- To be folded further, the remaining negative charges are neutralized by other factors including linker histones, cations, and other positively charged molecules.
- Thus, the chromatin structure can be dynamically changed depending on the electrostatic state of its environment. This change in chromatin structure is critical for gene expression because it directly governs access to the DNA.
- In eukaryotic nucleus, the chromatin resembles a disordered assembly of 10-nm fibers.
- Thus, the basic structure of the chromosome is a liquid-like compact aggregation of 10-nm.

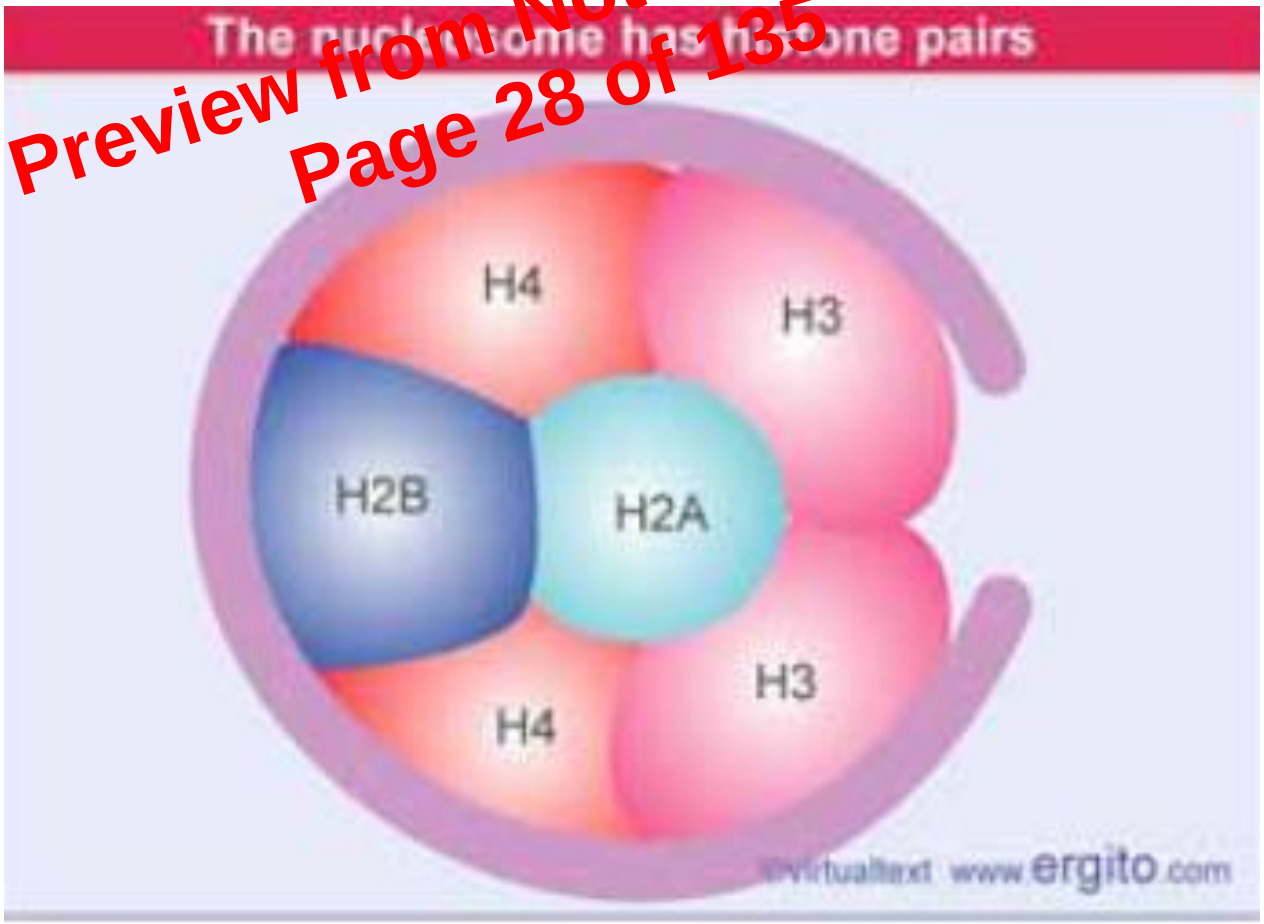
- Because chromatin is composed of an irregular and dynamic 10-nm fiber and does not have a crystal-like long-range order, chromatin in the cell is considered "liquid-like" rather than static solid-like substance. This liquid can take various structures including extended, folded, interdigi-tated, bent, looped and columnar structures.
- - The tail domains of histones H3 and H4 play crucial roles in forming these various structures .

- Facultative heterochromatin is euchromatin that will adopt heterochromatic properties in a developmentally controlled manner, suggesting temporal silencing of regions

of the genome.

- ✓ The 25–40 amino acid N-terminal domains of the core histones are highly conserved.
- ✓ They are mobile and flexible extending out of the nucleosome core in a manner that frees them for other interactions.

- CENP-A assembles into centromeres in the absence of DNA replication and can replace H3 in the nucleosome in vitro.
- H2A.Z histone variant. It is not found in cells of the inner cell mass but accumulates as the cells differentiate, being enriched in pericentric heterochromatin.
- –In euchromatin areas, H3 and H2A are replaced by H3.3 and H2AZ variants respectively.
- H2AX- represents 2–25% of H2A in a cell and is phosphorylated on serine-139 at the site of double-strand DNA breaks. H2AX foci suggest a mechanism for DNA damage detection and repair.



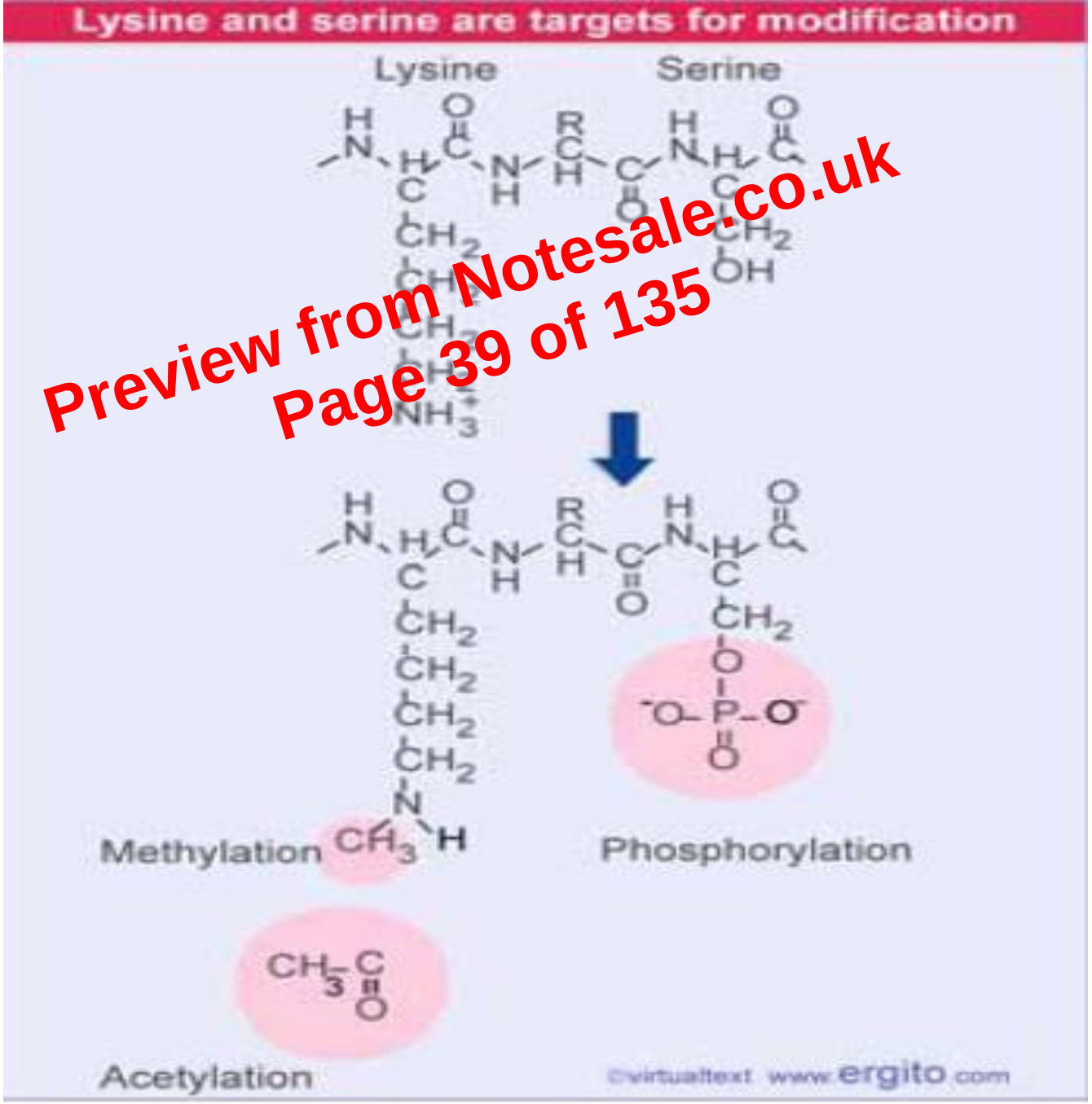
•Histone modifications

-All the histones are modified by covalently linking extra moieties to the free groups of certain amino acids.

-Modification sites are concentrated in the

N-terminal tails but can also occur at the C terminal tail.

-The modifications have important effects on the structure of chromatin and in controlling gene expression.



β-N-acetylglucosamine

- Many non-histone proteins are regulated via modification of their serine and threonine side chains with single β-N-acetylglucosamine (O-GlcNAc) sugar residues.

-Such reactions occurs in histones also. In mammalian cells, there appears to be only a single enzyme, O-GlcNAc transferase, which catalyses the transfer of the sugar from the donor substrate, UDP-GlcNAc, to the target protein.

- Histones H2A, H2B and H4 have been shown to

be modified by O-GlcNAc

- Non histone proteins associated with DNA in eukaryotic nucleus

-In addition to the core histones, the DNA scafford contain several proteins: large amounts of histone Hl (located in the interior of the fiber) and topoisomerase II

-Structural maintenance of chromosome (SMC) proteins.

-The primary structure of SMC proteins consists of five distinct domain (see fig below (a)).

-The amino- and carboxyl-terminal globular domains, N and C, each of which contains part of an ATP-hydrolytic site are connected by two regions of a-helical coiled-coil motifs that are joined by a hinge domain.

-The shape of the nucleosome shape is like a flat disk or cylinder, of 11nm diameter and a height of 6 nm

The length of the DNA is roughly twice the ~34 nm circumference of the particle. The DNA follows a symmetrical path around the

octamer. The DNA makes two turns around the cylindrical octamer where it "enters" and

"leaves" the nucleosome at points close to one another, the point where histone H1 is located .

- The ladder is generated by groups of nucleosomes. This shows that in chromatin, DNA is coiled in arrays of nucleosomes.
- When nucleosomes are fractionated on a sucrose gradient, they give a series of discrete peaks that correspond to monomers, dimers, trimers, tetramers etc.
-

->95% of the DNA of chromatin can be recovered in the form of the 200 bp ladder. Suggesting that almost all DNA is organized in nucleosomes.

-In their natural state, nucleosomes are closely packed, with DNA passing directly from one to the next. Free DNA is probably generated by the loss of some histone octamers during isolation.

-The average periodicity over the nucleosome is 10.17 bp/turn, and for DNA in solution is 10.5 bp/turn.

-The crystal structure of the core particle suggests that DNA is organized as a flat superhelix, with 1.65 turns wound around the histone octamer.

-Regions of high curvature occur at positions ± 1 and ± 4; corresponding to S6 and S8, and to S3 and S11, which are the sites least sensitive to DNAase I .

Two numbering schemes divide core particle DNA into 10 bp segments. Sites may be numbered S1 to S13 from one end; or taking S7 to identify coordinate 0 of the dyad symmetry, they may be numbered -7 to +7.

Illustration of nucleosomal positions relative to the DNA superhelix

-Using the nucleosomal digest, each eukaryotic chromosome contains many replicons. The incorporation of H3 at the centromeres is inhibited and CENP-A protein is incorporated in higher eukaryotic cells (in Drosophila Cid is used while in the yeast Cse4p is used). This occurs by the replication-independent assembly pathway because the replication-coupled pathway is inhibited for a brief period of time while centromeric DNA replicates

-Assembly is initiated when the tetramers bind to each of the daughter duplexes assisted by CAF-1 (an assessory protein). This is followed by binding of the two dimers (H2A·H2) to each tetramer to complete the histone octamer. Thus, the assembly of tetramers and dimers is random with respect to "old" and "new" subunits.

-Thus, the deposition of histone octamers on DNA is intrinsic. It is determined by structural features in DNA extrinsically resulting from the interactions of other proteins with the DNA and/or histones.

- Structural features of DNA affect placement of histone octamers. These include:

a). A·T-rich regions are arranged in such a way that the minor groove faces in towards the octamer.

-The location of DNA on nucleosomes can be described in two ways:

a). **translational positioning-** describes the location of a histone octamer at successive

turns of the double helix, which determines which sequences are located in linker

Regions.

-

-It describes the linear position of DNA relative to the histone octamer. Displacement of the DNA by 10 bp changes the sequences that are in the more exposed linker regions, but does not alter the face of DNA that is protected by the histone surface and which is exposed to the exterior.

-In particular, it determines which sequences are found in the linker regions. Shifting the DNA by 10 bp brings the next turn into a linker region. Thus, translational positioning determines which regions are more accessible (e.g to the micrococcal nuclease).