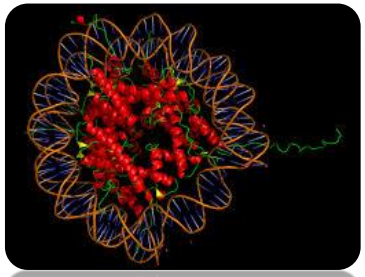
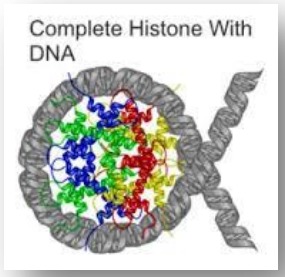
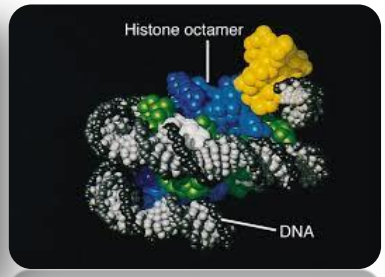
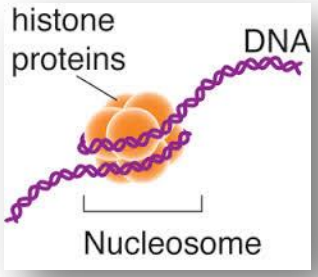
UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

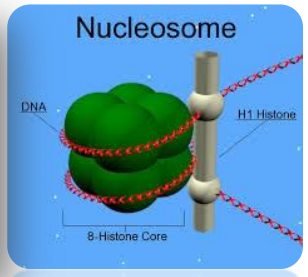
BIOC315W1

DNA Chemistry







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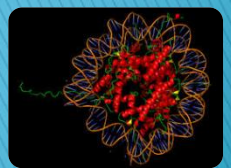
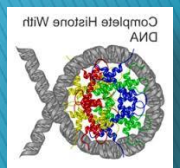
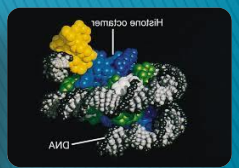
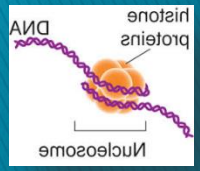
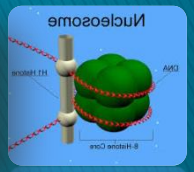
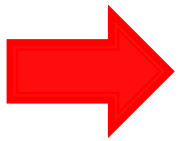
Histones and Nucleosomes

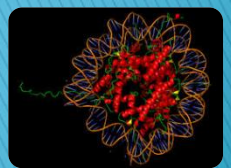



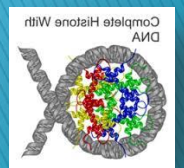


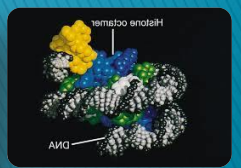


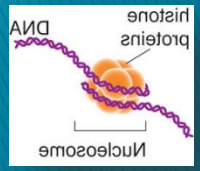


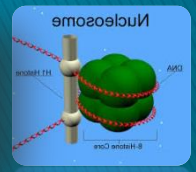




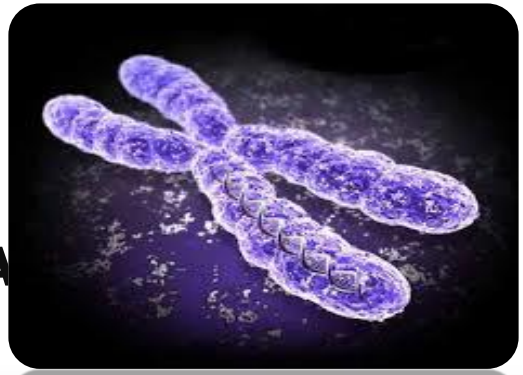

Complete Histone With
DNA


Histone octamer
DNA

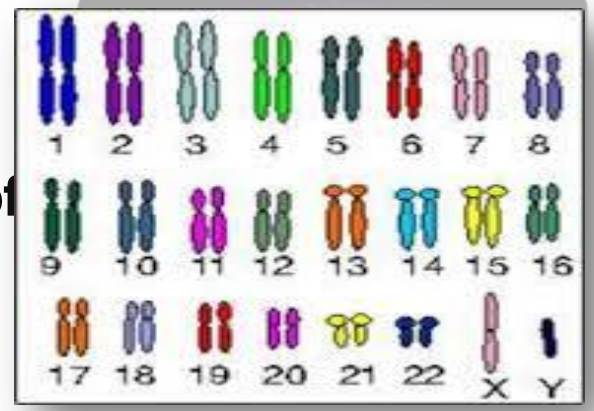

Histone
proteins
DNA
Nucleosome


Nucleosome

Chromosomes



- Each chromosome is made up of a single DNA molecule with an equal mass of protein.

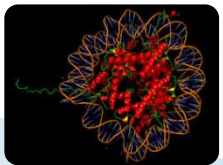
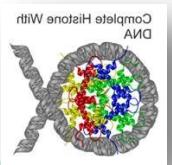


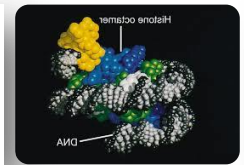
DNA + Protein = CHROMATIN

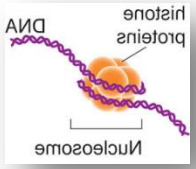
- Human genome has approx. 3.9 x 10^9 bp of DNA organized as 23 chromosome pairs.

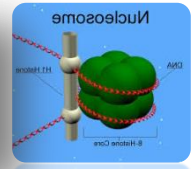
Table represents the haploid genome

	CHROMOSOMES	BASE PAIRS	LENGTH (mm)
Yeast (*S.cerevisiae*)	16	1.4 x 10^7	4.6
Fruitfly	4	1.7 x 10^6	56
Human	23	3.9 x 10^9	1300

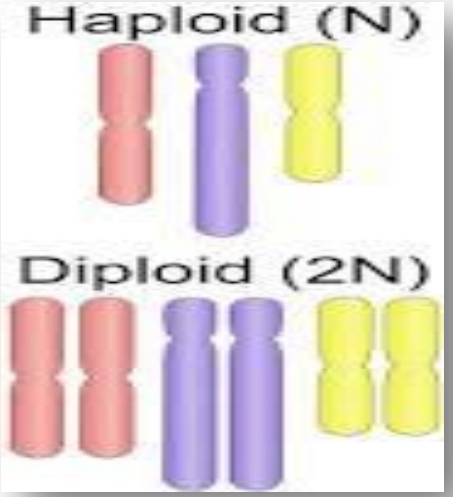






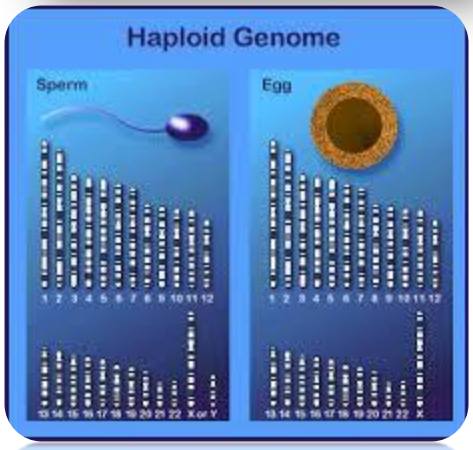
Chromosomes – contd…

- Uncoiled DNA from each chromosome – would measure between 1.7 – 8.5 cm long.



- Total length for haploid genome = 1300 mm

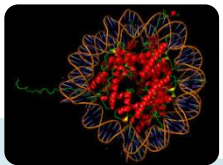
Diploid genome > 2 m (2600 mm)

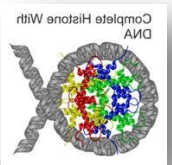


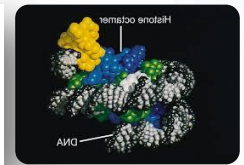
Q: How can >2 m of DNA be packaged into the nucleus of a cell?

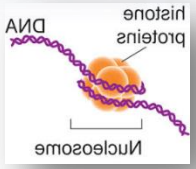
- Structural studies revealed 3 levels of folding.

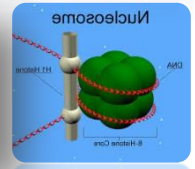
Look at proteins responsible - **HISTONES**



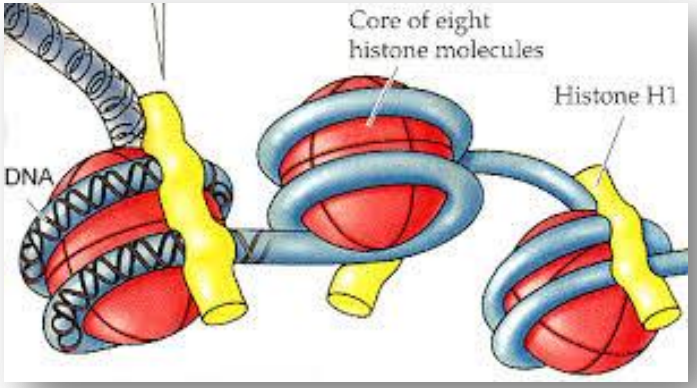




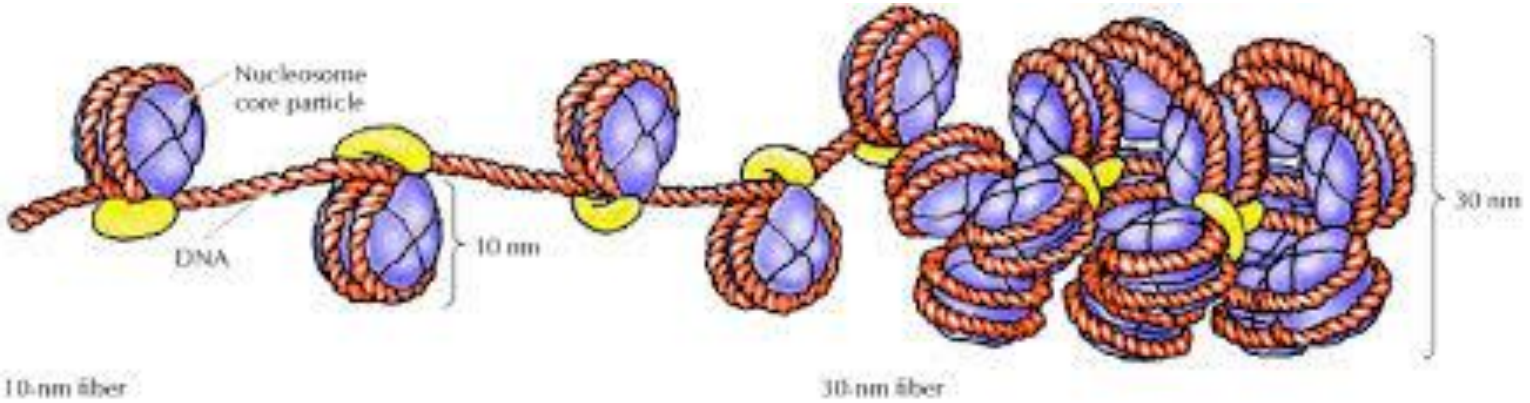




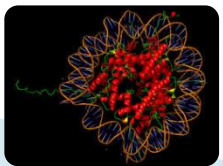
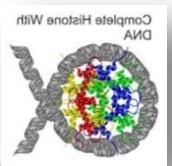
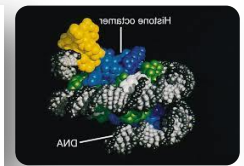
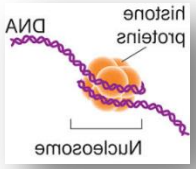
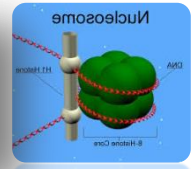
Histones



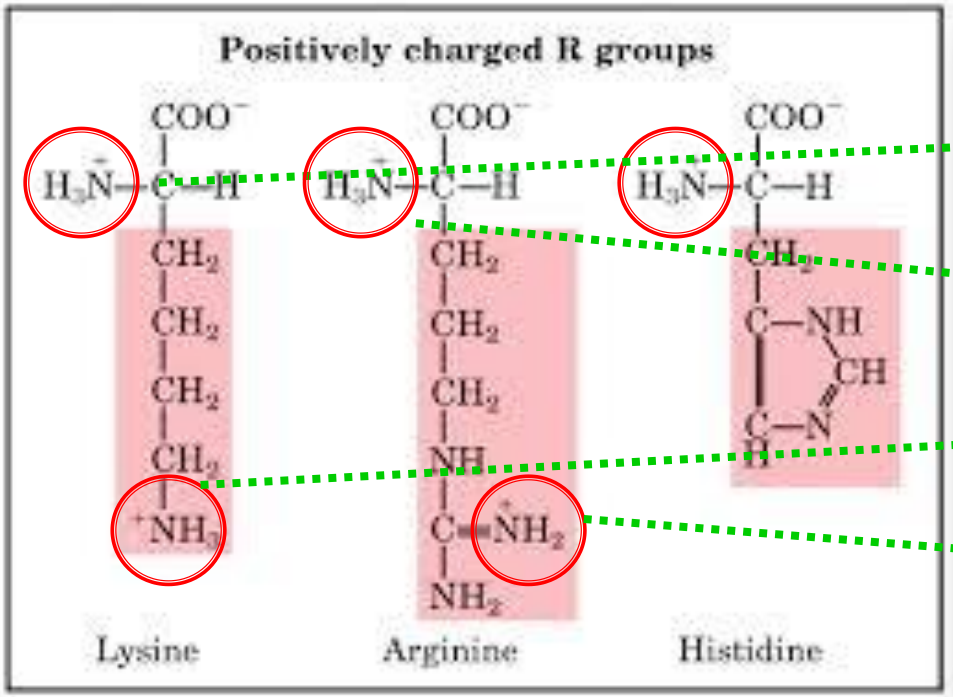
- Specialized positively charged (basic) proteins that help package chromosomal DNA into a compact structure.

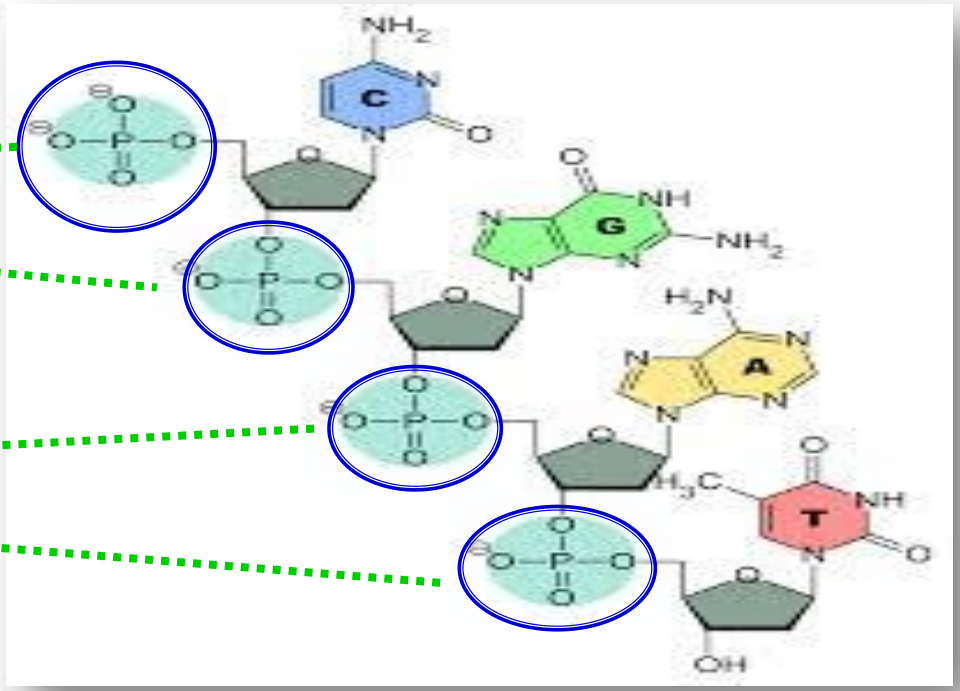


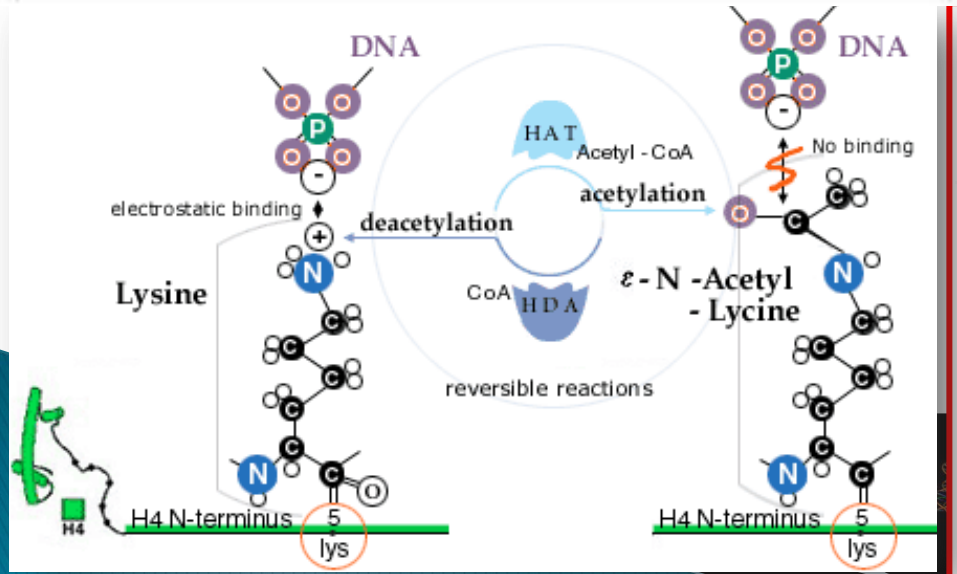
- Histones (+ve) will associate strongly with DNA (-ve) by ionic interactions.
- Chromatin mass is 50% DNA and 50% histones.
- DNA replication - large amounts of histones synthesized at 1:1 ratio.

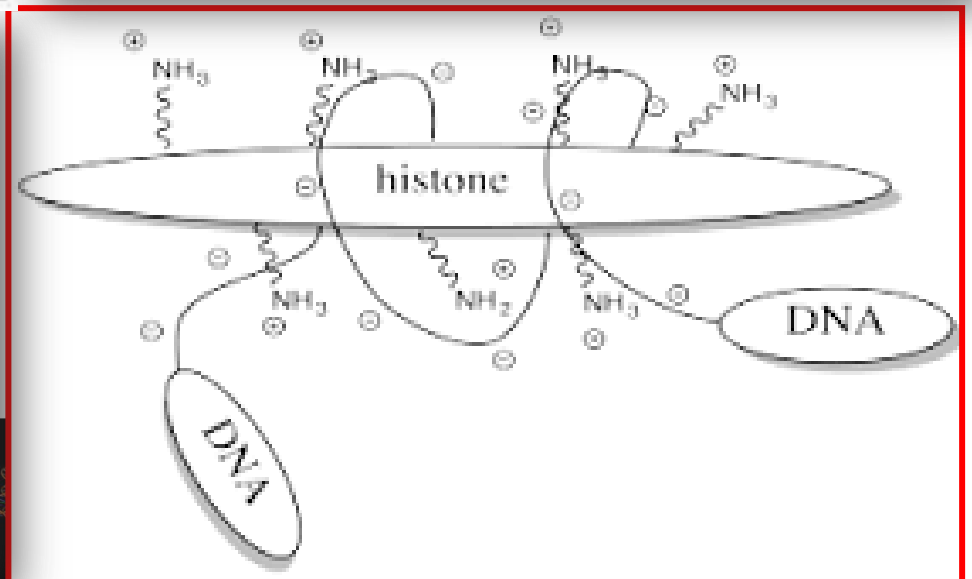






Histones *vs* DNA interactions



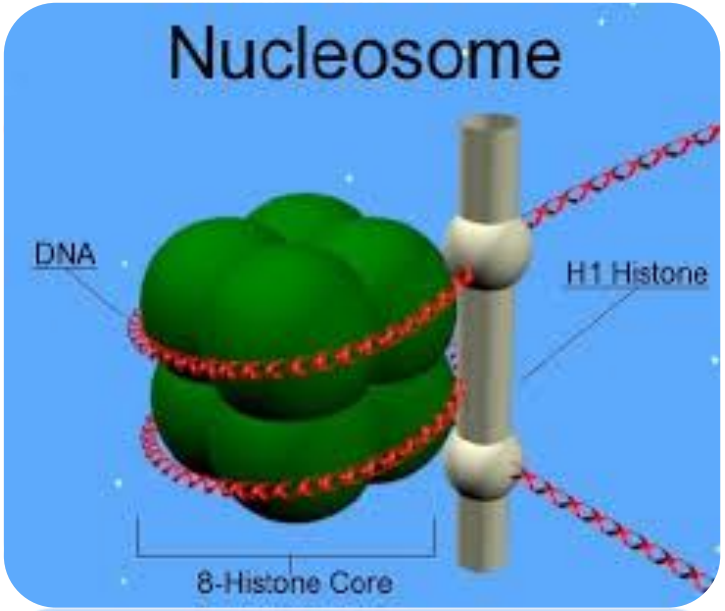




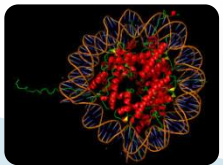
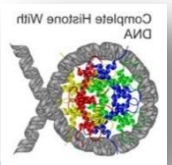
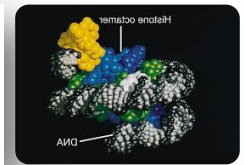
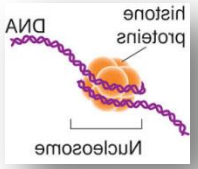


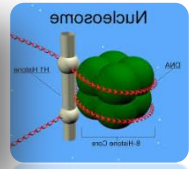
Histones *vs* DNA interactions

- Most condensed state (metaphase)- chromosome is 1.3-10 µm in length.
- Fundamental packaging unit of chromatin i.e. DNA wrapped around a core of histones is called the **NUCLEOSOME**.



- DNA of 1 chromosome → 10^6 nucleosomes.
- Nucleosomes associate to produce a compact supramolecular structure / complex.
- The resulting 30 nm chromatin fibers then condense to form chromosomes that are visible during cell division.



Light jogging vs longevity

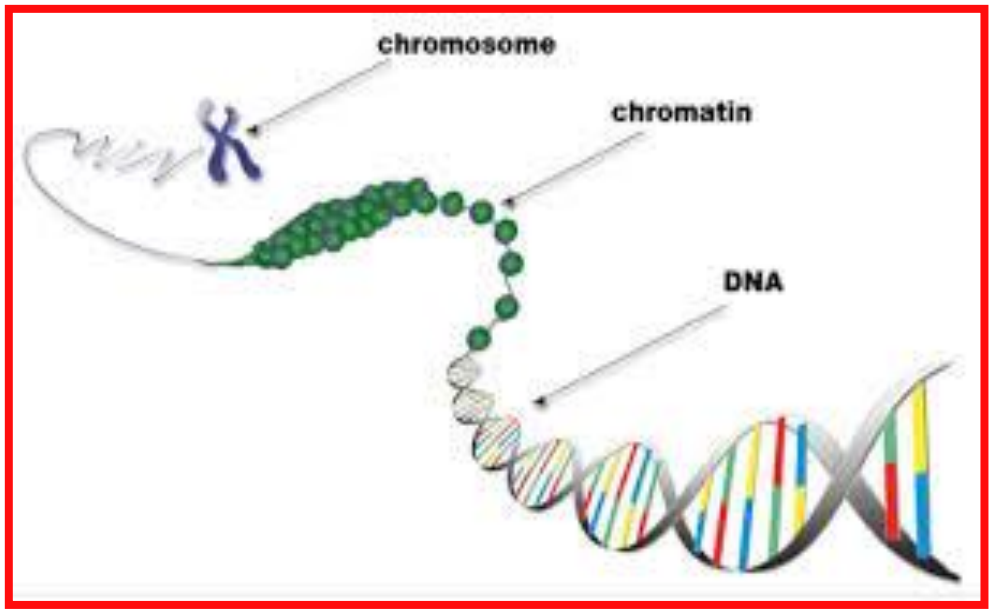
- **Light jogging in helpful but too much may be harmful for your longevity**

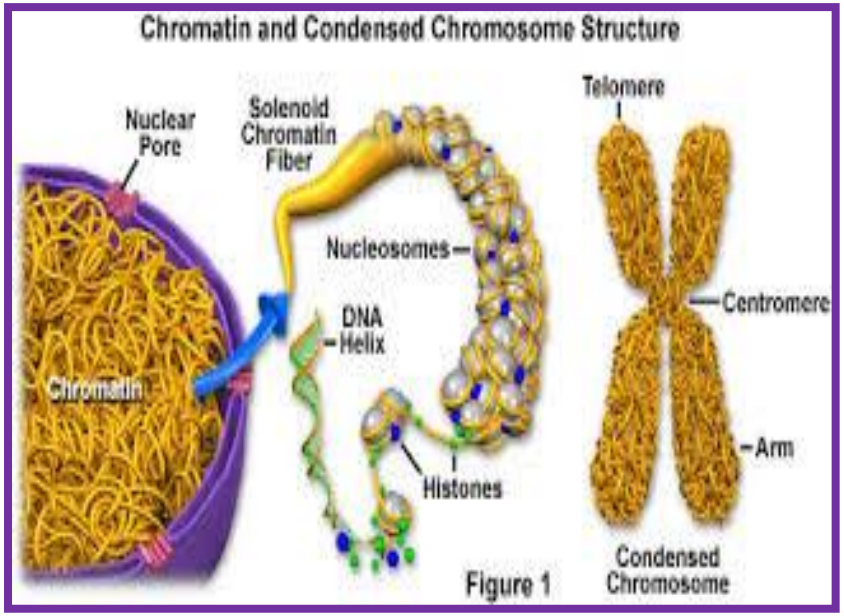
- ***Feb. 02, 2015*** **— Jogging may be best in small quantities according to a new study. The study, which tracked hours of jogging, frequency, and the individual's perception of pace, found that over the 12-year study strenuous joggers were as likely to die as sedentary non-joggers, while light joggers had the lowest rates of death.**

- ***Source:*** **American College of Cardiology and *Journal of the American College of Cardiology*, 2015; 65 (5): 411.**

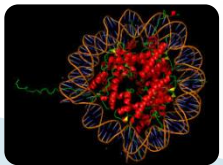
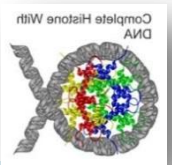
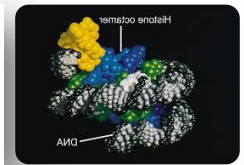
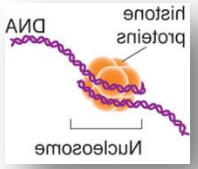
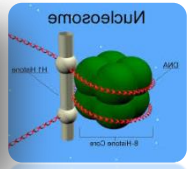
Histones *vs* DNA interactions

- The resulting 30 nm chromatin fibbers then condense to form chromosomes that are visible during cell division.

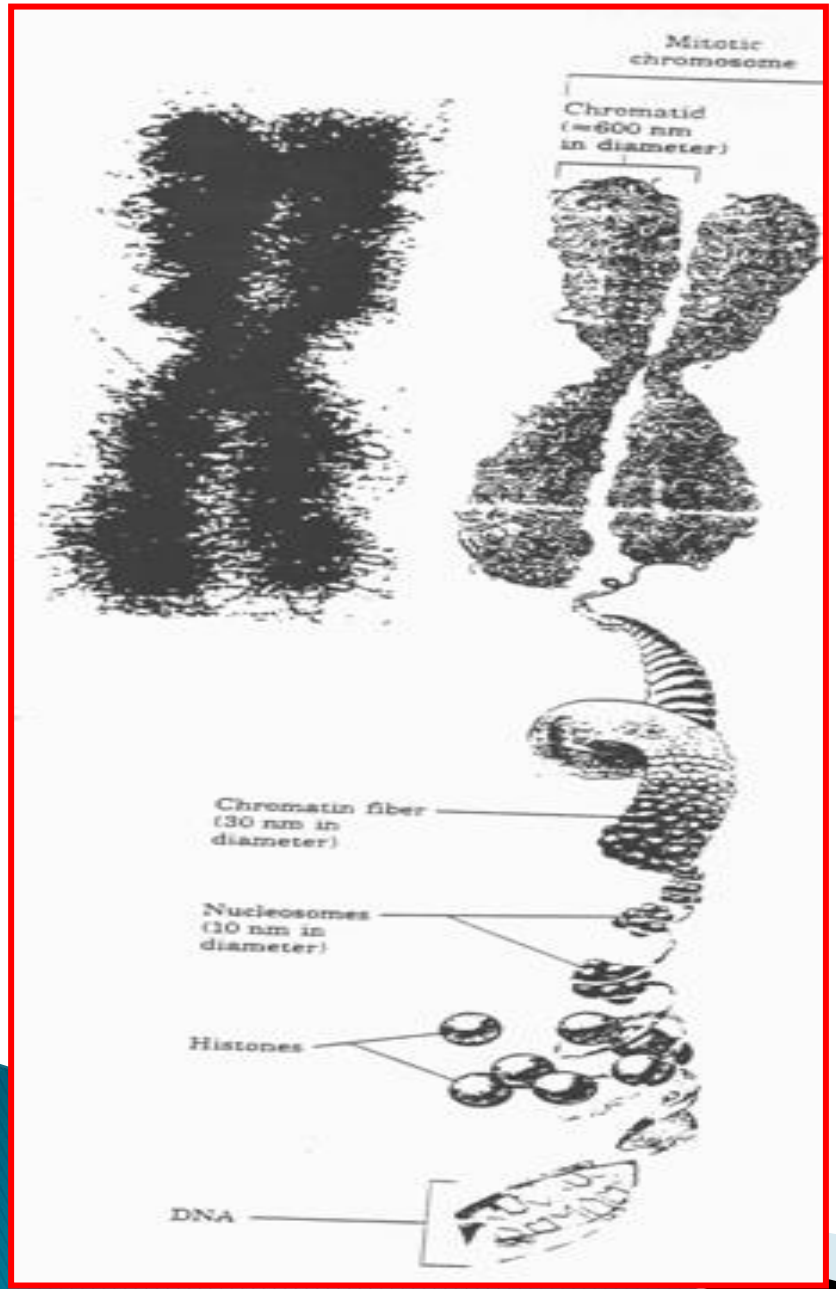




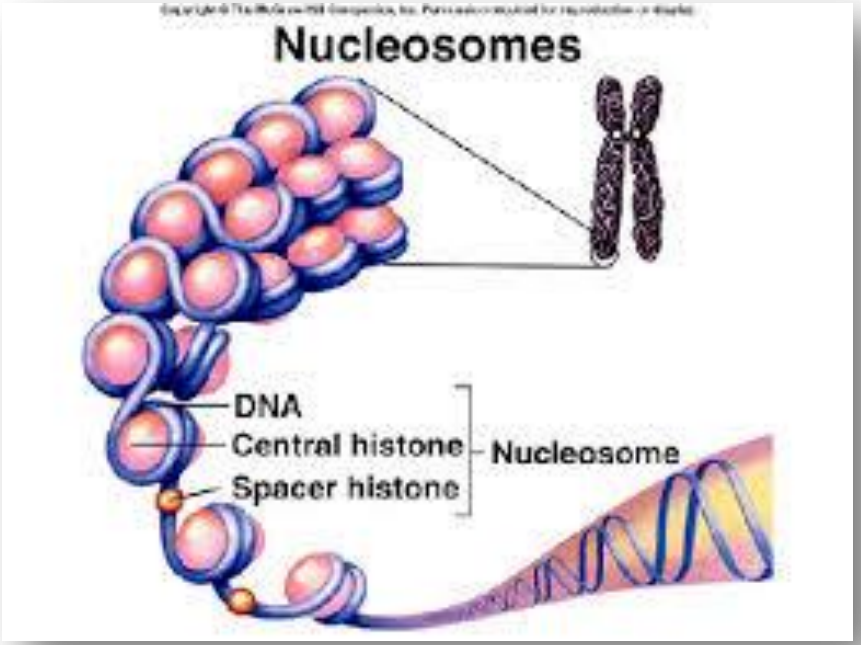
- DNA is tightly packed in nucleosomes.
- DNA (2 m fully stretched) is now approx. 200 nm and can fit into the nucleus of the cell.

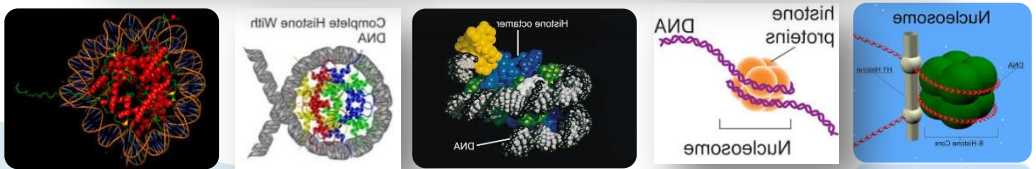






Chromosomes under EM



- DNA is wrapped around the histones to form many nucleosomes which associate to form a chromatin fiber that in turn folds and compacts into 2x chromatids= chromosome.



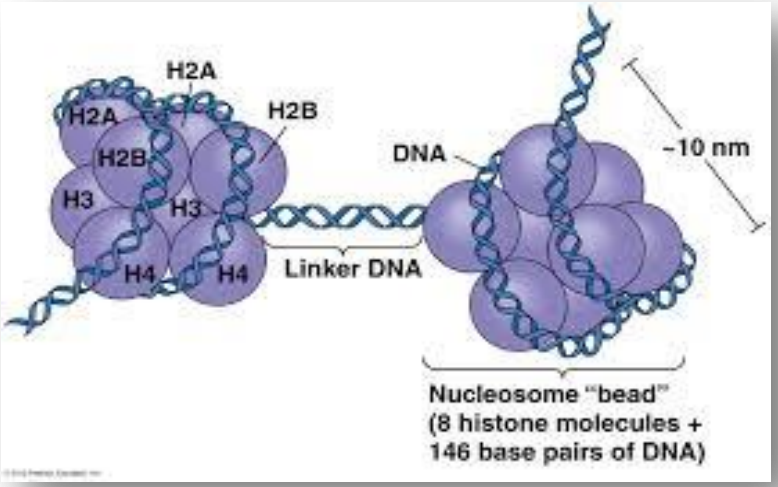


Types/classes of histones

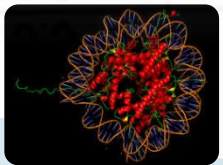
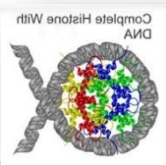
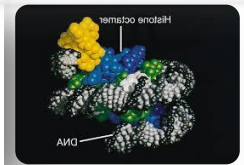
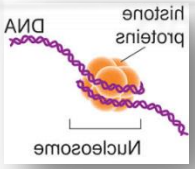
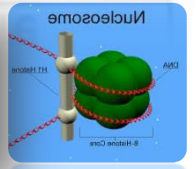
- According to the electrophoretic mobility histones are subdivided into 5 types / classes.

  H1, H2 (H2A and H2B), H3 , H4, H5.

- H2 (H2A and H2B), H3 and H4 are considered as CORE histones when H1 and H5 are called linker histones.

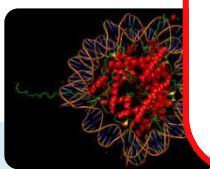


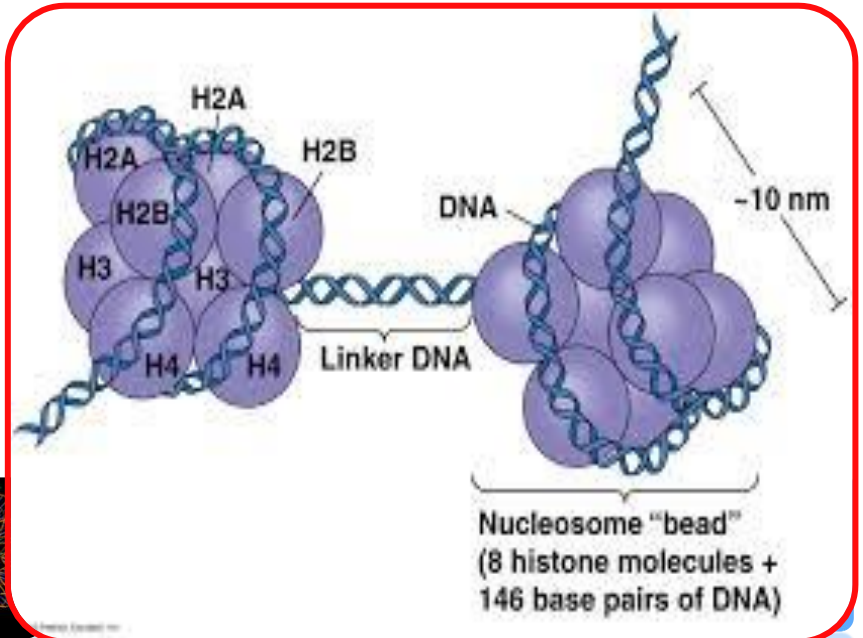
TYPE	NO.OF RESIDUES	MASS(kD)	% ARG	%LYS	LOCATION
H1	215	23	1	29	Linker
H2A	129	14.5	9	11	Core
H2B	125	13.8	6	16	Core
H3	135	15.3	13	10	Core
H4	102	11.3	14	11	Core

Assembly of histones

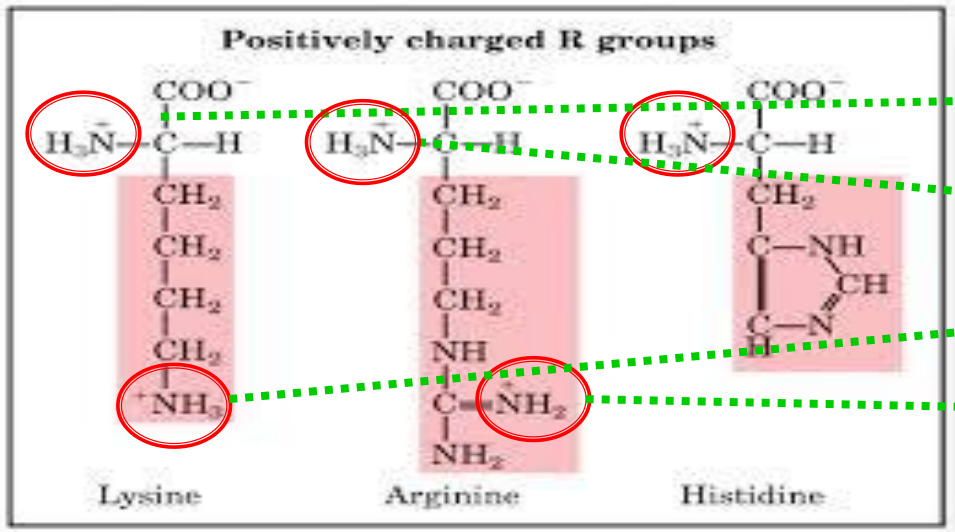
- Two of each of the core histones assemble to form one octameric nucleosome core which is approximately 63 Angstoms in diameter
- 146-147 base pairs of DNA wrap around this core particle 1.65 times in a left-handed super-helical turn to give a particle of around 100 Angstroms across.
- The linker histone H1 binds the nucleosome at the entry and exit sites of the DNA, thus locking the DNA into place and allowing the formation of higher order structure.
- The most basic such formation is the 10 nm fiber or beads on a string conformation. This involves the wrapping of DNA around nucleosomes with approximately 50 base pairs DNA separating each pair nucleosomes (also referred to as linker DNA).

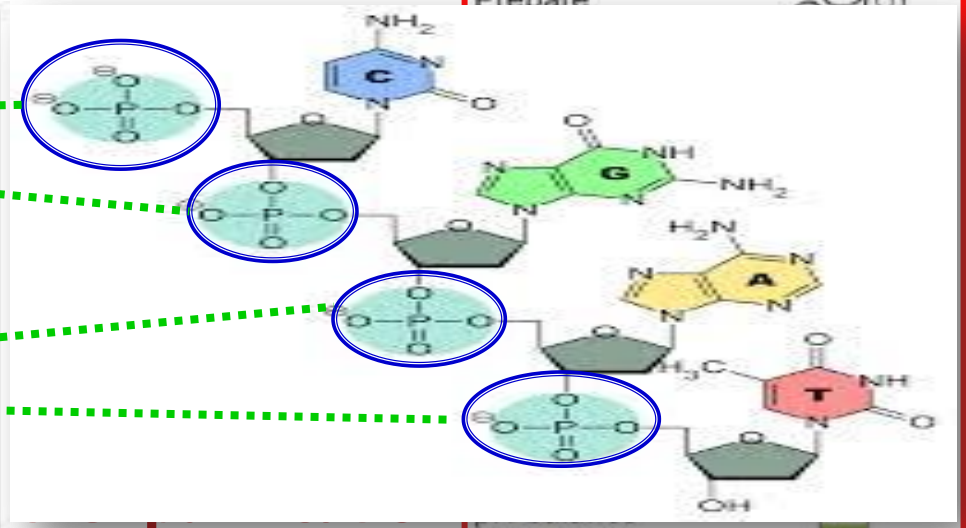


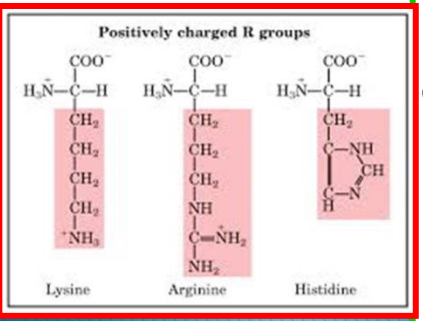


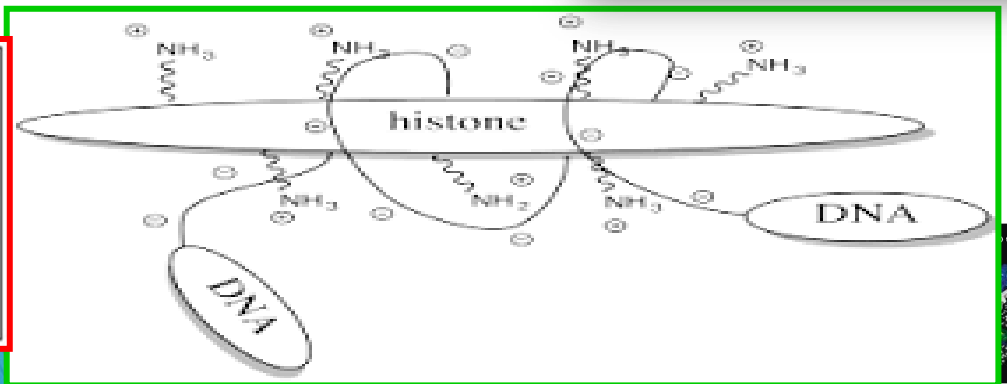
Isolation of histones from DNA

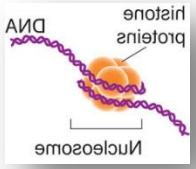
- **Histones are basic proteins with many positively charged arg and lys residues.**
- **Arg and lys have a free NH_3^+ group that binds tightly to the negatively charged backbone of DNA.**

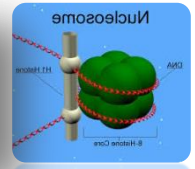




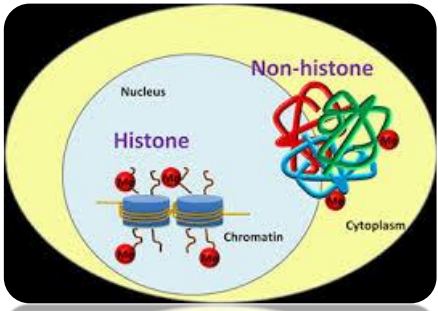






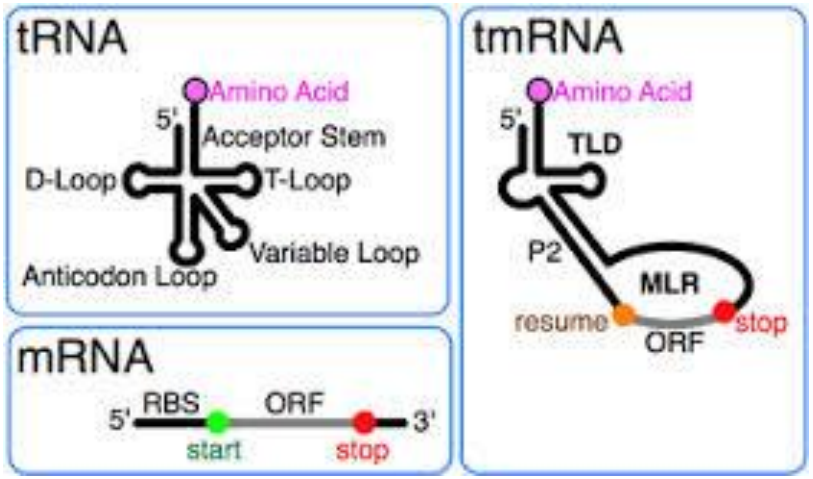


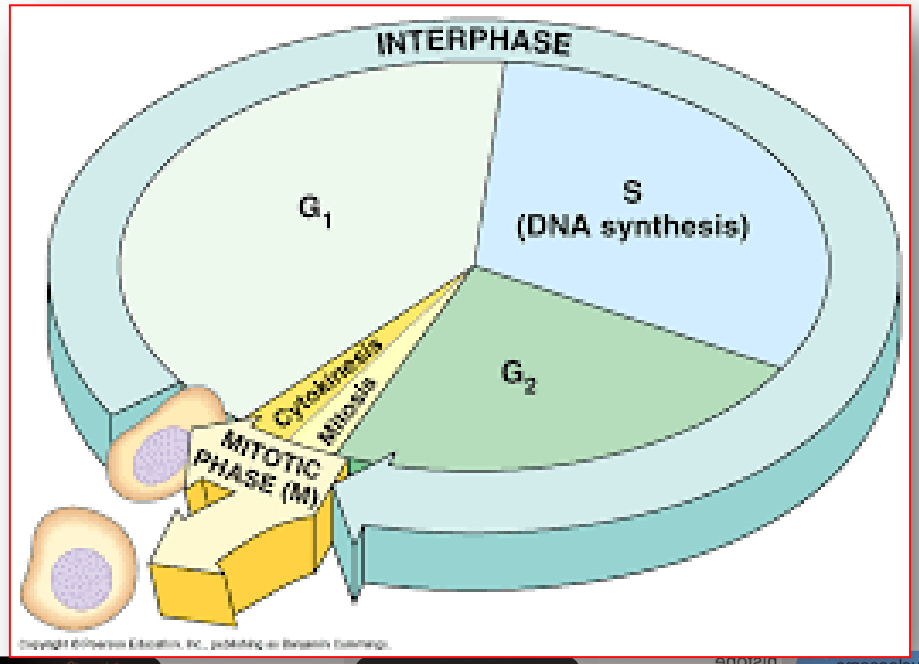
Non-histone proteins

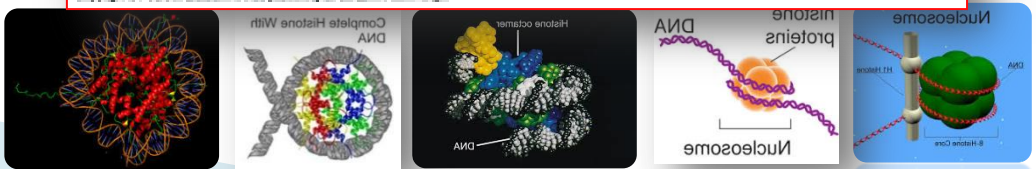


- Normally neutral and acidic proteins.

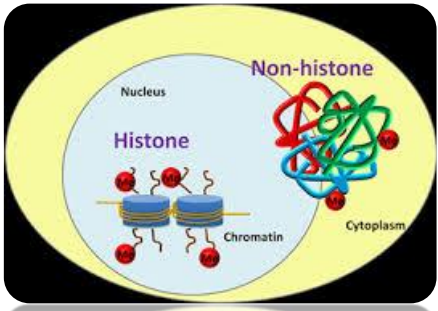
1. **RNA polymerase** - for synthesis of mRNA, tRNA, rRNA and small nuclear RNA (splice pre- mRNA)
2. **DNA polymerase** - responsible for replication of DNA (nuclear and mitochondrial) and DNA repair. Active in 'S' phase of cell cycle.





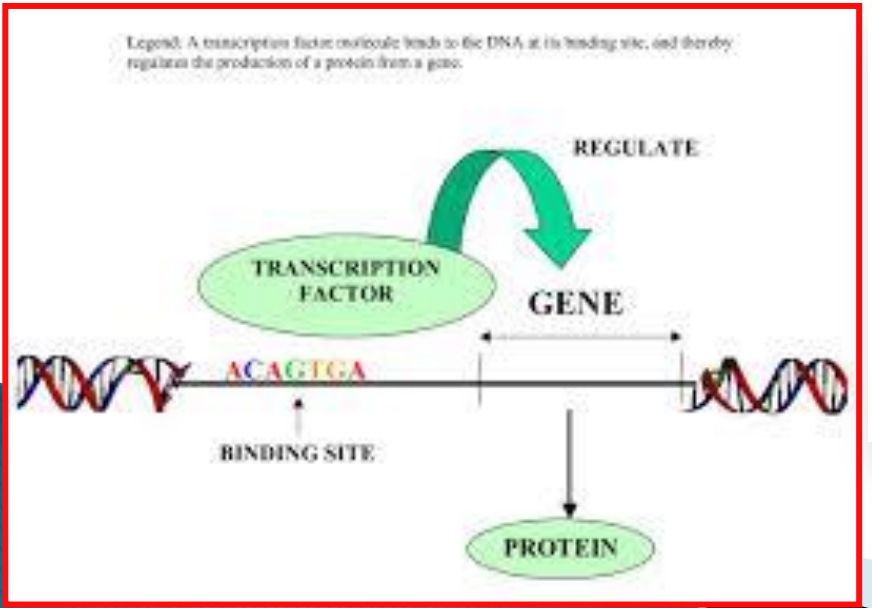


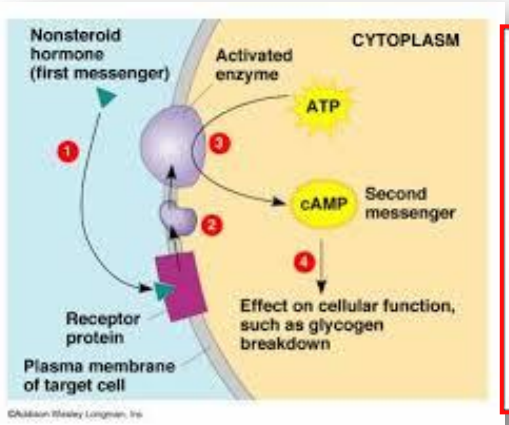
Non-histone proteins

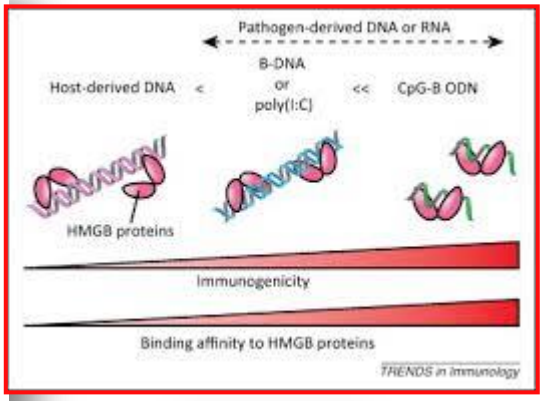


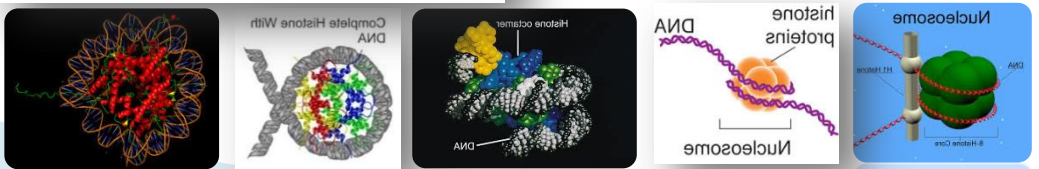
- Normally neutral and acidic proteins.

3. **Transcription factors** – that bind to upstream regulatory sequences in eukaryotic genes (turn genes on and off).
4. **Hormone Receptor Proteins** - bind to hormone receptor elements (HRE) eg. steroid hormones.
5. **HMG (High Mobility Group) Proteins** - highly conserved, low molecular weight (< 30 kD). Confers nuclease sensitivity to the erythrocyte globin gene.





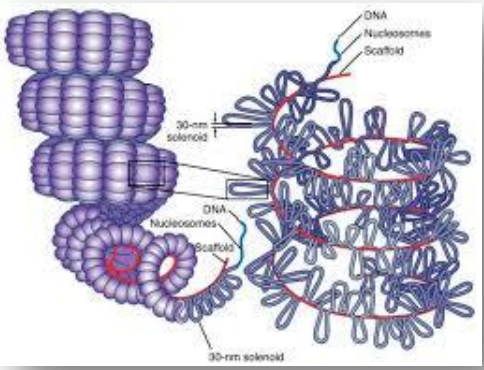


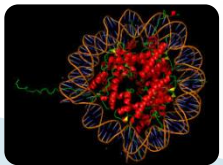


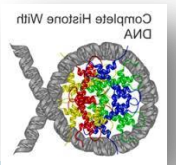
Histones are evolutionarily conserved

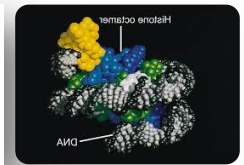
- Histone binding to DNA is not dependent on nucleotide sequence (DNA) but on the AA sequence of the histones.
- Most molecules conserved during evolution.
- Look at histone H4 from calf thymus and a pea seedling.

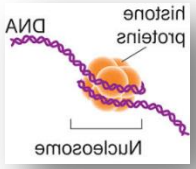
CALF THYMUS	POSITION	PEA SEEDLING
Valine- GUU GUC GUA	60	Ile –AUU AUC AUA
Lysine – AAA AAG	77	Arg – AGA AGG

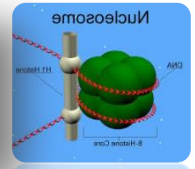












Histones are evolutionarily conserved- contd…

CALF THYMUS	POSITION	PEA SEEDLING
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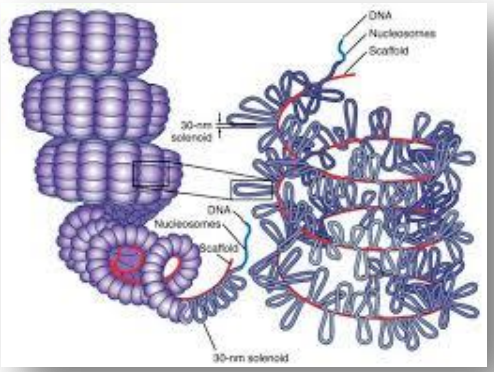
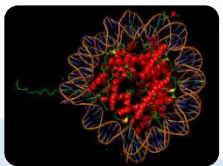
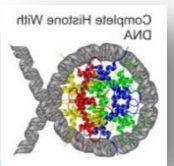
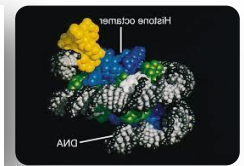
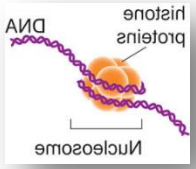
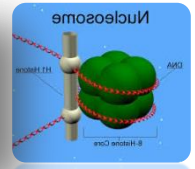


- H4 differs only by 2 AA residues in a chain of 102 AAs (25 arg and lys)
- Changes at position 60 : val →ile ; and position 77 : lys → arg
- H4 – most invariant of core histones- constant for 1.2 x 10^9 years.
- H3 of calf thymus and pea differs at 4 positions (little evolutionary change). H1 – most variable of the histones.



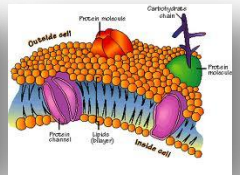


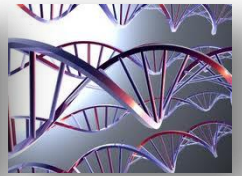


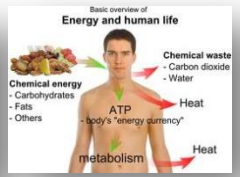
Overweight vs Brain Function

- **Overweight and obesity compromise the normal brain function.**
- ***Feb. 11, 2014*** — Jeremy D. Coplan, MD, professor of psychiatry at SUNY Downstate, led a multicenter team that visualized the molecule, N-acetyl-aspartate (NAA), using magnetic resonance spectroscopy. *NAA is associated with brain cell health. Overweight study participants exhibited lower levels of NAA in the hippocampus than normal weight subjects. The effect was independent of age, sex, and psychiatric diagnoses.*

Source: Science Daily



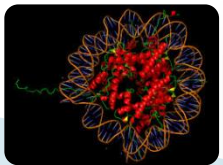


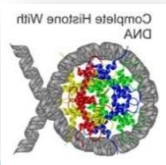


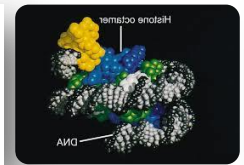
Levels of chromatin structure and organization

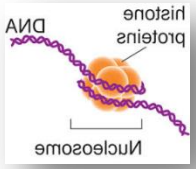
1. Nucleosome: First level

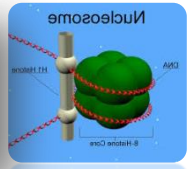
- Histones -discovered in 1884 by **Albrecht Kossel**, a German Biochemist who's main interest was on Genetics.
- First considered to be "packaging" material for DNA until 1990.
- Early 1990- regulatory functions histones are discovered.







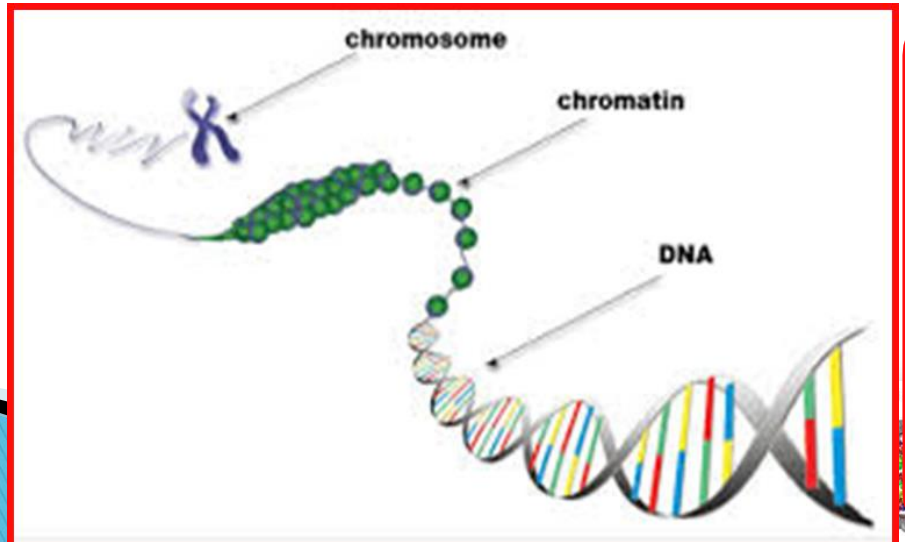


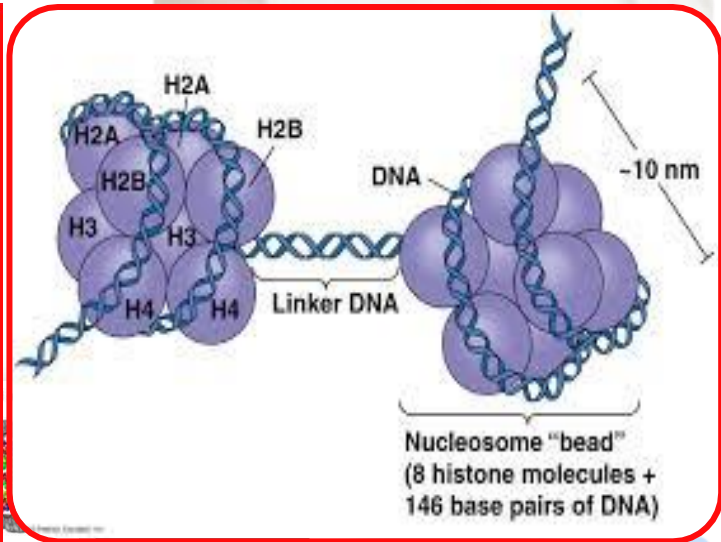


Levels of chromatin structure and organization

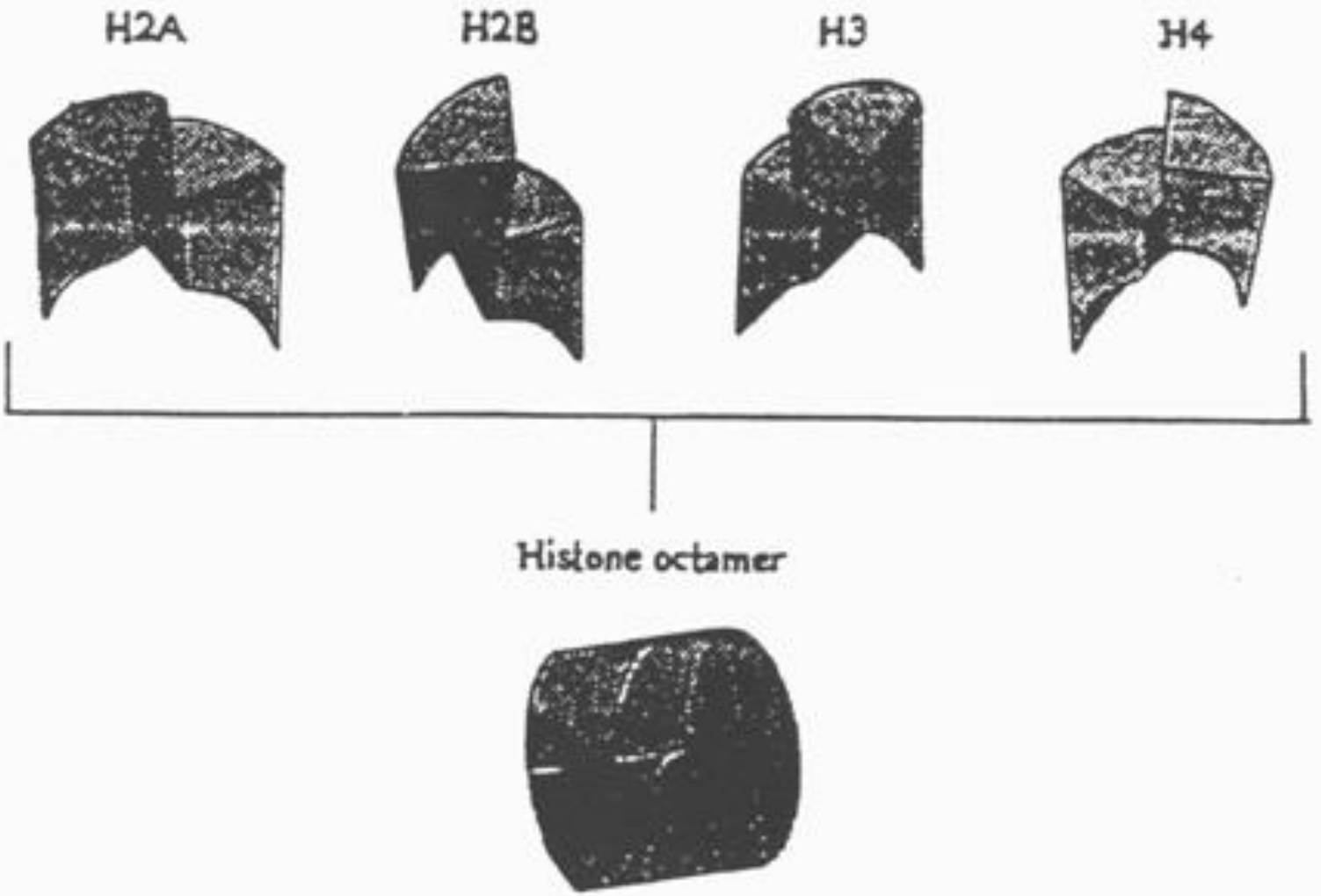
Q: How are histones arranged on DNA to form chromatin?

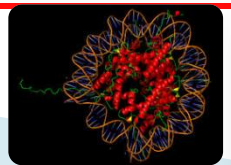
- **1974 – Roger Kornberg, an American Biochemist proposed that chromatin is made up of repeating units (200 bp DNA)**
- **There are 4 x 2 core proteins (H2A, H2B, H3, H4)**
- **Form an octameric complex.**

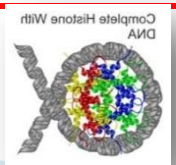


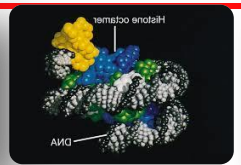


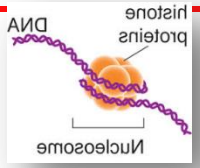
Histones under microscope

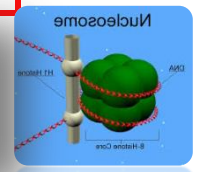










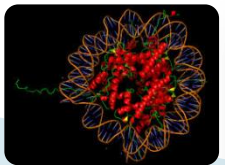


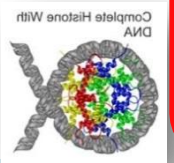
Structure of the nucleosome

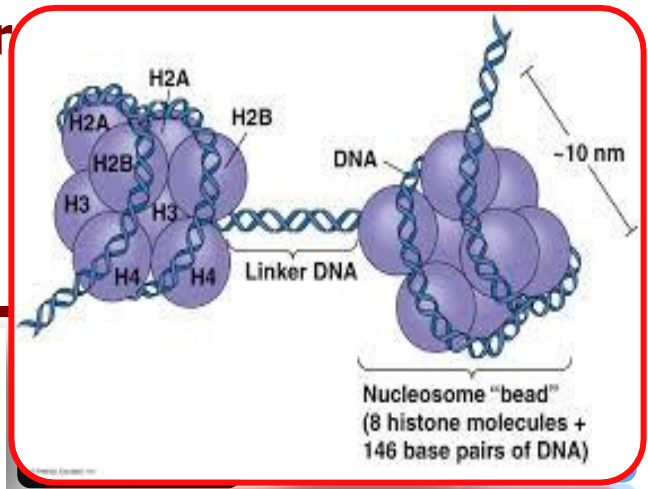
- H3 and H4 – centrally situated.
- H2A and H2B – found at either ends of the nucleosome.
- DNA (147 bp)- are wrapped around the octamer (about 2.85 turns of DNA)
- Form repeating chromatin units called NUCLEOSOMES (11 nm /110 Å)
- Linker DNA (54 bp / 50-200 bp) – between nucleosomes.
- Linker DNA is stabilised by histone H1 and H5.
- Histone H1- situated outside the nucleosome.

  - binds to nucleosome and to linker DNA and brings adjoining nucleosomes together for further coiling.

  - acts as a bridge and fastens DNA.

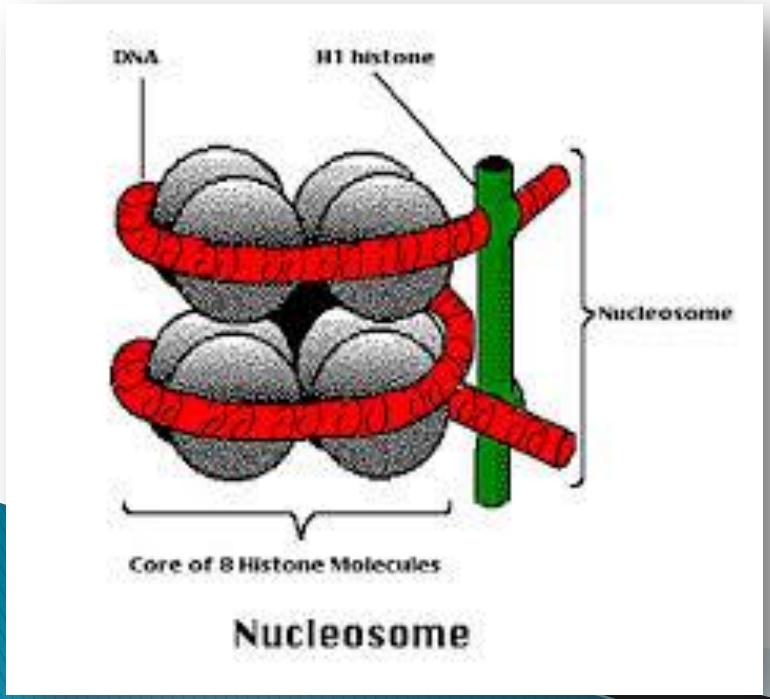


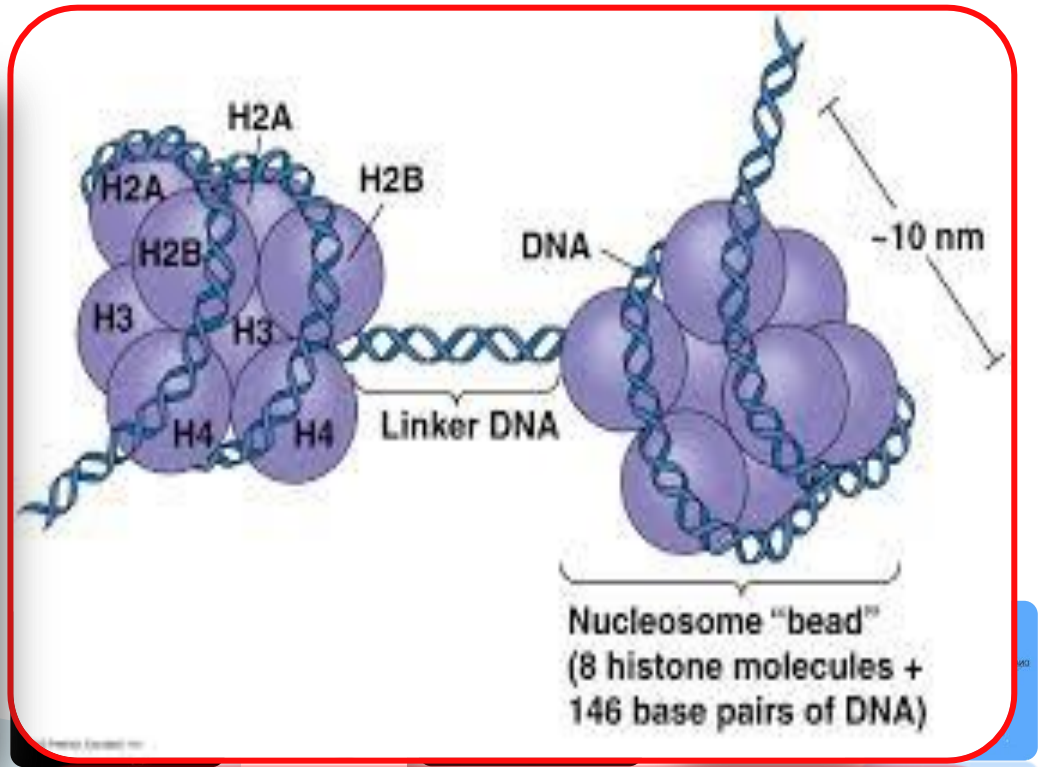




Structure of the nucleosome

- 70-80% of histones incorporated in core with remainder (N-terminals)- stick out of the nucleosome as a tail.
- Total mass of complex = 100 000 dal.
- Nucleosomes pack into a 30 nm fibre (0.1 cm long and spans the nucleus 100X)
- Higher order of packaging required to produce a compact structure.

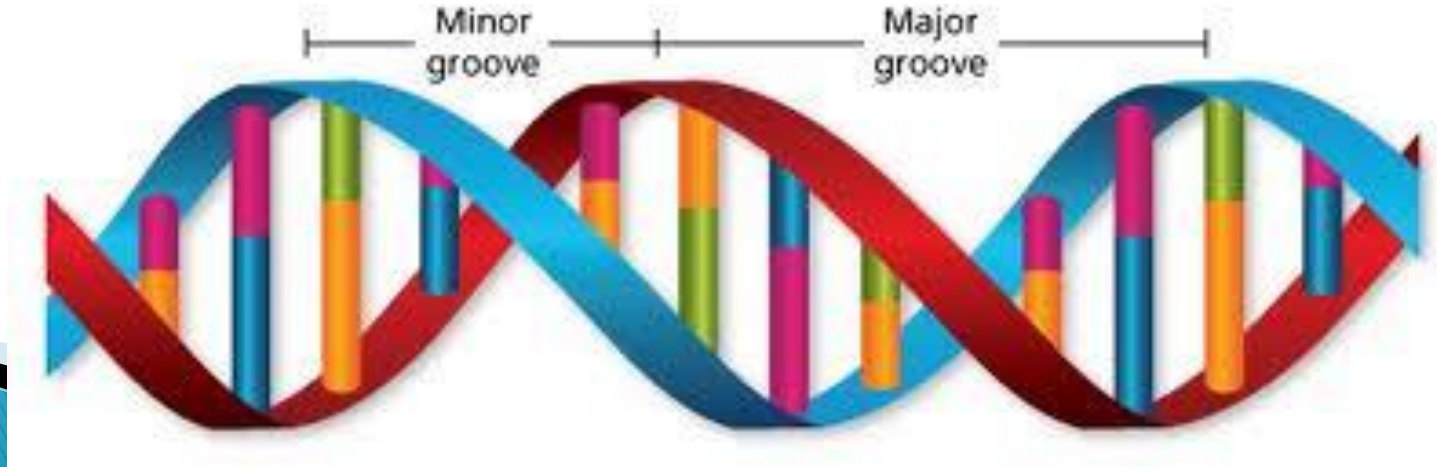




Structure of the nucleosome

***In vitro* studies have shown:**

- **Wrapping of DNA around histones requires removal of 1 helical turn of DNA.**
- **Topoisomerase enzyme - important in assembly of chromatin from the histones and DNA *in vitro*.**
- **DNA sequence plays some part in histone-DNA binding.**
- **Nucleosomes form where: (a) A=T bp are abundant**
  (b) minor groove of DNA
- **Cluster of 2/3 A=T pairs- get tight wrapping of DNA.**



Red wine *vs* body fat

Drinking wine may help you to burn fat

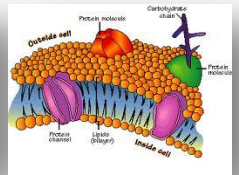
February 6, 2015:

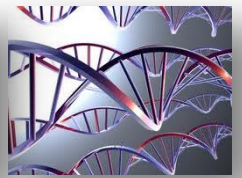
Source: **Oregon State University**

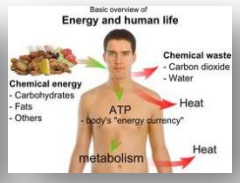
Summary:

- **Drinking red grape juice or wine - in moderation - could improve the health of overweight people by helping them burn fat better, a new study indicates. The findings suggest that consuming dark-colored grapes, whether eating them or drinking juice or wine, might help people better manage obesity and related metabolic disorders such as fatty liver.**

Ref: *Journal of Nutritional Biochemistry*, 2015; 26 (1): 82 DOI: 10.1016/j.jnutbio.2014.09.010

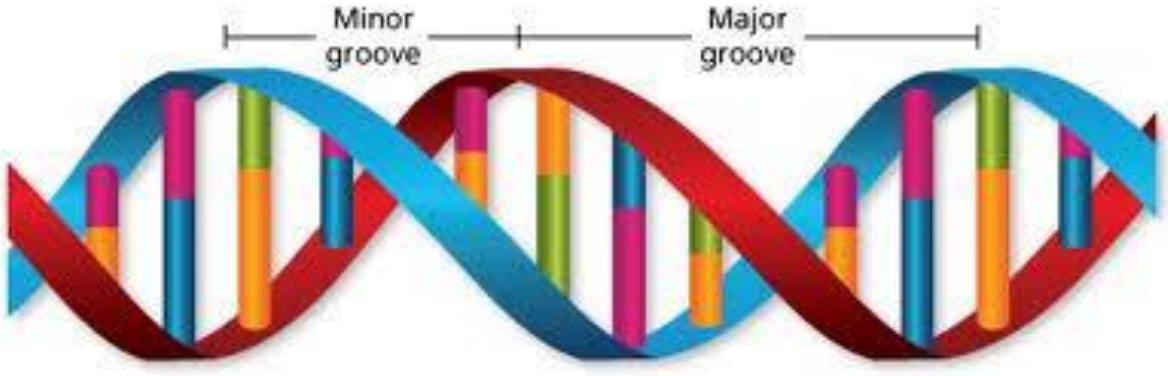


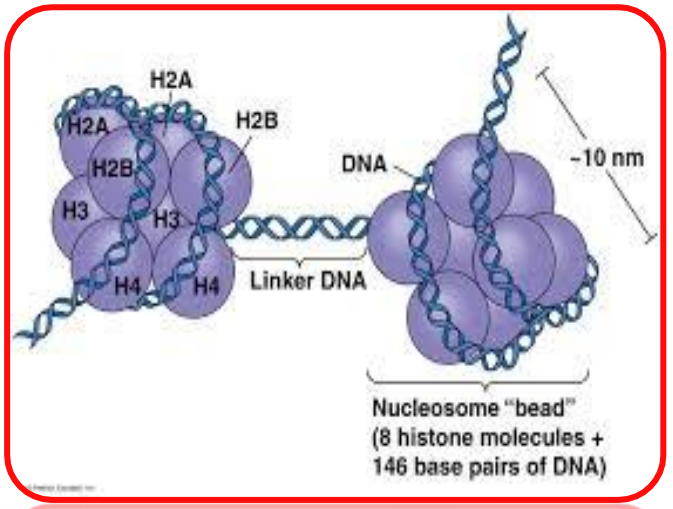


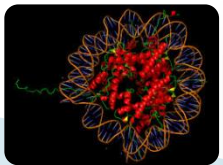


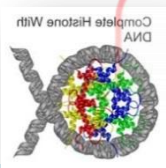
Structure of the nucleosome

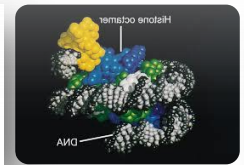
- Most contacts - by the H3-H4 tetramer and center of the DNA.
- H3- has α helical rods that fit into the minor groove of the DNA.
- H2A-H2B attach to the H3-H4 tetramer and then bind to the last half turn of the DNA helix.
- This stabilizes the nucleosome core.

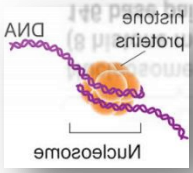


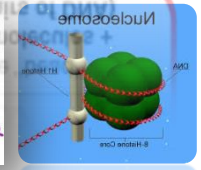








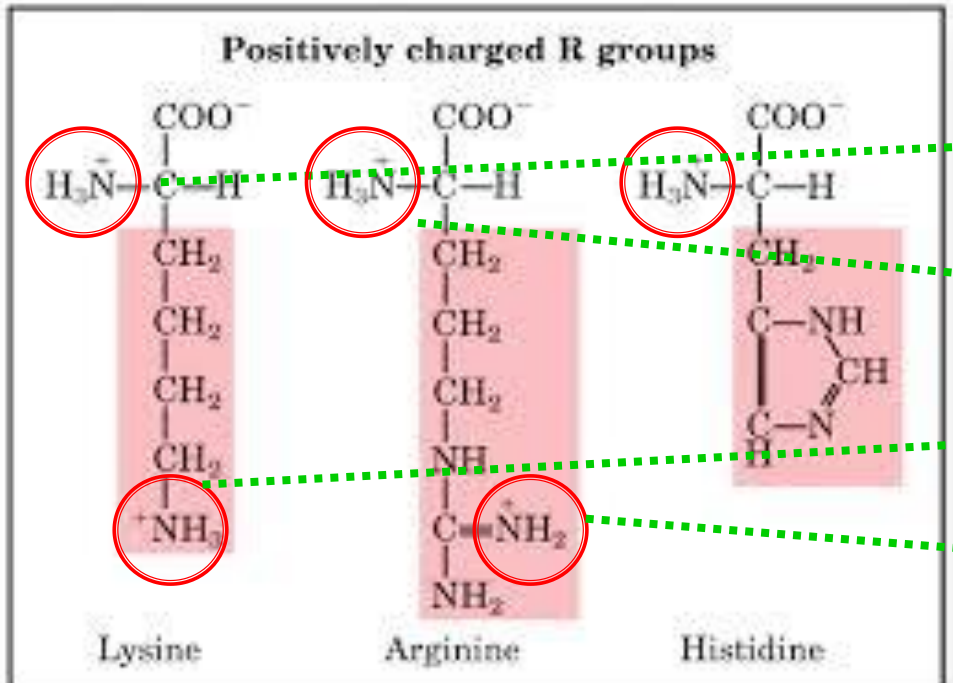


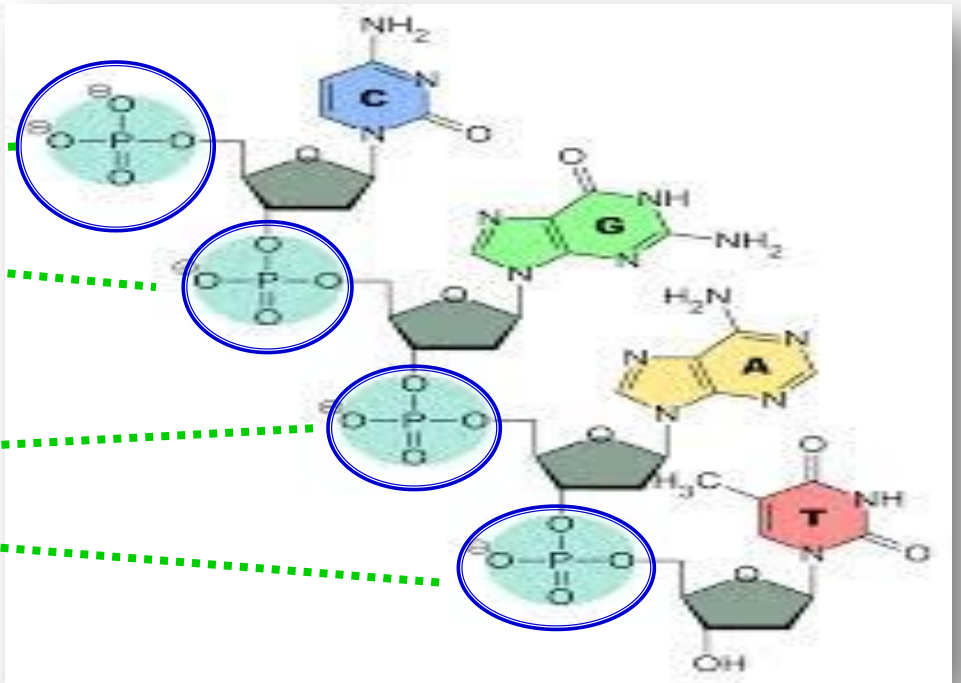


Histones-DNA interactions

Histones have 5 types of interactions with DNA.

1. **Helix-dipole interaction from α-helices in H2, H3, H4. This causes a net +ve charge accumulation at the point of interaction with the –vely charged phosphate groups of DNA.**
2. **H-bonds between DNA backbone and amine groups on main chain of histone proteins.**

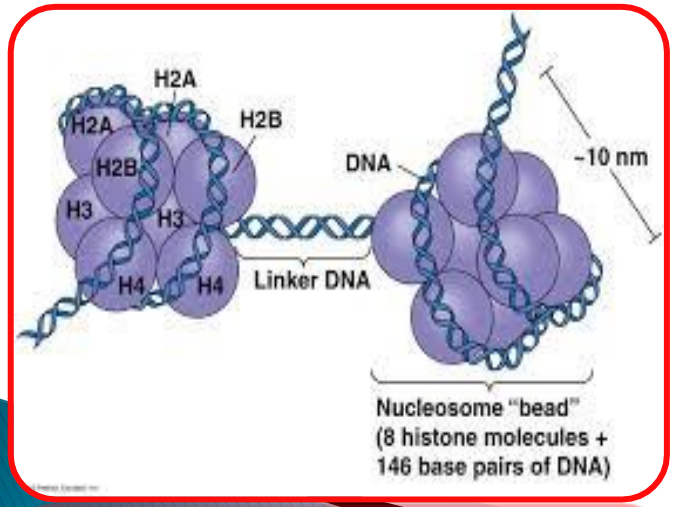


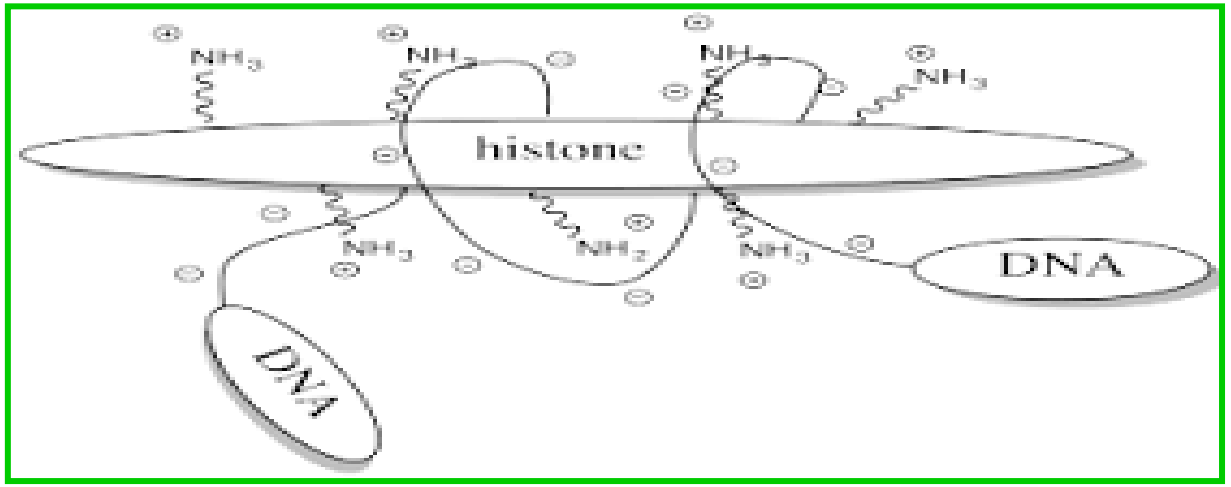


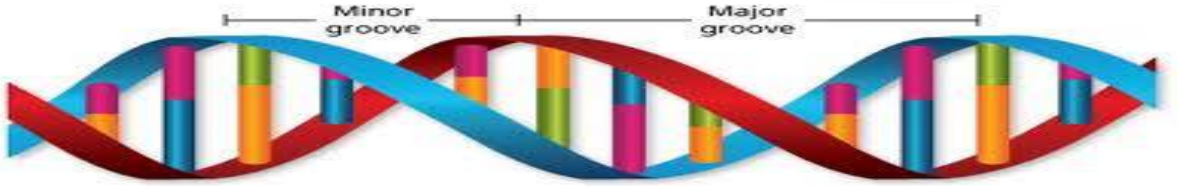
Histones-DNA interactions

Histones have 5 types of interactions with DNA.

3. **Non-polar interactions between histones and the deoxyribose sugars on DNA.**
4. **Salt bridges and H-bonds between side chains of basic AAs (lys/arg) and phosphate oxygen on DNA.**
5. **Non-specific minor groove insertions of H3 and H2B N-terminal tails into 2 minor grooves each on the DNA molecule.**



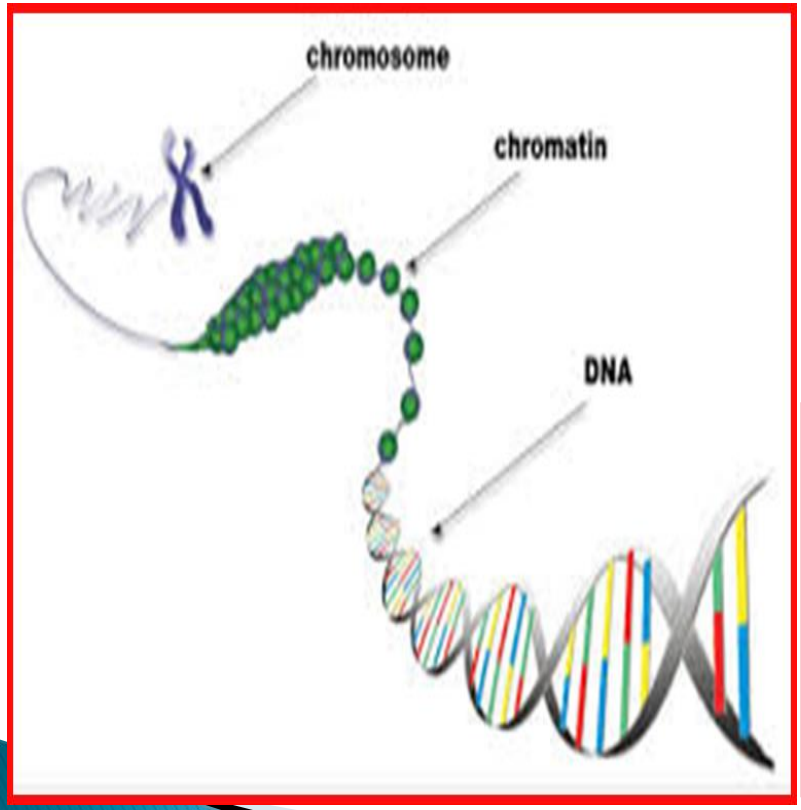


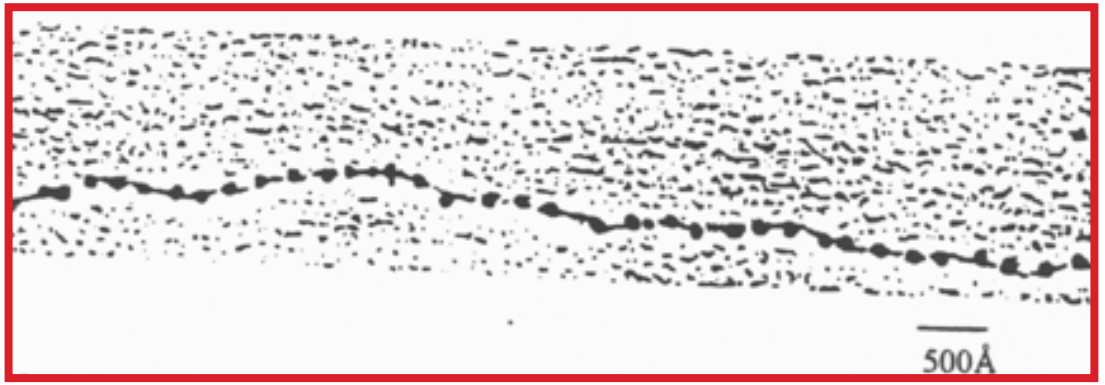


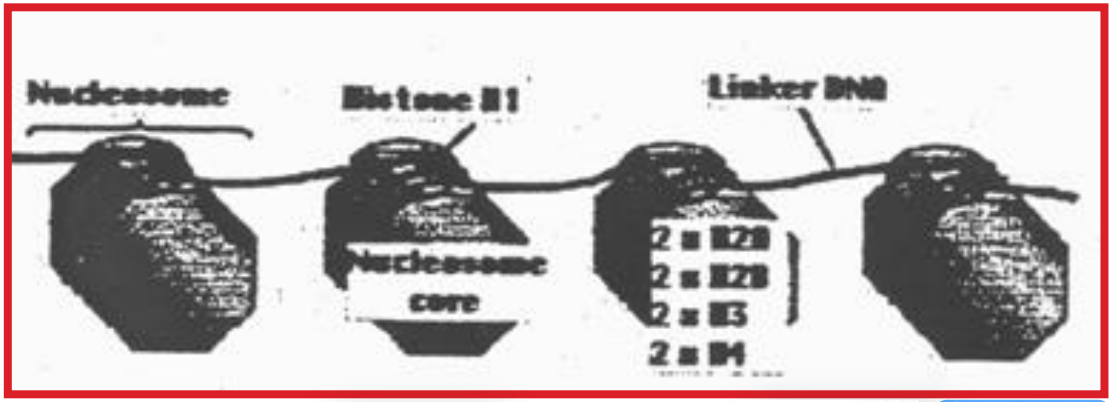
Chromatin organization expt.

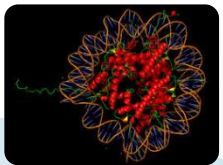
1. **X-Ray Diffraction studies – showed a regular chromatin fibre repeated every 100Å**

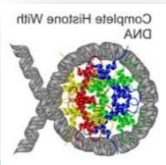
2. **Electron Microscopy – showed that chromatin of 100Å in diameter are connected like beads on a string**

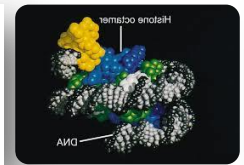


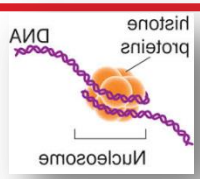


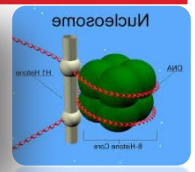








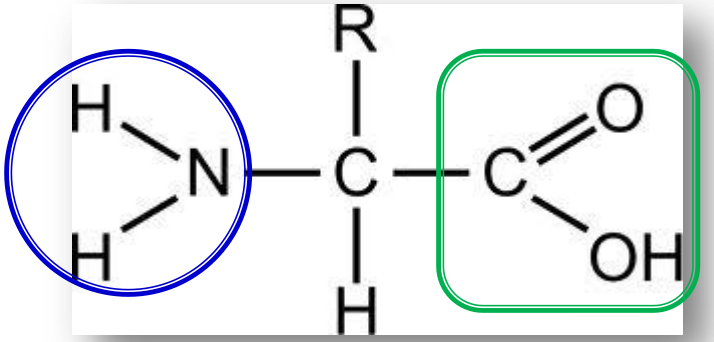


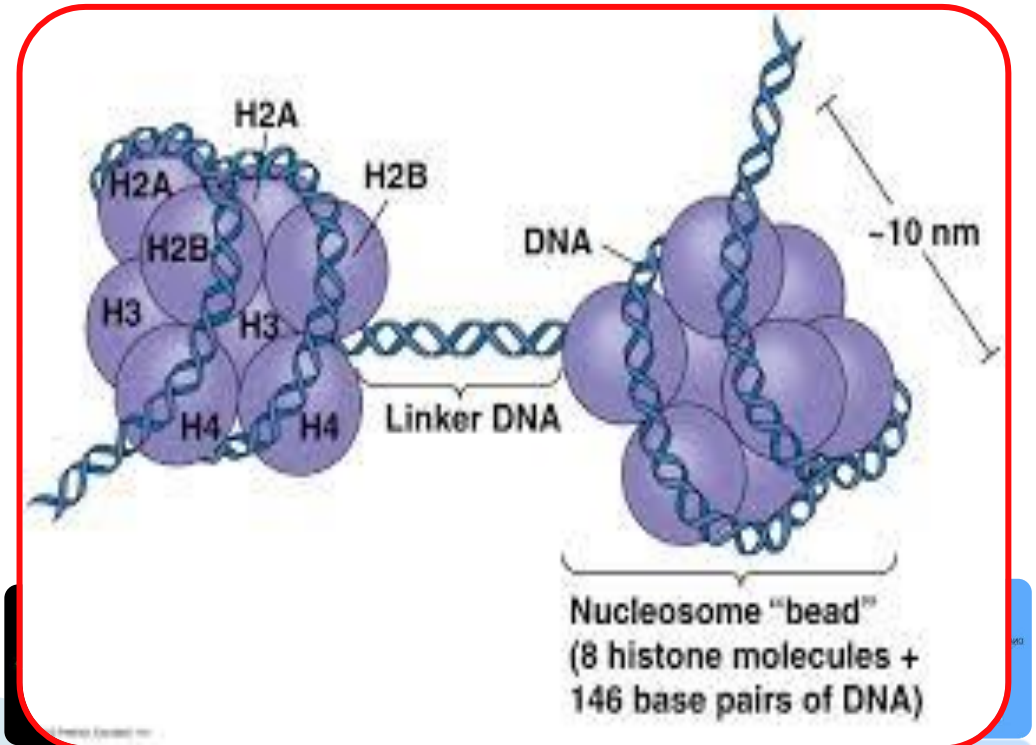


Chromatin org. expt.-contd.

3. Chemical Cross-linking Experiments – indicated that histones H3 and H4 associate to form a tetramer $(H3)_2(H4)_2$.

- **Group 2 histones can be treated with a H_2O soluble carbodiimide to cross link proteins**
- **Cross links occur between –COOH and NH_2 groups.**
- **Group 2 histones can be dissociated with SDS, followed by PAGE- produces 8 bands.**



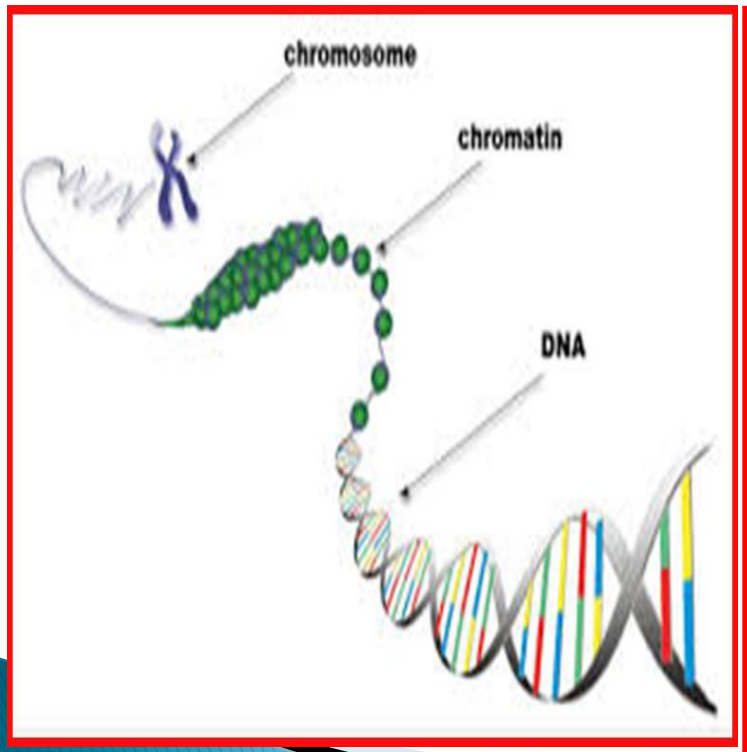


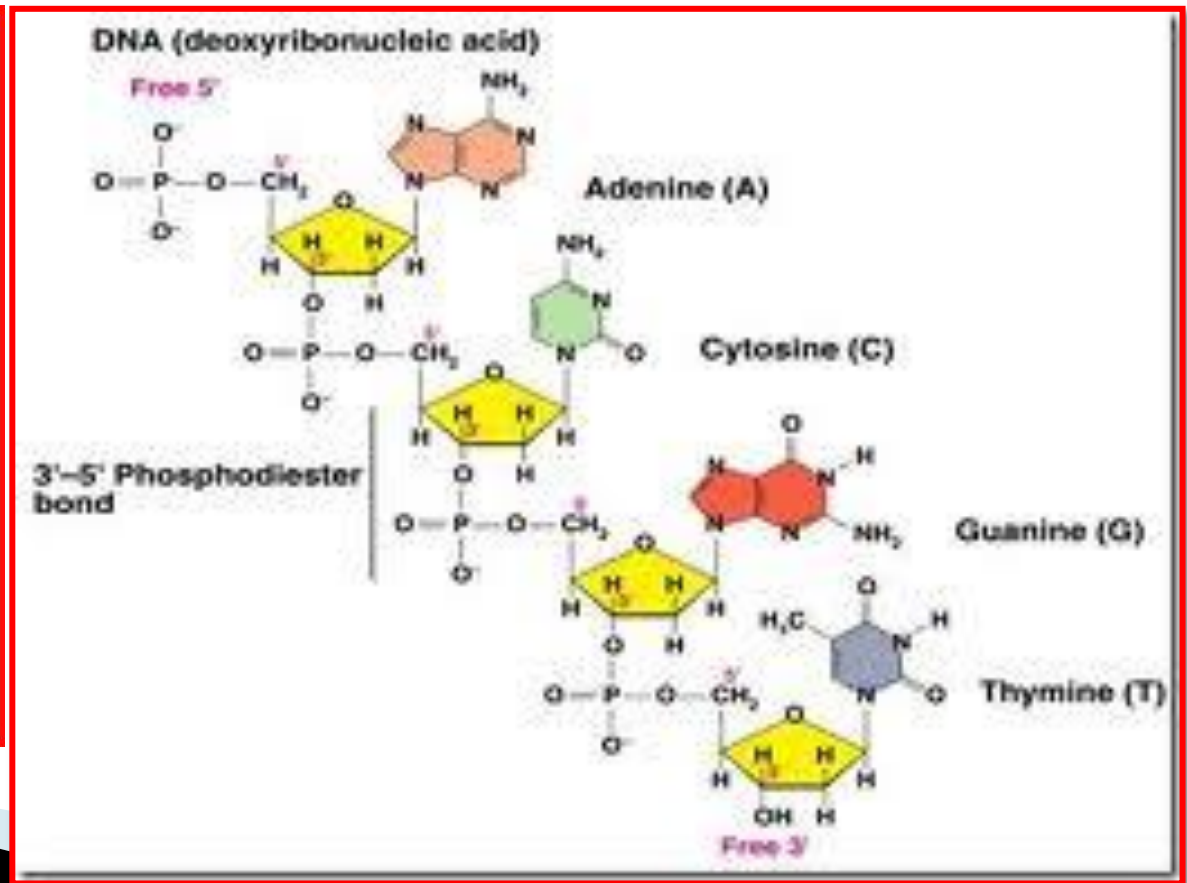
Chromatin org. expt.-contd.

4. Nuclease Digestion Experiments - Free DNA can be cleaved at any of its phosphodiester bonds by enzymes :

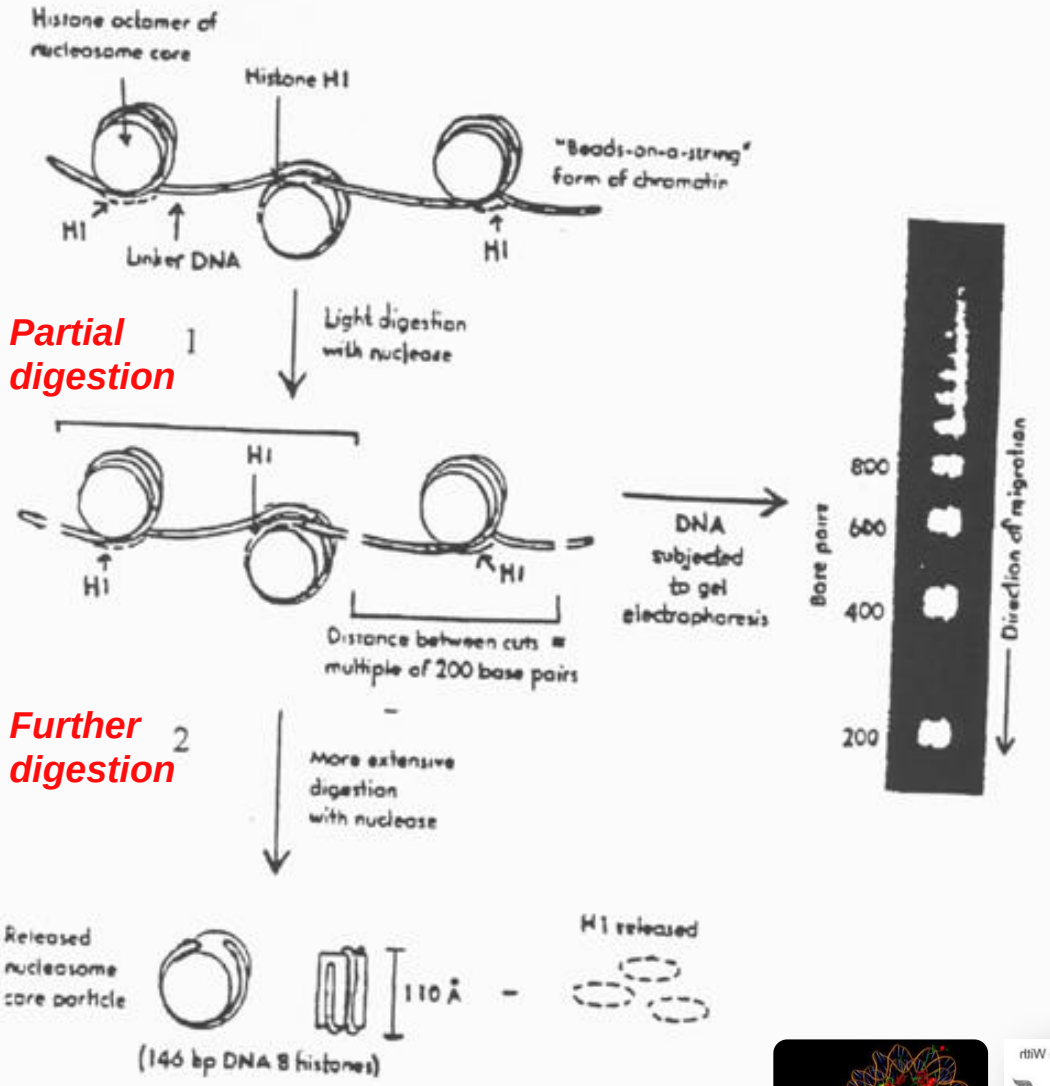
(a) Micrococcal nuclease

(b) Pancreatic Deoxyribonuclease 1 (DNAse 1)



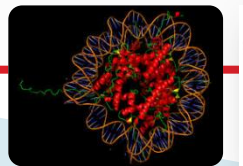
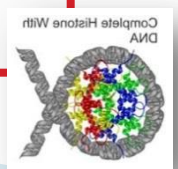


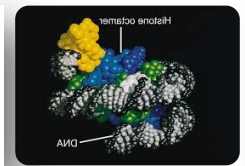
Chromatin org. expt.-contd.

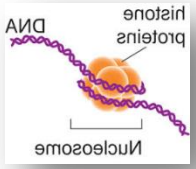


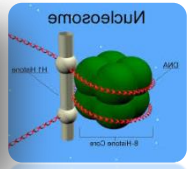
(a)Micrococcal nuclease digestion: Protein associated DNA is protected. Linker DNA is exposed.

i) Partial digestion - nucleosomes containing H1 and 200 bp intermediates of DNA are produced. This produces a "ladder effect" on agarose gel electrophoresis.

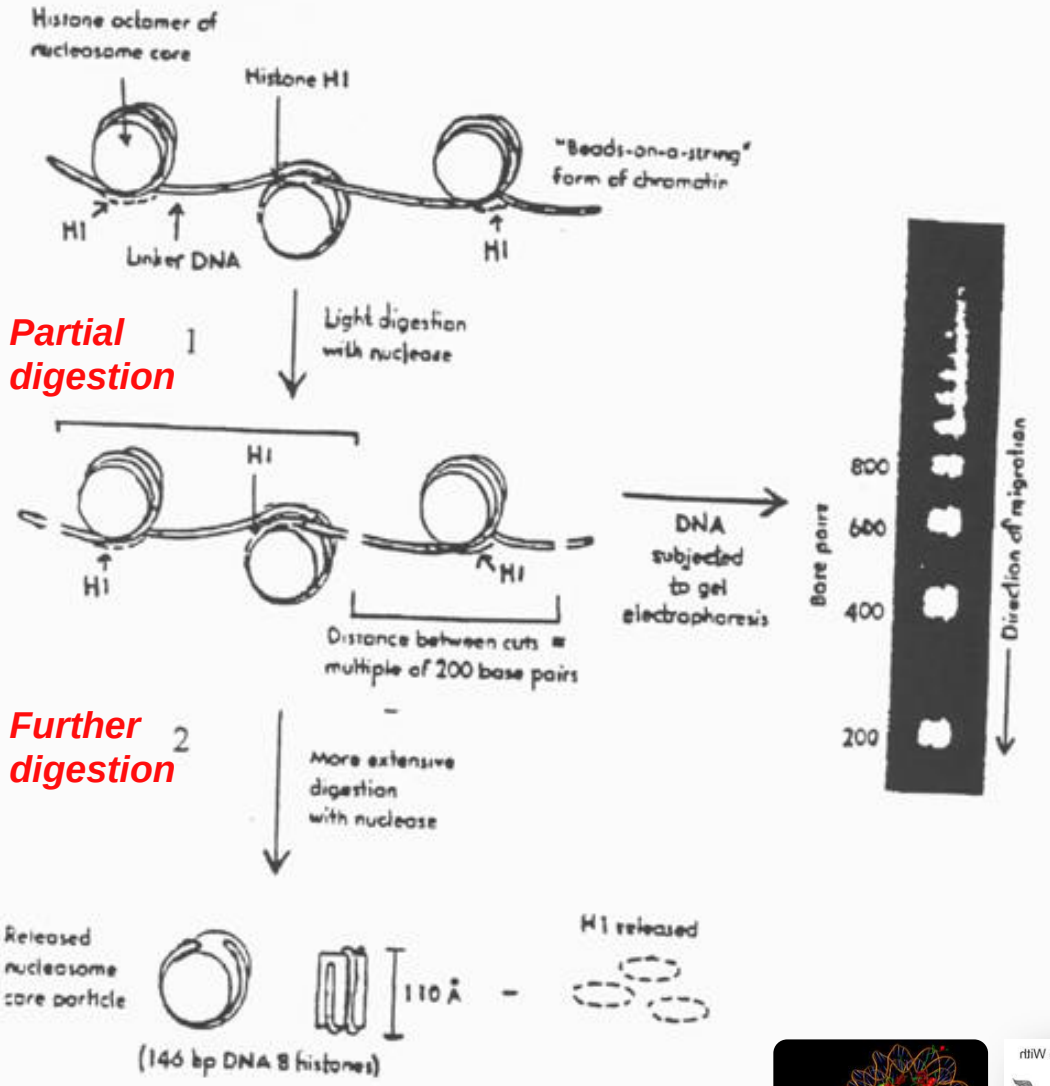





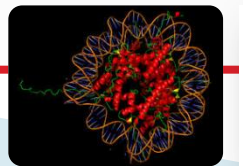
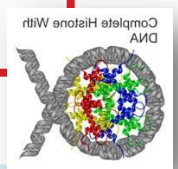


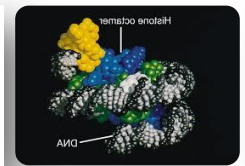


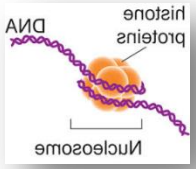
Chromatin org. expt.-contd.

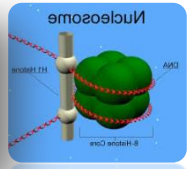


ii) Further digestion - trims DNA and releases histone H1 together with nucleosome core particles containing 146 bp of DNA.

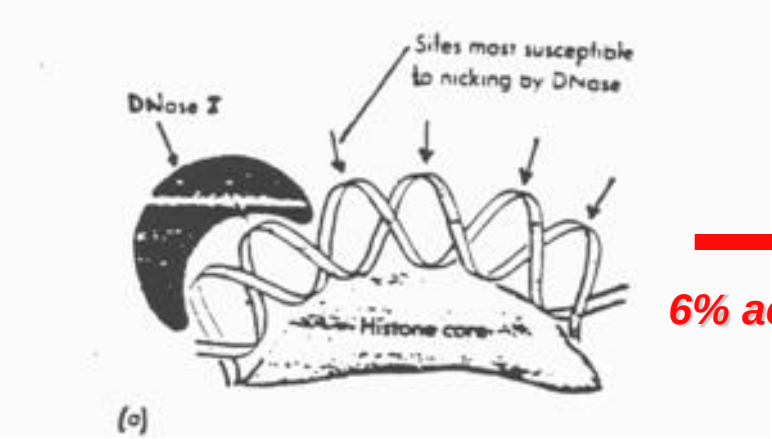






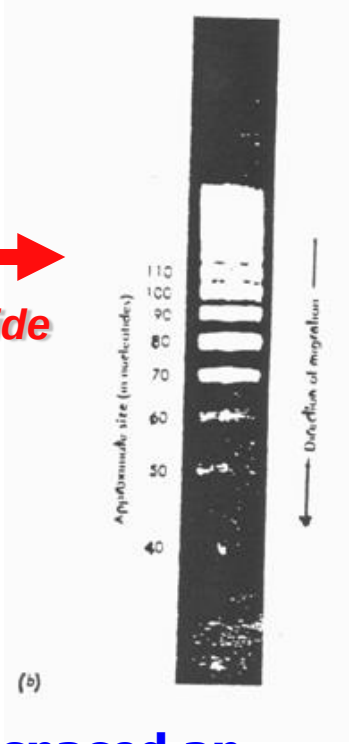
Chromatin org. expt.-contd.

(b) PANCREATIC DEOXYRIBONUCLEASE 1 DIGESTION (DNAse 1)

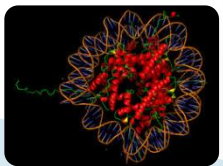
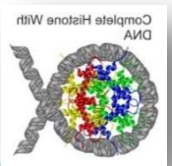


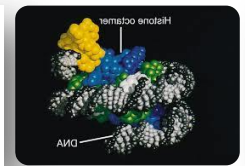
PAGE

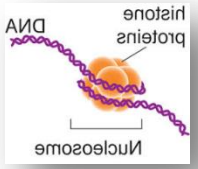
6% acrylamide gel +99% formamide

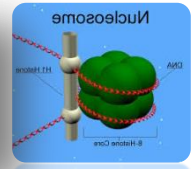


- Nuclease digestion studies first suggested that DNA is wrapped around the outside of nucleosome.
- DNA in contact with histones are protected.
- The sites most susceptible (exposed) to DNase 1 are spaced an average of 10 nucleotides apart on each DNA strand.
- DNase 1 cleaves1 strand without cleaving the second.
- Digested DNA shows a ladder of bands differing by about 10 nucleotides in length after fractionation on a polyacrylamide gel under denaturing conditions.





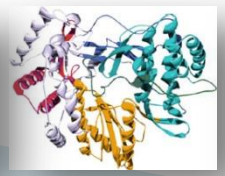


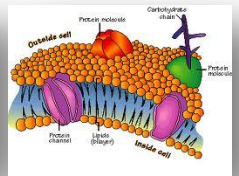
Added fructose vs Type 2 diabetes

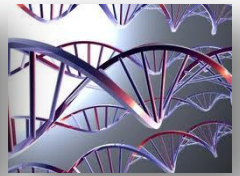
- **Added fructose is principal driver of type 2 diabetes**

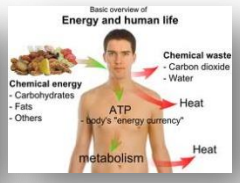
January 29, 2015: **Recent studies have shown that added sugars, particularly those containing fructose, are a principal driver of diabetes and pre-diabetes, even more so than other carbohydrates. Clinical experts challenge current dietary guidelines that allow up to 25 percent of total daily calories as added sugars, and propose drastic reductions in the amount of added sugar, and especially added fructose, people consume.**

Source: ***Elsevier health science***





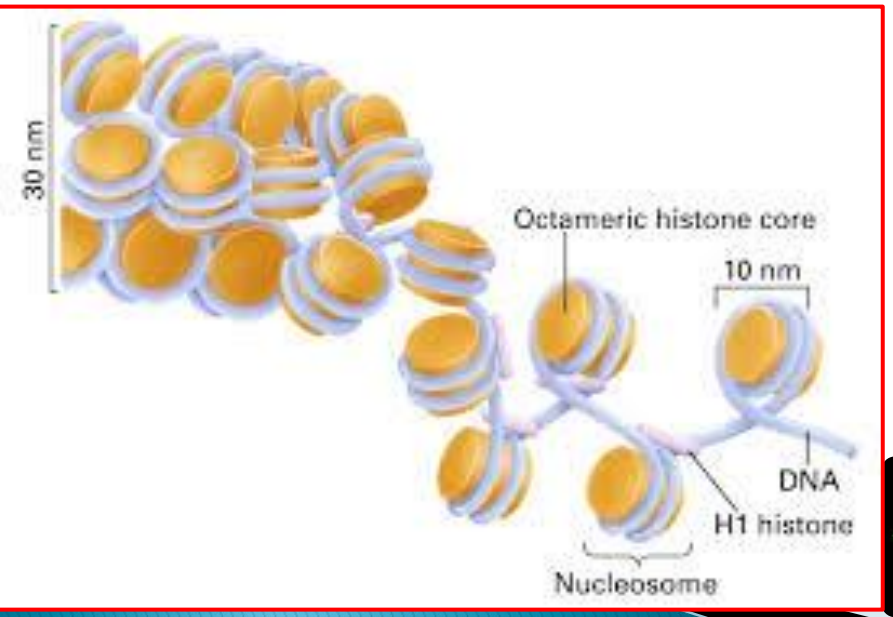


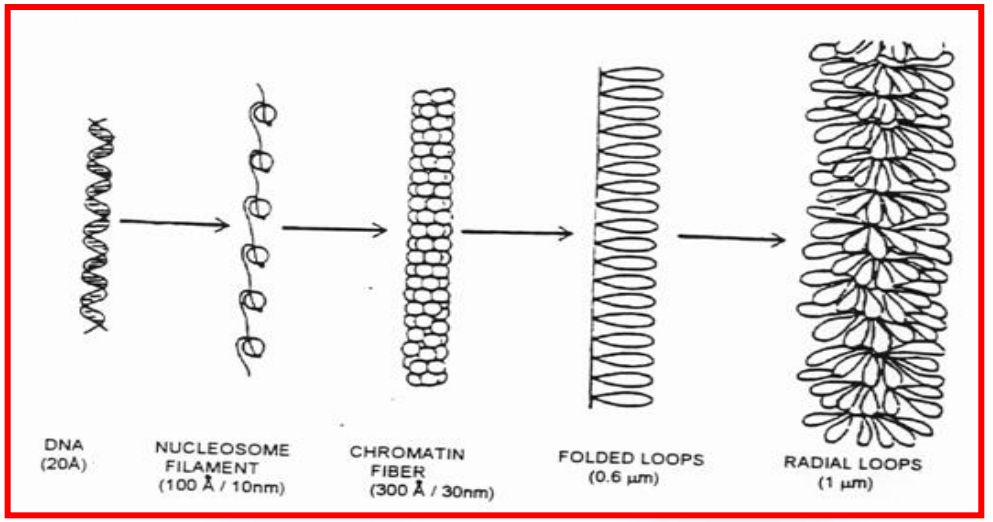


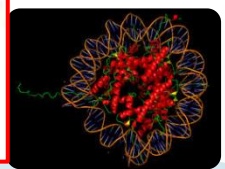
Second level of chromatin organization

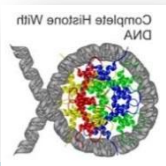
300Å FILAMENTS:

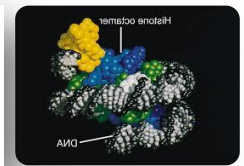
- As [salt] increases, H1 containing nucleosome filament folds into a "zig-zag" conformation.
- At physiological [salt] – chromatin forms a 300Å thick filament and nucleosomes are visible.
- This suggests that nucleosomes interact via contacts between H1 histones.

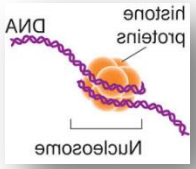


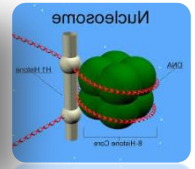








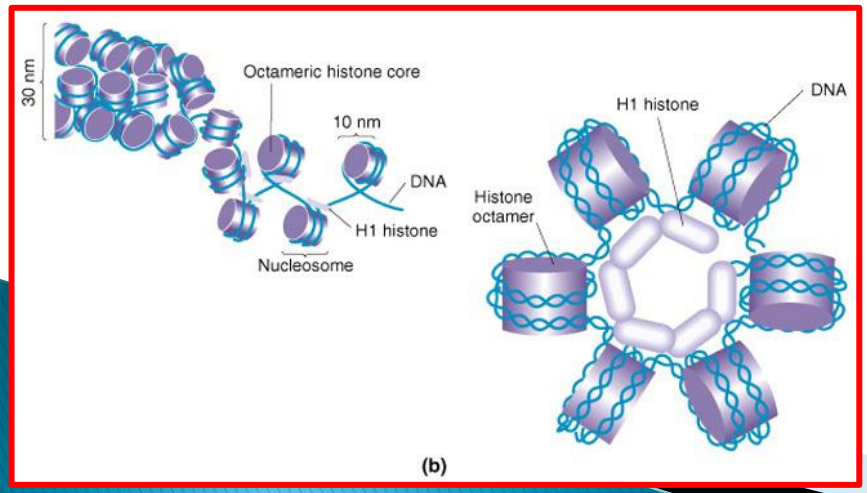


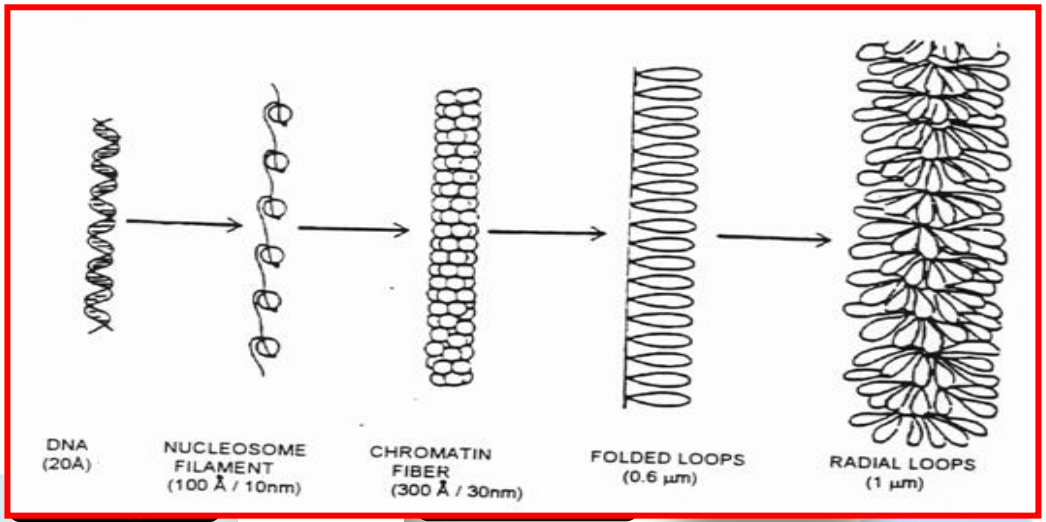


Second level of chromatin organization

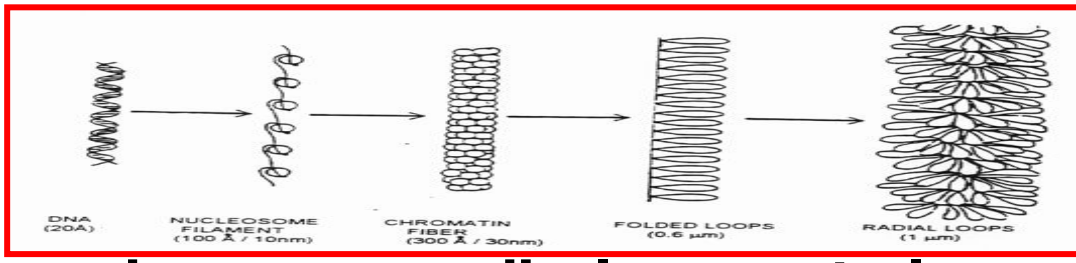
300Å FILAMENTS:

- Evidence suggests that the 300Å filament is formed by the winding of the 100Å filament into a solenoid with about 6 nucleosomes per turn.
- Solenoid - stabilized by the H1 molecules, each made up of a conserved nucleosome-binding globular core, and a variable, extended N and C terminal arms.
- These arms are thought to contact adjacent nucleosomes.



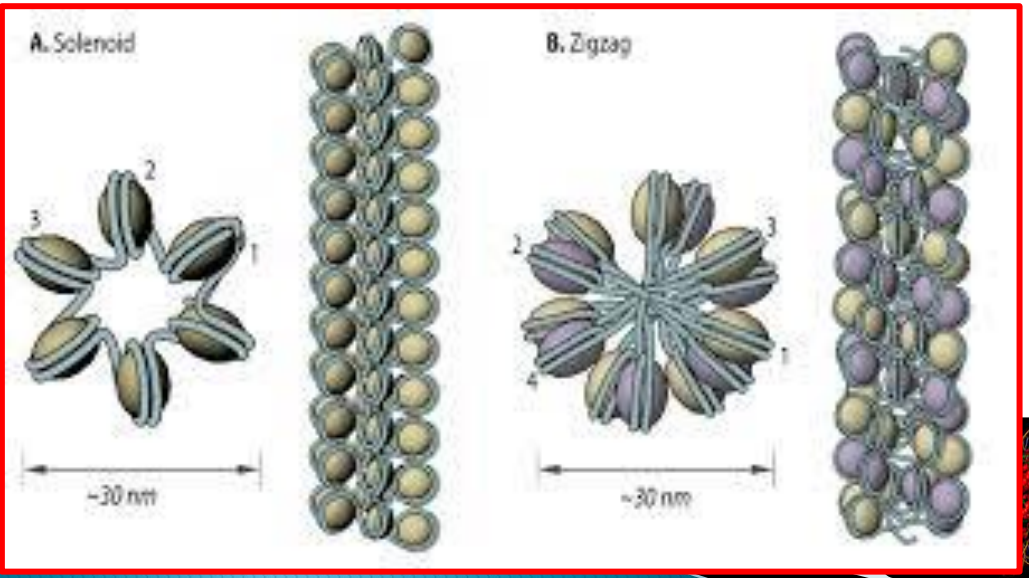


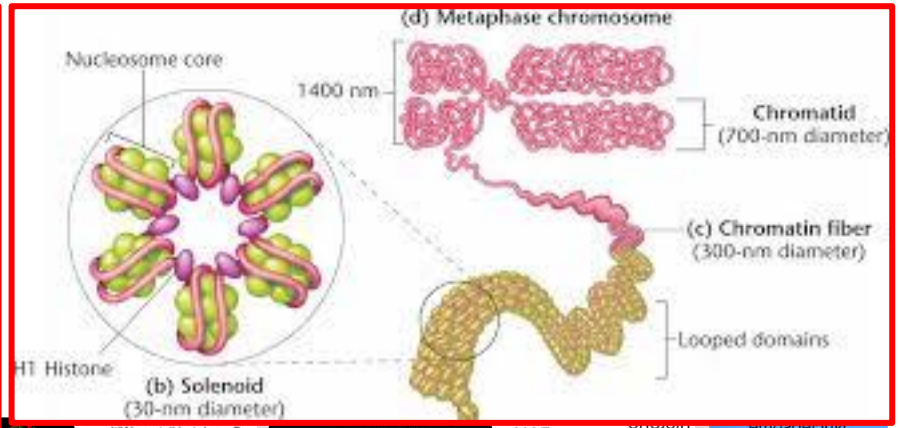
Third level of chromatin organization

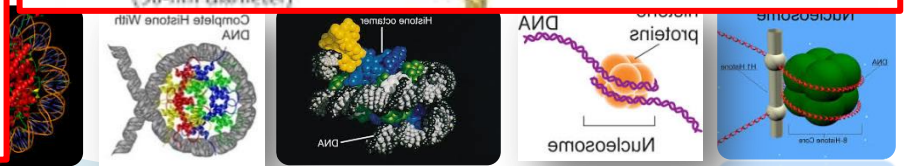


RADIAL LOOPS:

- Histone depleted metaphase chromosomes display a central fibrous protein "scaffold" surrounded by a "halo" of DNA.
- "Halo" of DNA is made up of loops of DNA that enter and leave the scaffold at the same point.
- The loops range in length from 15-30 μm (45-90 kbp).
- Condensed 300Å filaments are about 0.6 μm long.



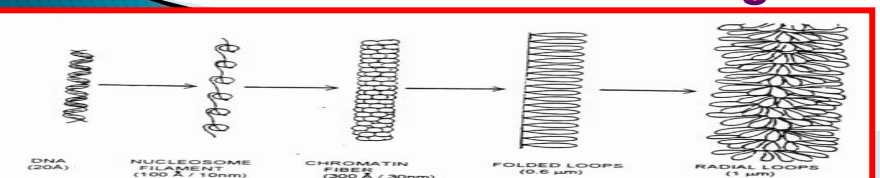


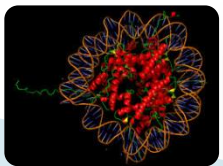


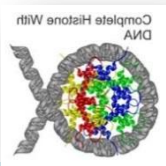
Third level of chromatin organization

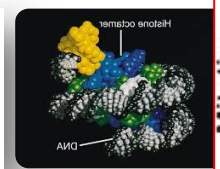
RADIAL LOOPS:

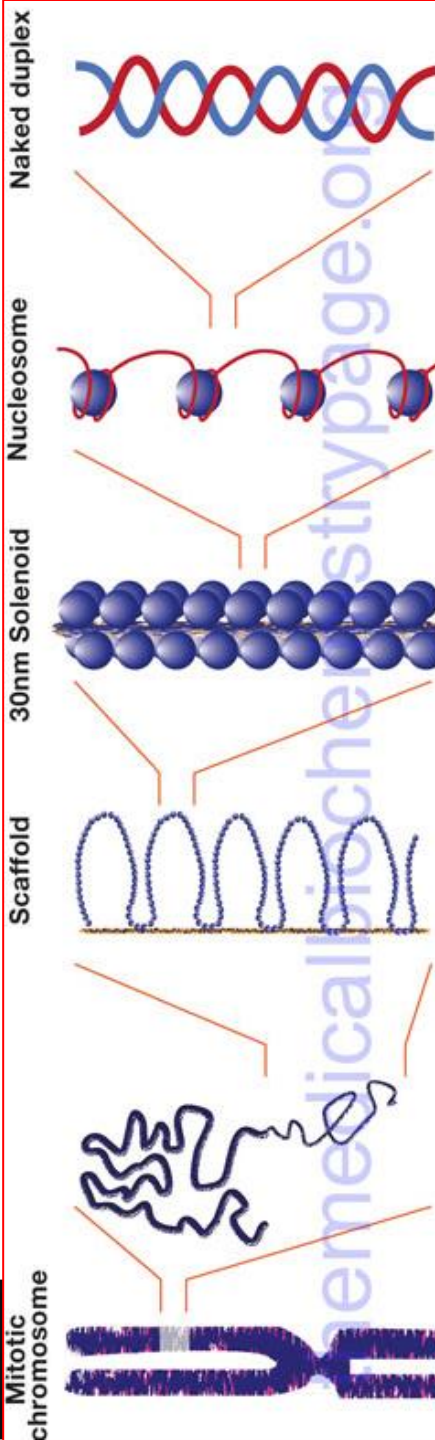
- EM – suggests that chromatin fibers are radially arranged.
- These radial fibers are loops about 0.3 μm in diameter. Fibers double up to form a loop (2 x 0.3 μm) and scaffold diameter is about 0.4 μm.
- Hence predicted diameter of metaphase chromosome is about 1.0 μm .
- Radial loop model accounts for DNA's observed packing ratio in chromosomes. Exact mechanism is not known???
- Scaffold appears to contain several proteins e.g. H1, topoisomerase II (hence the important relationship between DNA unwinding and chromatin assembly)



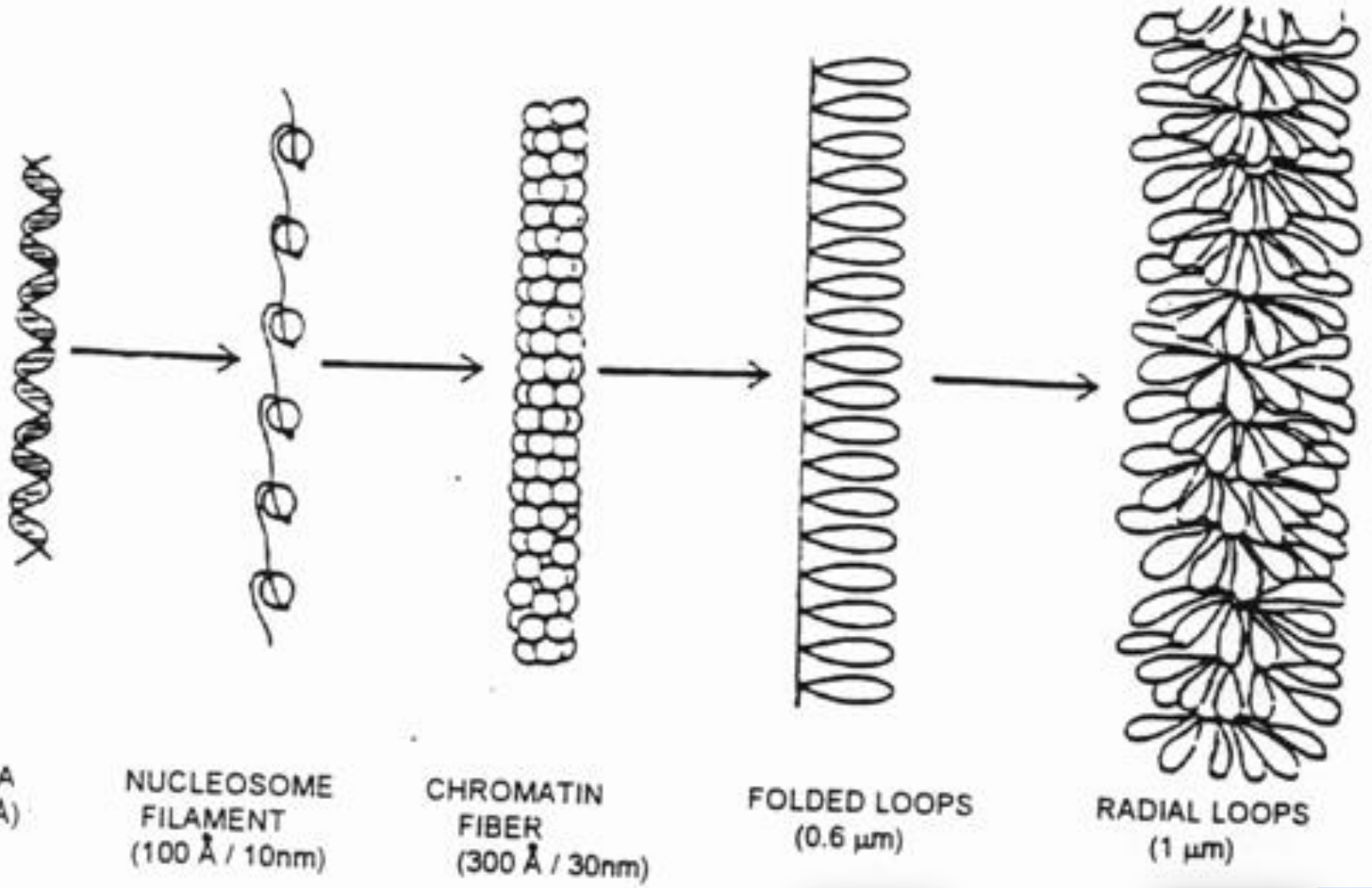


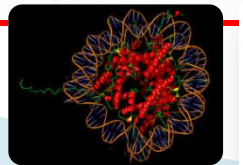
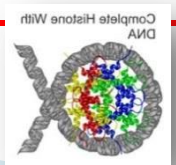


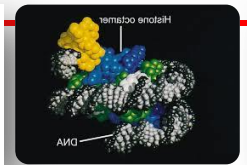


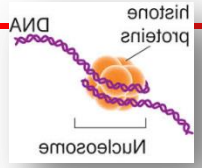


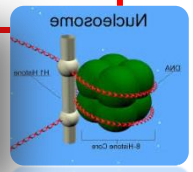
Levels of chromatin organization



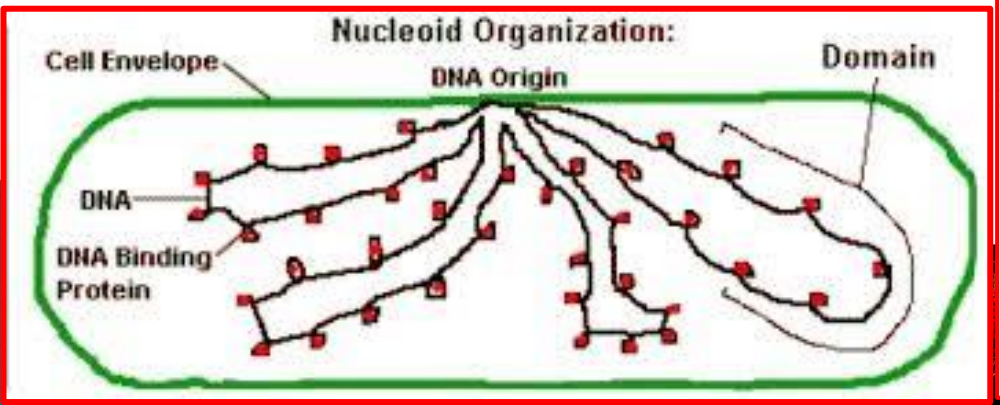


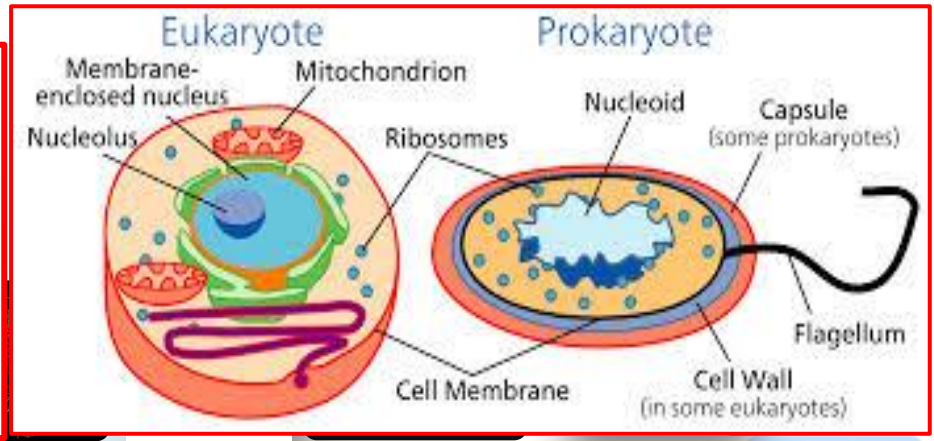




Bacterial DNA packaging

- Although histones are only found in eukaryotes, prokaryotic DNA is also packaged with proteins in a condensed form.
- Some of these proteins are referred to as "histone-like" proteins, because they resemble eukaryotic histones.
- Most prokaryotes have no nucleosome-like particles. Most of DNA is not associated with protein.
- Bacterial DNA is attached to a "scaffold" in large loops of about 100 kb.
- Arrangement of bacterial chromosome is called a **NUCLEOID**.

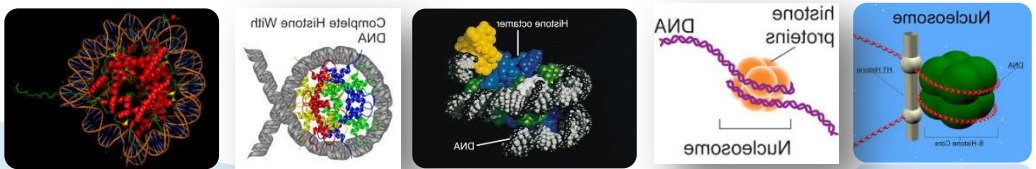




Histone modifications

- Although AA primary sequence / structure of histones is stable, individual histone molecules do vary in structure due to chemical modifications that occur later to individual amino acids.
- Degree of modification depends upon species, tissue and stage of cell cycle.

Modifications include:	
1. Acetylations (lys)	2. Methylations (lys/ arg)
3. Phosphorylations (ser/ thr)	4. ADP-ribosylations
5. Glycosylations	6. Ubiquitinations (lys)
7. Sumoylation	8. Citrullination (arg)



Histone modifications-contd…

- Reversible modifications occur an the amino terminal tail domains –especially on H3 and H4.
- Modifications occur in regions of chromatin that are active in gene transcription.

1. **Acetylation:**

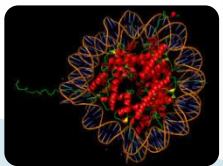
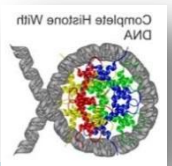
   Addition of acetyl groups to lys. Neutralizes +ve charge.

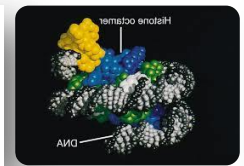
2. **Methylation:**

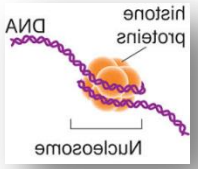
   Addition of methyl groups to arg /lys. Neutralises +ve charge.

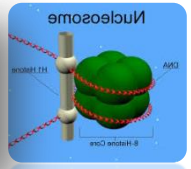
3. **Phosphorylation:**

   Addition of phosphate groups to ser /thr. Increases –ve charge. Uses kinase enzymes.







Histone modifications-contd…

4. **ADP-Ribosylation:**

   Aaddition of ADP-ribose groups.

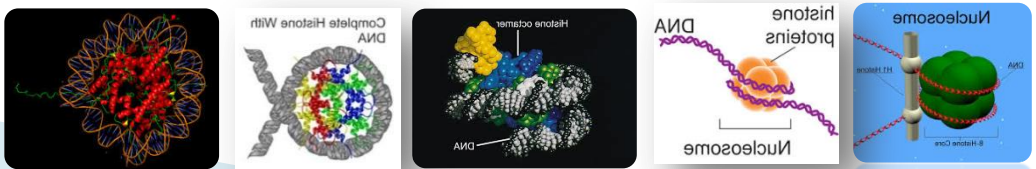
5. **Glycosylation:**

   Addition of carbohydrate residues e.g. mannose.

   Evidence seen for glycosylation of H1, H2A, H2B, H3 by fucose and mannose.

6. **Ubiquitination:**

   Addition of ubiquitin on lys. Ubiquitinated H2B usually located in transcriptionally active chromatin.



Histone modifications-contd…

7. **Sumoylation**:

   Addition of sumo proteins on histone H4. Suggested that this modification mediates gene silencing by recruitment of histone deacetylase enzyme and heterochromatin protein 1 (HP 1).

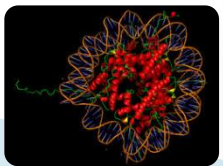
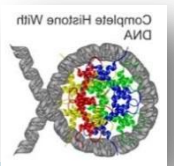
8. **Citrullination /'deimination':**

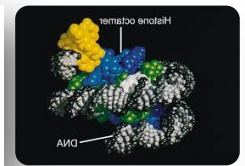
   Modification of arg to citrulline.

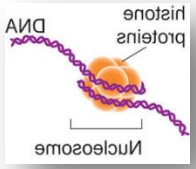
   Catalysed by the peptidylarginine deaminase (PAD 8) enzyme.

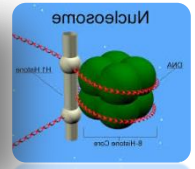
   Can also convert methylated arg to citrulline (demethylation) by the PAD 4 enzyme.

   Reaction uses water and produces NH_4^+ as a side product.





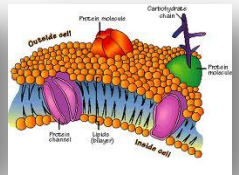


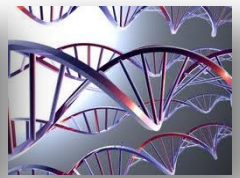
Sugary drinks vs early menstruation

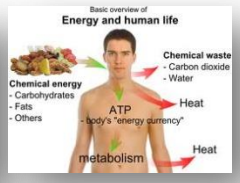
- **Sugary drinks linked to earlier onset of menstrual periods**

- ***January 27, 2015:*** **Girls who frequently consume sugary drinks tend to start their menstrual periods earlier than girls who do not, according to new research. The findings are important not only because of the growing problem of childhood obesity in a number of developed countries, but also because starting periods earlier is linked to an increased risk of breast cancer later in life.**

- ***Source: Oxford University Press***

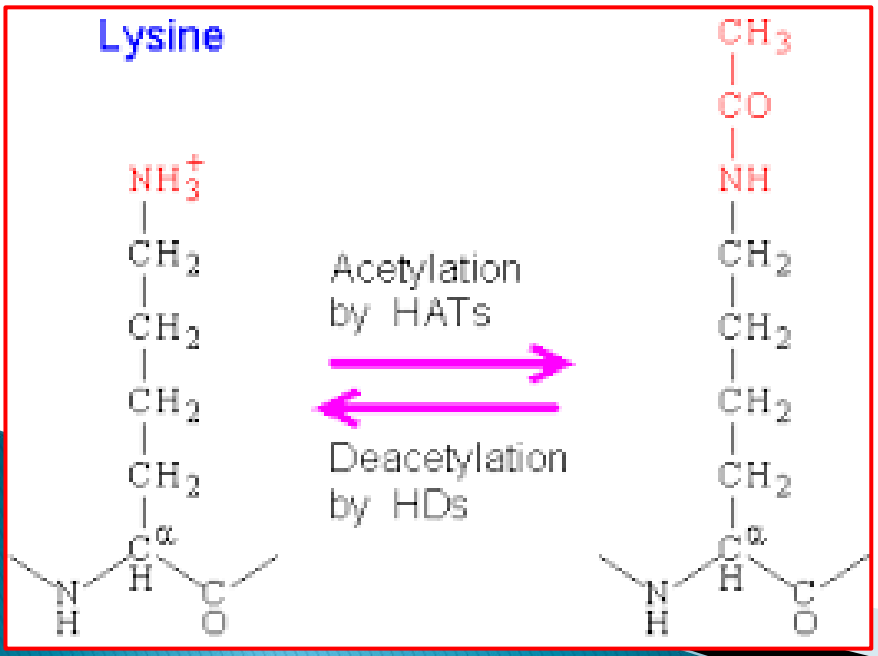


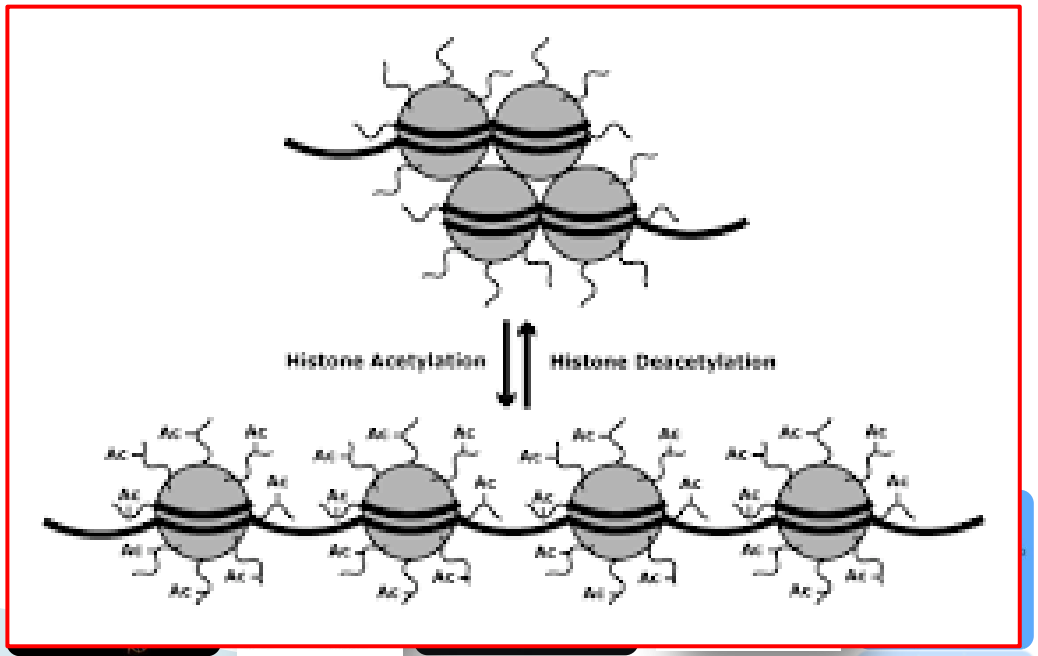




1. Acetylation of histones

- One of the most studied covalent modification.
- Closely linked to transcriptional activation.
- Acetylation occurs "post-translationally" and reversibly on the ε-NH_3^+ group of lys. Lys is embedded in the tails of the core histones.
- Histone are more acetylated in active genes and less acetylated in silenced genes.



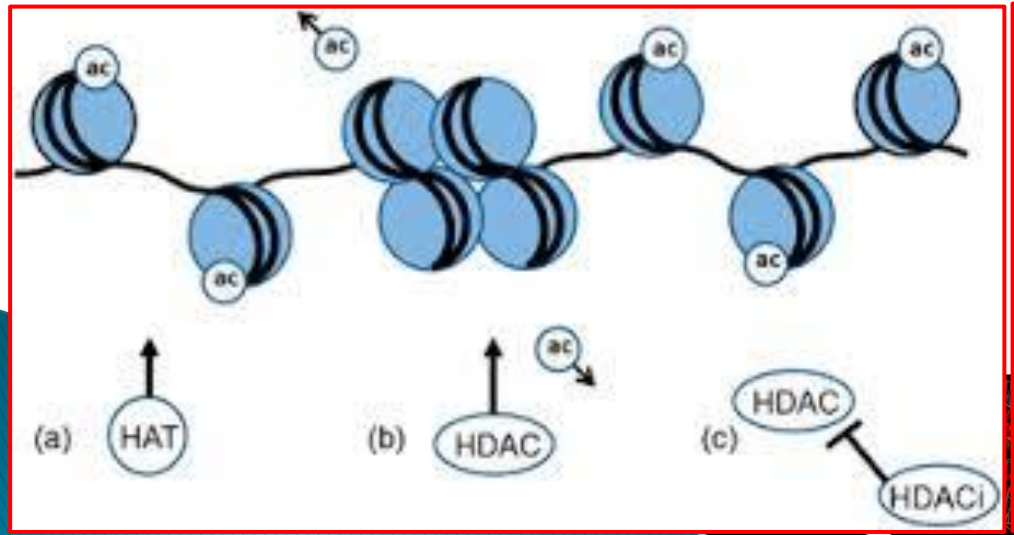


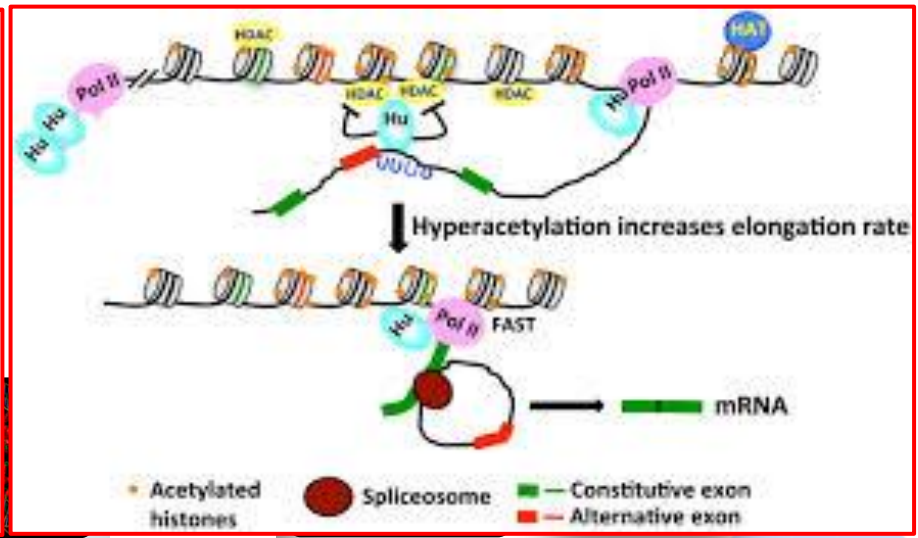
1. Acetylation of histones

TYPE OF ACETYLATION

1. **Hypoacetylation :** **Strong internucleosomal interactions. Histone tails constrain the DNA.**
2. **Hyperacetylation :** **Weak internucleosomal interactions. Histone tails do not constrain the DNA which becomes accessible to transcription factors.**

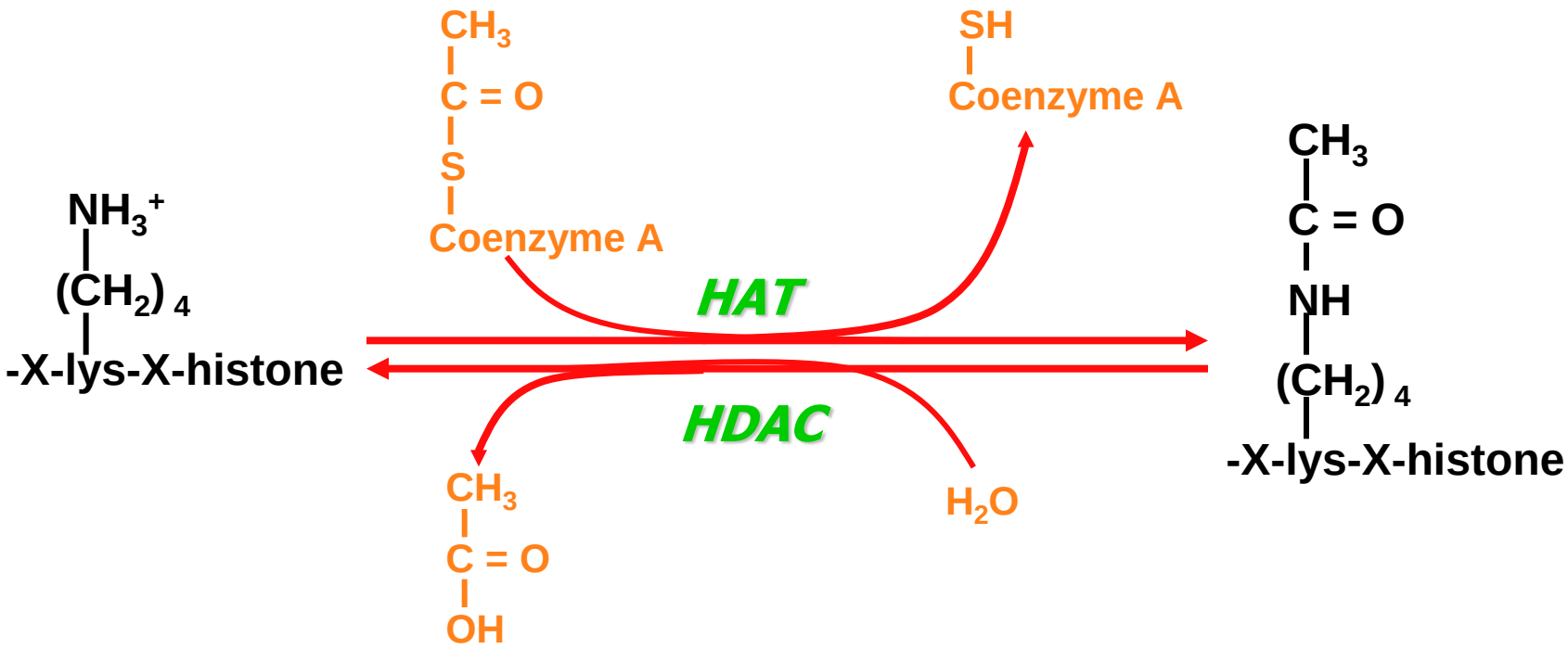
- ❑ **Most modifictions occur on histones H3 and H4.**
- ❑ **Histones are transiently stored in an acetylated form but deacetylated upon chromatin incorporation.**





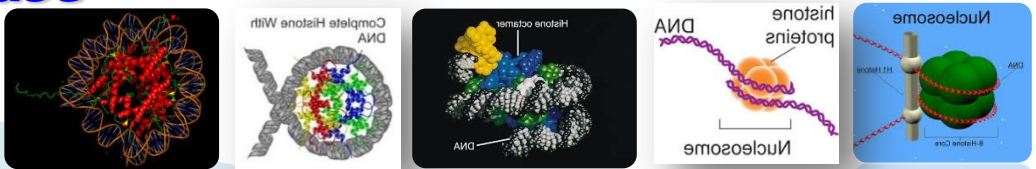
1. Acetylation of histones

- The acetyl group(CH_3CO-) from Acetyl CoA binds to the NH_3^+ of lys. CoASH is released.
- Reaction is catalysed by *Histone acetyl transferase (HAT)*



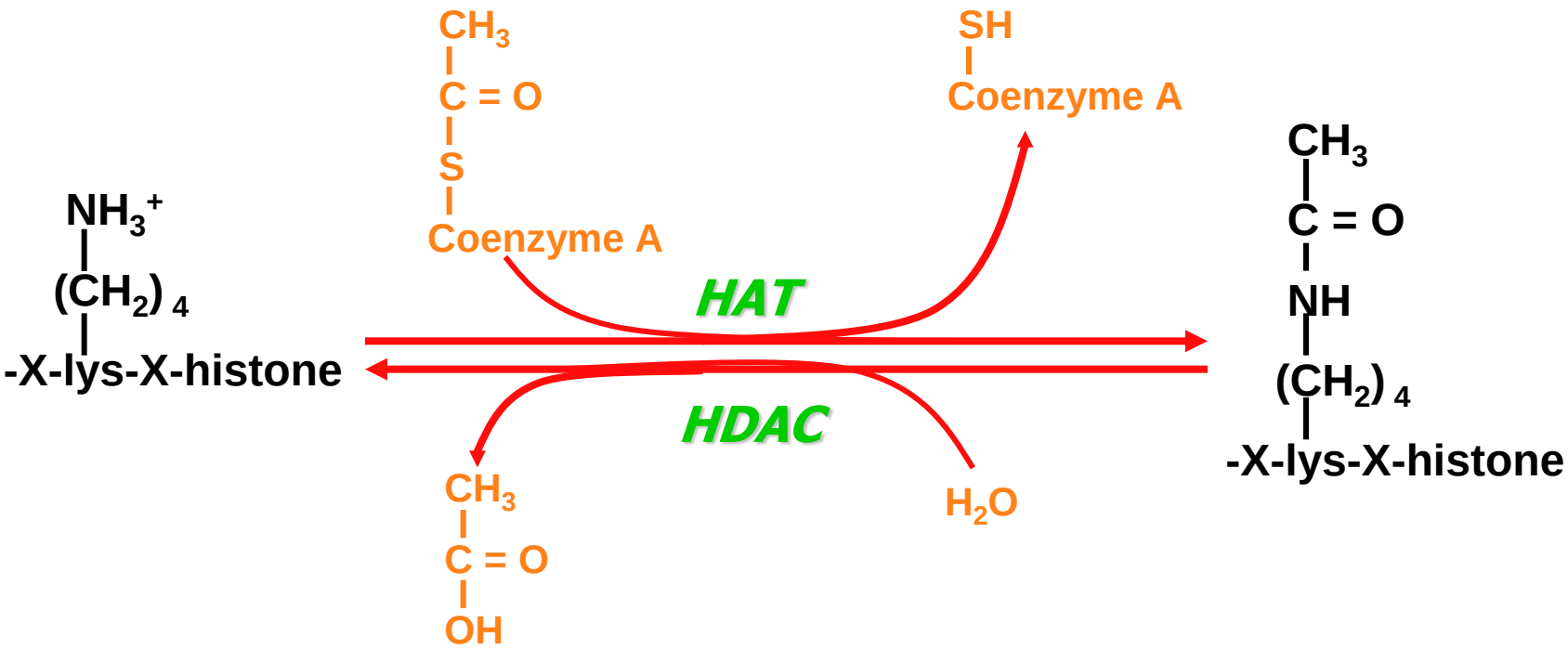
HAT = Histone acetyl transferase

HDAC = Histone deacetylase

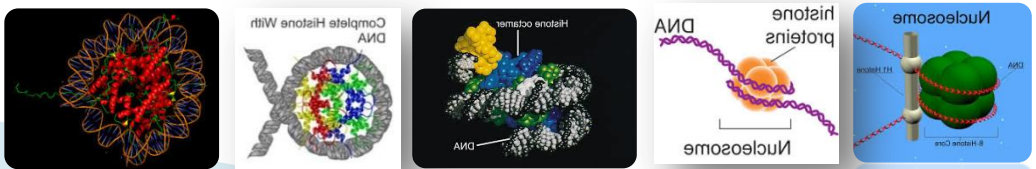


1. Acetylation of histones

- The acetyl group(CH3CO-) neutralises the +ve charge on lysine.
- →increases hydrophobicity and loosens the DNA coil.
- Acetylation enhances transcription.

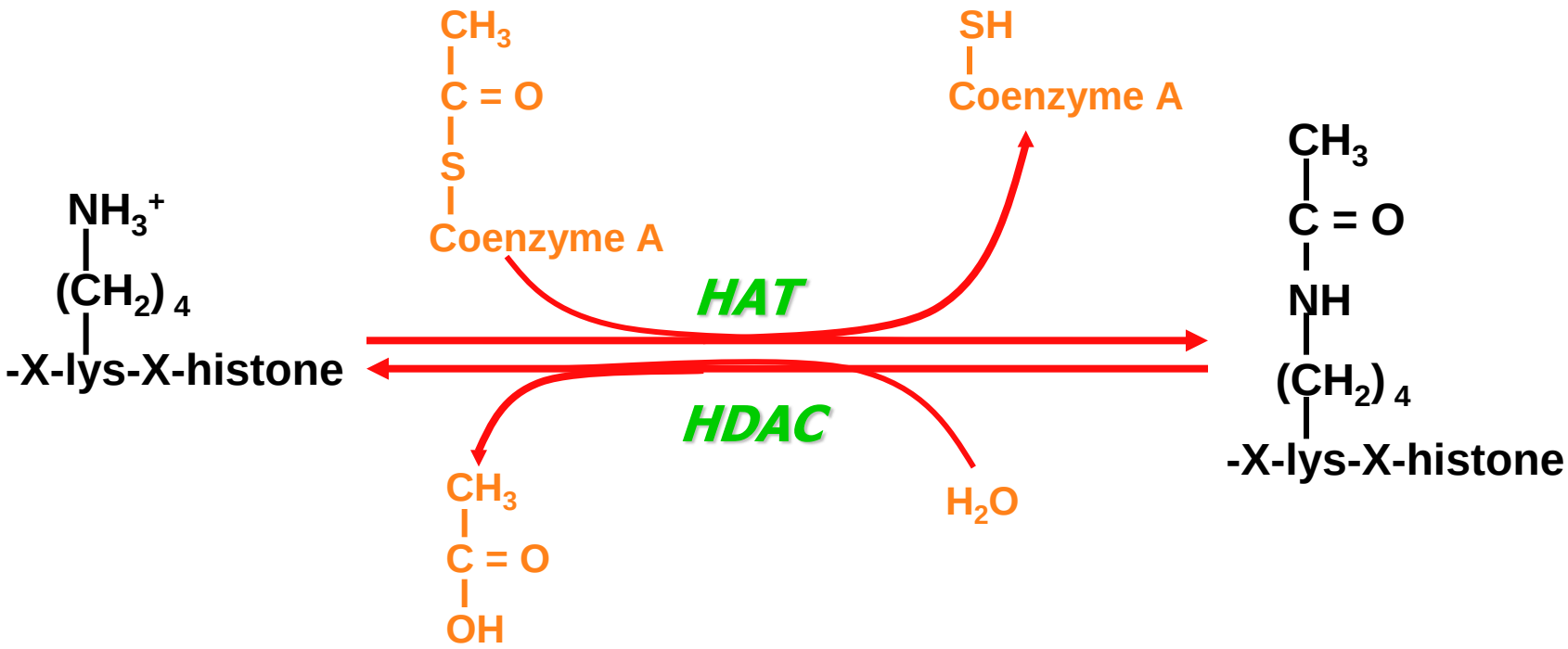


- Most species histone H3 is acetylated at lys 9, 14, 18 , 23.
- Acetylation - lys 9 plays a role in histone deposition and chromatin assembly.

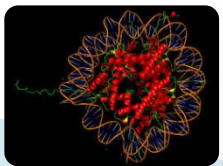
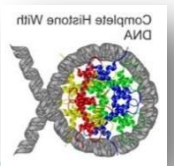


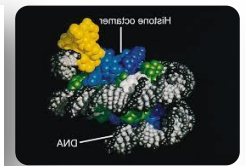
Deacetylation of histones

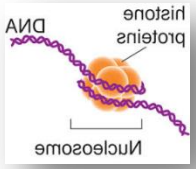
- Removal of acetyl group(CH3CO-) by the addition of water.
- Reaction is catalysed by Histone deacteylase (HDAC).
- Re-establishes +ve charge on the histone lys residue.

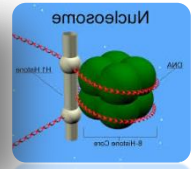


- DNA wraps tightly around the histones.
- Deacetylation represses transcription

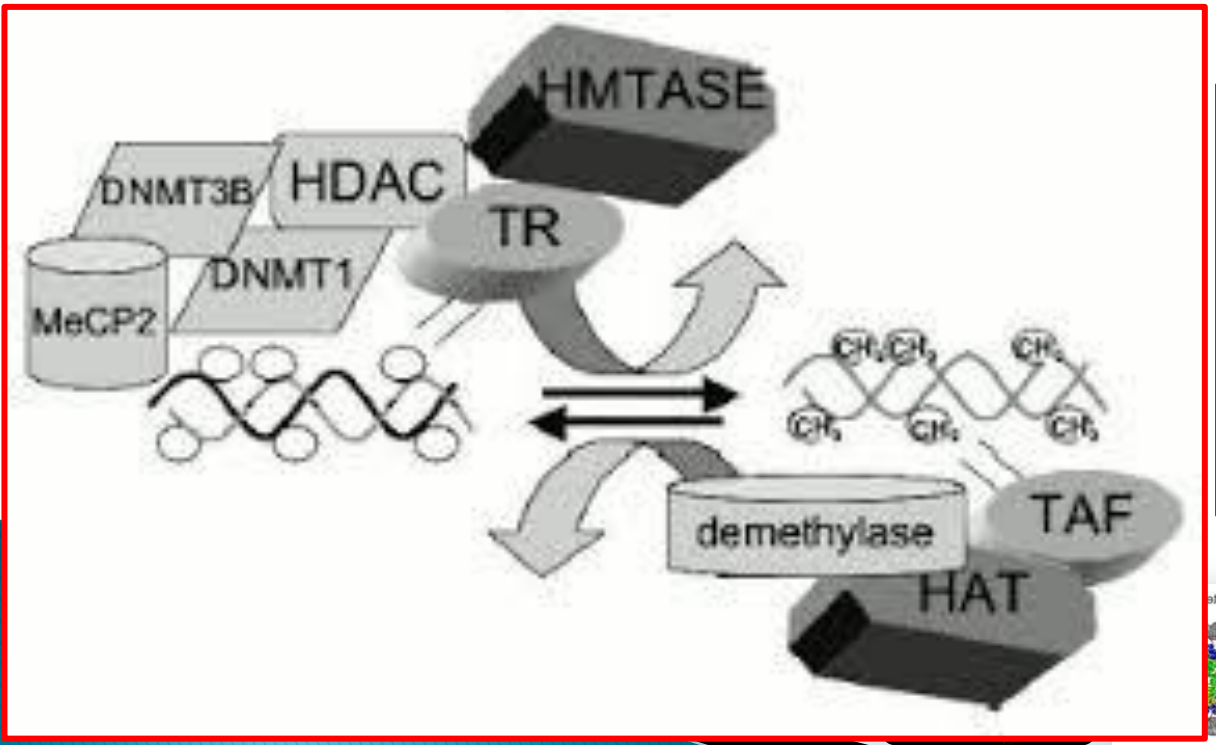


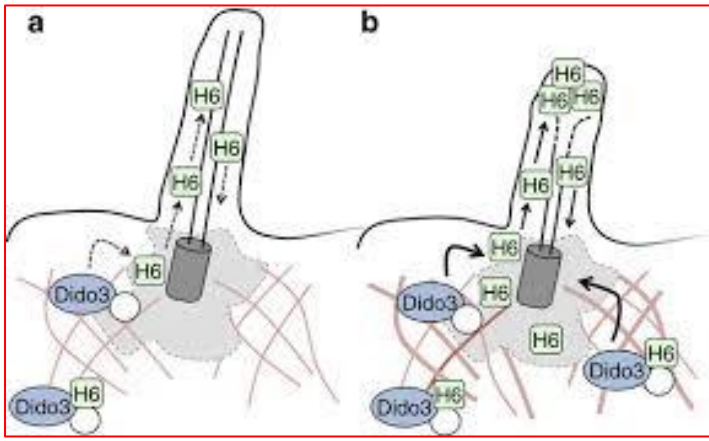


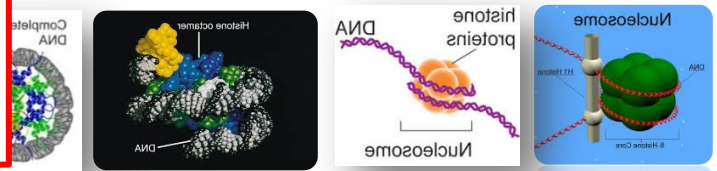


Eq. steady-state of histones Ac.

- *Equilibrium steady-state histone acetylation* - is achieved by the opposing activities of HAT and HDAC.
- Both enzymes have subunit p48.
- Histone chaperone (CAF1) is active in replication coupled chromatin assembly and repair.
- It forms a complex with p48 and histones H3 and H4.

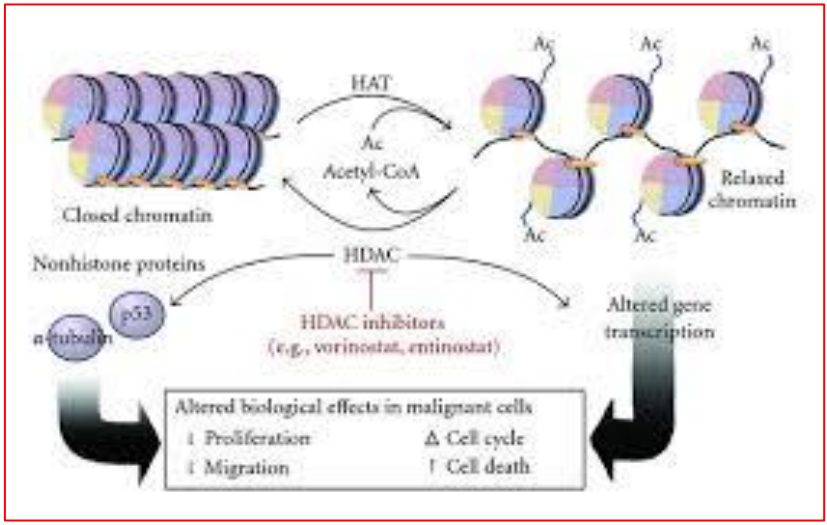






Types of HATs

- Get 2 types of HATs :



(i) Type A HAT:

- Localised in nuclei.
- Acetylate nucleosomal histones in reactions related to transcriptional activation.

(ii) Type B HAT:

- located in cytoplasm.
- Acetylate newly synthesised histones before chromatin assembly during DNA replication.
- First identified in yeast.

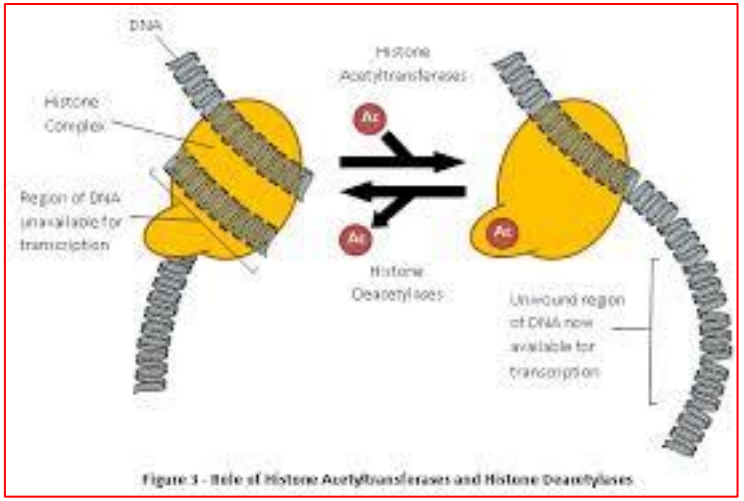
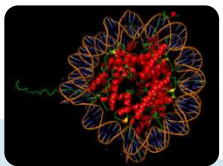
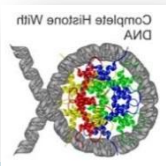
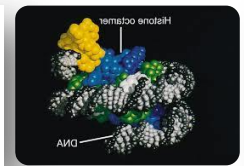
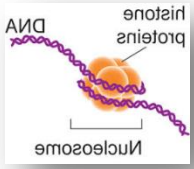


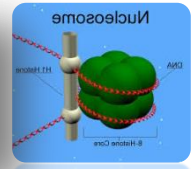
Figure 3 - Role of Histone Acetyltransferases and Histone Deacetylases







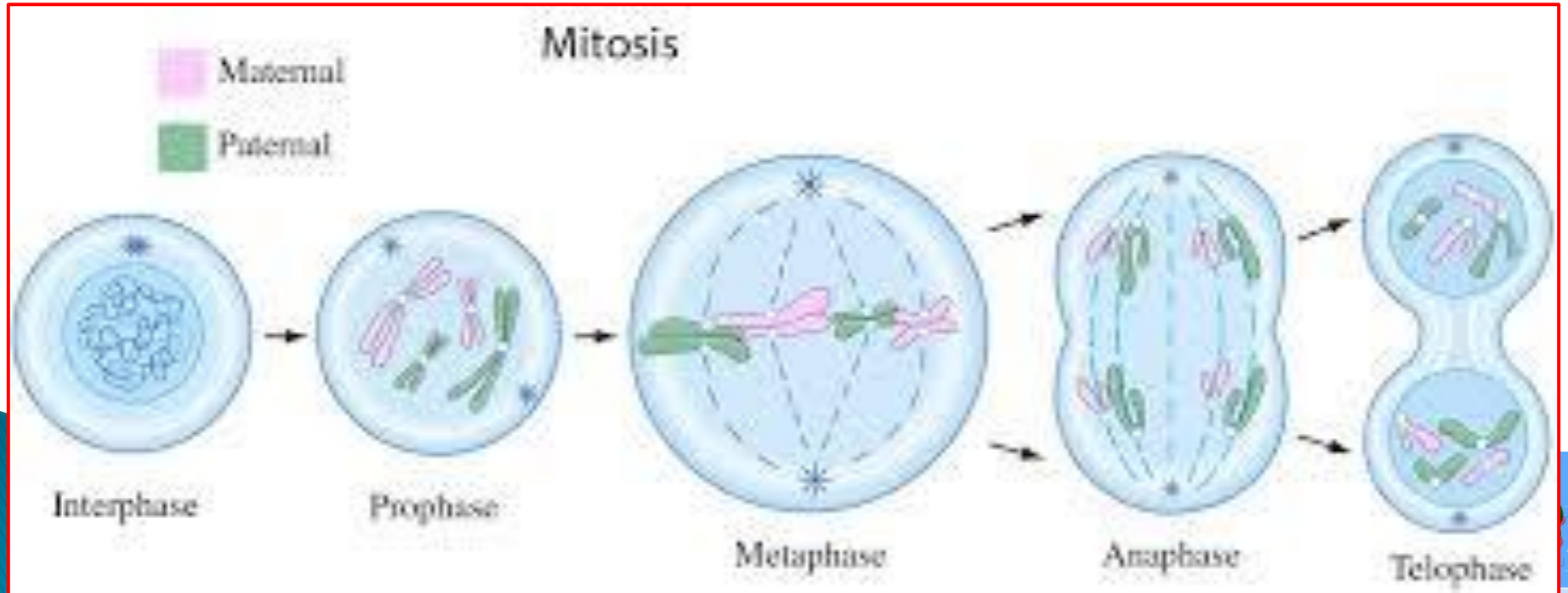




Acetylation states inheritance

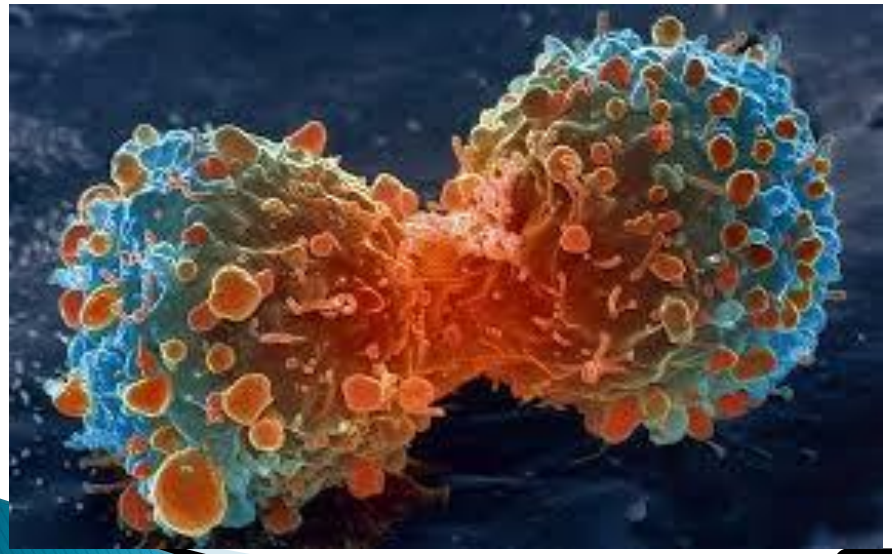
Are specific acetylation patterns inherited throughout cell division?

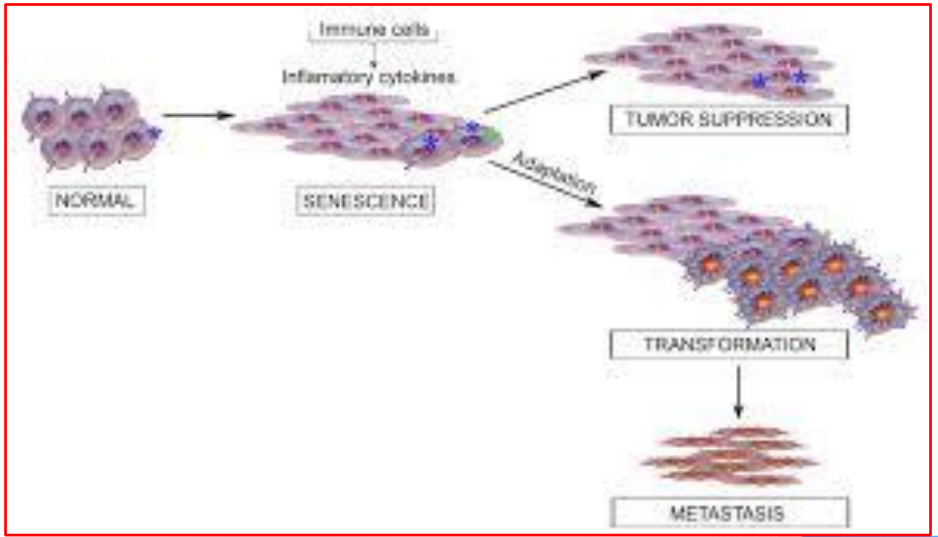
- **Mitosis – distributes histones acetylated at specific loci between daughter chromosomes.**
- **Specific HATs are also distributed between daughter cells .**
- **HATs acetylate adjacent nucleosomes and hence spread over the entire chromatin domain.**

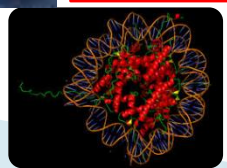


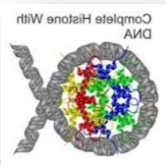
Clinical links of HATs & HDACs

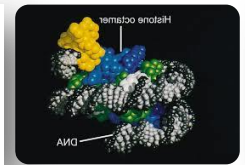
- Alteration in chromatin structure is important in gene transcription and possible cancer suppression.
- HAT promote gene expression. During acetylation the DNA coil is loosened and genes are available for expression.
- HDAC inhibit gene expression. During deacetylation the DNA is tightly wrapped and genes cannot be accessed and are hence repressed.

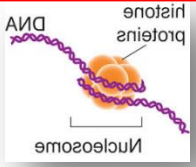


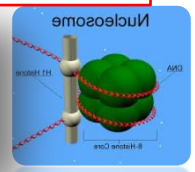






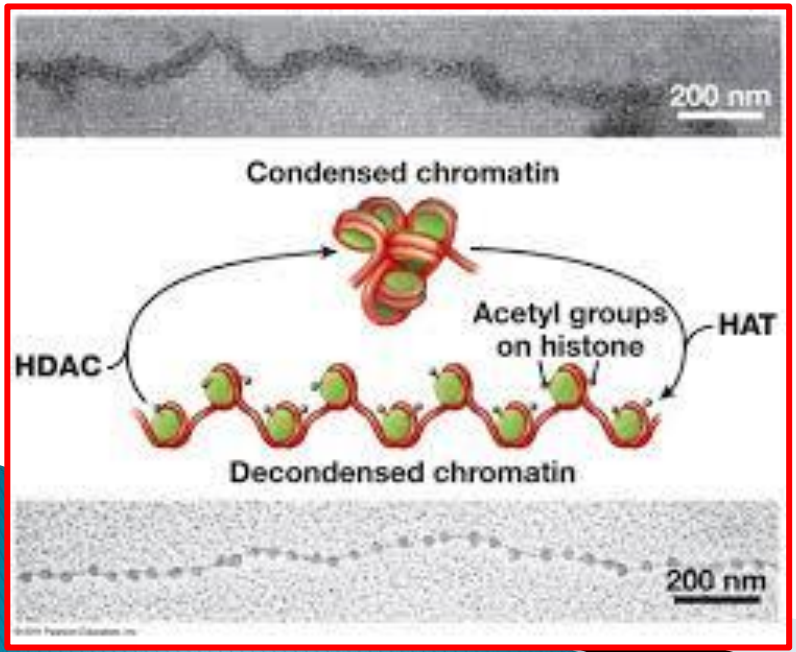


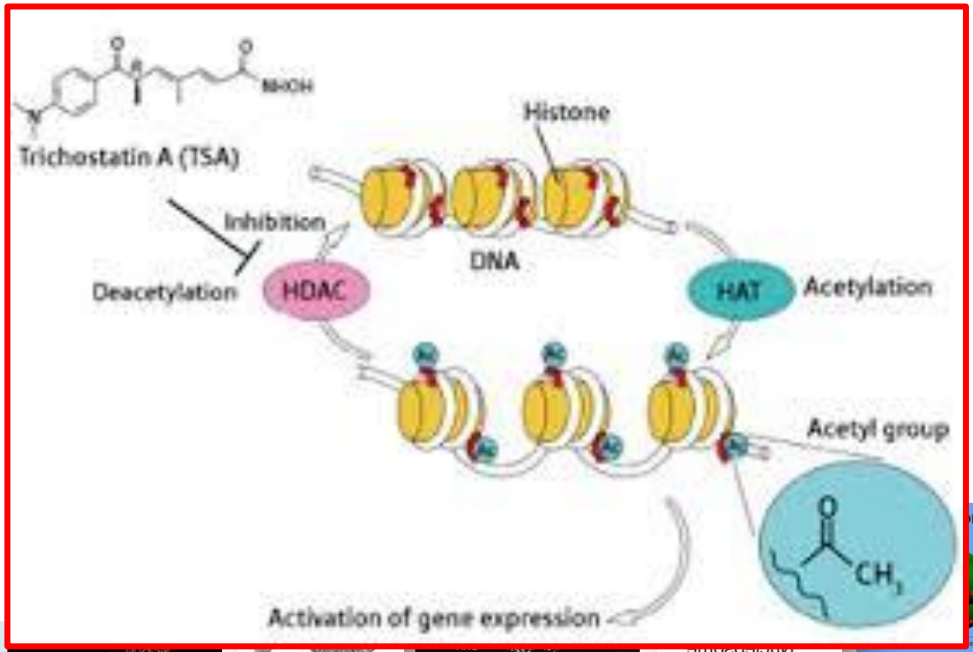




Clinical links of HATs & HDACs

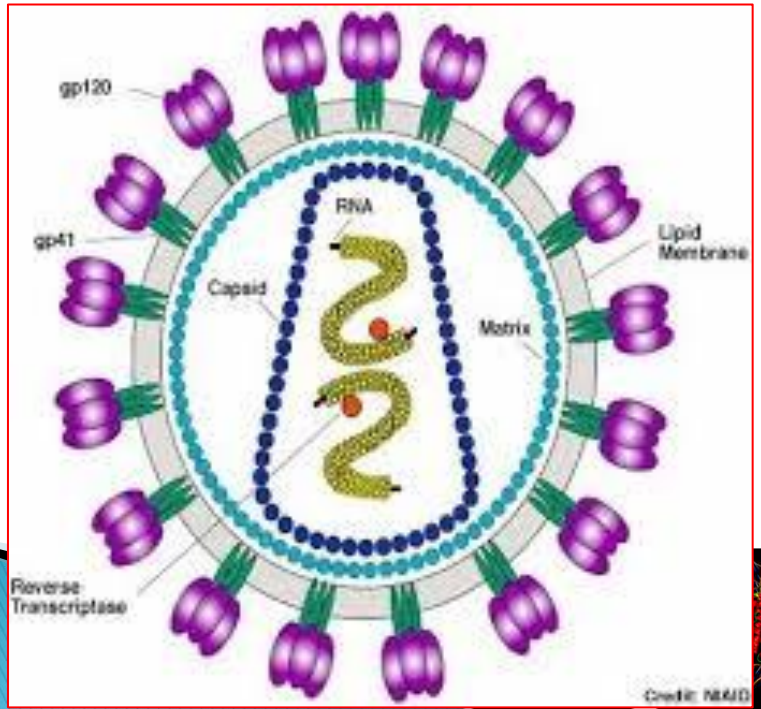
- The 2 enzymes provide a balance between activation and inactivation of genes.
- Any disruption of this balance leads to disease states e.g. cancer.
- Drugs are being developed to modify the activity of these enzymes.
- The drug, *Trichostatin A (TSA),* is a potent and reversible inhibitor of HDAC.

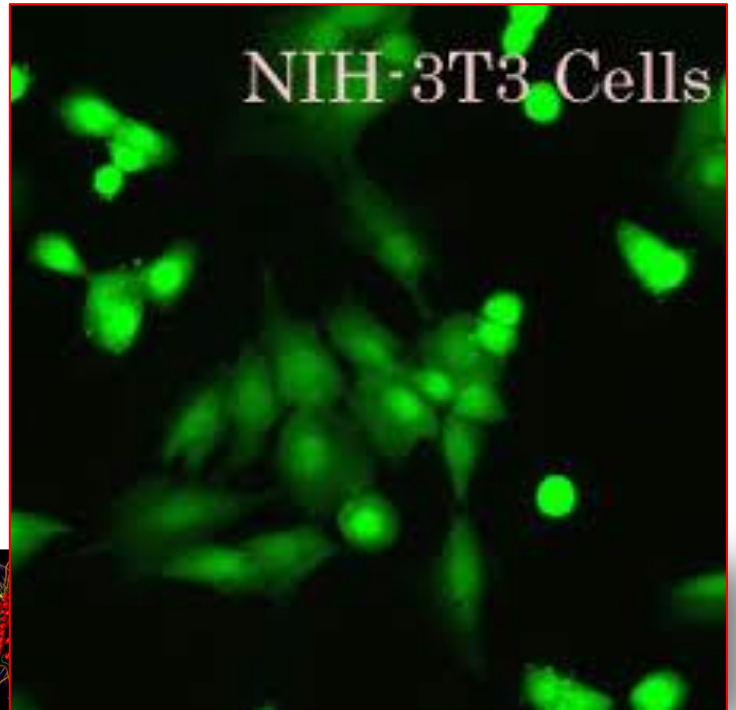


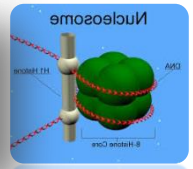


Clinical links of HATs & HDACs

- In HIV-1 cells TSA induced transcriptional activation.
- In NIH-3T3 cells you get reversion of oncogenic ras-transformed cells to normal using TSA.
- It also showed immunosuppressive activity in a mouse model.
- TSA is a useful tool for induction of hyperacetylation and elucidation of the role of histones in gene expression.

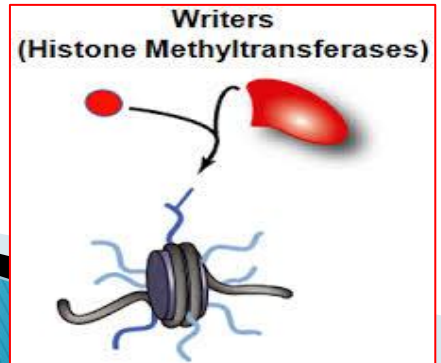


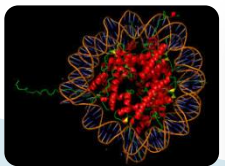


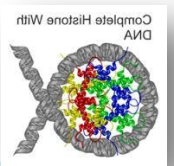


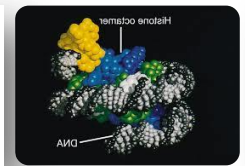
Histone methylation

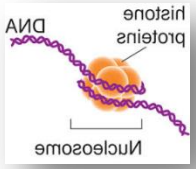
- Methylation - least understood post-translational modification.
- Evidence exists that methylation of H3 at lys 4 / 9 might influence chromatin transcription.
- Methylation at lys 4 →with active gene expression.
- Methylation at lys 9 →heterochromatin assembly in mice and yeast. Associated with inactive genes.
- Methylation by *Histone Methyltransferases (HMTases)* - important in maintaining chromatin structure.
- Absence of this enzyme could result in a looser chromatin with impaired recruitment of chromatin-associated proteins.

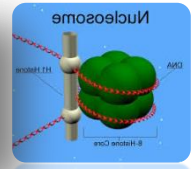






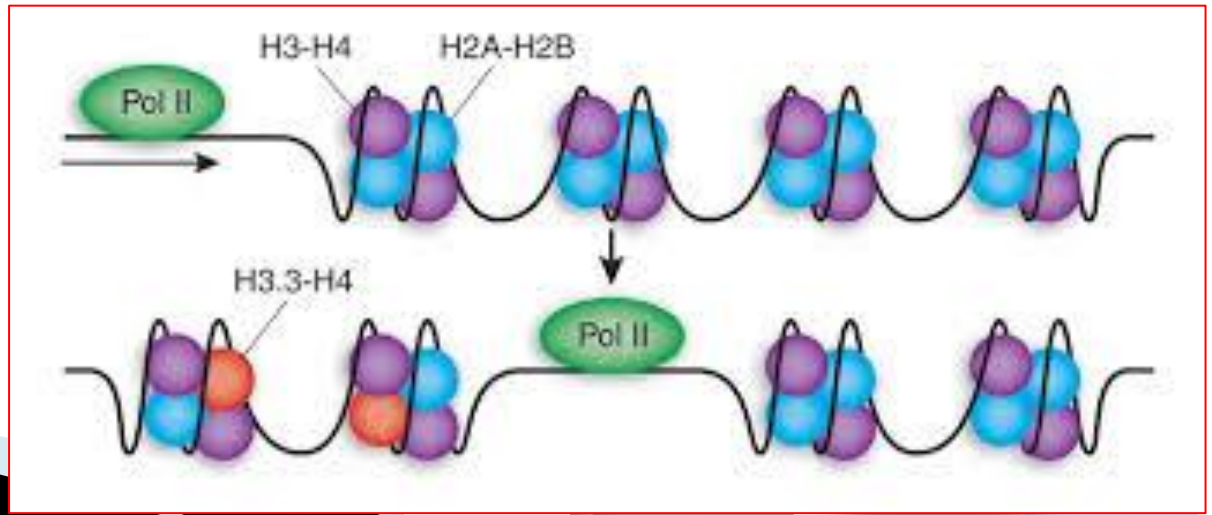






Roles of histones

- Histones were for many years simply viewed as spools around which DNA is wrapped for packaging in the nucleus.
- Last 15 years →histones play an active role in regulating the form of chromatin structure and gene expression.
- Isolated pure forms of histones are of interest in DNA:PROTEIN interaction studies and to investigate the structure and function of chromatin.
- They also serve as substrates for protein kinases.
- Histones H3 and H4 play active roles in modulating gene expression.

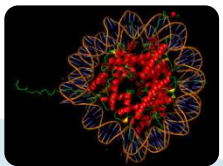


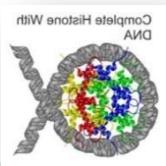
Types of chromatin

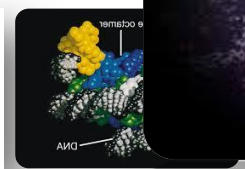
(a)Heterochromatin : Highly condensed chromatin and not genetically expressed because genes are transcriptionally inactive / silent. (seen throughout cell cycle)

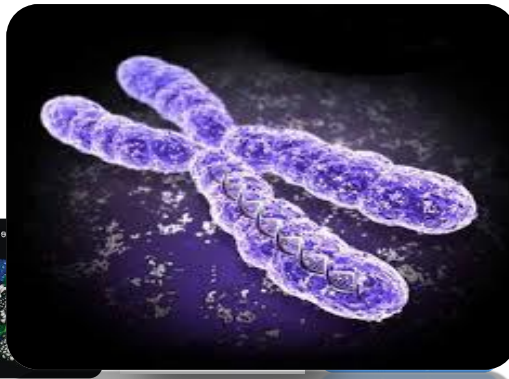
(b) Euchromatin : Loosely condensed chromatin that is genetically expressed. Genes are transcriptionally active. (seen only during interphase of cell cycle)

- **Some regions of chromosomes eg. Centromeres are almost always in the form of heterochromatin = Constitutive Heterochromatin**
- **Regions that are euchromatic / heterochromatic depending on tissue / cell type = Facultative Heterochromatin**
- **Heterochromatin is inherited in an epigenetic manner.**





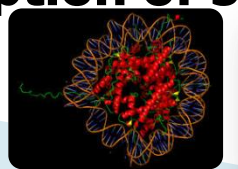
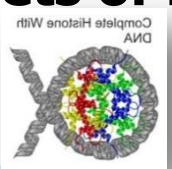


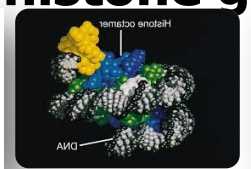


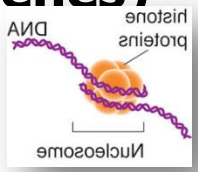
Histone synthesis & gene reiteration

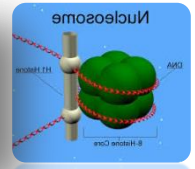
- Histones synthesised during the short 'S' phase is required in massive amounts for chromatin assembly.
- Small amounts are synthesised for repair processes only.
- Histone mRNA has no poly(A) tail.
- Ensures rate of histone synthesis parallels that of gene transcription.
- Multiple copies of histone coding genes i.e multiple reiteration of genes.
- Only identically repeated genes code for the proteins.
- This organisation permits sensitive control of histone synthesis.

(co-ordinate transcription of sets of histone genes)

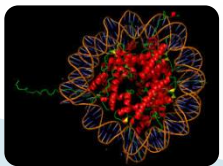
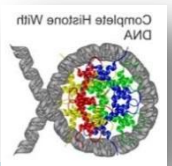


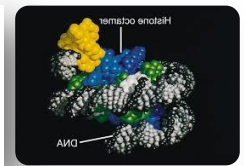


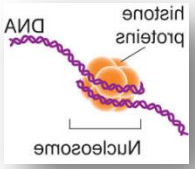


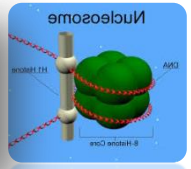
Histone synthesis & gene reiteration

- Almost all histone genes sequences lack introns (non-coding sequences).
- Significance??
- Organisation and length of h-genes occur as species clusters as repeats or randomly.
- No relationship between genome size and total number of histones
- Eg. Birds / mammals have about 10-20 copies of each of 5 histone genes. Drosophila has about 100 copies. Sea Urchins have several hundred copies
- Histone gene expression efficiency varies with different species.

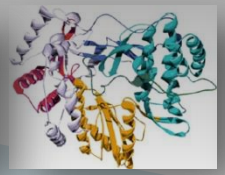



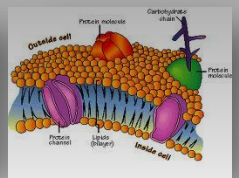


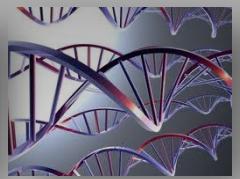


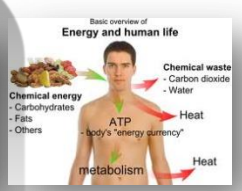


The End




Protein molecule
Carbohydrate chain
Outside cell
Protein molecule
Protein channel
Lipids (bilayer)
Inside cell




Basic overview of
Energy and human life
Chemical waste
- Carbon dioxide
- Water
Chemical energy
- Carbohydrates
- Fats
- Others
ATP
- body's "energy currency"
Heat
metabolism
Heat