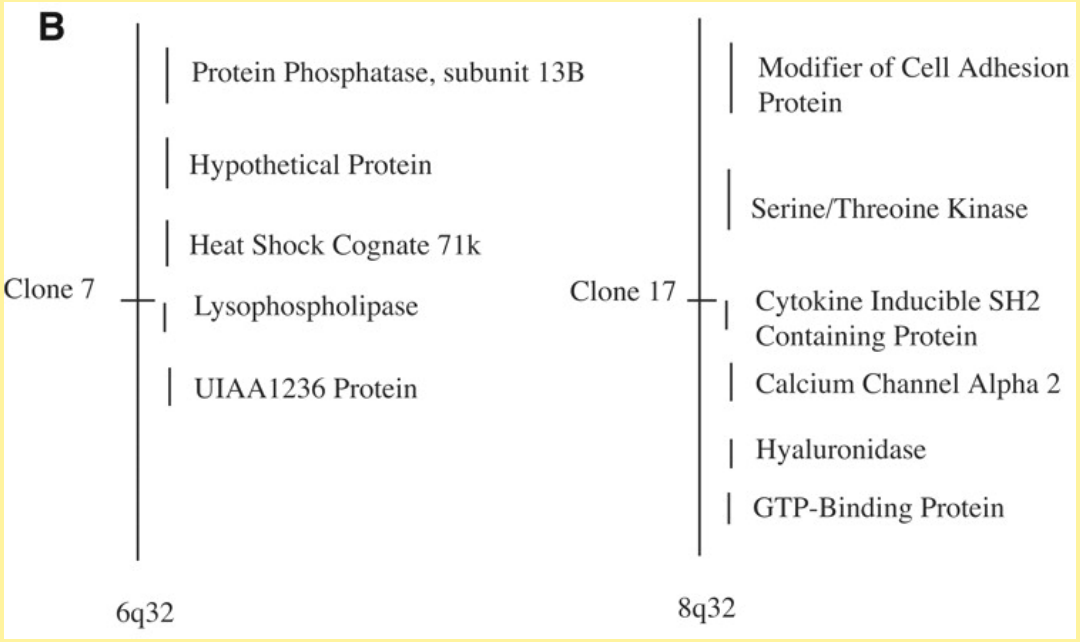
PCR was used to identify areas of DNA methylation in both vinclozolin and untreated male rats.

In both A) and C) we can see differences in DNA methylation between the vinclozolin-treated rats and the controls.

Overall, 25 regions were found to differ in their DNA methylation state between the control and the experimental samples:

Some were found to have methylation where there was none before, and others were lacking their usual methylation.

Figure 3B: DNA methylation changes



These are some genes that are found within two of the altered regions of DNA methylation.

The link between these genes and the sexual defects found in the mice are not obvious.

It is also not obvious what might cause these particular regions of DNA methylation to change.

Conclusions from the paper…

Exposure of a pregnant female rat to two pesticides during the period of pregnancy when her offspring is undergoing sexual determination has negative effects on her offspring, including:

- Decreased sperm cell number and viability
- Increased incidence of male infertility

These negative effects of exposure were observed not only in the male born from the treated female, but in all subsequent generations of male mice that were tested.

The effects of the treatment on reproduction correlated with altered DNA methylation patterns in the cells involved in sexual reproduction.

Are there any other ways to explain this data?

- Could it be due to a change in the DNA itself after treatment?
  - It's possible, but unlikely. The chance of a mutating the DNA in the same way so many times is very, very small. There are hotspots for mutations, but the frequencies observed here are more than 10X higher than anything that's been seen in hotspots before.
- How about a different type of epigenetic change?
  - This is possible, but since the authors give direct evidence for a change in DNA methylation, this seems like a likely possibility. More experiments could be done to test this.
- What about chance?
  - The authors have repeated their experiment several times, and used statistical tests to show that it is very unlikely to see these results by chance.

Questions that remain…

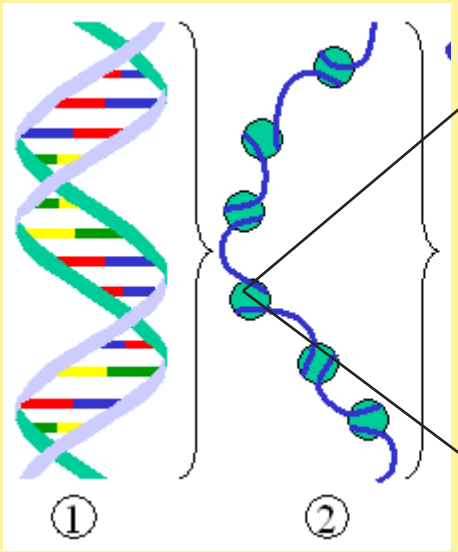
- Are we seeing a direct effect from these pesticides upon DNA methylation, or are there steps in between that we're not seeing? If so, what are they?
- The doses used in this study are higher than the trace amounts of pesticide that people and animals are usually exposed to.
  - Do trace amounts of pesticide have the same effect, but on a smaller scale ?
  - Are these effects additive over multiple generations?
- What other studies would you like to see done?

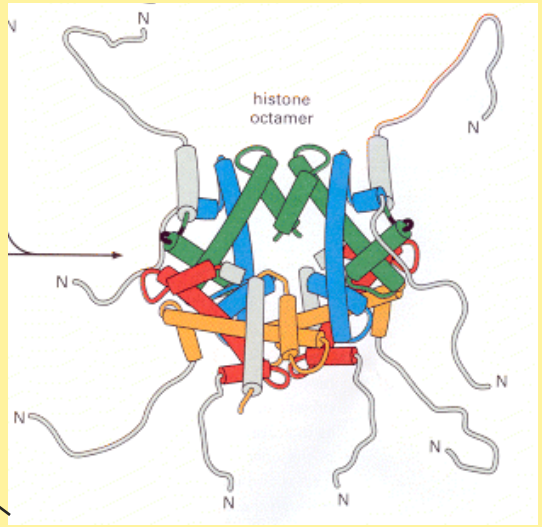
My conclusions…

I think it is very important to teach children and young adults basic science in the context of environmental health, so that as they mature, they can make educated decisions concerning, not only how we affect the environment, but how it affects us.

THANKS!!!

A closer look at DNA packaging…

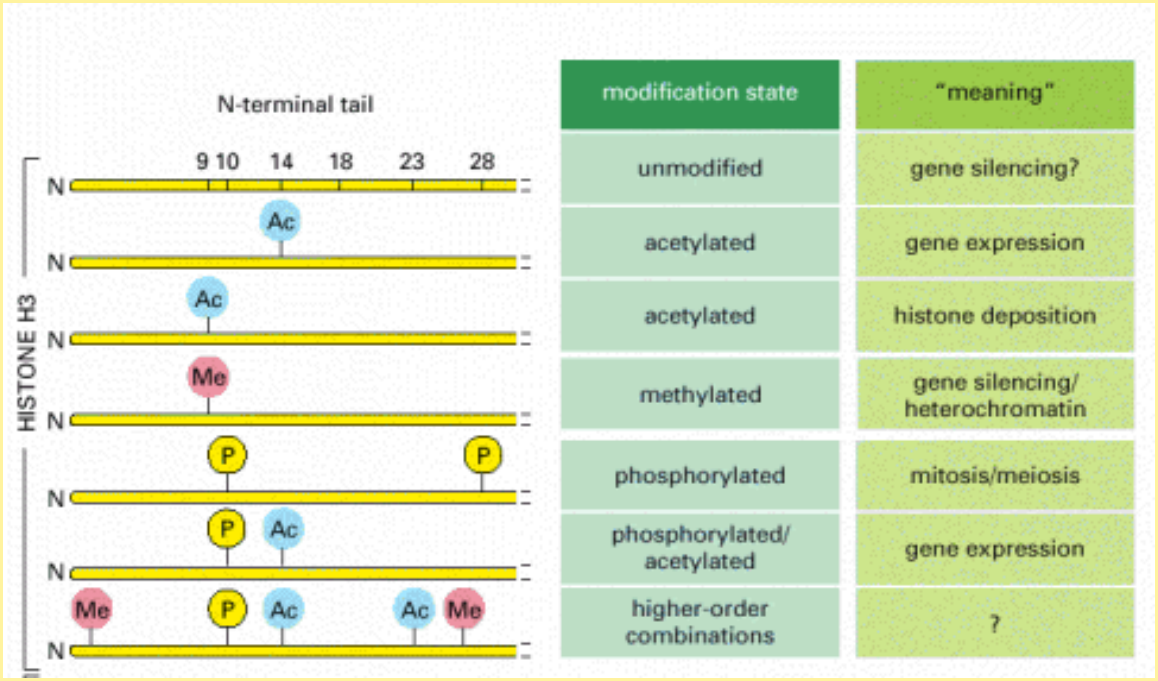




- DNA is found in the nucleus of eukaryotic cells. (1)
- The double helix of DNA is wrapped around a core of proteins called **histones**, creating the appearance of beads on a string (2) This is further twisted into more compact structures.
- **Histones** have protein "tails" that extend outside the core, and are accessible to **chemical modification**.

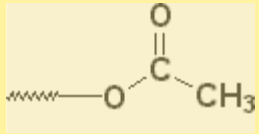
What kind of chemical modification, and what does it do?

The "Histone Code"



modification state	"meaning"
unmodified	gene silencing?
acetylated	gene expression
acetylated	histone deposition
methylated	gene silencing/ heterochromatin
phosphorylated	mitosis/meiosis
phosphorylated/ acetylated	gene expression
higher-order combinations	?

A variety of chemical modifications to histone tails have been observed, including:



- Acetylation ( )
- Methylation ($-CH_3$)
- Phosphorylation ($-PO_4$)

The effect of these modifications on the genome depends on both what type of chemical group it is, and where it lies on the tail.

Common effects of this type of code are to tag genes for transcription or silencing.

Alberts, et al., Molecular Biology of the Cell, 4th edition