Chromatin Structure

Biochemistry 201
Advanced Molecular Biology
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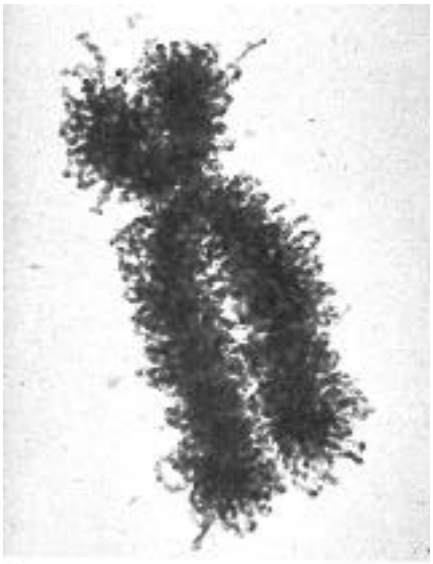
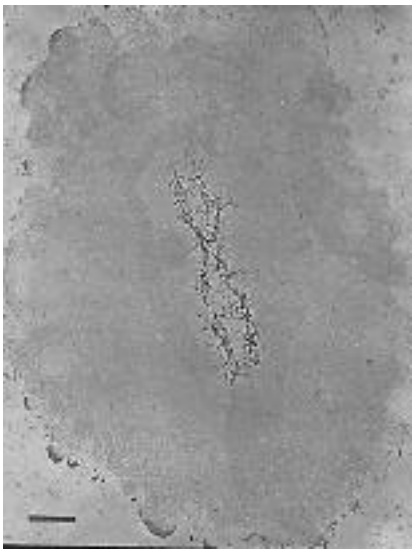
Introduction

The supercoiling of prokaryotic DNA is induced and maintained by the action of the enzyme DNA gyrase. The supercoiling of eukaryotic DNAs results primarily from the folding of the DNA about histone proteins to form chromatin.

The formation of chromatin is the first step in a series of folding events that reduce the length of DNA 10,000 fold from its extended form into a compact mitotic chromosome. An average human mitotic chromosome 5 microns in length contains 5 cm of DNA.





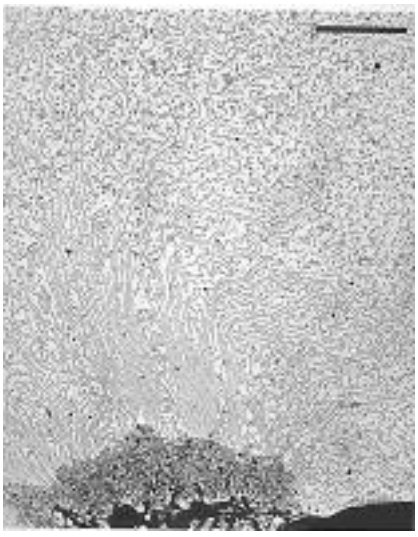


FIGURE 1-14
An electron micrograph of a human chromosome. Chromosome XII from a HeLa cell culture. [Courtesy of Dr. E. Du Praw.]

In the electron microscope, chromosomes appear to be composed of fibers 250 to 300 Å in diameter.

When chromatin is spread under more dissociating conditions (low ionic strength) these fibers extend into 100 Å diameter threads.

The 100 Å fibers are about 6 to 10 times longer than the 300 Å fibers from which they are derived.

The final level of unfolding of the chromosome into DNA requires the removal of the histone proteins themselves.

Histones and Chromatin Structure

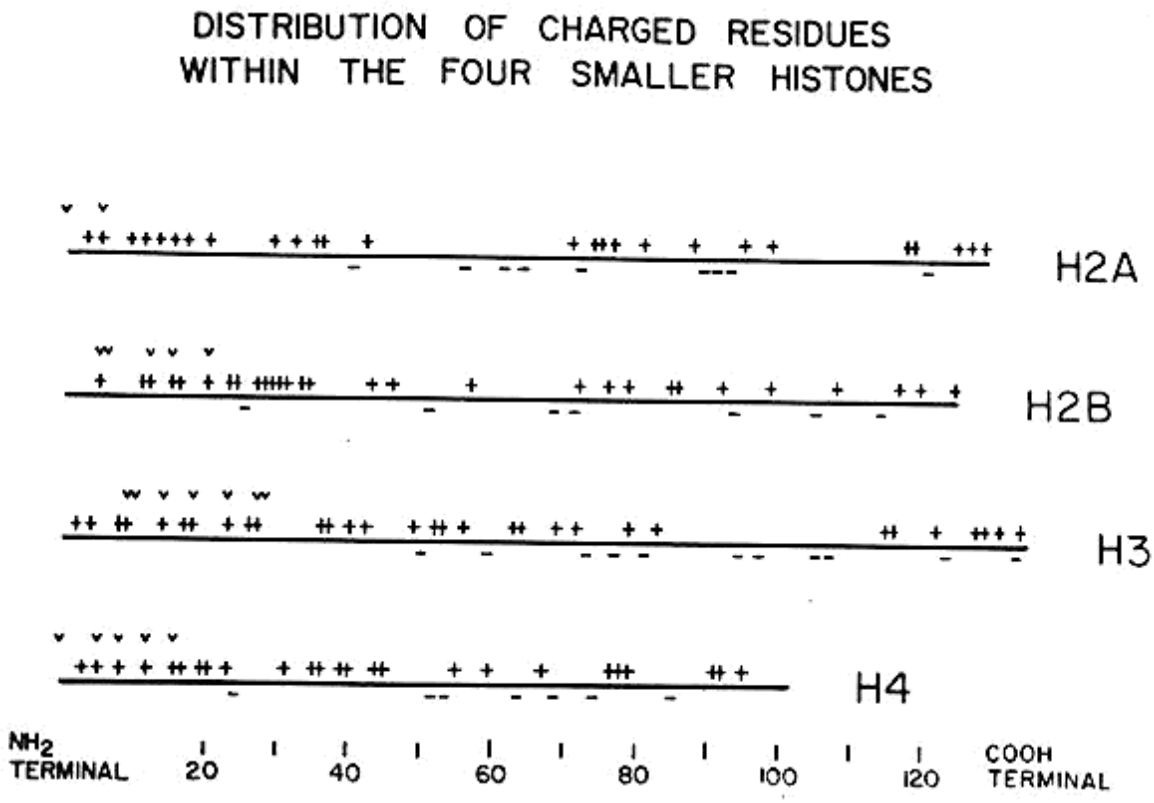
Histones can be resolved into five major classes by biochemical fractionation procedures and by several physical properties.

1. Histone H1, the most unusual histone, is larger than all the others (20,000 daltons) is very rich in the amino acid lysine, and has the lowest affinity for DNA.

2. Histone H1 can be dissociated from chromatin at ionic strengths as low as 0.5 M NaCl, while 2.0 M salt is needed to remove the other four classes of histone.

3. Histone HI has an unusual distribution of amino acids. Both the N-terminus and the C-terminus are rich in basic residues while the center of the molecule is richer in hydrophobic residues and has an equal number of acidic and basic amino acids.

4. Physical studies indicate that the center of the molecule folds into a beta-pleated sheet structure while the ends of the molecule remain extended in a random coil conformation.



There are four other histones called H2A, H2B, H3, and H4 for historical reasons. These histones are among the most highly conserved protein sequences known. The sequence of histone H4 from cows and pea plants differs by only 3 out of 104 residues and these are very conservative amino acid substitutions. This conservation speaks to

the important nature of the structure of the histones and suggests that an alteration of even a single amino acid side chain is either lethal or strongly selected against.

Histone-histone associations

Physical studies indicate that the predominant structural feature of these four histones is the presence of alpha-helical segments in the hydrophobic regions. These histones also tend to associate with each other in a very specific pattern of interactions.

The interactions were first investigated by studying physical parameters, since the amounts of helical structure in each histone increased upon associating with another histone molecule. The pattern of associations is:

H3=======H4
Ç Ç
Ç Ç
Ç Ç
H2A=====H2B

Histones H3 and H4, the most arginine rich histones, interact very strongly with each other and form a very specific complex, a tetramer. Histones H2A and H2B also interact with each other strongly and can form primarily dimers and some higher oligomers.

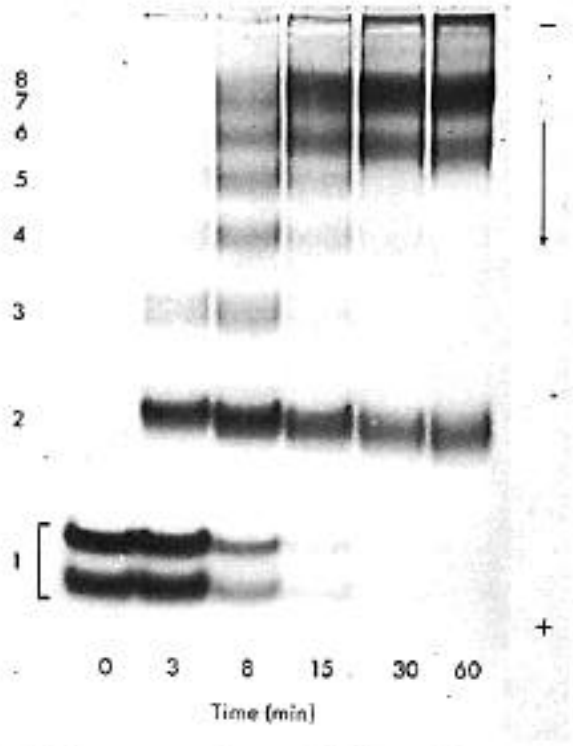


Figure 3. *A time-course of cross-linking of histone octamer (free of H1 and DNA) with dimethyl suberimidate at pH 9, I=2.0, in the presence of 0.5mM phenylmethylsulphonyl fluoride. At the protein concentration used the octamer is partially dissociated to hexamer and dimer (29, 29) as indicated by the final cross-linked products. (The broadening and slightly increased mobility of the dimer (and other) bands with increasing time is probably the result of progressive intramolecular*

The first direct evidence that such associations existed within chromatin came from the work of Roger Kornberg and Jean Thomas. By gently extracting histones from chromatin at very high protein concentration it was possible to isolate specific complexes of histones of even larger size. Octamers are the largest specific histone complex and they consist of 2 molecules each of histones H2A, H2B, H3, and H4. An octamer of histone interacts with an equal weight of DNA (160-200 base pairs) in chromatin.

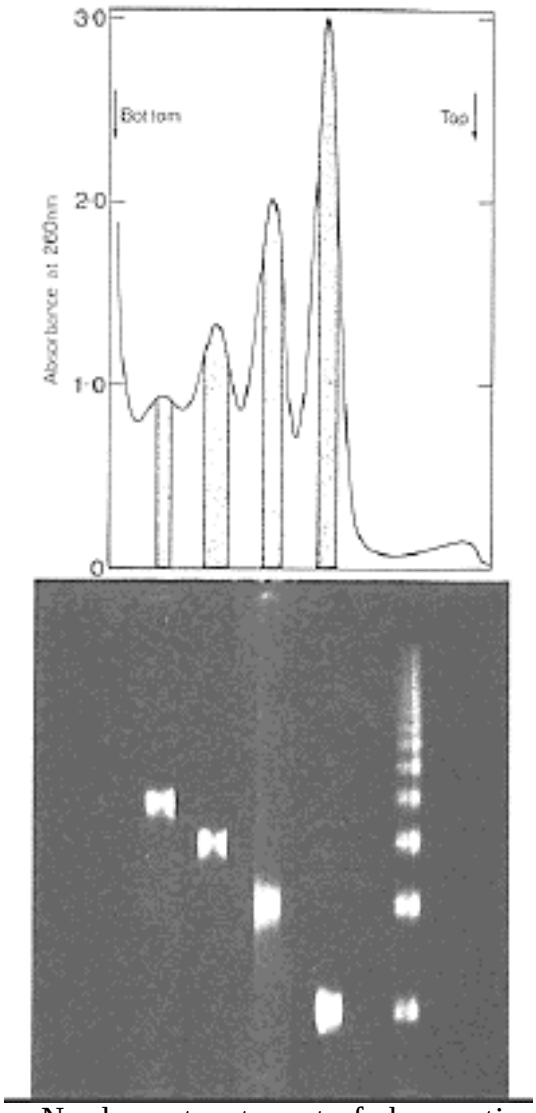
Nucleosomes

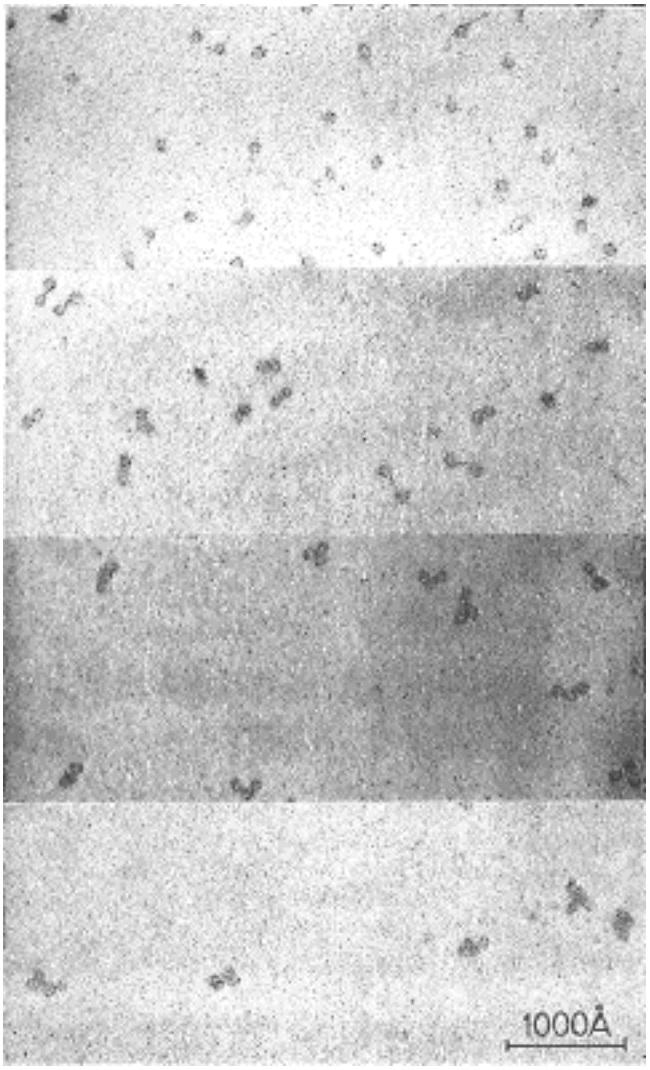
Chromatin is composed of repeating subunits that were

based on an octamer of histones and 200 base pairs of DNA, referred to as a nucleosome.

Evidence for a repeating nucleosome structure includes:

- Low angle X-ray diffraction indicating a 110 Å repeating unit.

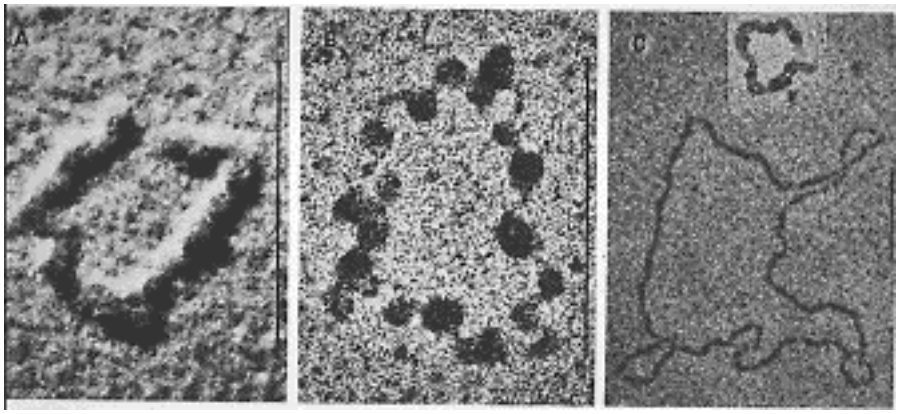




- Nuclease treatment of chromatin cleaves DNA at 200 base pair intervals.

- Nucleosomes can be visualized in the electron microscope as beads on a string.

Chromosome Structure



- The DNA of the virus SV40 replicates in the nucleus and is coated with histones in a manner quite similar to the host cell chromatin. When SV40 chromatin is spread at low ionic strength, one can see the beaded appearance and separation of the adjacent nucleosomes.

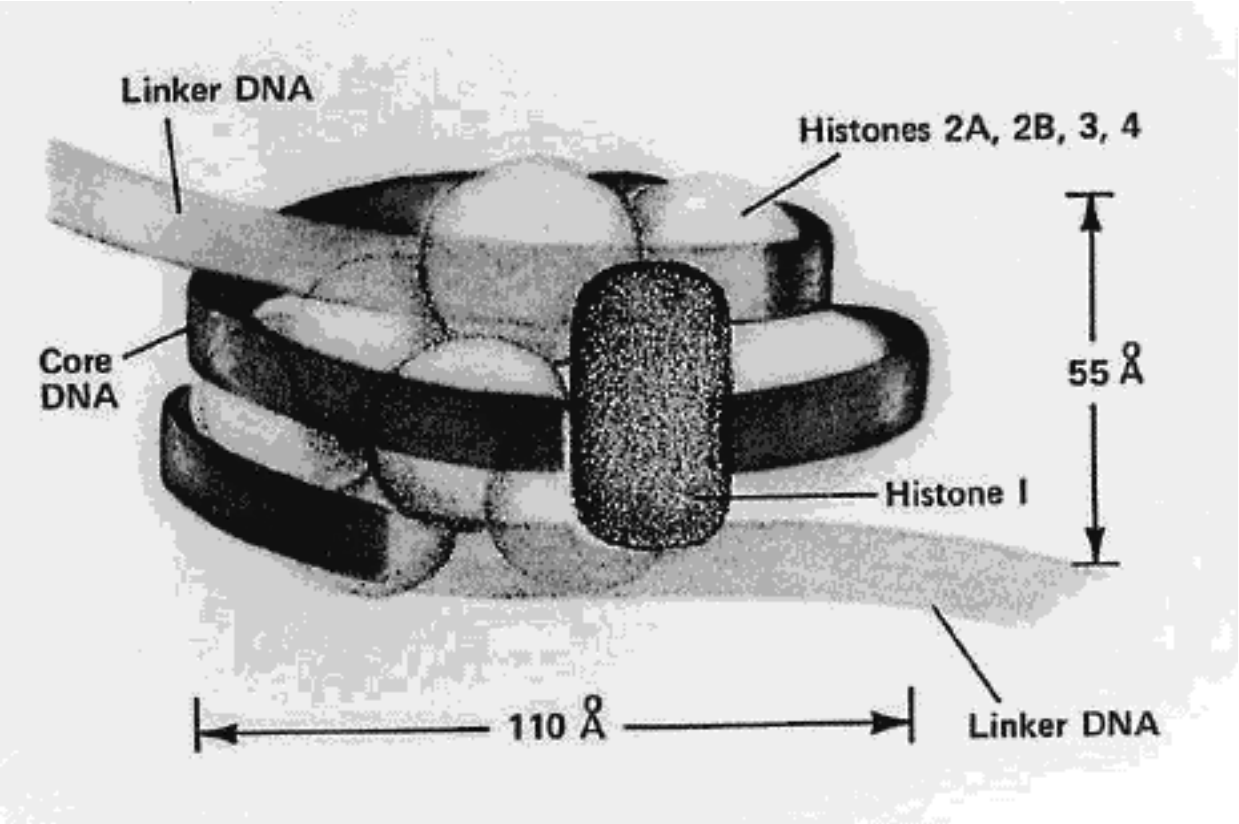
- The DNA between the nucleosomes is very sensitive to micrococcal nuclease and is referred to as linker DNA.

- The resistant DNA with its associated histone octamer is referred to as the nucleosome core particle or sometimes just the core particle.

- Folding of SV40 DNA into chromatin results in the compaction of the circumference of these two circles by 7 fold. About 200 base pairs of DNA which by itself is 680 Å in length is converted into 100 Å diameter nucleosome structure.

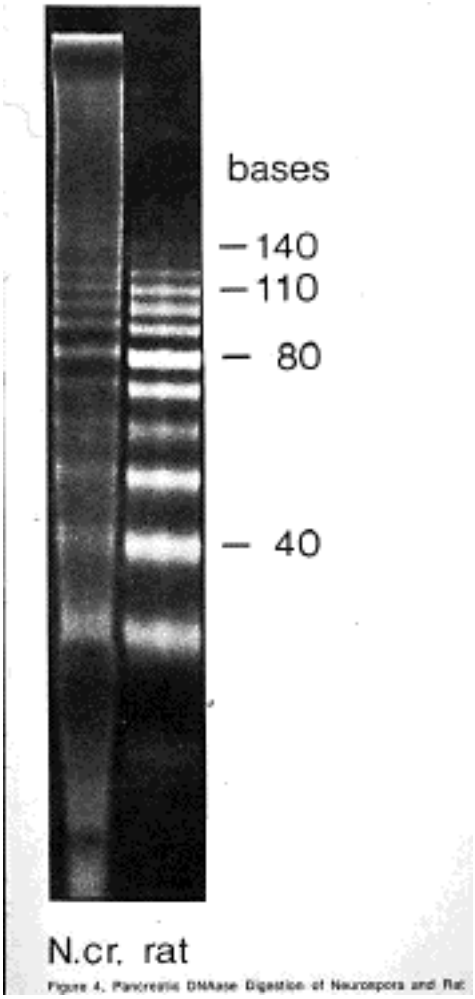
Evidence that the DNA is wrapped on the outside of the core histone came from experiments in which either chromatin or isolated core particles are cleaved with another endonuclease, called pancreatic DNAse. Pancreatic DNAse, unlike the micrococcal nuclease, does not cleave preferentially in the linker region between nucleosomes. Instead it nicks only one strand of the DNA helix. The lengths of these fragments differ by about 10 bases, each fragment being about 10 bases longer than the fragment before it.

Chromosome Structure



Finch, Klug and Lutter proposed the wrapping of DNA around the histone octamer based on these patterns of sensitivity to DNAse. Core particles contain about 140 base pairs of DNA that they proposed was wrapped in two turns about the octamers.

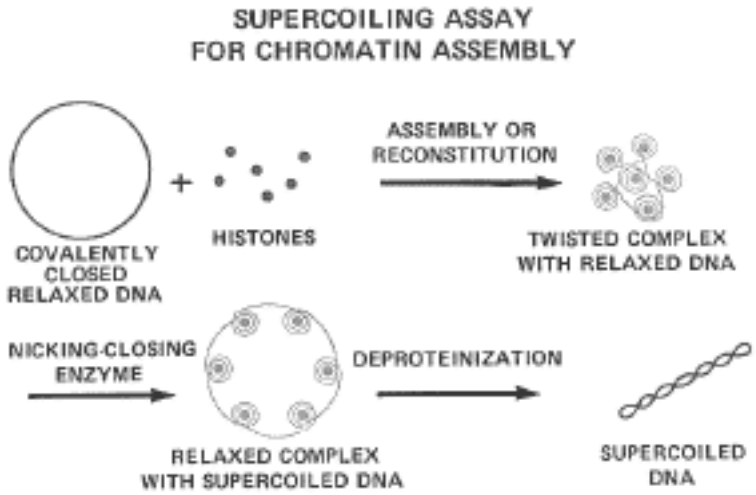
Chromosome Structure



The isolation of core particles has also allowed the crystallization of the nucleosome and a low resolution 3-dimensional picture has been obtained.

The histone octamer is in the core. The 140 base pairs of DNA associated with octamer is known as the core DNA. Together they are known as the core particle. The linker DNA joins one nucleosome to the other.

Histone H1 is associated with the linker DNA.



Nucleosomes and Supercoiling of Eukaryotic DNA

Reconstitution of core histones with circular SV40 DNA in the presence of a DNA topoisomerase shows the formation of beaded nucleosome structure and the induction of 1.25 supertwists per nucleosome core particle.

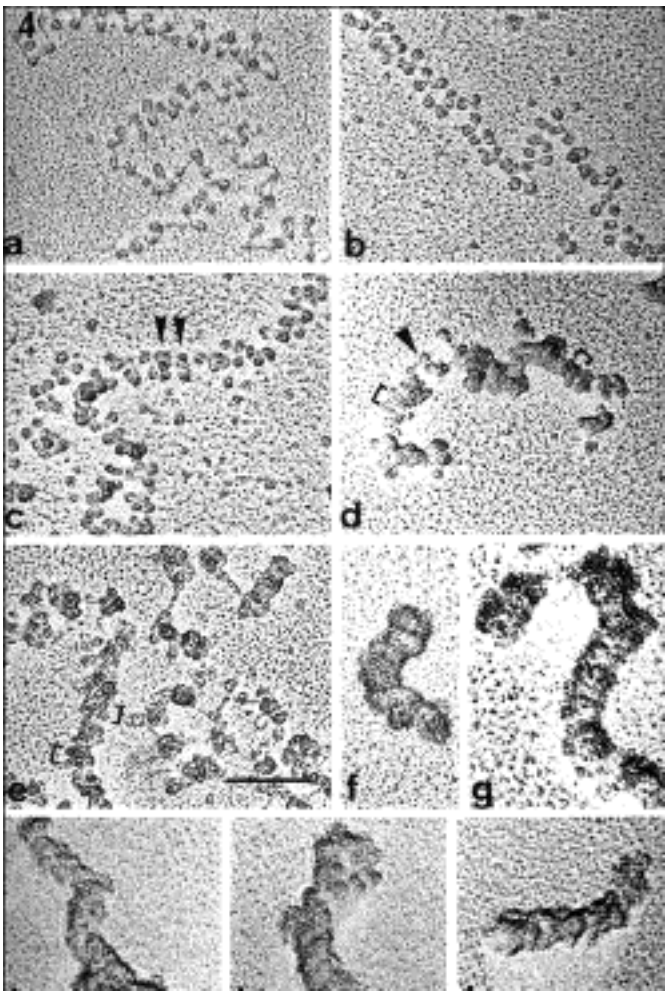
The winding of DNA twice about the histone core in a left-handed way should cause a writhe of two negative twists for 200 base pairs in the nucleosome or 1.75 negative twists for 140 base pairs of core particle DNA.

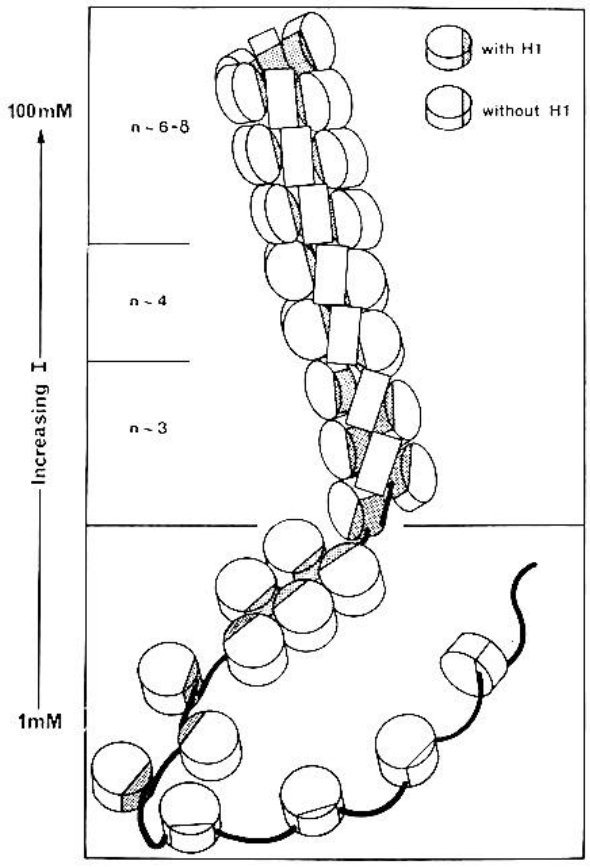
The difference in observed and expected number of writhes is due to a change in the pitch of the DNA helix on the nucleosome. Instead of 10.4 base pairs per turn of the helix in solution the pitch of the helix as it wraps around the nucleosome is tighter by 4% being exactly 10.0 base pairs per turn. This slightly increased pitch means that over the 140 base pairs of DNA that would normally have 13.5 helical turns in solution, would have 14.0 helical turns on the nucleosome.

Higher Order Folding of Chromatin

The specificity of histone H1 for twisted DNA suggests that it might be involved in higher order folding of the chromosome. The interaction of histone H1 with the

linker region between nucleosomes suggests that it might also have a role in stabilizing the 110 Å fiber.





This is supported by the fact that if histone H1 is removed from chromatin in 0.6 M NaCl, the chromatin assumes the same beaded appearance that it does in low ionic strength.

Finch and Klug obtained electron microscopic evidence for a repeating pattern within the 300 Å fiber suggesting that the nucleosome beads themselves are further wound in a helical pattern that they referred to as the solenoid.

This higher repeating pattern contained six nucleosomes per turn of the solenoid and hence resulted in the packing of 1200 base pairs, or 4100 Å of DNA into 100 Å rise of the solenoid.

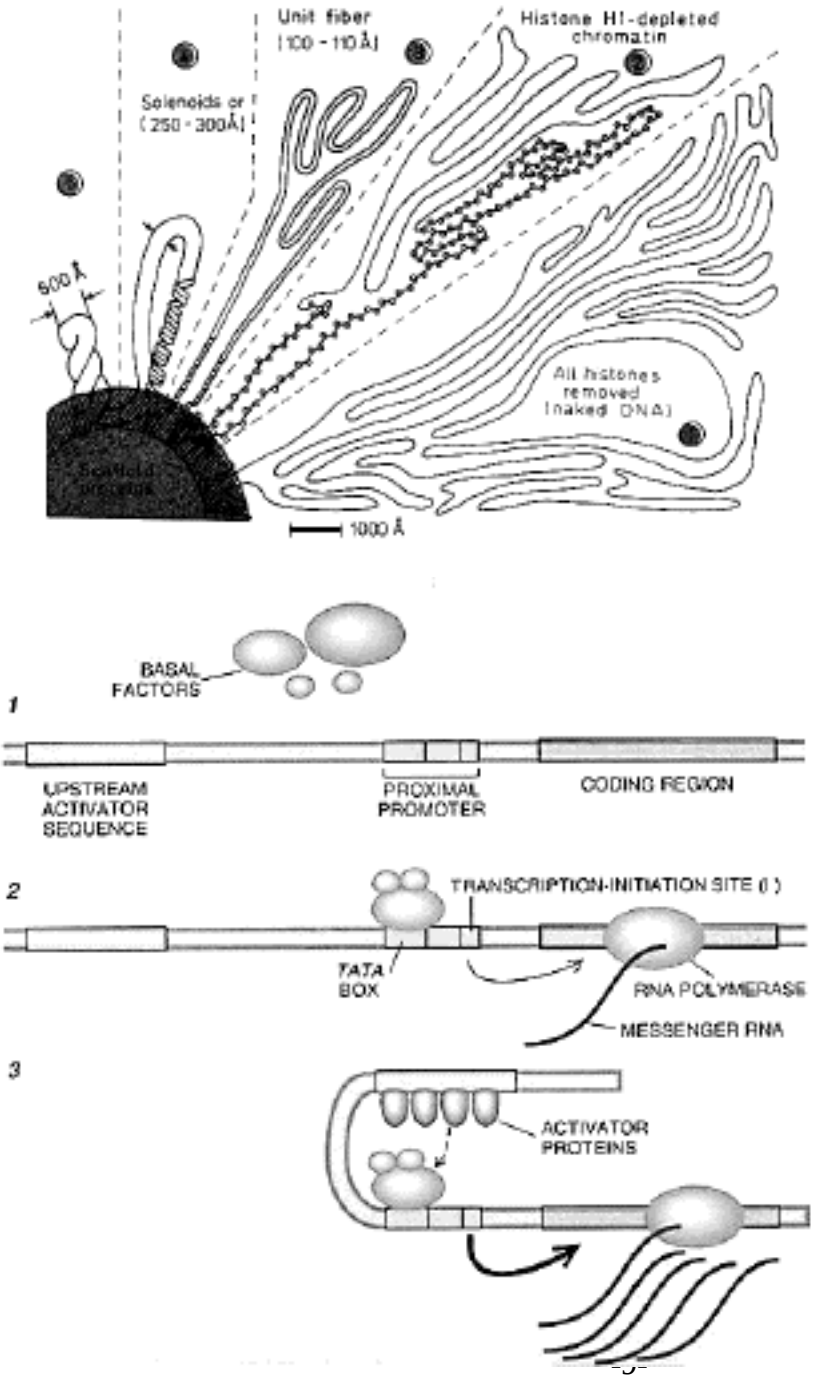
This is an additional six-fold packing of DNA on top of the seven-fold packing into the 110 Å fiber suggesting an overall compaction of DNA 40 fold into the 300 Å diameter solenoid.

Chromosome Structure

The formation of this higher order structure required the presence of both Mg^{++} and histone H1.

Our ultimate understanding of the eukaryotic chromosome will depend on knowing the nature of the this folding and even higher order of folding of the 300 Å fiber into a mitotic chromosome. The regulation of this folding will be essential to understanding the processes of transcription, replication, repair, recombination and the metabolism of DNA.

Regulation of Chromatin Folding



During DNA replication and gene expression the chromatin structure is largely unfolded and no evidence of nucleosome structure is found. Transcribed genes or newly replicated DNA is highly sensitive to nuclease digestion. These genetic regions are also associated with modified forms of histones including acetylated and phosphorylated forms.

The N-terminal lysines of histones that are associated with metabolically active genetic regions are highly modified, primarily by acetylation.

Acetylation of the N-terminal region of histones decreases their affinity for DNA and alters the structural properties of the nucleosome so that they are in a less condensed and more extended conformation.

Another modification of histones, the phosphorylation of up to eight serine and

threonine amino acids of histone H1 is correlated with the highly condensed form of the chromatin fibers seen in the mitotic chromosomes.

Within 10 minutes after mitosis, all of these phosphate residues are removed, before the beginning of the next cell cycle.

So far evidence linking histone modification with gene expression and DNA replication is many circumstantial, being primarily in the form of correlations rather than causal in nature.

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