1. DNA & RNA Structure, and Chromatin: A Dynamic Structure

LECTURE 13-15

BIO 1140 A – Introduction to Cell Biology

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1-1: DNA & RNA STRUCTURE

Key Questions That We Will Try to Answer As We Go Along

- What is the structure of DNA and how do we know?
  - Polymers, there are many different structures and they are all dynamic
- Is DNA always the genetic material?
  - No
- Is the DNA structure always the same?
  - No, so why bother with a defined structure? : So we have a model to explain and understand in teaching.
- Is the DNA structure in the cell relevant?
  - It's the DNA protein structure that is relevant: the DNA will wrap and supercoil to fit inside the nucleus.
- Is the genome what we think it is?
  - It is not full of genes only.
- Without chromatin, would it be necessary to invent it? (next section)
  - Chromatin = protein DNA complexes

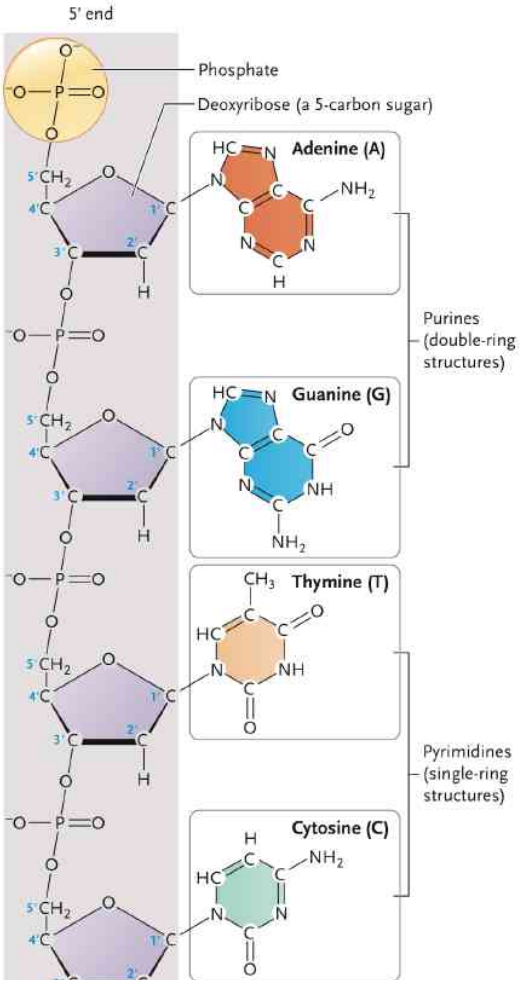
NUCLEIC ACIDS: USEFUL REVIEW INFORMATION (F37-F39)

- Two types of nucleic acid: DNA (stores hereditary information) and RNA (variety of functions in protein synthesis.)
- A nucleo**tide** consists of :
  a. A nitrogenous base formed from rings of carbon and nitrogen
     i. Bases are heterocyclic aromatic rings (attach to 1' carbon)
  b. A five carbon ringed shaped sugar (attach to 4' carbon)
  c. One to three phosphates (attach to 5' carbon)

The nitrogenous bases link covalently to **a five-carbon sugar, either ribose or deoxyribose:**

- Ribose: OH at 2' position (this 2' prime hydroxyl can bond allowing RNA more dynamic structure)
- Deoxyribose: H at 2' position

- The numbering system
  - The nitrogenous base carbons are numbered 1,2,3,4… while the sugar carbons are numbered 1',2',3',4'…
- **If this was RNA what would change?**
  - Uracil replaces thymine (uracil has no –Methyl group)
  - Ribose replaces deoxyribose (2' OH)



BASIC BUILDING BLOCKS FOR DNA & RNA

- Two types of nitrogenous bases: purines (double ring) and pyrimidines (single ring)
  - Purines: adenine (A) and guanine (G)
  - Pyrimidines: thymine (T), cytosine (C), and uracil (U) – in RNA

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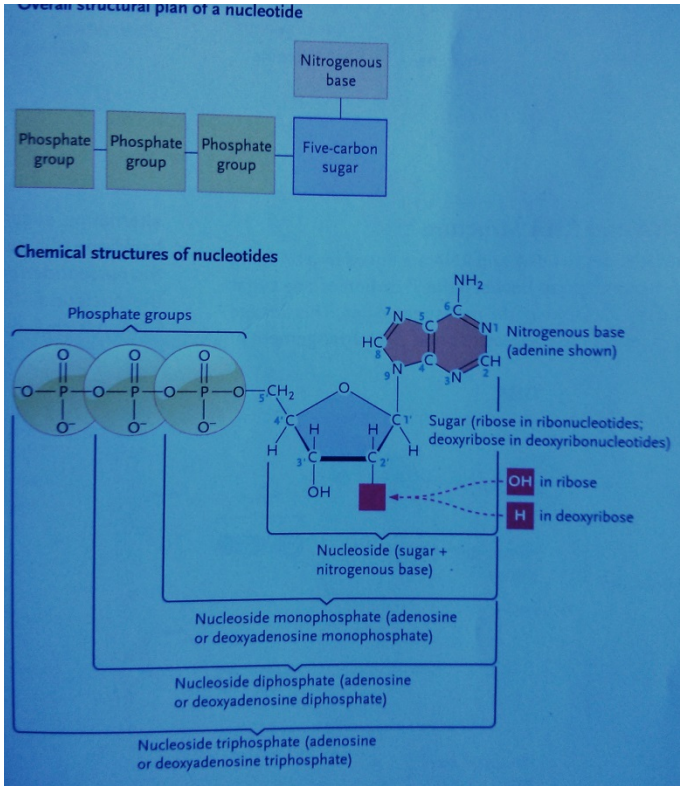
- **A base + a pentose sugar = nucleoside.**
  - if the sugar is a deoxyribose, it is a deoxyribonucleoside (deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine)
- **A nucleoside + phosphate = deoxynucleotide**
  - (deoxyadenosine 5'-monophosphate, deoxycytidine 5'-diphosphate, etc.)
- **There are 5'-monophosphates, 5'-diphosphates, 5'-triphosphates**
  - (deoxyadenosine 5'-monophosphate (dAMP), deoxadenosine 5'-diphosphate dADP), deoxadenosine 5'-triphosphate dATP).



DNA AND RNA STRUCTURE

- Nucleotides in DNA and RNA are linked by a phoshodiester bond between the 5' carbon phosphate groups and 3' carbon of two sugars in line.
- Arrangement of alternating sugar and phosphate groups forms the backbone of nucleic acid.
- The nitrogenous bases project from this backbone

DNA DOUBLE HELIX

- In cells, DNA is found as double helix (or called double stranded DNA), two nucleotide chains wrapped around each other

GENETIC MATERIAL: ALMOST ALWAYS DNA

- A brief summary of the evidence
  1. Transformation experiments (1920s, 1940s)
  2. Chargaff's rules (Late 1940s)
  3. Phage infection experiment (1950s)
  4. X-ray analysis (Late 1950s-60s and 70s)
     - Model Building
     - The modern "synthesis"- DNA structures

1. TRANSFORMATION EXPERIMENTS

GRIFFITH'S EXPERIMENT

- Working with streptococcus bacteria, 2 types: a lethal type(S) that had a bacterial capsule and a non-lethal type (R) with no capsule
- Injected mouse with S. form of bacteria, killed it
- Inject with R form, not killed
- Heat killed the S form cells
- When he injected live R Cells with heal-killed S cells, he found that the living R cells could be converted to virulent S cells due to some factor from the dead S cells. –In some way, living R bacteria had acquired the ability to make a capsule, they had *Transformed*
- Transforming principle!! We changed something in the harmless bacteria to cause it to become lethal.

- Yet, he couldn't figure out for certain which macromolecule was responsible for this transforming principle (lipids, carbs, DNA, proteins?
-

AVERY-MACLEOD MCCARTY EXPERIMENT

Treatment*	Activity	Transformation
None	None	Yes
"Trypsin"	protease	Yes
"Chymotrypsin"	protease	Yes
"Ribonuclease"	RNAse	Yes
"Deoxyribonuclease"	DNAse	None

* I have given them their modern names, Proteases break down protein, RNAses/DNAses break down RNA/DNA

- repeated mouse experiment this time adding specific enzymes to test which macromolecule was responsible for transformation
- only the deoxyribonuclease was able to destroy the transformation process = DNA responsible for transforming principle
- Despite breaking down the proteins/RNA you can still transform the cell so the transforming principle doesn't occur at those levels

Caveats

- **Purity:** They had to make their own extracts, No controls to show purity, no way to make pure enzymes
- Many who believed that protein was the hereditary material argued that their impure enzymes could not have fully removed all protein from the culture and the remaining proteins were in fact responsible for the transformation
- If they did this with another organism where RNA is genetic material or it could have stopped the transformation at the RNAse level and not DNAse level thus indicating that for that organism the genetic material was RNA
- If protease removed the transforming capability than that could have been the transforming principle (there are some cases where protein can change phenotype of cell in a genetic, inheritable manner)

2. CHARGAFF'S EXPERIMENTS

- Chargaff used harsh chemicals to degrade DNA into the four bases then quantified the amount of each. The experiment gave two discoveries, now summarized as **Chargaff's Rule:**
  - The number of Adenine bases is equal to the number of Thymine bases, and number of Cytosine bases is equal to Guanine bases. A=T and C=G.
  - The proportion of A:T and C:G holds true for both strands.
- For example in humans A= 30.9% and T= 29.4%; G=19.9% and C=19.8%.
- How can we explain this result?
  - We now know that Chargaff's rule is correct because we know that A-T base pairs occur and C-G base pairs occur in DNA

Conversely how can we explain DNAs where the Rule does not apply?

- single stranded DNA do not require equal ratios of base pairs
- find the answer, has something to do with chemistry of strands and the fact that he was looking for the 4 bases individually
- many of the bases in our DNA are modified after replication, and they would be "lost" in this analysis

3. PHAGE INFECTION EXPERIMENT

HERSHEY AND CHASE

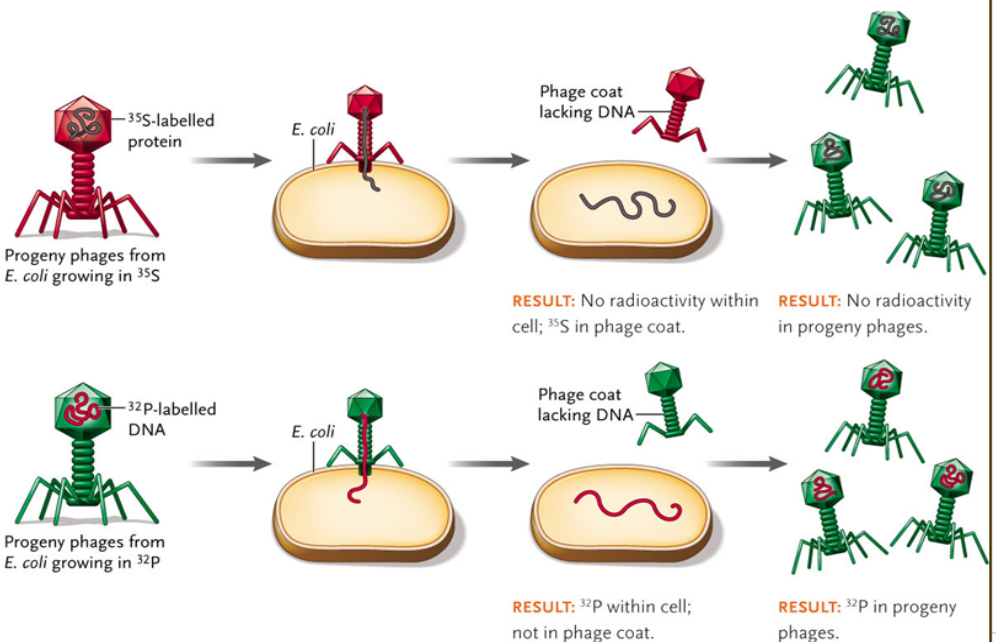
- Studied E.Coli bacteria infected by bacteriophages T2. Viruses infect the cell with viral nucleic acids so these scientists decided to study viruses, and they only have a few genes since they are so small
- DNA on the inside and protein forming outer structure. Therefore either the protein or the DNA must be genetic material
- They prepared two batches of phages, one with the protein radioactively tagged, the other with DNA tagged. Proteins were tagged with an isotope of sulfur, DNA tagged with isotope of phosphorous
    - PROTEIN: They then infected bacteria with protein labeled phages, they found no radioactivity within the progeny phages, but they found it in the material removed by blending (the protein coats)
    - DNA: In the second set, they infected bacteria with DNA labeled phages, they found radioactivity in the progeny phages but none in the coats removed by blending.

Figure 12.3
The Hershey and Chase experiment demonstrating that DNA is the hereditary molecule.

QUESTION: Is DNA or protein the genetic material?

EXPERIMENT: Hershey and Chase performed a definitive experiment to show whether DNA or protein is the genetic material. They used phage T2 for their experiment; it consists only of DNA and protein.

1. They infected *E. coli* growing in the presence of radioactive ^{32}P or ^{35}S with phage T2. The progeny phages were either labelled in their protein with ^{35}S (top), or in their DNA with ^{32}P (bottom).
2. Separate cultures of *E. coli* were infected with the radioactively labelled phages.
3. After a short period of time to allow the genetic material to enter the bacterial cell, the bacteria were mixed in a blender. The blending sheared from the cell surface the phage coats that did not enter the bacteria. The components were analyzed for radioactivity.
4. Progeny phages analyzed for radioactivity.



CONCLUSION: ^{32}P, the isotope used to label DNA, was found within phage-infected cells and in progeny phages, indicating that DNA is the genetic material. ^{35}S, the radioisotope used to label proteins, was found in phage coats after infection but was not found in the infected cell

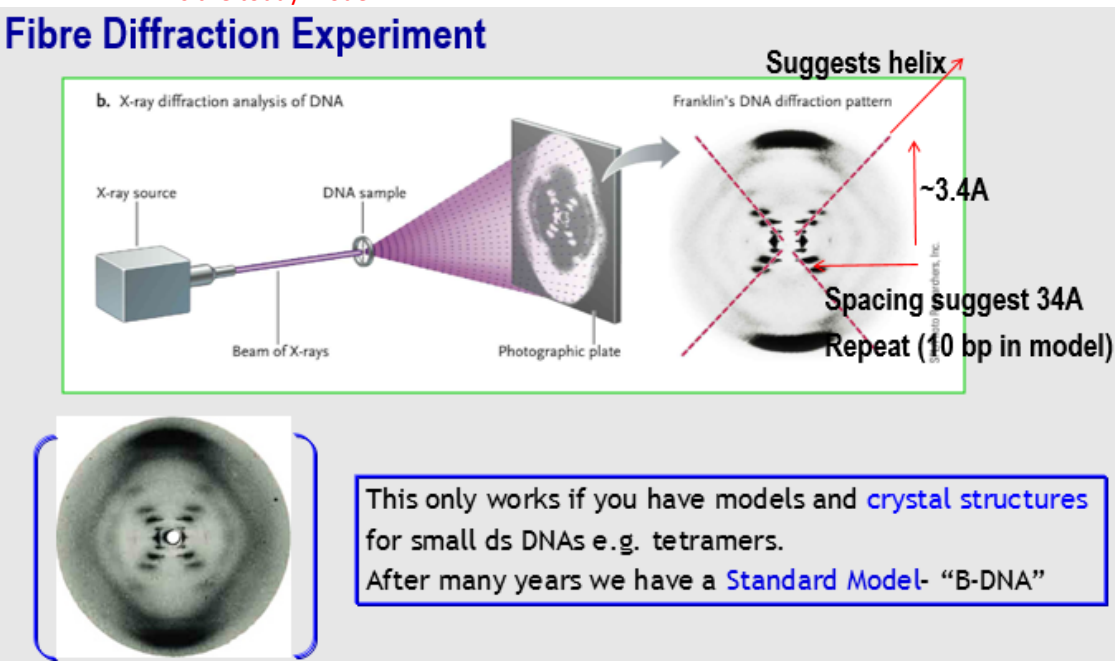
- RESULT: the virus's protein doesn't enter the cell, ONLY it's DNA, therefore the DNA is the genetic material of the phage, not protein.

Caveats

- A lot of viruses, this experiment wouldn't work, only worked because of this viruses special properties (i.e. its phage and protein coat)

4. X-RAY - FIBRE DIFFRACTION EXPERIMENT

- In X-ray diffraction, an x-ray beam is directed at a molecule in the form of a regular solid, ideally in the form of a crystal
- Within the crystal, regularly arranged atoms bend and reflect the X-rays into smaller beams that exit the crystal at definite angles determined by the arrangement of atoms in the structure of the crystal.
    - Rosalind Franklin correctly deduced that the shape of DNA was a staircase like double helix.
    - The distance between bases is 3.4 angstroms, the cross patterns suggest it is a helical structure
    - 10 spots (in a better pic) suggests that the molecule repeated every 10 units (base pairs)
- This only works if you have models and crystal structures for small ds DNAs e.g. tetramers.
    - They used a sample of DNA molecules pulled out into a fibre.
- After many years we have a Standard Model– "B-DNA"
- Watson and crick made the model that is still often made today
- Their model was inaccurate though
- The only way for model to be accurate is to have better information by actually crystalizing the base pairs, and the more base pairs that are crystalized the better
- B-DNA is the today model



Caveats

- Not enough information to build the model of DNA we have today, but enough to make small DNA with few base pairs (e.g. tetramers)

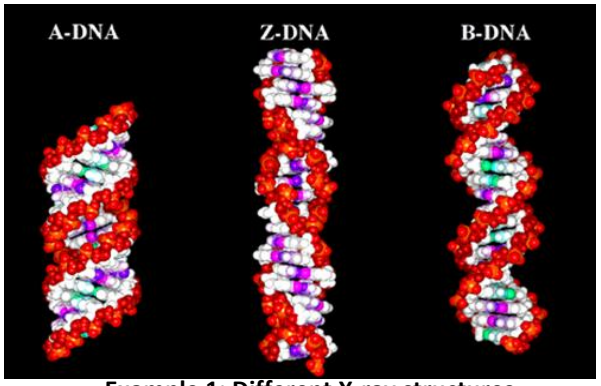
DNA STRUCTURE

- Natural DNA forms a right-hand helix
  - (If you look along the length of the helix from the top [red arrow], the strands spiral down and to the right).
- Note: you do see reproductions where the DNA is left-handed, even in leading journals or advertisements or posters!!
- The two strands are "anti-parallel" (read 5' to 3'but in opposite directions).
- The distances between the coils of the double helix isn't the same (major and minor grooves)
- Major groove where regulation occurred (proteins can interact with DNA), structure suggests metabolic function
- Directional! 5' → 3'
- If there are different versions of DNA, why have a standard model? Because it gives us a starting point which we can use as a comparison point for DNA

DNA STRUCTURE IS NOT ALWAYS THE SAME

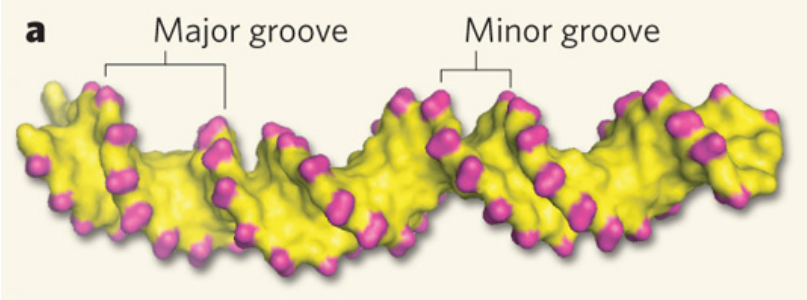
Why not?

- Different X-ray fibre patterns (A,B,C,D, Z), under different ionic conditions
- The vast majority of DNA within the cell is the normal DNA structure. However in nature DNA can form three structures, A-, B- and Z-DNA. A and B chromosomes are very similar, forming right-handed helices, while Z-DNA is a more unusual left-handed helix with a zig-zag phosphate backbone. Z-DNA is thought to play a specific role in chromatin structure and transcription because of the properties of the junction between B- and Z-DN



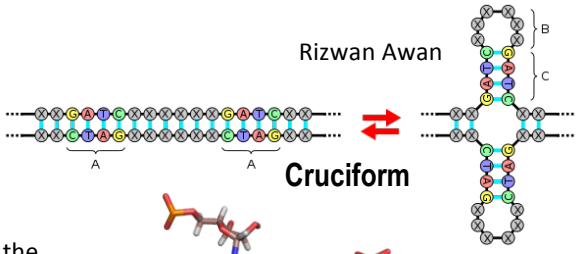
Example 1: Different X-ray structures

- Z DNA is found in GC –rich DNA and is left-handed!
- Z DNA has been associated with some binding proteins and transcription
- These in vitro results suggest that microheterogeneity can occur in the DNA structure!
  - **Microheterogeneity:** variation in chemical structure of the DNA without producing major change in its properties
  - Proteins can help change structure even more (topoisomerase, helicase, etc.)



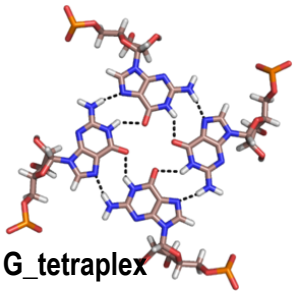
Example 2: AT-rich DNA and narrower

- If you have double stranded RNA it looks like A-DNA (double stranded RNA from a viral infection, perhaps)
- DNA-RNA hybrid (1 strand DNA, 1 strand RNA) looks like A-DNA → this is happening during **transcription**
- Z-DNA is left handed → only left handed one!! You see this in a test tube when you have a **high G and C** content in DNA
- Experiment designed recently distinguishing between left and right handed DNA and the experiment showed there is left handed DNA
- Having a AT rich or GC rich DNA will cause different structures (go to extremes – put ALL AT or ALL GC in a strand)
- Different forms of DNA occurring when different metabolic functions occur
- Why do we use the B-model as the standard? We need a model to work with for research and explain concepts, Strawmann hypothesis

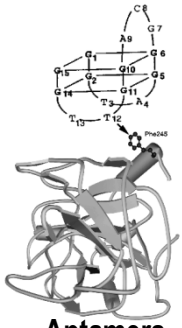


Cruciform

- There are a variety of "strange structures" but do they "exist" in nature.
- Cruciform with hairpin structures
- G-tetraplex
  - There is reasonable evidence that there is G-tetraplex at the end of our DNA strands
- DNA aptamers**
  - DNA Aptamers are sequences of DNA that can make structures that can act as protein by forming specific recognition sites
- DNA structure can change with time
- DNA has many structures and is dynamic, changes based on required function
- Example Question: tell me why you think DNA is dynamic and give me two examples?



G_tetraplex



Aptamers

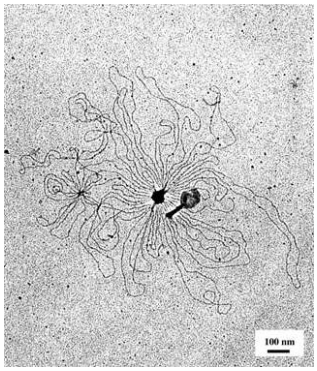
What can we conclude about DNA structures?

- DNA has many structures and is dynamic.
- Example Question: tell me why you think DNA is dynamic and give me two examples?

THE BASIC OR ARCHETYPAL STRUCTURAL FORMS FOR DNA AND RNA.

Type of Organism	Genetic Material	Organization
"Bacterium"	dsDNA(s) circular	Protein/DNA Nucleoid
"Eukaryote"	dsDNA linear	Protein/DNA Chromosome
Mitochondrion	dsDNA circular	Protein/DNA
Chloroplast	dsDNA circular	Protein/DNA
Virus	ss or ds DNA circular or linear	Protein/DNA
	ss or ds RNA circular or linear	Protein/DNA
Viroid	ssRNA, circular	None ?

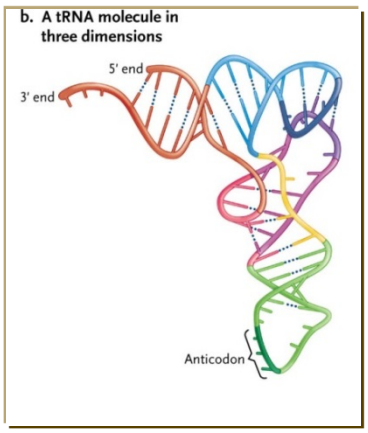
*Protein/DNA structure: refers to how is the DNA organized (DNA is 2m long, MUST be folded into space of 10um)
RNA viruses would not undergo transformation after being exposed to RNAses and so would disprove Avery McCarthy Experiments
Viroid: small, can infect plants, no protein RNA structure, ex. would not work in Avery McCarthy experiments

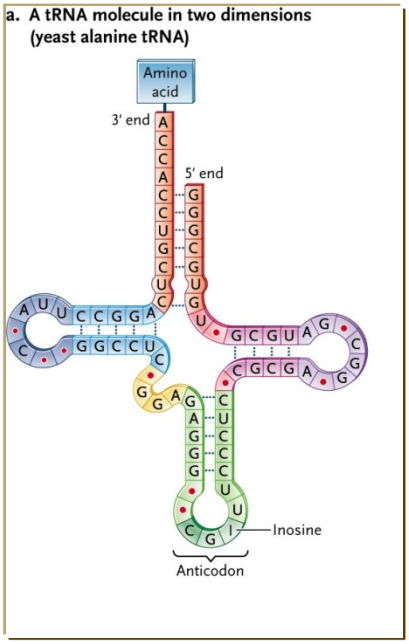


- DNA released from bacteriophage (bacterial virus). You can see individual ds DNA!
  - Directly infer that DNA strands directly bind to blobs which are proteins
  - If we took that cell and broke it open, the white blob is the protein DNA complex, and we can see some of the plasma/DNA's going out and coming back into that giant blob
- DNA released from chromosome. After you remove histones you can see individual ds DNA! **
- We can conclude that there are many structures for DNA & DNA-Protein in the cell. This is not surprising because...
- We can't really see DNA moving in and out of chromosome because it's so much larger and more complicated
  - ** in future lectures I will refer to the protein core as the Matrix or scaffold – two most common names for eukaryotes, proteins that help organize the DNA
- DNA must be folded, when folded there are repulsions between the phosphate, to solve this there are histones proteins

RNA STRUCTURES ARE HIGHLY ORGANIZED

- **Primary** (5' GGGCGUG...etc.)
  - Sequence of nitrogenous bases (linear)
- **Secondary**: the folding of proteins into pleats, loops, etc. due to polar interactions (i.e. hydrogen bonding) (2D)
- **Tertiary**: further folding of the proteins but with distant groups and more powerful interactions (supercoiling due, H bonding, etc.) (3D)
- **Quaternary:** multiple peptide chains forming a protein unit
- Red dots are modified bases (pic right)





- Model of a group II intron showing
- Extensive pairing with itself (p295).
- This is a secondary structure.

IS THAT ALL THERE IS?

- DNA can be extensively modified in the cell. These modifications are normally post replication.
- While there are many modifications we will only discuss (later) the methylation of cytosine. 5-methylcytosine (5MeC) is implicated in the regulation of eukaryotic genes.
- RNA can be extensively modified in the cell. These modifications are normally post transcription.
- While there are many modifications we will only discuss (later) the methylation of guanosine and polyadenlylation of mRNA; RNA editing that changes cytosine to uridine; and some modifications in tRNA that affect structure.

WHAT LESSONS CAN WE DRAW FROM THESE EXAMPLES?

- -chemistry is important for structure and function
- -tertiary structure is important for correct binding of proteins
- -structures are dynamic, they are affected by many processes that change the need for genes being expressed or not, different metabolic functions, folding
- -can we predict the tertiary structure?
    - Only by observing similar classes of molecules, not by straight up looking at the primary structure
- We can conclude that there are many structures for DNA, DNA-Protein, RNA, & RNA-Protein in the cell.
- This is not surprising because cell is **DYNAMIC**!

Quiz

- **DNA and RNA structures**
    1) **What is the possible role of transformation *in vivo?***
        - Pickup up DNA, adaptation (i.e. evolution),
        - Recycling: DNA is composed of carbon, nitrogen and are reused once organism dies
    2) **Is DNA always the same structure?**
        - No, the structure of DNA is dynamic, because: crystallographic studies of fibre diffraction: form A, B, Z (B is classic form)—one example
        - during transcription DNA structure comes apart, allowing for transcription/replication
        - after DNA rep, DNA can be modified
    3) **What is microheterogeneity?**
    4) **What are the different possible RNA structures?**
        - primary, secondary and tertiary, via modification of bases

1-2: CHROMATIN, A DYNAMIC STRUCTURE

THE BASIC PRINCIPLES

- DNA packaging involves **specific proteins.**
- Packaging is achieved via the use of **repeating subunits.**
- More compact structures are based upon **simpler structures.**
- Subunit assembly must be **dynamic** to allow for change and disassembly that allows functions like transcription, replication.
- More compactness= less activity (ex. Less transcription)
- **Protein modification** (s) can affect chromosome structure.
    - **In eukaryotes this is called the "histone code"**
        - The **histone code** is a hypothesis that the **transcription of genetic information encoded in DNA is in part regulated by chemical modifications to histone proteins**, primarily on their unstructured ends. Histones associate with DNA to form nucleosomes, which themselves bundle to form chromatin fibers, which in turn make up the more familiar chromosome**. Histone tail modifications correlate very well to chromatin structure and both histone modification state and chromatin structure correlate well to gene expression level**s. The critical concept of the histone code hypothesis is that the **histone modifications serve to recruit other proteins by specific recognition of the modified histone** via protein domains specialized for such purposes, rather than through simply stabilizing or destabilizing the interaction between histone and the underlying DNA. **These recruited proteins then act to alter chromatin structure actively or to promote transcription.**

- **Viruses**: Use basic proteins that may be part of the virus particle (capsid).
- **Bacteria:** Use basic proteins such as "HU", IHF", "H-NS" to form a series of loops called a **nucleoid.**
    - Why are all these proteins basic?
      (have a positive charge at neutral pH, amino acids are basic)
- **Eukaryotes:** Use basic proteins called histones AND a hierarchical organization
    - Specific regions of the histone are basic and they interact with the DNA which is a polyanion (negatively charged phosphate groups) and can interact with positively charged histones.

DNA ORGANIZATION IN EUKARYOTES AND PROKARYOTES

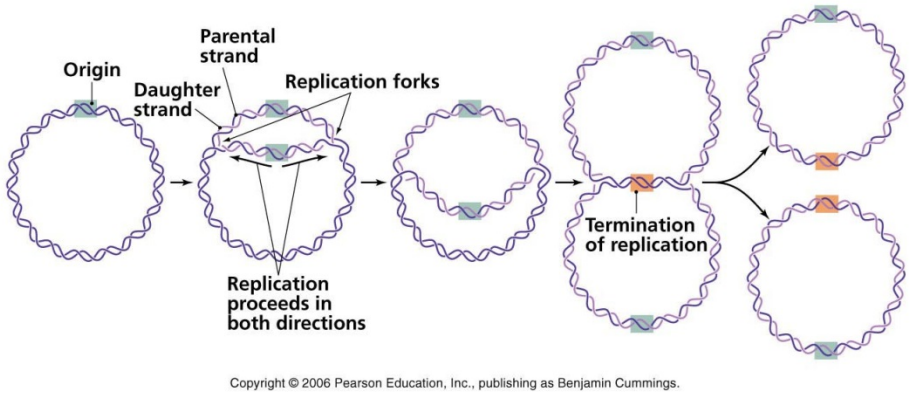
- ***It's a fundamental property of the genome to be organized, to be folded in some way inside the nucleus. Now it's becoming clear that there is more to the genome than the sequence. We have to describe how the genome is organized, figure out the mechanisms involved in the organization, and then figure out how the organization contributes to function. Tools are being developed to really address these questions in a systematic fashion."***
- Fundamental property of all living organisms

- Histones pack eukaryotic DNA at successive levels of organization i.e. **hierarchical organization**
- Many non-histone proteins have key roles in the regulation of gene expression
- DNA is organized more simply in prokaryotes than in eukaryotes
- DNA-protein interactions follow the same **basic principles**
    - ***To get "real" we must understand DNA-protein complexes and how they change with time and development within the cell! The structure is DYNAMIC!***

PROKARYOTIC CHROMOSOMES

THE BACTERIAL CHROMOSOME

- Sizes range from 500 to 10,000 kilobase pairs (kb) (a kb= 1000 base pairs)
- Closed, circular molecule of DNA packed into **nucleoid**
- Replication begins from a single origin (ori), proceeds in both directions (pg. 163)



- **Plasmids** (in many bacteria) replicate independently of the host chromosome

THE BACTERIAL NUCLEOID

- DNA + basic proteins such as "HU", "IHF", "H-NS", "Fis", "StpA"
- 40 and 50 supercoiled loops of DNA/genome in E. coli
- The loops are secured by protein-protein and protein-DNA interactions (I will call this the "**matrix**": protein core as the matrix or scaffold.(Proteins that help organize the DNA)
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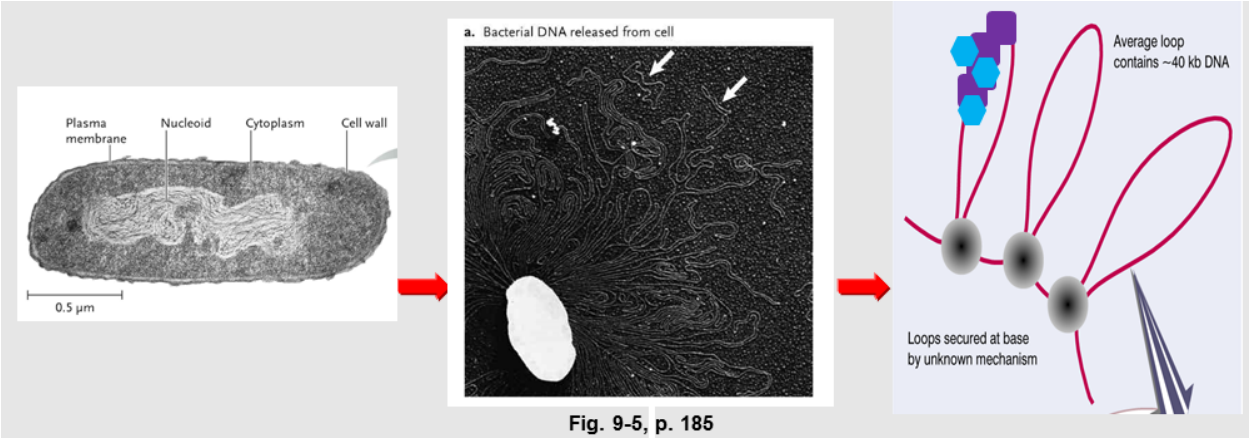


Fig. 9-5, p. 185

- The black circles are proteins, the blue and purple blocks are proteins
- Supercoiled and topologically independent (i.e. if u cut one of the loops, the other loops still remain in coil structure, if you affect for a function (ex. Transcription) one loop it does not affect neighbouring loops)

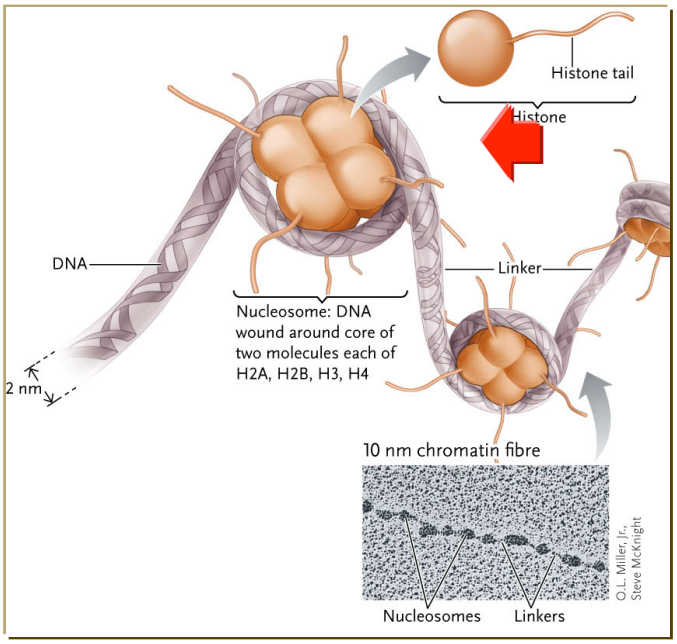
EUKARYOTIC CHROMOSOMES

- Consist of DNA complexes with **histone** and **non-histone** proteins, called **chromatin**.
- DNA wraps around a **nucleosome (diameter of 10nm)**

- Primary level of folding : nucleosome, DNA wrapped around a complex of 8-proteins, 2 copies of each = 4 histones
- Nucleosome structure folds into solenoid, more folding leads to metaphase chromosome (X shape thing)
- **Linker** DNA connects adjacent nucleosomes
    - Binding of histone H1 causes nucleosomes to package into a coiled structure (**solenoid**)
- Non-histone proteins help control the expression of individual genes
- ***In a human cell 6,000,000,000 bp -haploid content-(6 x 10^6kb or ~ 2m of DNA) is packaged into a nucleus with a diameter of 10µm! Thus "how can we fit this DNA into the** nucleus **and AT THE SAME TIME allow the DNA to be replicated and transcribed??***

NUCLEOSOMES AND CHROMATIN FIBER

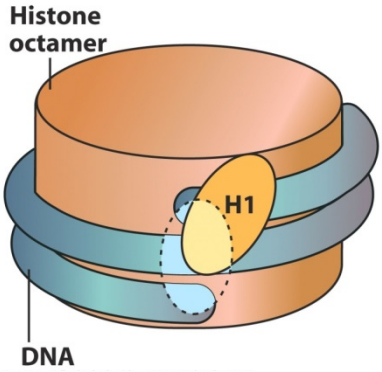
- **Nucleosomes form a left-handed helix.(**if you look along the length of the helix from the top [red arrow], the strands spiral down and to the left)
- ***What is the difference between nucleosome & core nucleosome? *****
    - *Nucleosome: ~200 bp of dsDNA plus 2 copies each of the four histones H2A, H2B, H3 and H4 plus one copy of H1. It includes the linker DNA.*
    - *Core Nucleosome: ~146bp of dsDNA plus 2 copies each of the four histones H2A, H2B, H3 and H4.* ***Does not include linker DNA or linker histone H1.***
    - *Unlike the other histones, H1 does not make up the nucleosome "bead"(core nucleosome). Instead, it sits on top of the structure, keeping in place the DNA that has wrapped around the nucleosome. H1 is present in half the amount of the other four histones, which contribute two molecules to each nucleosome bead. In addition to binding to the nucleosome, the H1 protein binds to the "linker DNA" at the entry/exit of the DNA strand on the nucleosome*



NUCLEOSOME STRUCTURE IS CONSERVED

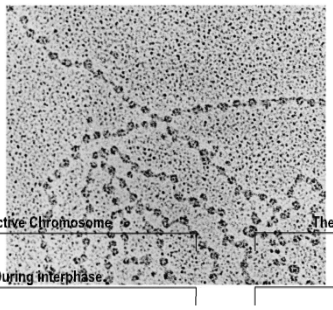
Nucleosome parameters**

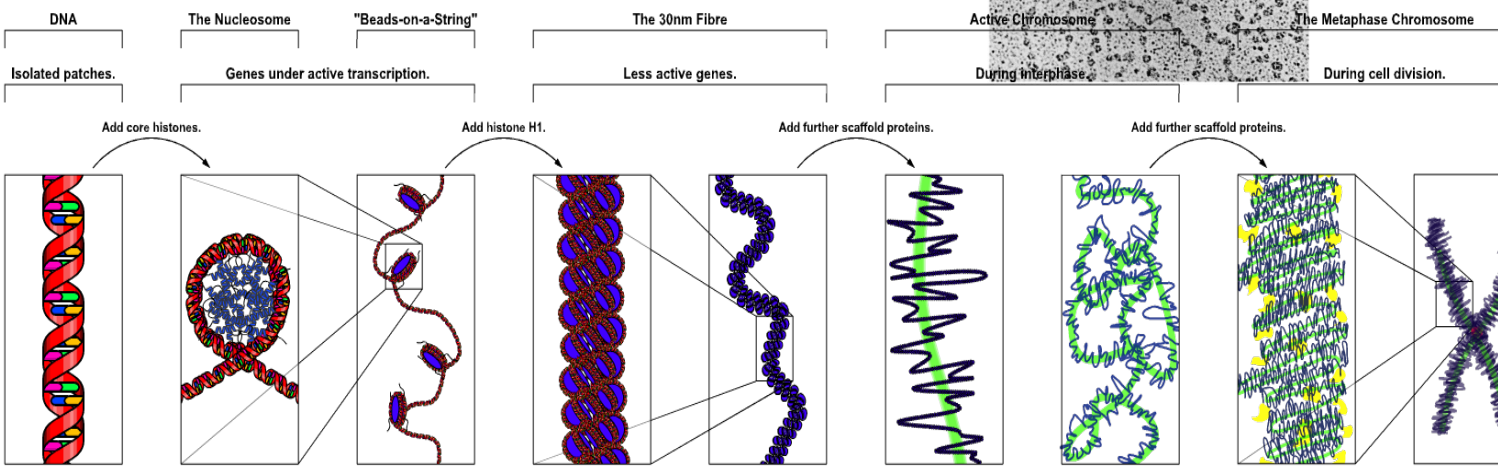
- 2 each of 4 histones named H2A, H2B, H3 and H4 (the "octomer")
- DNA (~146 bp) is wrapped around the outside
- adding histone H1 brings nucleosomes together to form the 10nm fibre
- sequences that are distant can now be closer together
    - Two regions that were 80 bp apart to be brought into close proximity. Thus, two sequences that are far apart can interact with the same regulatory protein to control gene expression.
    - Linear representation they are far apart, but in 3D



configuration they are folded and now closer together(i.e. see the heads of the strands in this pic (blue strands))

- Electron Micrograph of *Drosophila melanogaster* chromatin showing nucleosomes and 10nm fibre (diameter). This is the famous picture of "beads on a string".
- ***You see this only if H1 is removed, why??***
  - We can see linker DNA, because the function of H1 is to bring

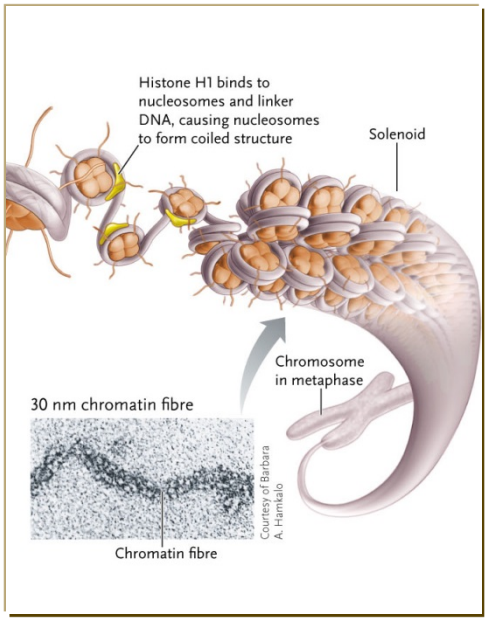




adjacent nucleosomes together.

THE SOLENOID (OR 30 NM FIBRE)

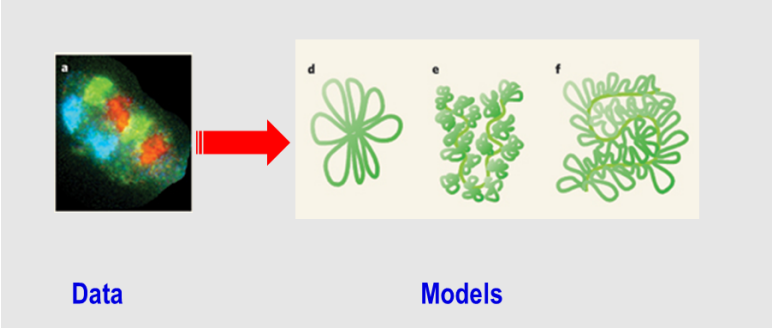
- Binding of H1 causes nucleosomes to package into a coiled structure 30 nm in diameter, the 30 nm fibre or solenoid
- 6-8 nucleosomes per turn
- Structure not well defined, debated
- **Sequences that are more distant can now be closer together!**
- **The next hierarchical level of organization is to fold these solenoids into loops….**



HIGHER ORDER STRUCTURES

"Looped Structure" And The "Chromosome"

- **While the previous structures are well defined at several levels, there are higher ordered structures which are less well defined (WHY!).**
- **What are they and what is the evidence for them?**



- The loops in the models above are made up of solenoids.

a, Territories can be visualized using chromosome-specific fluorescent probes. In this mouse liver nucleus, chromosome 12 (red), chromosome 14 (green) and chromosome 15 (blue) were painted. Different chromosomes are found in different territories within the nucleus.

d–f, Higher-order organization of chromosome territories.

d, In plants, the chromatin fibre forms a rosette-like structure.

e, In higher eukaryotes chromatin forms interconnected megabase-sized domains.

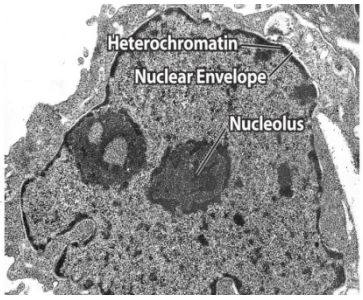
f, Other models suggest looping of the fibre from a central backbone.

- Modifications of the histone drive change from lowest hierarchical form to highest (nucleosomes to chromosome

General Properties of Chromatin In Higher Order Structure

Heterochromatin	Euchromatin
•more highly condensed •Genes are generally inactive •DNA is more highly methylated. •5 methyl cytosine (more later) •15% or higher of genome is heterochromatin ** •Centromeric and telomeric •regions •Highly enriched in DNA repeats Heterochromatin can be constitutive or facultative; heterochromatin and euchromatin can change by a process called chromatin remodelling.	•Less condensed, more dispersed •Genes are generally active •DNA is less highly methylated.

- **constitutive heterochromatin**, containing few genes and formed principally of repetitive sequences located in large regions coincident with centromeres and telomeres, from
- **Facultative heterochromatin** composed of transcriptionally active regions that can adopt the structural and functional characteristics of heterochromatin, such as the inactive X chromosome of mammals.
- Barr Body- strong heterochromatic region, an X inactivation (XY in males instead of XX)
    - In females, one of the X is totally inactivated by heterochromatization, complete inactivation, most highly condensed chromosome

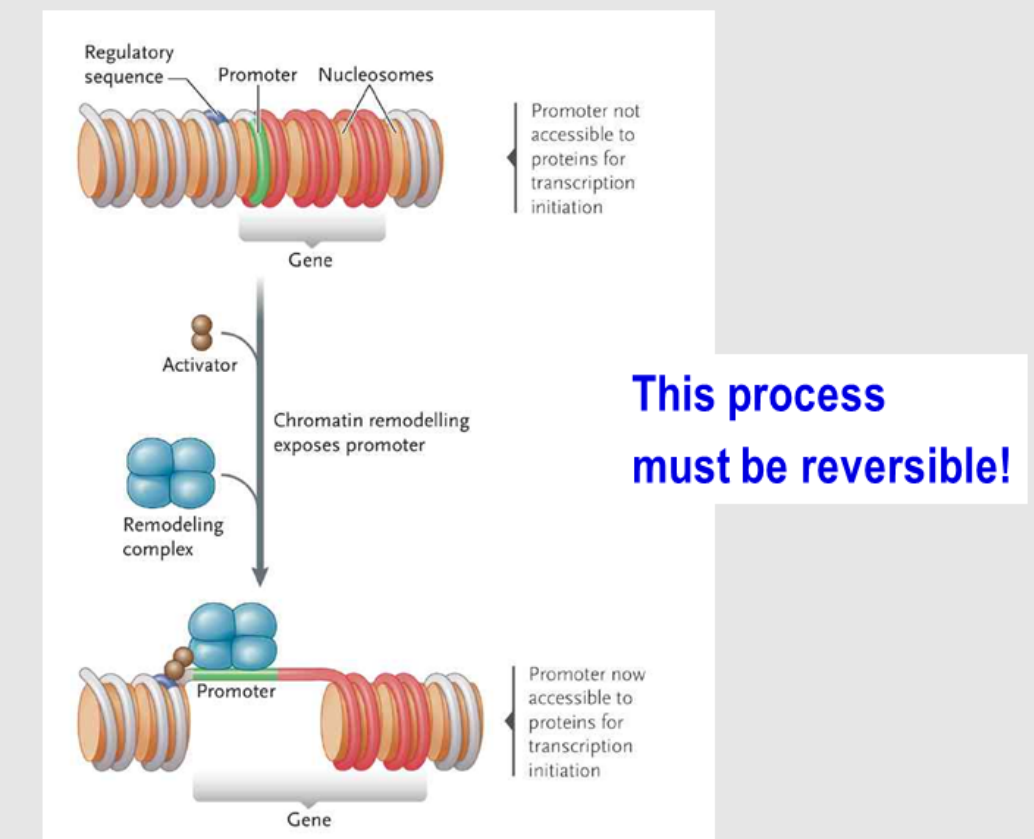


CHROMATIN REMODELLING

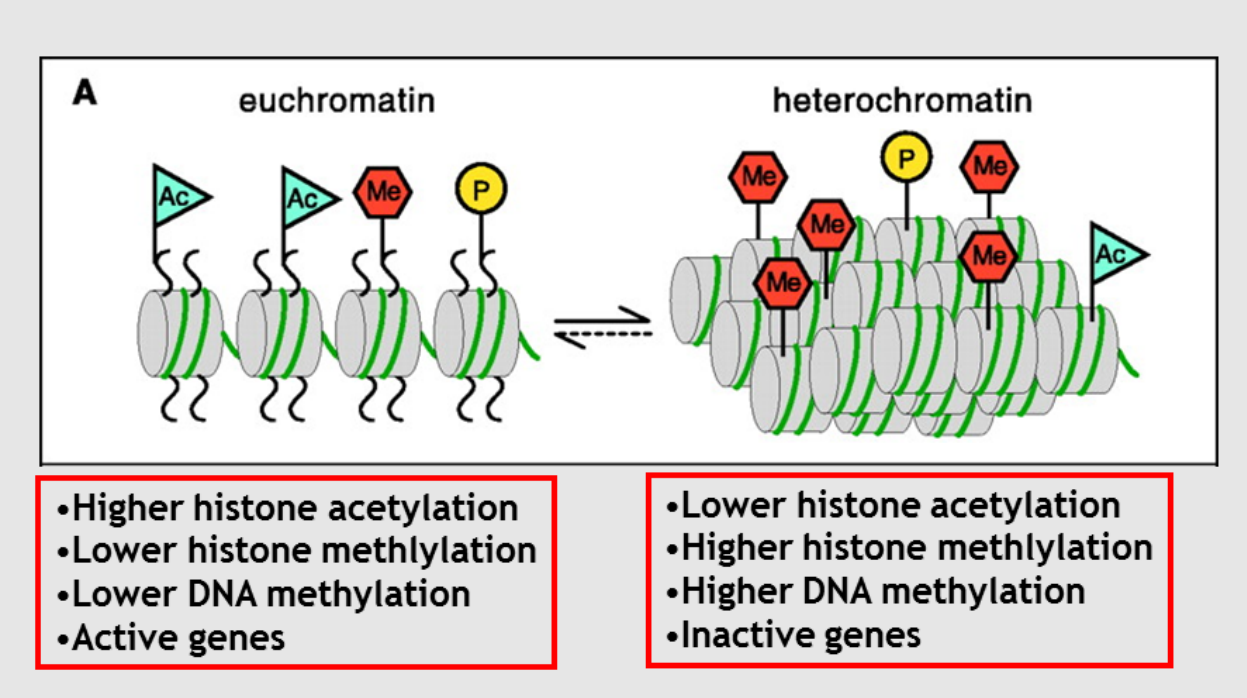
- Eukaryotic DNA wraps around **histones**, to form **nucleosomes**, **etc**. **Promoters are less accessible!**
- **Chromatin remodeling** makes gene promoters more accessible
- **Non histone proteins** help control the expression of individual genes by modifying histone association to DNA (tighten or loosen-ex. if gene is in more extended euchromatin it is more accessible.)
    - Activators –proteins that recruit remodeling complexes that displace nucleosomes and expose promoter
    - Activators recruit enzymes modify histones and loosen their association with DNA. The best studied system is **acetylation of Lysine**. This can be reversed by de-acetylation enzymes
        - Other modifications can include methylation of Lys, methylation of Arg and His, phosphorylation of Ser and His.
- These modifications affect the "tails" (amino termini) on the surface of the nucleosome.
- **The influence of these modifications on transcription is known as the "HISTONE CODE"**

HISTONE CODE: IMPORTANCE

- Post-translational modifications of histones introduce meaningful variations into chromatin and provide a regulatory platform for controlling.....transcription... repair & ...replication.
- Histone modifications, together with factors responsible for adding ...., interpreting... & removing ...modifications, regulate ...outputs of our genomes...the basis of the 'histone code hypothesis'.
- As recent evidence starts to link the miswriting, misinterpretation and mis-erasing of histone modifications to oncogenesis, we further propose that misregulation of the histone code leads to deregulated gene expression and perturbation of cellular identity, and is therefore a major contributor to cancer initiation, progression and/or metastasis (=the development of secondary malignant growths at a distance from the primary site.)



THE HISTONE CODE AND CONSEQUENCES



- This section of the course describes the effects at the level of chromatin where the change from one state to the other affects many genes in the region affected. This is a large scale effect. In some cases an entire chromosome can be silenced – called "genomic imprinting". See text p334.
- Localized methylation can also occur and effect (inactivate) only one (or a few) genes. In this case the chromatin structure is not changed!! This is discussed in the section of Regulation. See text p333-4.
- Wiki:
  - Histone methylation is generally associated with transcriptional repression.
  - However, methylation of some lysine and arginine residues of histones results in transcriptional activation. Examples include methylation of lysine 4 of histone 3 (H3K4), and arginine (R) residues on H3 and H4.

CHROMATIN SUMMARY

- The structure of chromatin influences transcription, replication, repair and recombination
- Chromatin remodeling can convert accessible (euchromatin) to inaccessible (heterochromatin) and is reversible
- Remodeling is influenced by alterations of the cell physiology (signals that regulate genes)
- Remodeling influences the expression of many genes, thus the "transcriptome": the summary of all the messages or all the RNAs that are made at one time.
- **The histone code hypothesis** states that the sum of modifications to the "tails" of the histones mediates transcriptional changes by mediating change in structure
- Histone modification and histone variants can play a role in the regulation of gene expression by mediating the compactness of the DNA strand.