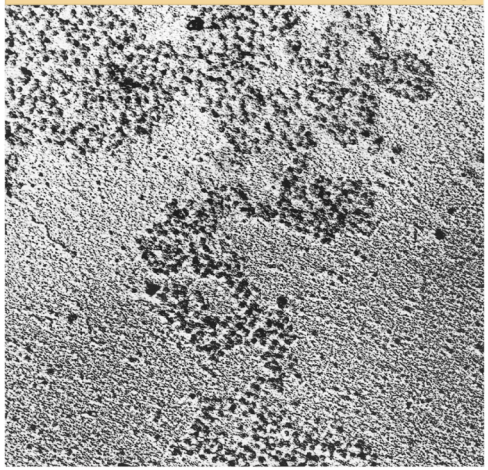
Chapter 29: Nucleosomes

- The **nucleosome** is the basic structural subunit of chromatin
  - consisting of ~200 bp of DNA and an octamer of histone proteins.
- **Histones** are conserved DNA-binding proteins that
  - form the basic subunit of chromatin in eukaryotes.
  - Histones H2A, H2B, H3, H4 form an octameric core around which DNA coils to form a nucleosome.
  - Histone H1 is external to the nucleosome.
- A **nonhistone** is
  - any structural protein found in a chromosome except one of the histones.
- Nucleosomes are the "beads" of the "beads on a string" description of Chromatin

Chromatin is a mass of beads on a string

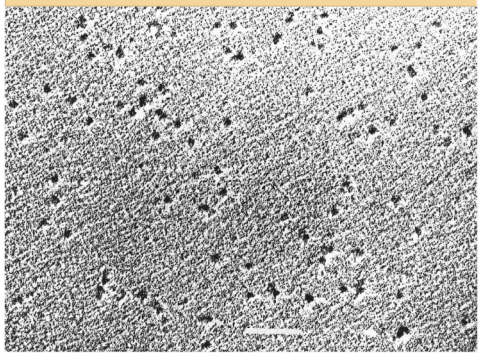


29.2 The Nucleosome Is the Subunit of All Chromatin

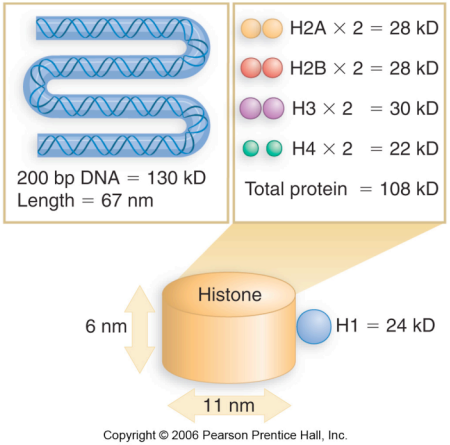
- A nucleosome contains
  - ~200 bp of DNA,
  - two copies of each core histone (H2A, H2B, H3, H4) = the protein octamer
  - and one copy of H1.
- DNA is wrapped around the outside surface of the protein octamer.
- **Micrococcal nuclease** is an endonuclease that cleaves DNA
- Micrococcal nuclease, in chromatin, cleaves DNA preferentially between nucleosomes
  - releases individual nucleosomes from chromatin as 11S particles.

Individual nucleosomes are released by digestion of chromatin with micrococcal nuclease.

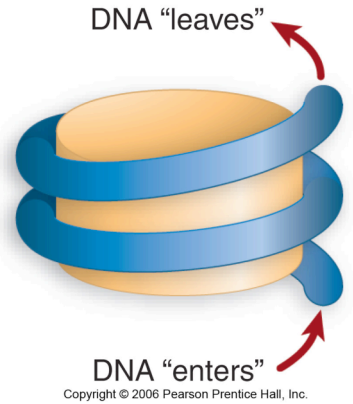
Nucleosomes are 10Å beads



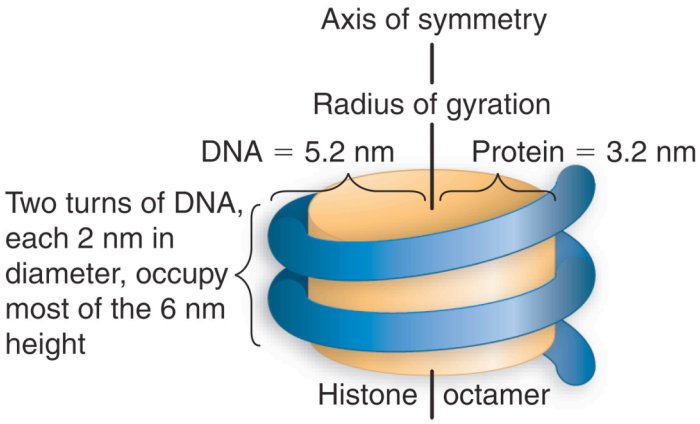
A nucleosome has 200 bp DNA and core histones



The nucleosome has two turns of DNA



The nucleosome is a flat cylinder



29.3 DNA Is Coiled in Arrays of Nucleosomes

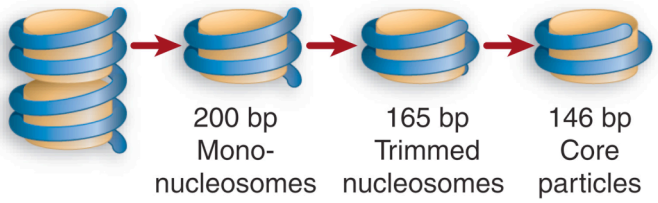
- >95% of the DNA is recovered in nucleosomes or multimers when micrococcal nuclease cleaves chromatin.
- The length of DNA per nucleosome varies for individual tissues in a range from 154–260 bp.

29.4 Nucleosomes Have a Common Structure

- **Nucleosomal DNA** is divided into
  - the core DNA
  - and linker DNA
  - depending on its susceptibility to micrococcal nuclease.
- is associated with linker DNA
  - may lie at the point where DNA enters and leaves the nucleosome.
- The **core particle** is
  - a digestion product of the nucleosome that retains the histone octamer and has 146 bp of DNA; its structure is similar to that of the nucleosome itself.
- **Core DNA** is
  - the 146 bp of DNA contained on a core particle that is generated by cleaving the DNA on a nucleosome with micrococcal nuclease.
- **Linker DNA** is
  - all DNA contained on a nucleosome in excess of the 146 bp core DNA.
  - Its length varies from 8–114 bp,
  - it is cleaved by micrococcal nuclease to leave the core DNA.

Micrococcal nuclease initially cleaves between nucleosomes. Mononucleosomes typically have ~200 bp DNA. End-trimming reduces the length of DNA first to ~165 bp, and then generates core particles with 146 bp.

Micrococcal nuclease trims nucleosomes to core particles

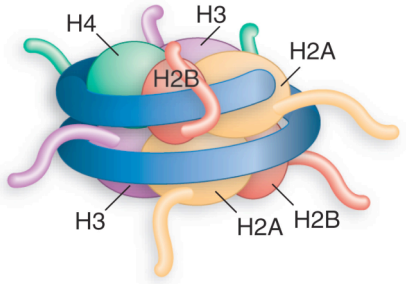


29.7 Organization of the Core Particle

- The **histone fold** is
  - a motif found in all four core histones in which three α-helices are connected by two loops.
- The histone octamer has
  - a kernel of a $H3_2 \cdot H4_2$ tetramer associated with two H2A · H2B dimers.
- Each histone is extensively interdigitated with its partner.
- All core histones have the structural motif of the histone fold.
- N-terminal tails extend out of the nucleosome.

In a symmetrical model for the nucleosome, the $H3_2$-$H4_2$ tetramer provides a kernel for the shape.

The nucleosome has histone pairs

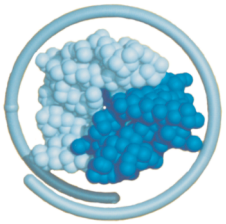


The crystal structure of the histone core octamer is represented in a space-filling model with the $H3_2$-$H4_2$ tetramer shown in light blue and the H2A-H2B dimers shown in dark blue.

DNA surrounds the histone octamer

Only one of the H2A-H2B dimers is visible in the top view, because the other is hidden underneath.

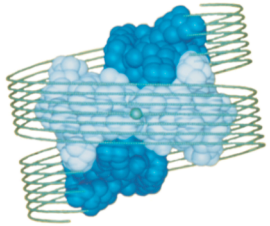
The potential path of the DNA is shown in the top view as a narrow tube (one quarter the diameter of DNA).



The crystal structure of the histone core octamer is represented in a space-filling model with the $H3_2$-$H4_2$ tetramer shown in light blue and the H2A-H2B dimers shown in dark blue.

The potential path of the DNA is shown in the side view by the parallel lines in a 20 Å wide bundle.

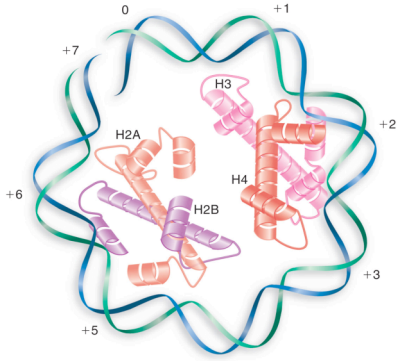
DNA turns twice around the nucleosome



All four core histones contact DNA.

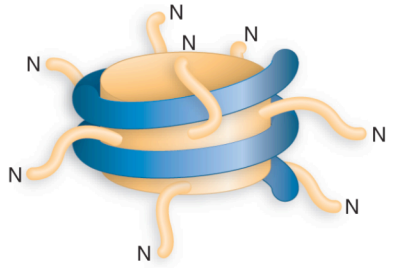
The structure of a "half nucleosome" shows the contacts with one turn of DNA.

Histones contact DNA by the histone fold



The N-terminal histone tails are disordered and exit from the nucleosome between turns of the DNA.

Histone tails emerge between DNA turns



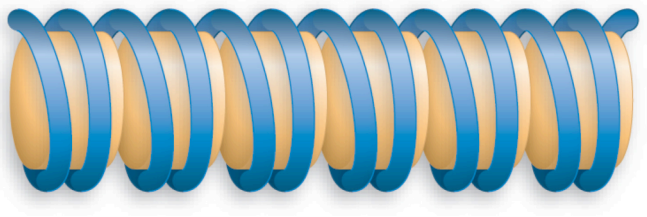
29.8 The Path of Nucleosomes in the Chromatin Fiber

- **10 nm chromatin fibers** are
  - consist of a string of nucleosomes.
  - a linear array of nucleosomes
  - generated by unfolding from the natural condition of chromatin (the 30nm fiber).

- The **30 nm fiber** is
  - the basic level of organization of nucleosomes in chromatin.
  - is a coiled coil of nucleosomes.
  - 30 nm fibers have six nucleosomes/turn, organized into a solenoid.
  - Histone H1 is required for formation of the 30 nm fiber.

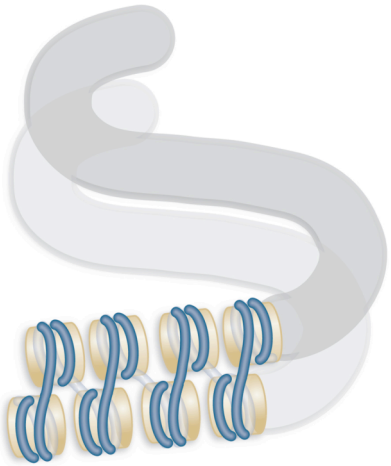
The 10 nm fiber is a continuous string of nucleosomes.

10 nm fiber consists of nucleosomes



The 30 nm fiber is a helical ribbon consisting of two parallel rows of nucleosomes coiled into a solenoid.

The 30 nm fiber is a solenoid



29.9 Reproduction of Chromatin Requires Assembly of Nucleosomes

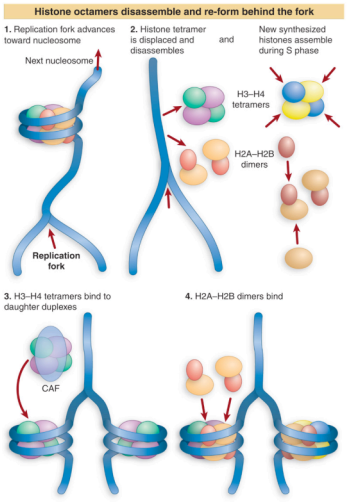
- Histone octamers are not conserved during replication
- H2A · H2B dimers and $H3_2 \cdot H4_2$ tetramers are conserved.
- There are different pathways for the assembly of nucleosomes during replication and independently of replication.
- Accessory proteins are required to assist the assembly of nucleosomes.
- CAF-1 is an assembly protein that is linked to the PCNA subunit of the replisome;
  - it is required for deposition of $H3_2 \cdot H4_2$ tetramers following replication.
- A different assembly protein and a variant of histone H3 may be used for replication-independent assembly.

Replication fork passage displaces histone octamers from DNA.

They disassemble into H3-H4 tetramers and H2A-H2B dimers.

Newly synthesized histones are assembled into H3-H4 tetramers and H2A-H2B dimers.

The old and new tetramers and dimers are assembled with the aid of CAF-1 at random into new nucleosomes immediately behind the replication fork.

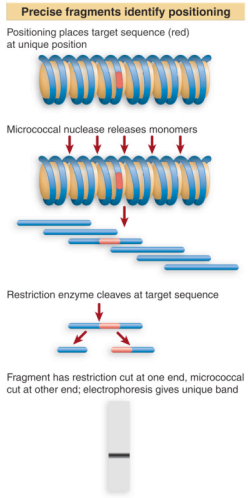


29.10 Do Nucleosomes Lie at Specific Positions?

- **Nucleosomes** may form at specific positions as the result either of the local structure of DNA or of proteins that interact with specific sequences.
- The most common cause of **nucleosome positioning** is the binding of proteins to DNA to establish a boundary.
- **Nucleosome positioning** describes the placement of nucleosomes at defined sequences of DNA instead of at random locations with regards to sequence.
  - Positioning may affect which regions of DNA are in the linker and which face of DNA is exposed on the nucleosome surface.

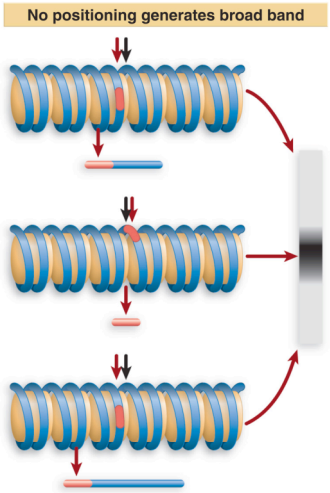
Nucleosome positioning places a specific restriction site at unique position relative to the linker sites cleaved by micrococcal nuclease in all copies of the genome.

In other words, a piece of DNA is wound the same way around the nucleosomes in all copies of the gene in an organism.



If nucleosome positioning did not exist, a restriction site would lie at all possible locations in different copies of the genome.

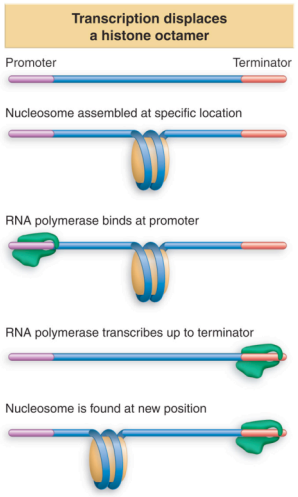
Fragments of all possible sizes are produced when a restriction enzyme cuts at a target site (red) and micrococcal nuclease cuts at the junctions between nucleosomes (black).



29.11 Histone Octamers Are Displaced by Transcription

- Nucleosomes are found at the same frequency when transcribed genes or nontranscribed genes are digested with micrococcal nuclease.
- Some heavily transcribed genes appear to be exceptional cases that are devoid of nucleosomes.
- RNA polymerase displaces histone octamers during transcription in a model system, but octamers reassociate with DNA as soon as the polymerase has passed.
- Nucleosomes are reorganized when transcription passes through a gene.

A protocol to test the effect of transcription on nucleosomes shows that the histone octamer is displaced from DNA and rebinds at a new position.

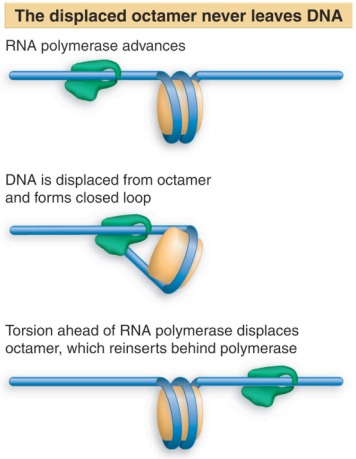


RNA polymerase displaces DNA from the histone octamer as it advances.

The DNA loops back and attaches (to polymerase or to the octamer) to form a closed loop.

As the polymerase proceeds, it generates positive supercoiling ahead.

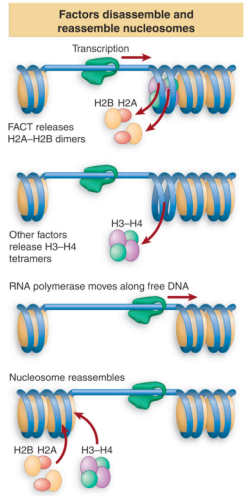
This displaces the octamer, which keeps contact with DNA and/or polymerase, and is inserted behind the RNA polymerase.



Histone octamers are disassembled ahead of transcription to remove nucleosomes.

They reform following transcription.

Release of H2A·H2B dimers probably initiates the disassembly process.

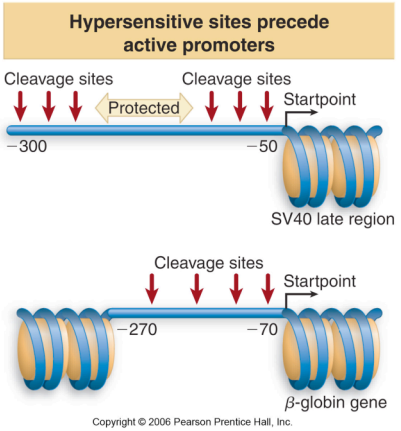


29.12 DNase Hypersensitive Sites Change Chromatin Structure

- A **hypersensitive site** is
  - a short region of chromatin detected by its extreme sensitivity to cleavage by DNAase I and other nucleases
  - it is an area from which nucleosomes are excluded.
- Hypersensitive sites are found at the promoters and enhancers of expressed genes.
- They are generated by the binding of transcription factors that displace histone octamers.

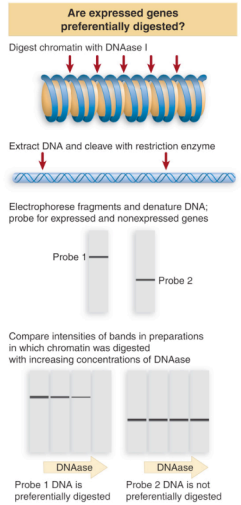
The SV40 gap includes hypersensitive sites, sensitive regions, and a protected region of DNA.

The hypersensitive site of a chicken β-globin gene comprises a region that is susceptible to several nucleases.

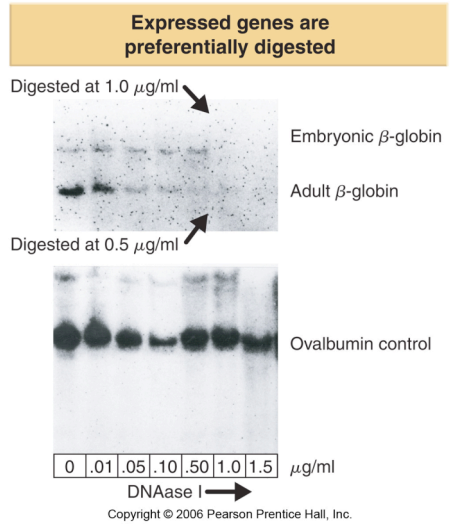


A domain containing a transcribed gene is defined by increased sensitivity to degradation by DNase I.

Sensitivity to DNase I can be measured by determining the rate of disappearance of the material hybridizing with a particular probe.



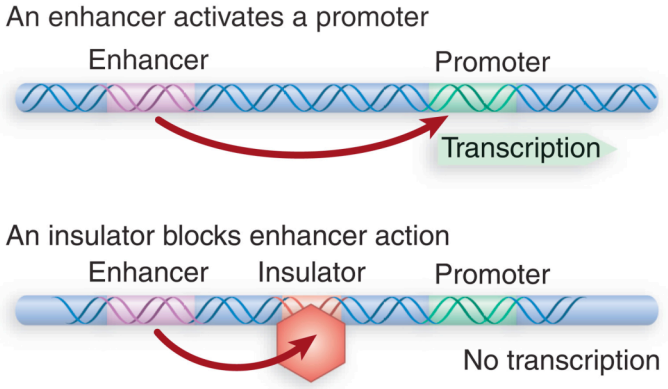
In adult erythroid cells, the adult β-globin gene is highly sensitive to DNAase I digestion, the embryonic β-globin gene is partially sensitive (probably due to spreading effects), but ovalbumin is not sensitive.



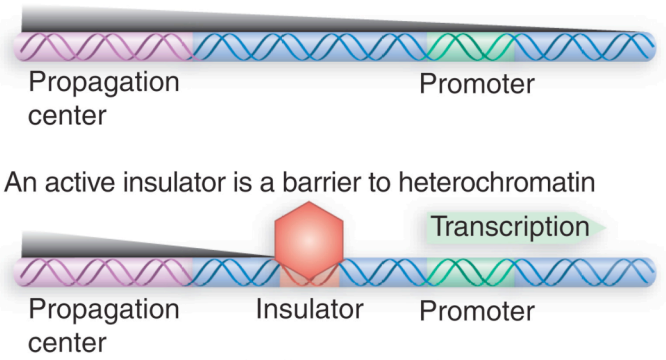
29.14
Insulators Block the Actions of Enhancers and Heterochromatin

- An **insulator** is a sequence that prevents an activating or inactivating effect passing from one side to the other.
- Insulators are able to block passage of any activating or inactivating effects from enhancers or silencers.
- Insulators may provide barriers against the spread of heterochromatin.
- Two insulators can protect the region between them from all external effects.

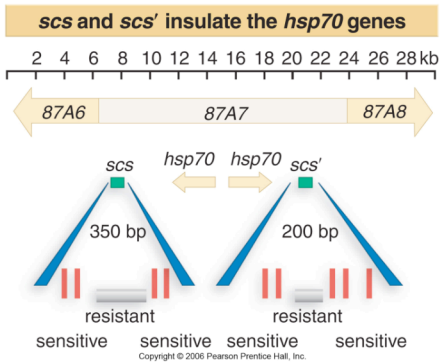
An insulator may block an enhancer



An insulator may block heterochromatin



Specialized chromatin structures (SCS) that include hypersensitive sites mark the ends of a domain in the *D. melanogaster* genome and insulate genes between them from the effects of surrounding sequences.

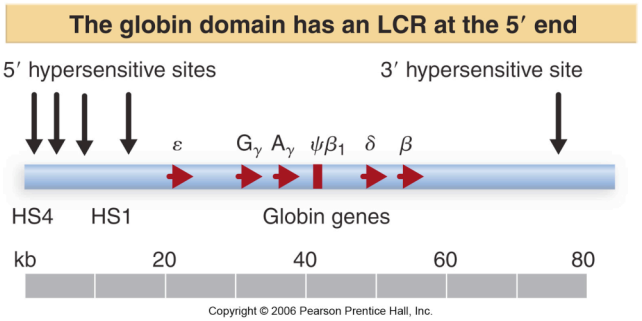


29.15 An LCR May Control a Domain

- The **locus control region (LCR)** is
  - required for the expression of several genes in a domain.

- An LCR is
  - located at the 5' end of the domain and consists of several hypersensitive sites.

- A globin domain is
  - marked by hypersensitive sites at either end.
- The group of sites at the 5' side constitutes
  - the LCR and
  - is essential for the function of all genes in the cluster.



- Domains may possess three types of sites:
  - •insulators to prevent effects from spreading between domains;
  - •MARs to attach the domain to the nuclear matrix;
  - •and LCRs that are required for initiation of transcription.
- An enhancer may act on more than one promoter within the domain.