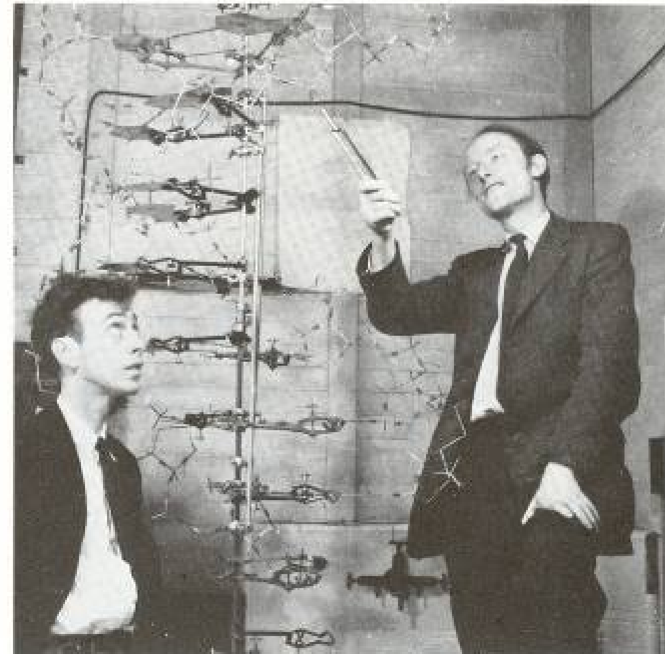
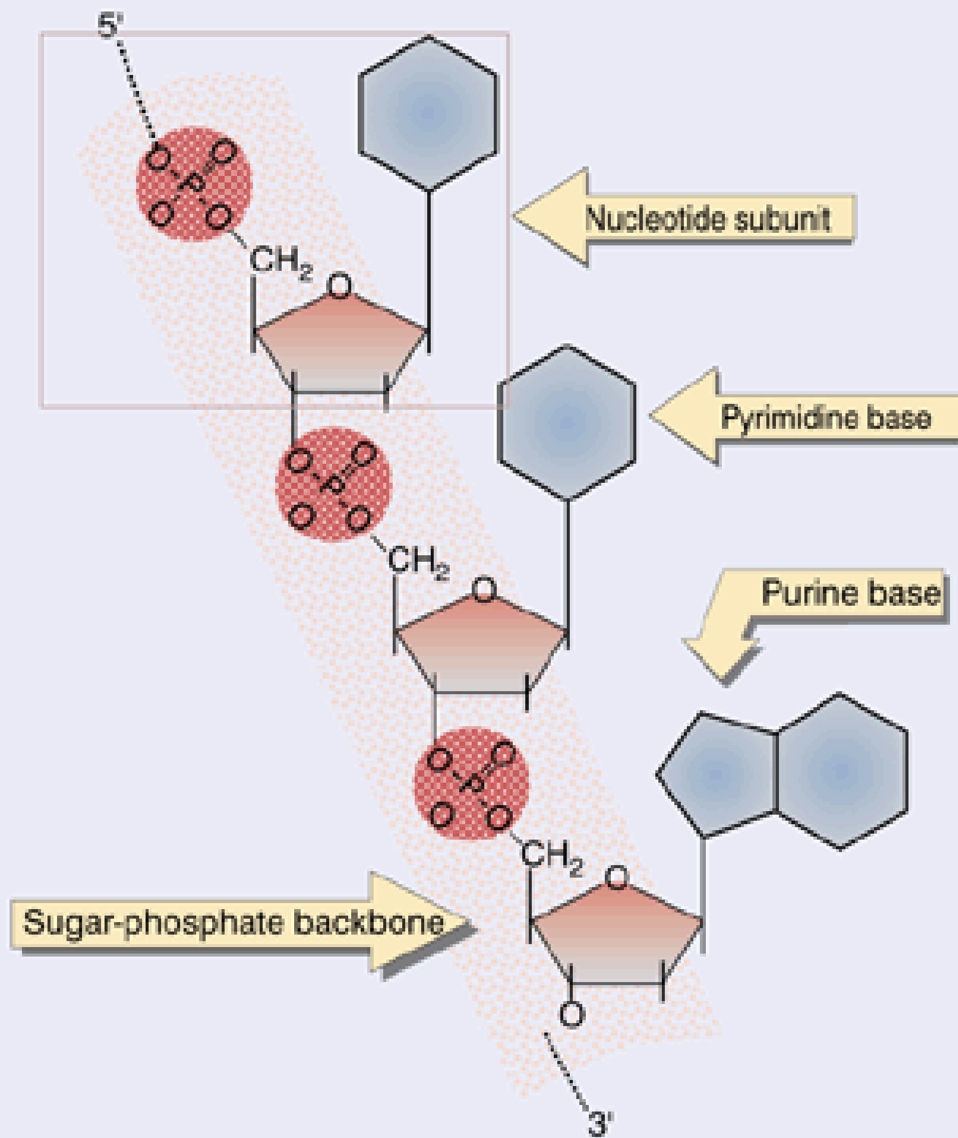
Chromatin

Structure and modification of chromatin

Chromatin domains



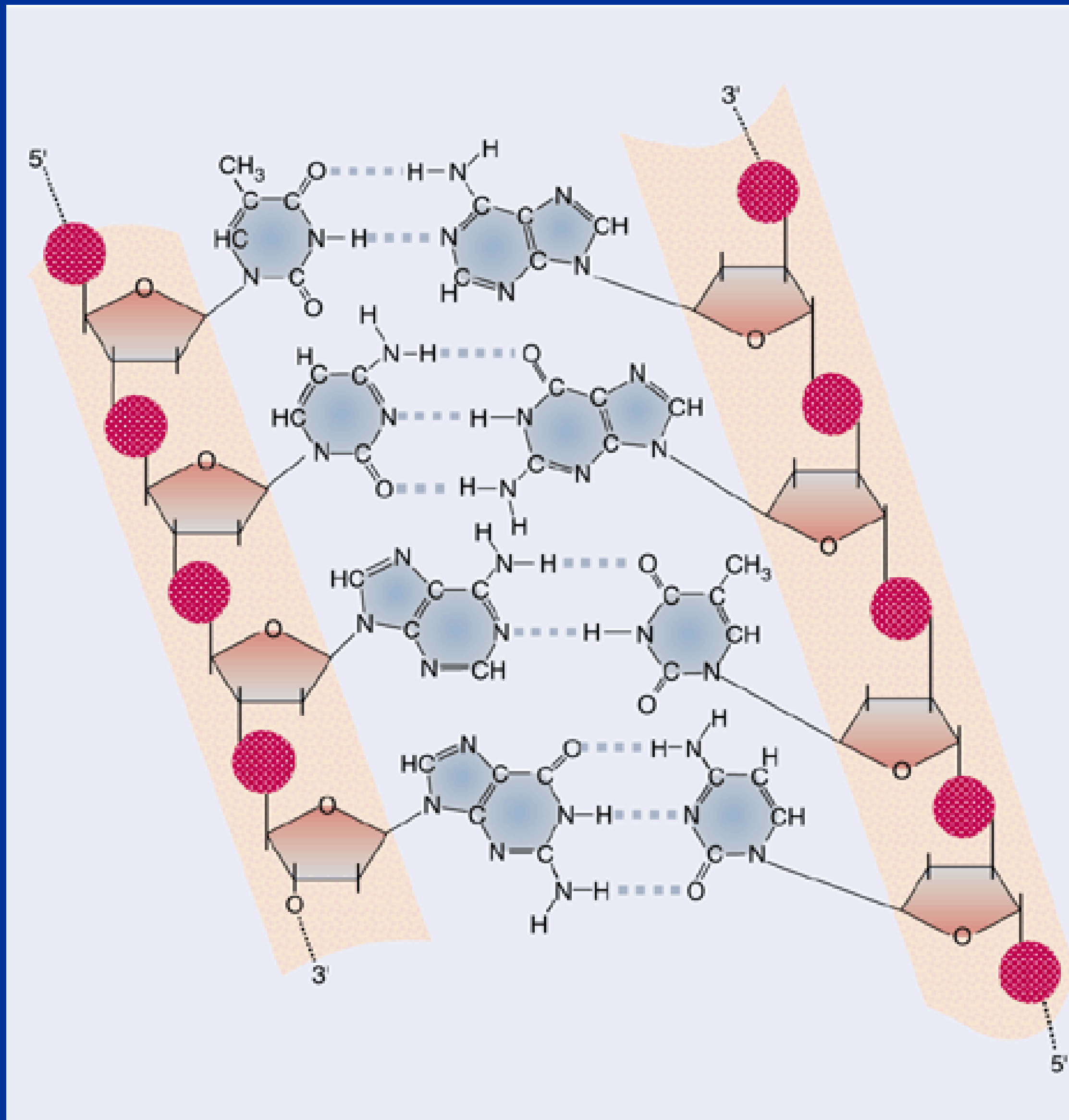
▲ **Figure I-12** (*left*) James D. Watson (1928–) and (*right*) Francis H. C. Crick (1916–) with the double-helical model of DNA they constructed in 1952–1953. *From J. D. Watson,* The Double Helix, *Atheneum, p. 215, copyright 1968 by J. D. Watson. Courtesy of A. C. Barrington Brown.*



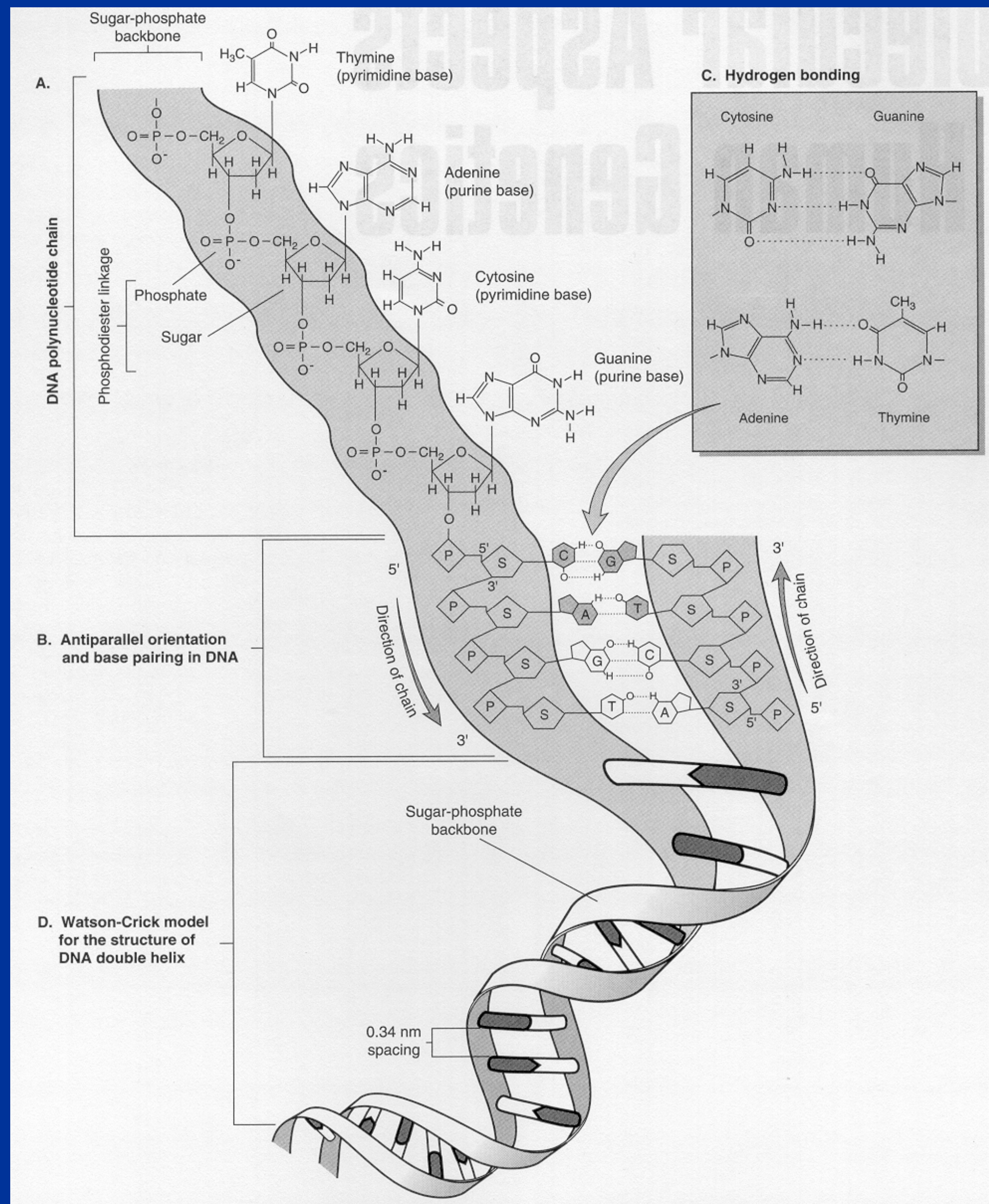
DNA

consensus 5´→ 3´

DNA



DNA



RNA

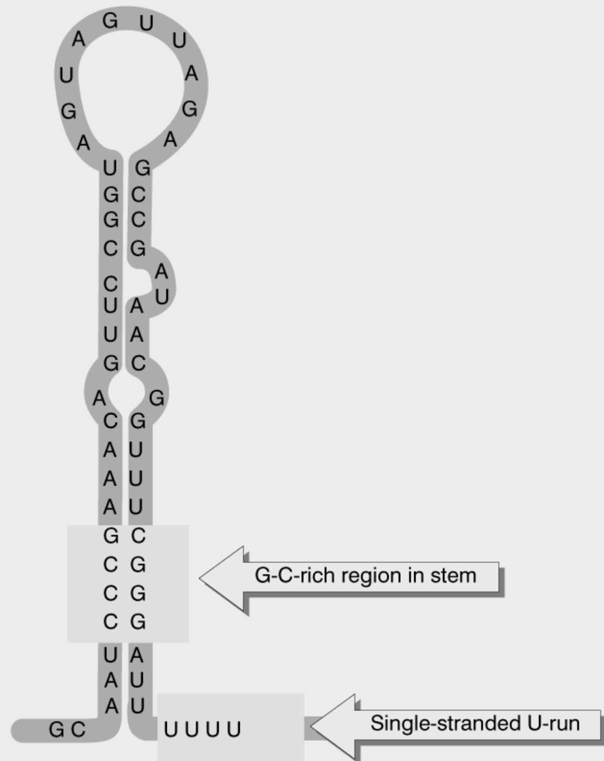
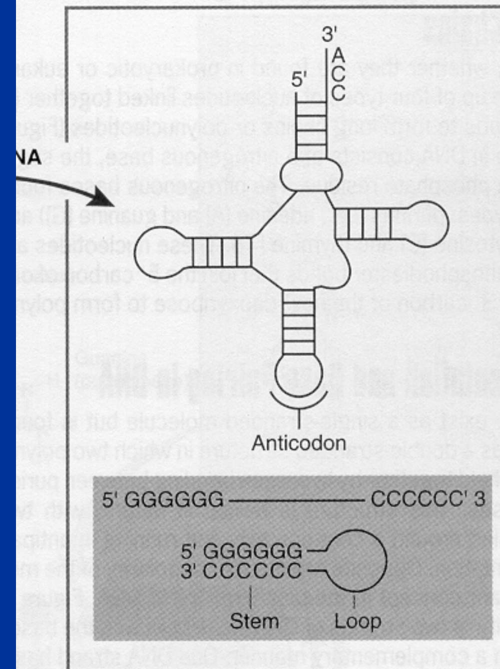
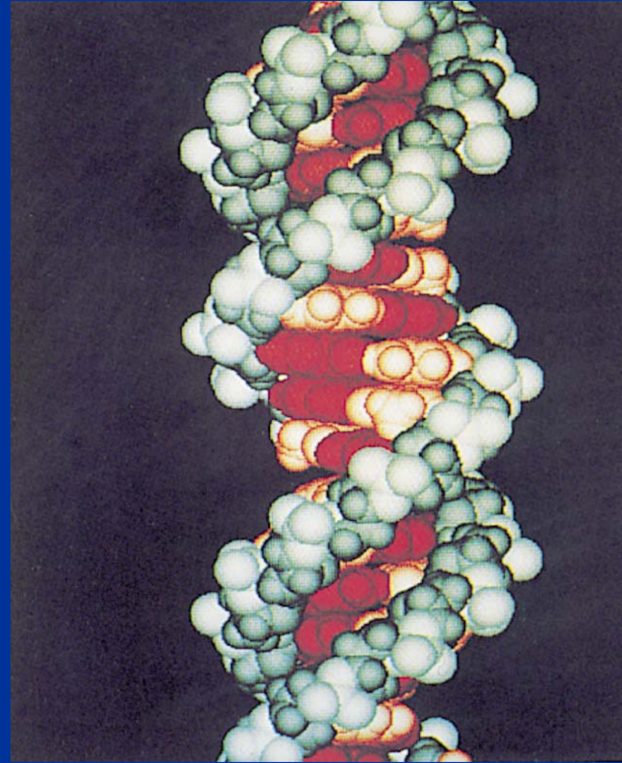


Figure 9.27 Intrinsic terminators include palindromic regions that form hairpins varying in length from 7 to 20 bp. The stem-loop structure includes a G-C-rich region and is followed by a run of U residues.

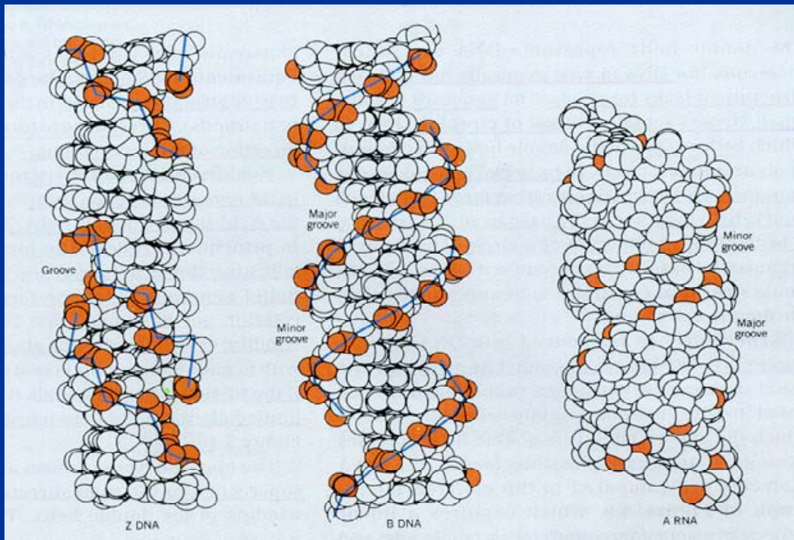


- ss RNA forms secondary structures with ds hairpins

ds forms of nucleic acids



Form ds	coiling	bp/turn	rotation/bp	diameter
A	R	11	34,7°	2,3 nm
B	R	10	43,0°	1,9 nm
Z	L	12	30°	1,8 nm

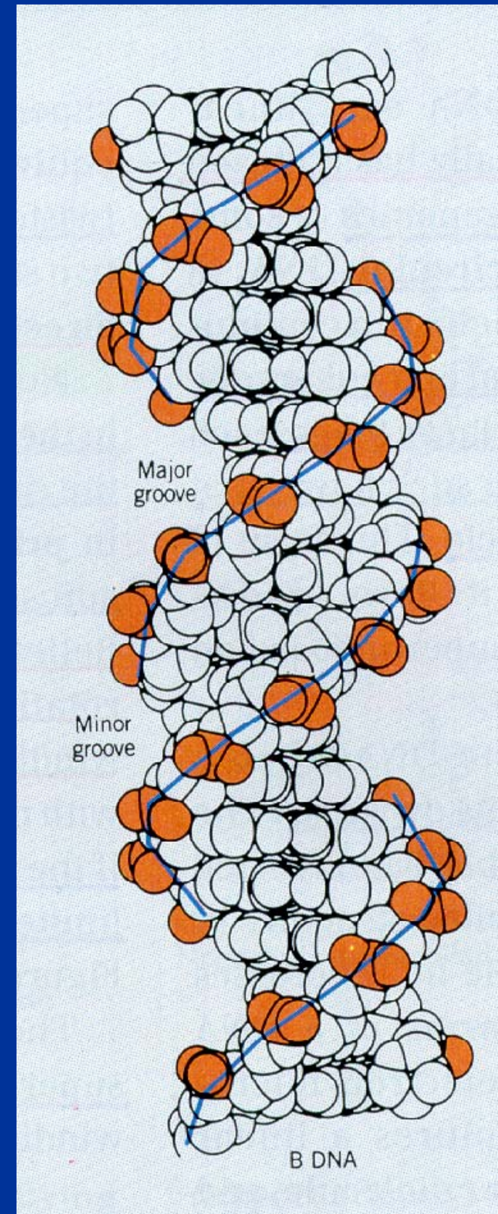


ds forms of nucleic acids

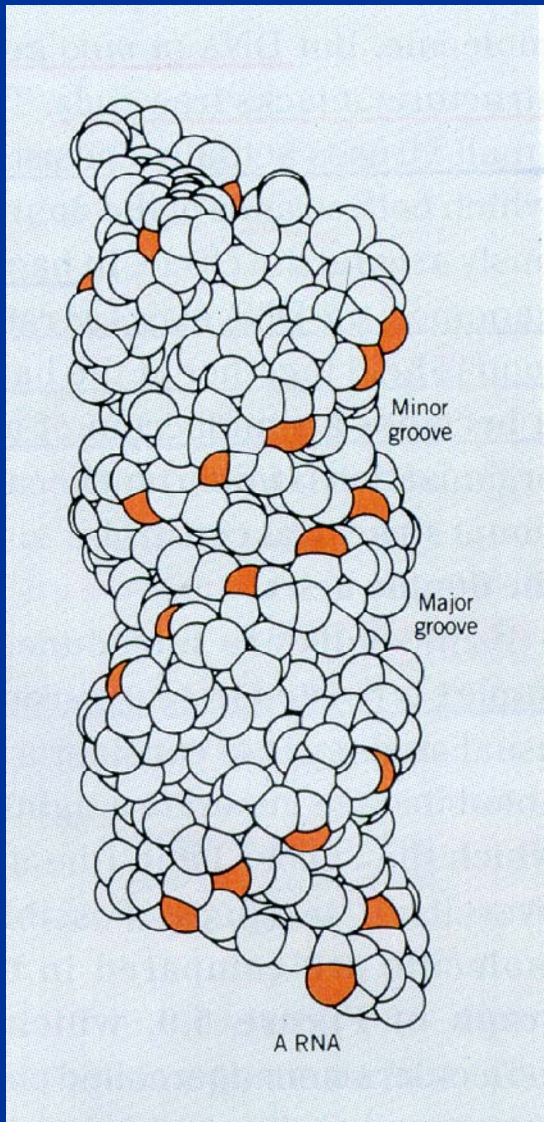
Form	coiling	bp/turn	rotation/bp	diameter ds
A	R	11	34,7°	2,3 nm
B	R	10	43,0°	1,9 nm
Z	L	12	30°	1,8 nm

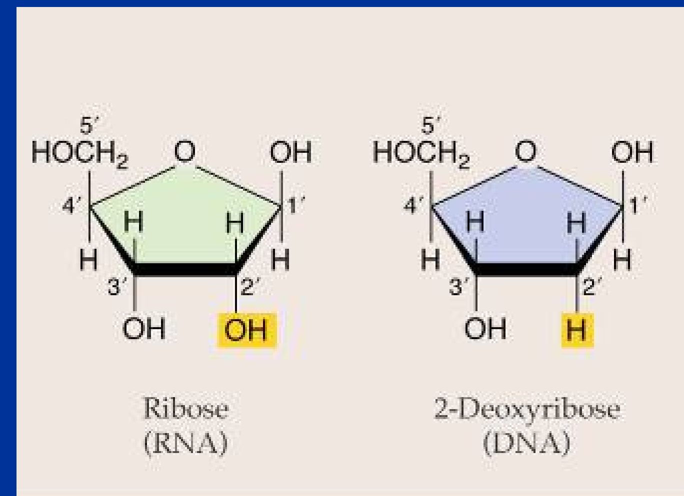
B form

- prevailing dsDNA form *in vitro*
- *in vivo* transitions to other forms
- major groove – point of interaction with sequence-specific DNA-binding proteins

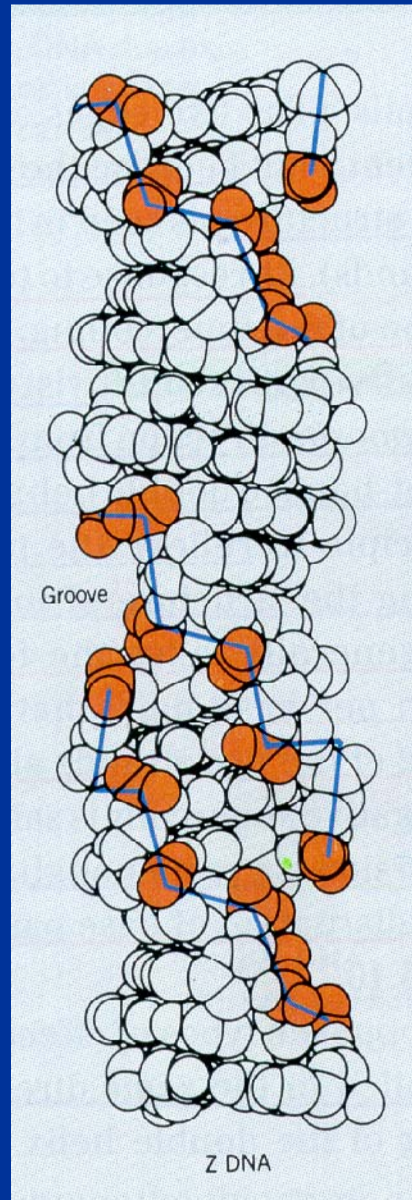


A form





Z form (Zigzag)



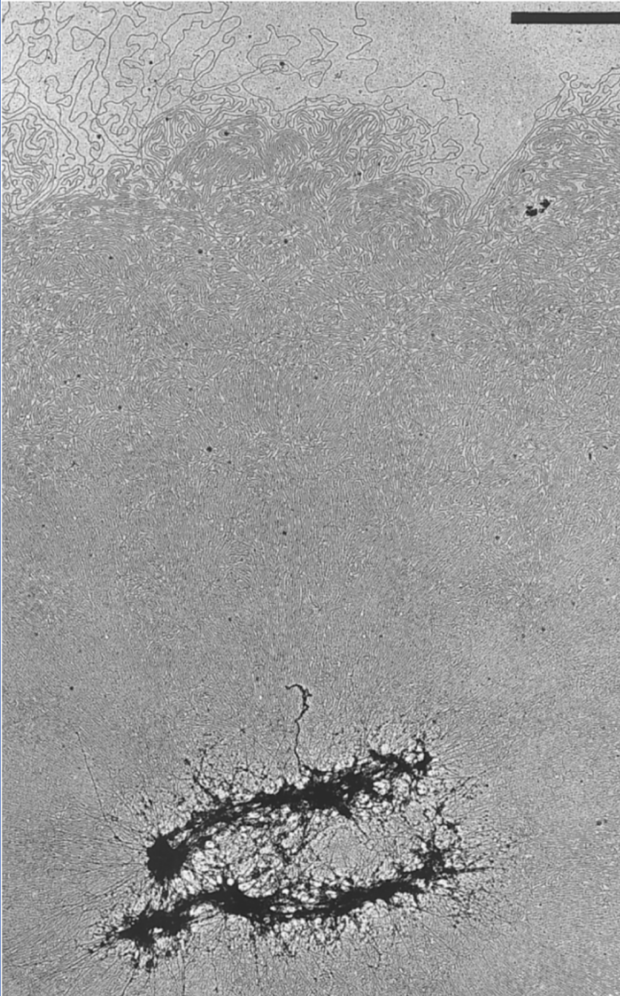


Figure 18.7 Histone-depleted chromosomes consist of a protein scaffold to which loops of DNA are anchored. Photograph kindly provided by Ulrich K. Laemmli.

supercoiling

- positive – double helix overwound, tightens the structure
- negative – loosens the structure, reduces rotation per bp, local disruption of base-pairing

chromatin proteins

- neutralize the negative charge of DNA molecule
- help condensation (packaging) of chromatin
- form structures which enable formation of other loops and domains
- affect gene expression

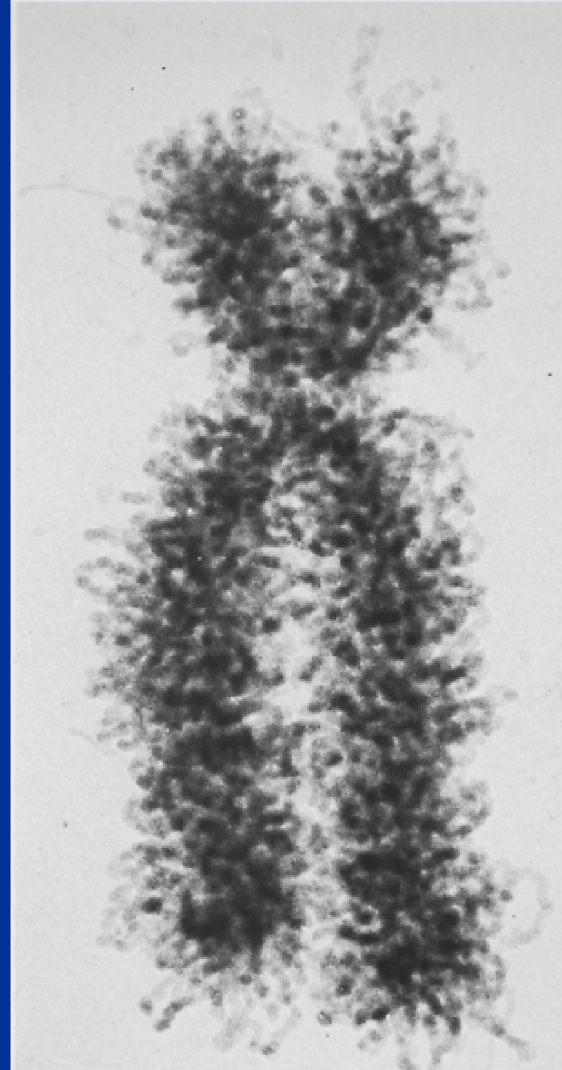
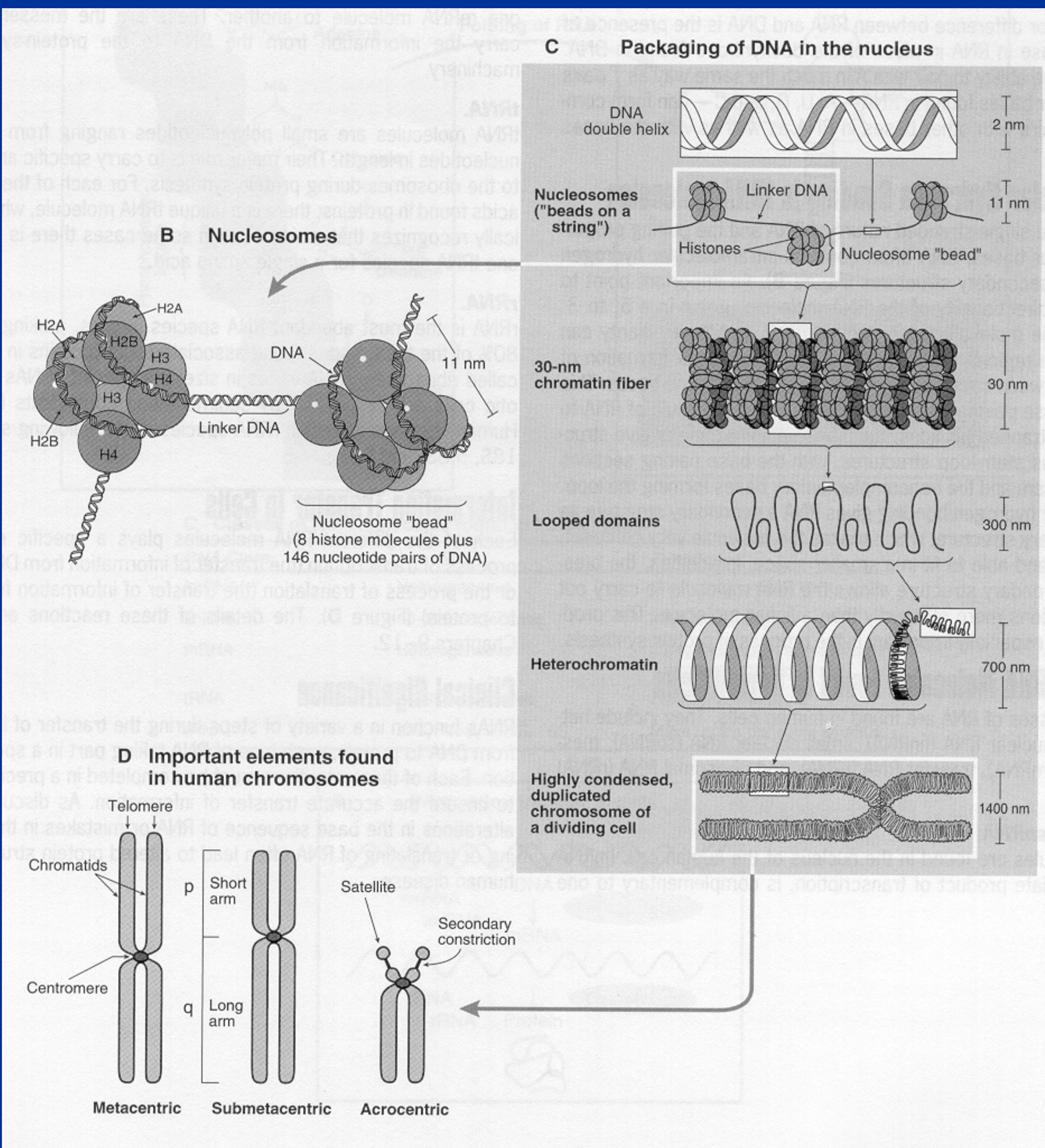


Figure 18.9 The sister chromatids of a mitotic pair each consist of a fiber (~30 nm in diameter) compactly folded into the chromosome. Photograph kindly provided by E. J. DuPraw.



hierarchy of chromatin organization

Institute of Experimental Botany, Academy of Sciences of the Czech republic

1st level of chromatin - nucleosomes

2 nm DNA coils around histone octamer („beads") formed by small basic proteins
histons H2A, H2B, H3, H4

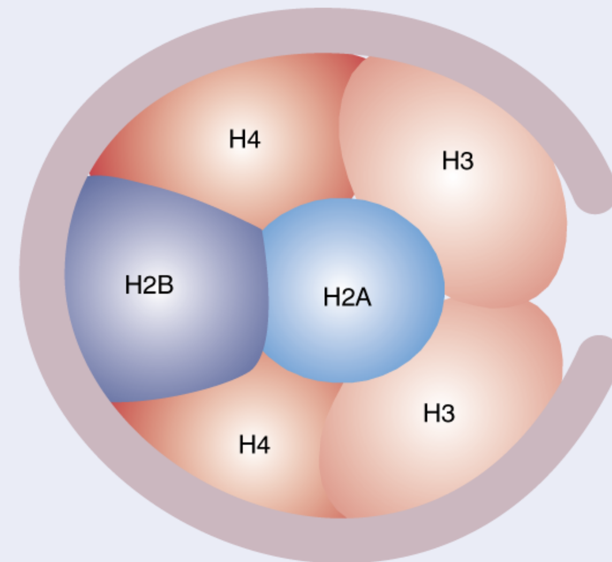
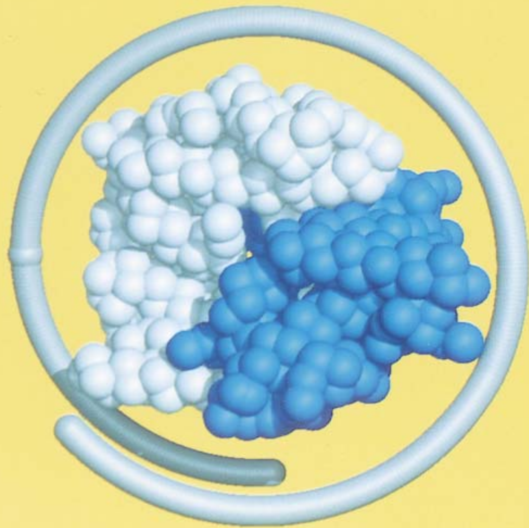
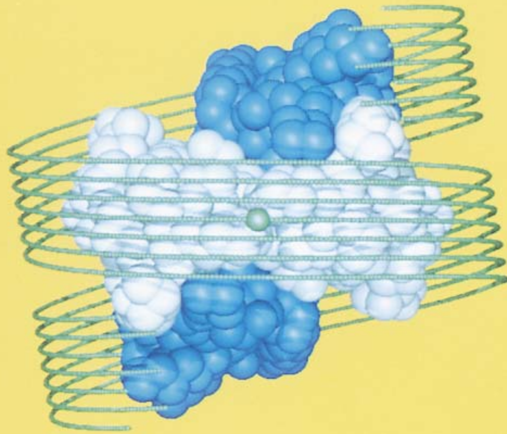


Figure 19.21 In a symmetrical model for the nucleosome, the $H3_2$-$H4_2$ tetramer provides a kernel for the shape. One H2A-H2B dimer can be seen in the top view; the other is underneath.

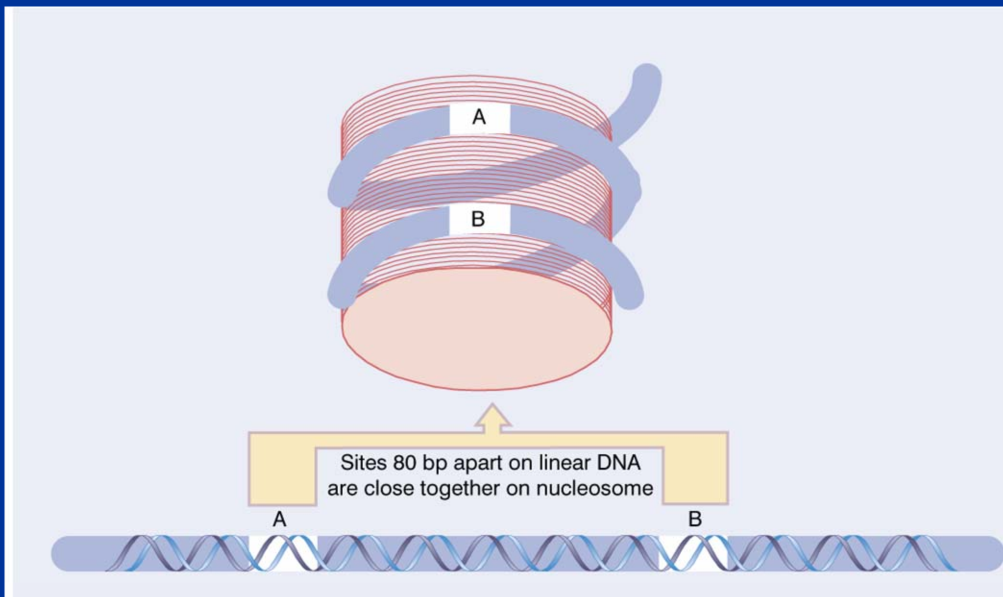


Side view



1st level of chromatin - nucleosomes

1st level of chromatin - nucleosomes



a protein could contact sequences on DNA that lie at different ends of nucleosome

sites 80 bp apart on linear DNA are close together on nucleosome

1st level of chromatin - nucleosomes

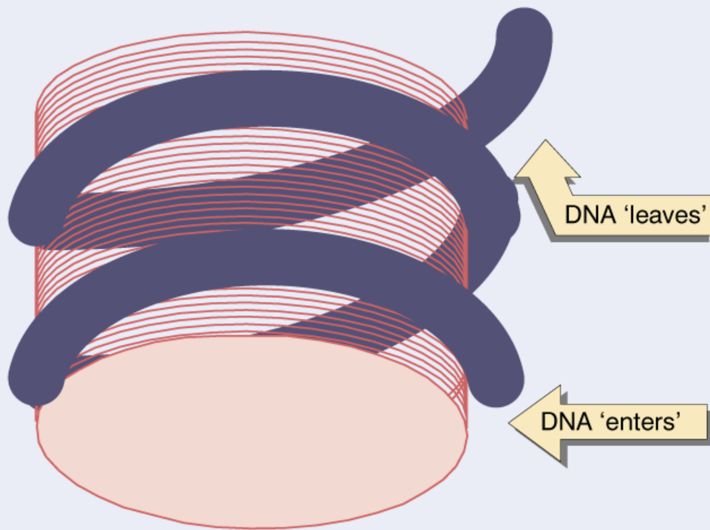


Figure 19.4 The nucleosome may be a cylinder with DNA organized into two turns around the surface.

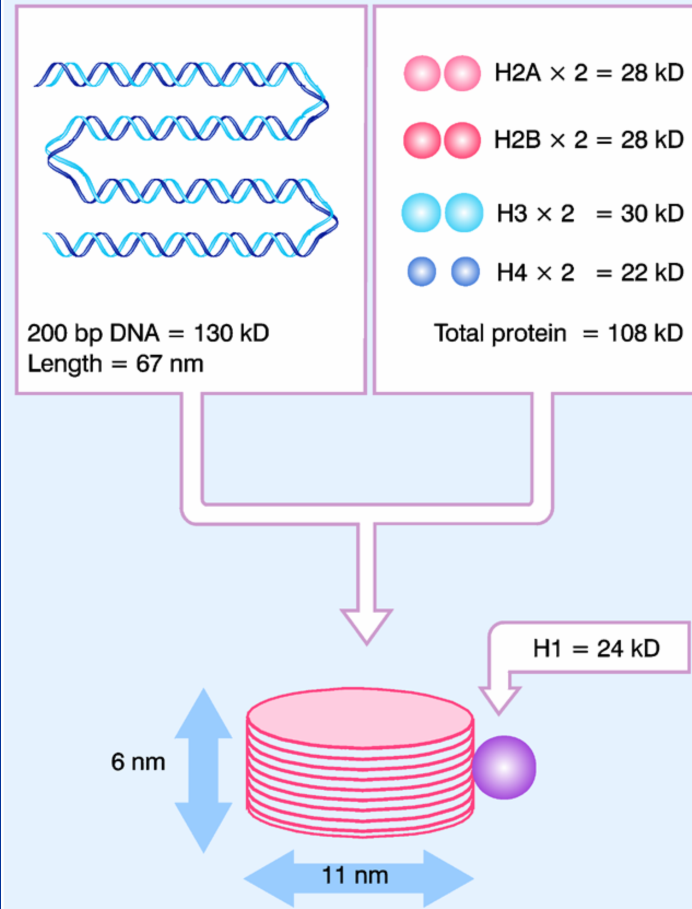


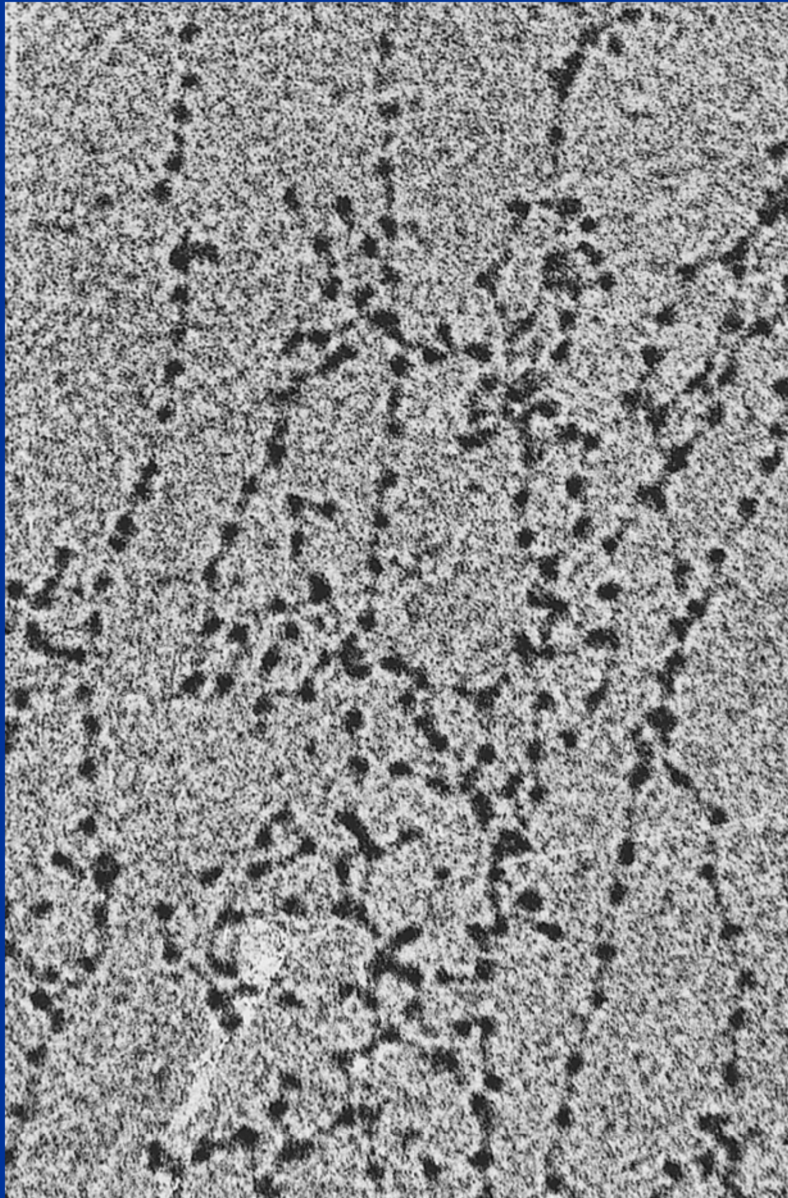
Figure 19.3 The nucleosome consists of approximately equal masses of DNA and histones (including H1). The predicted mass of the nucleosome is 262 kD.

Figure 19.18 The 10 nm fiber is a continuous string of nucleosomes.

1st level of chromatin - nucleosomes

10 nm fiber – „beads on string"

cca 200 bp/nucleosome

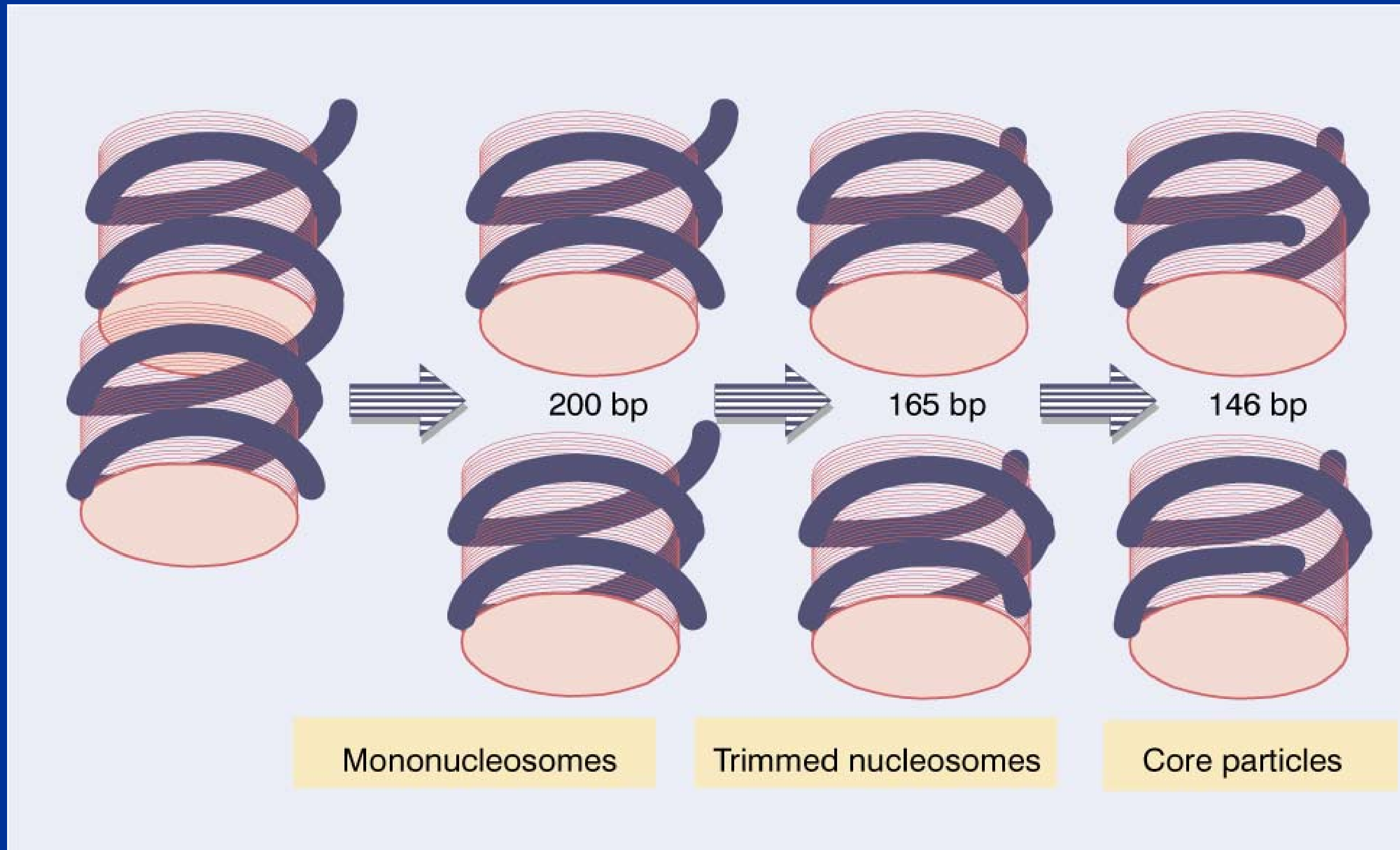


1st level of chromatin - nucleosomes

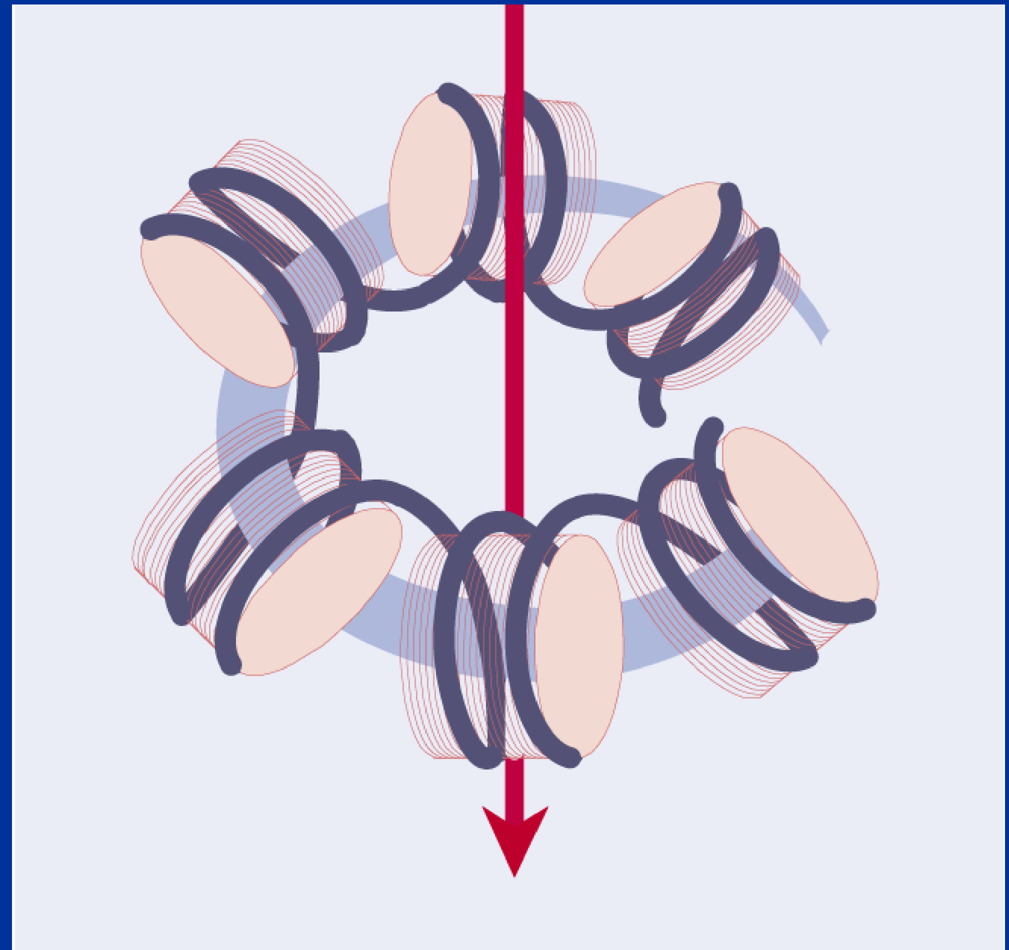
10 nm - „beads on string"

packing ratio ~ 6

1st level of chromatin - nucleosomes



2nd level of chromatin - solenoid



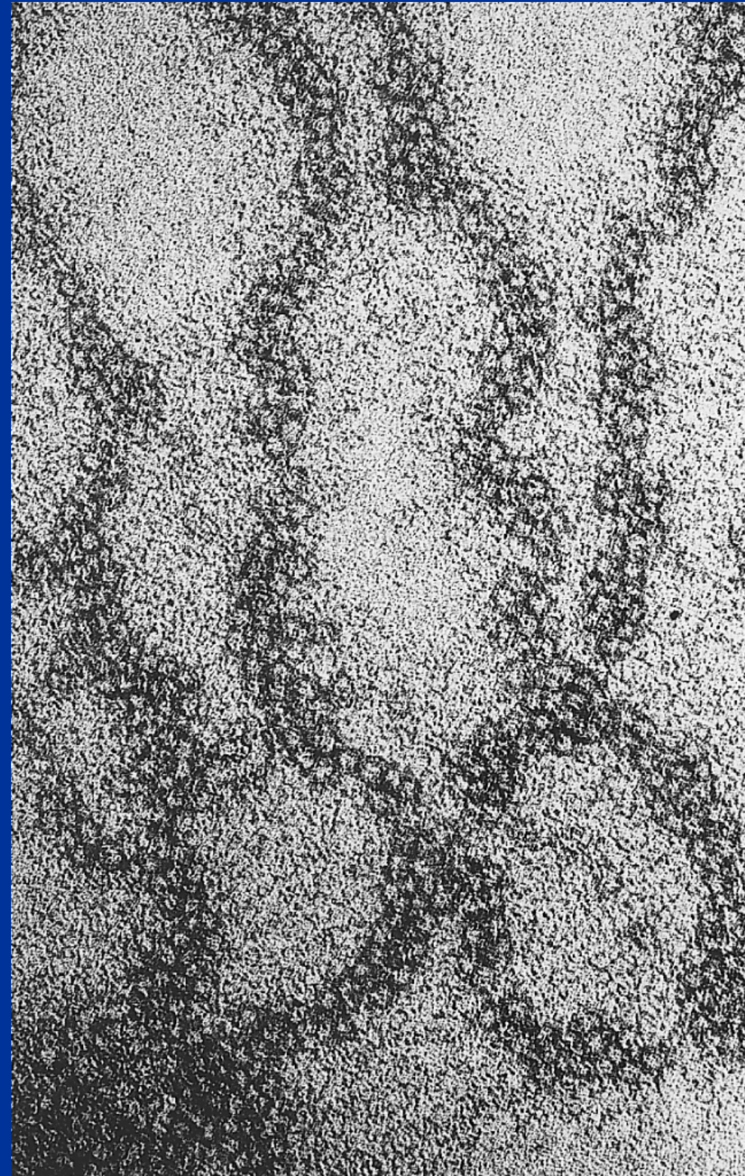
30 nm fiber

6 nucleosomes per one turn of helical structure

2nd level of chromatin - solenoid

requires histone H1 and other (non-histone) proteins

packing ratio ~ 40



2nd level of chromatin - solenoid

histone H1 -
„linker“ histone

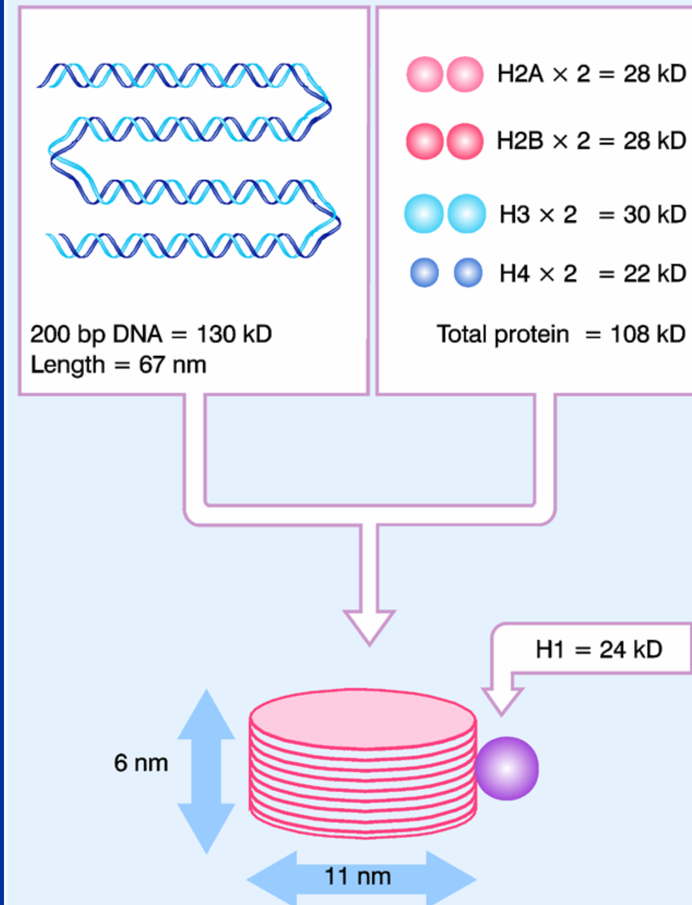
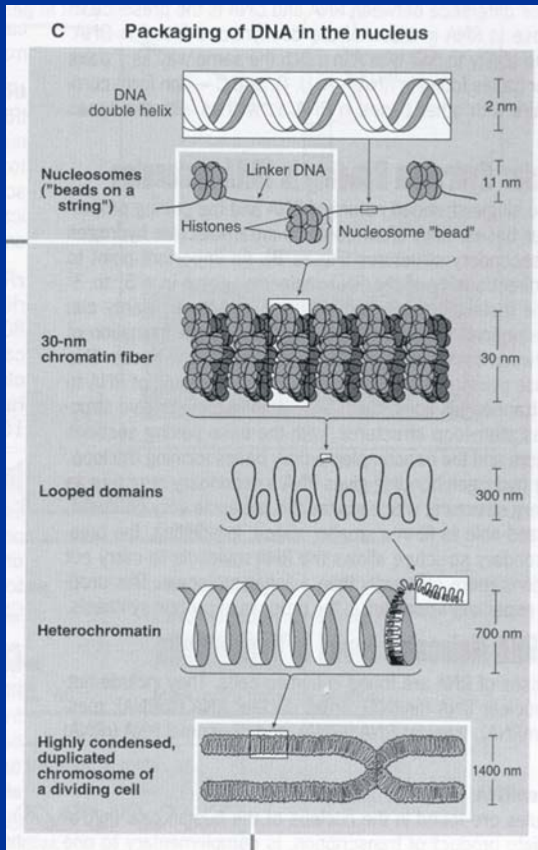


Figure 19.3 The nucleosome consists of approximately equal masses of DNA and histones (including H1). The predicted mass of the nucleosome is 262 kD.

higher order chromatin



due to accessory proteins

difference in packing ratio:

euchromatin ≥ 1000

mitotic chromosomes ≤ 10 000

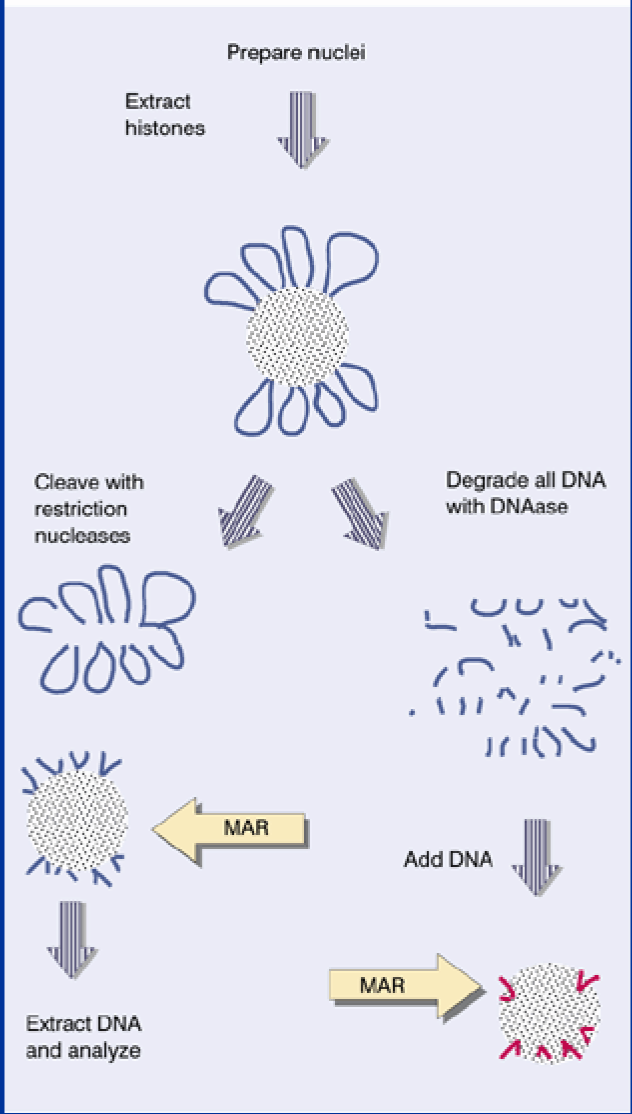
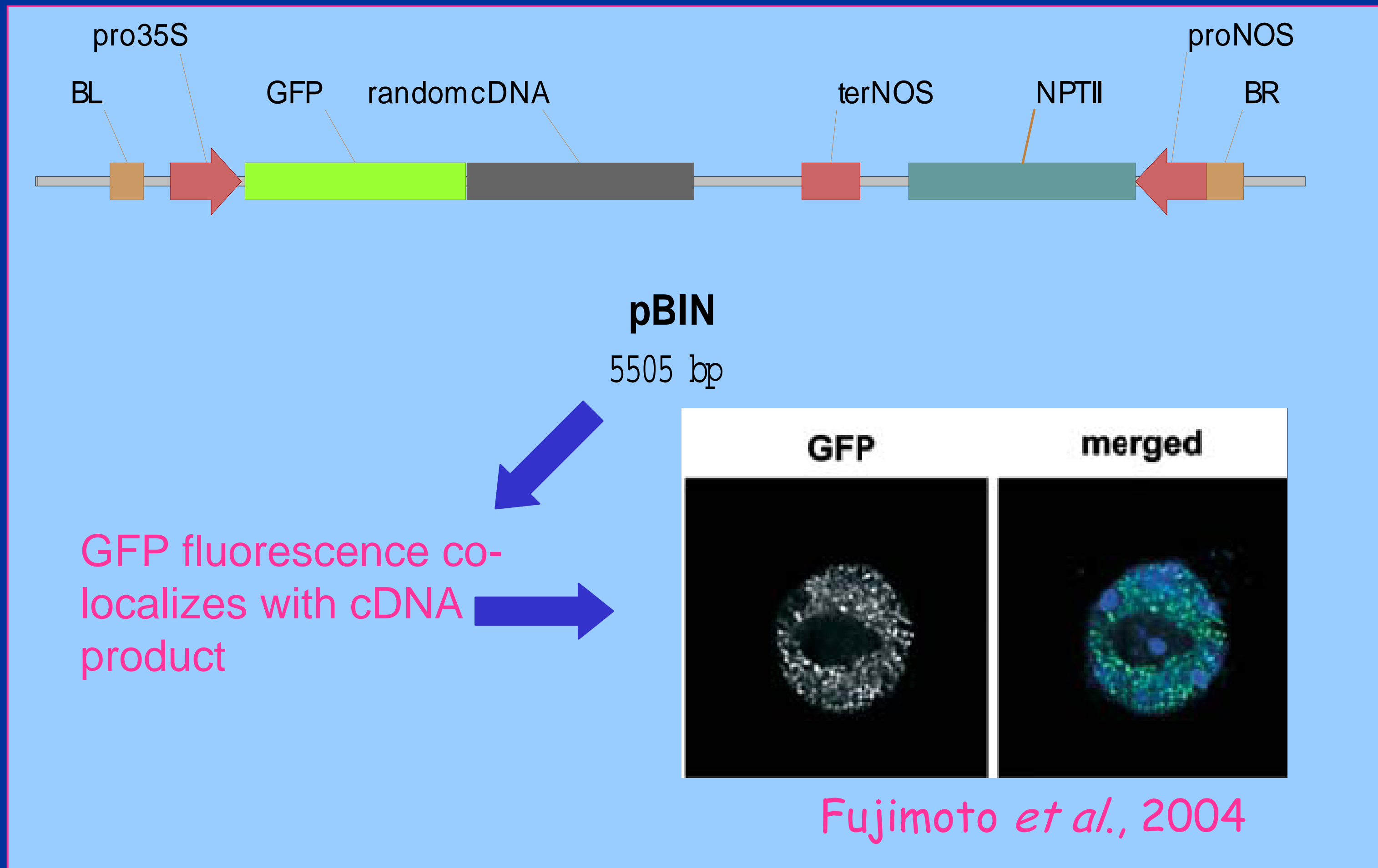


Figure 18.8 Matrix-associated regions may be identified by characterizing the DNA retained by the matrix isolated *in vivo* or by identifying the fragments that can bind to the matrix from which all DNA has been removed *in vivo*.

nuclear matrix associated regions

searching for MAR-binding proteins

26



Fujimoto *et al.*, 2004

- MARs

(matrix attachment regions)

also SARs (scaffold attachment regions)

  - anchor coding (functional) regions to proteinaceous scaffold of chromosomes or to nuclear matrix of interphazic nuclei
  - AT rich, recognized by topoisomerase II
  - every 3 kb to 100 kb
  - MAR sequences placed near transgene increase the transgene expression and decrease the variability of expression among idependent transformants

genome is a collection of loops)

- LBARs

(loop basement attachment regions)

  - more permanent in nature, give an „address“ to each gene
  - organize genomes to big loops (distance 20 kb to 100 kb)

MARs´ influence on transgenes

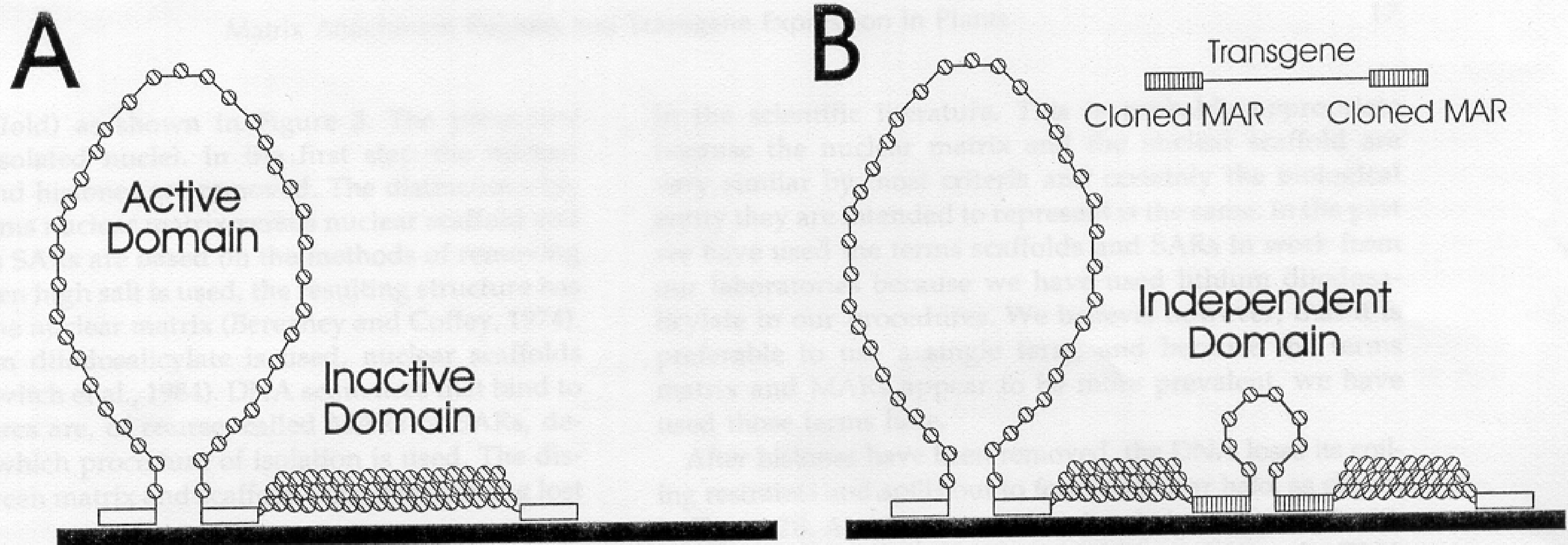
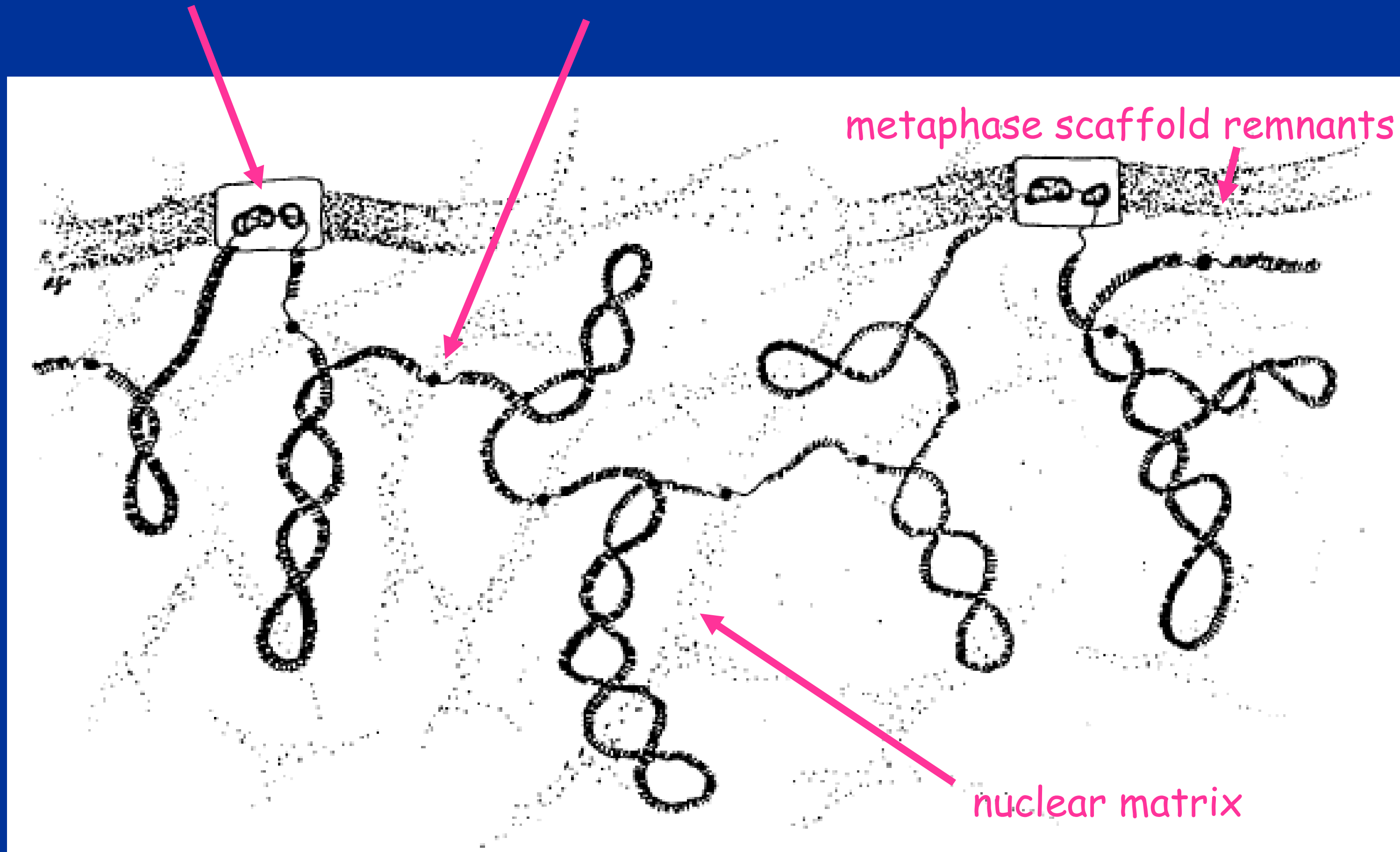


Figure 1. Models depicting the organization of chromatin into active and inactive loop domains and the formation of independent transgenic loop domains. A, MAR sequences (open boxes) interact with nuclear matrix fiber (filled bar) to form two loop domains. The active domain is depicted as an 11-nm nucleosome fiber and the inactive domain as a 30-nm fiber formed by supercoiling of the 11-nm fiber. B, An independent domain formed by the integration of MAR-flanked transgene into the inactive domain.

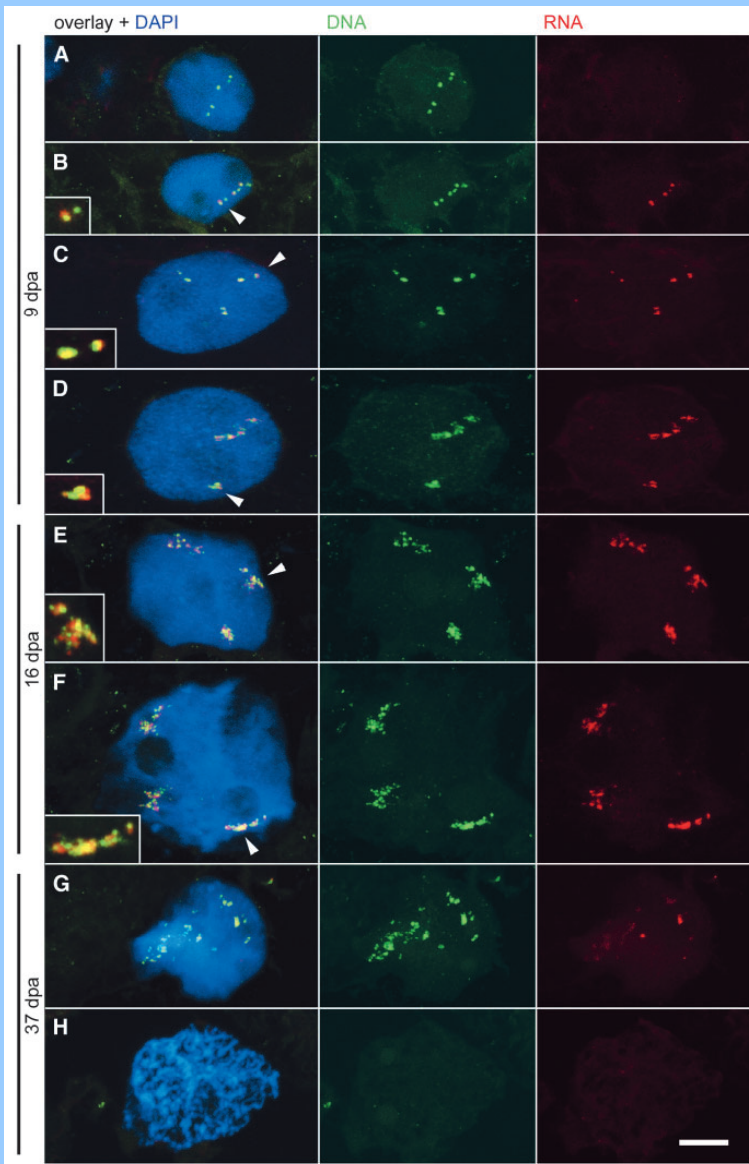
LBARs and MARs model



Paul A.-L., Ferl R.J., 1999

Chromatin modification and remodelling

- activly transcribing chromatin contains hyperacetylated histones and is DNaseI sensitive
- in active chromatin, the distances between nucleosomes are shorter
- chromatin has a repressive effect on gene expression
- DNA methylation coincides with transcriptional inactivation



Chromatin decondensation during transcription

Wegel *et al.*, 2004

1. Covalent posttranslational modification of chromatin

dynamic chromatin changes

- linker histone

  (H1) basic amino and carboxy-termini

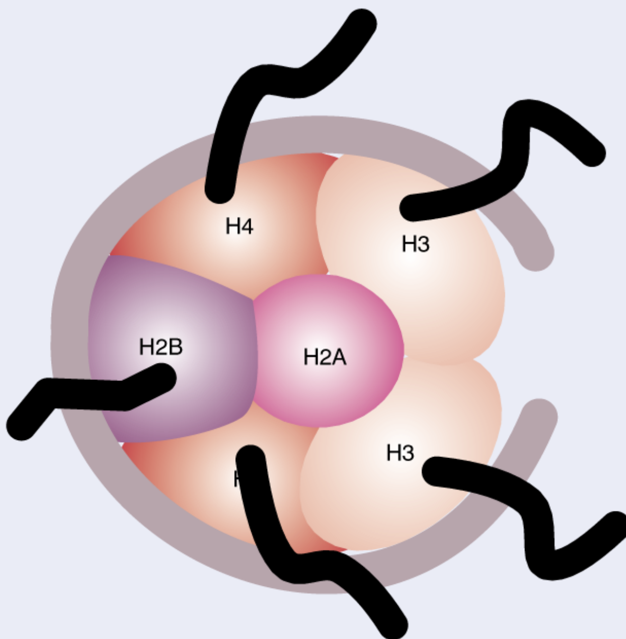
  interacts with both histones and DNA

  phosphorylation of H1 at the start of mitosis, later reversed

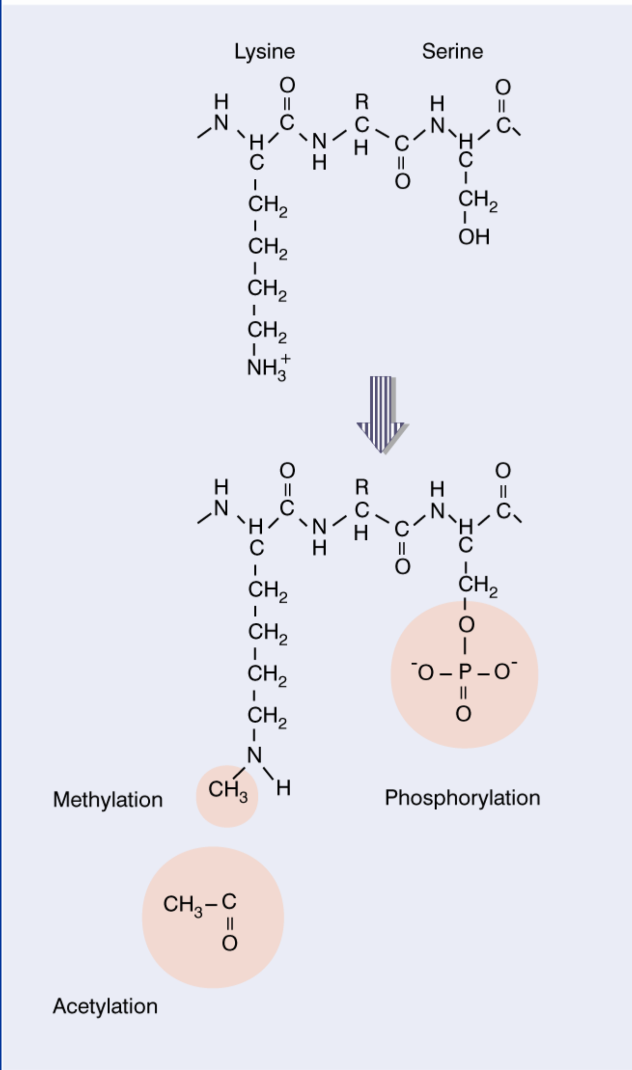
  related to chromatin remodelling (? affinity to chromatin/DNA?)

1. Covalent posttranslational modification of chromatin

Figure 19.24 The globular bodies of the histones are localized in the histone octamer of the core particle, but the locations of the N-terminal tails, which carry the sites for modification, are not known, and could be more flexible.



- nucleosomal histones



1. Covalent posttranslational modification of chromatin

1. Covalent posttranslational modification of chromatin

- nucleosomal histones

Acetylation

opens chromatin structure of whole domains, affects numerous nucleosomes, prevents higher order chromatin structure

ADP ribosylation

molecul similar to ssDNA can locally disrupt chromatin structure

Ubiquitinylation

peptide (76 aa) marks protein for degradation

? nucleosome loss in actively transcribed genes

Methylation

‼ of histones, no structural changes known

Covalent posttranslational modification of chromatin during cell cycle

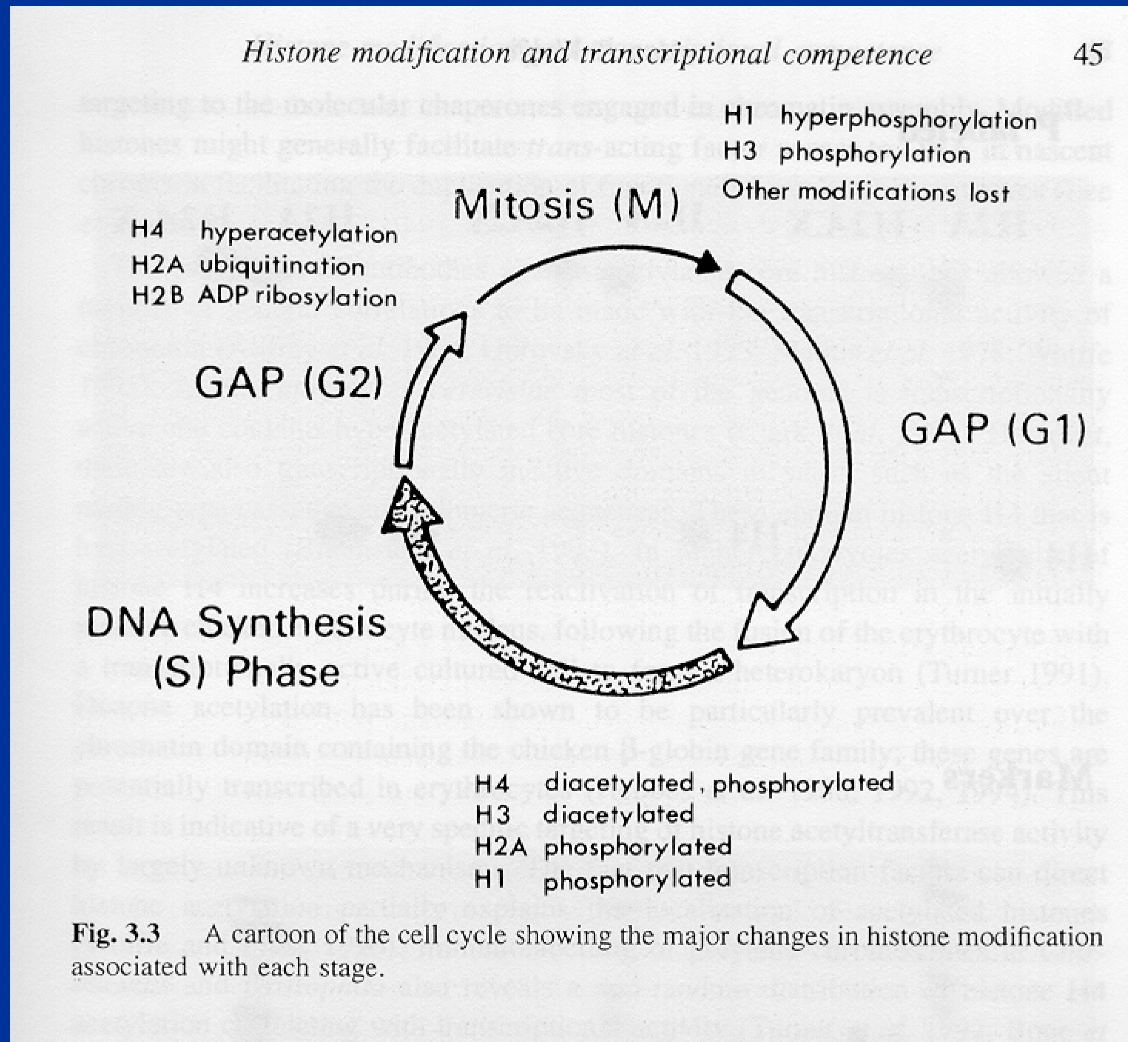


Fig. 3.3 A cartoon of the cell cycle showing the major changes in histone modification associated with each stage.

Covalent posttranslational modification of chromatin and differentiation

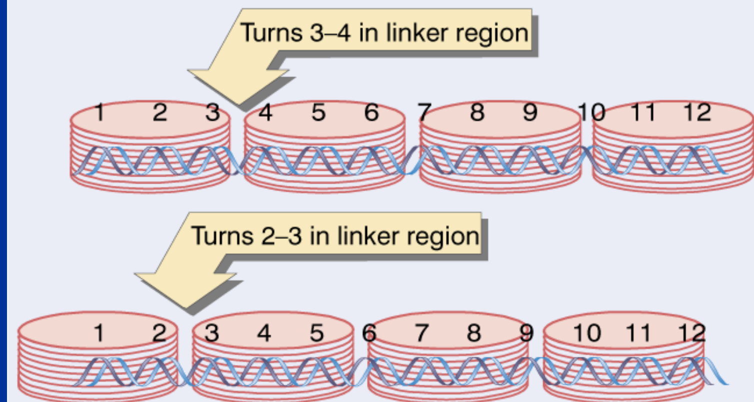
Changes in histone acetylation are important for maintaining stable activity or inactivity of a gene during development (mainly early stages – embryogenesis) and for epigenetic imprinting

Nucleosomal structure must be re-established by equally modified proteins which form nucleosomal structure on both daughter chromatids

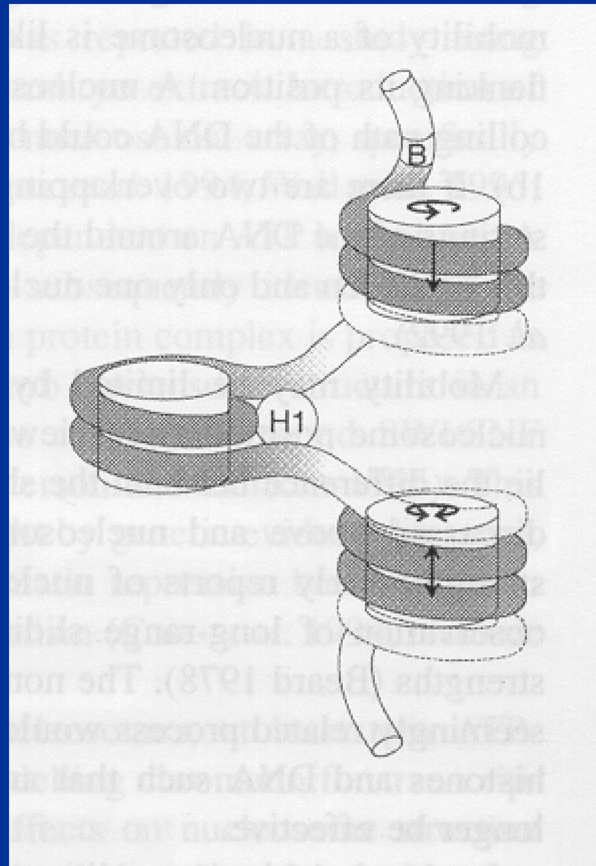
2. Nucleosome positioning

- Nucleosome positioning along DNA sequence is not random
- Enables transcriptional modulation
- Nucleosome mediates contact of physically distant sites
- Nucleosome positioning is affected by DNA sequence

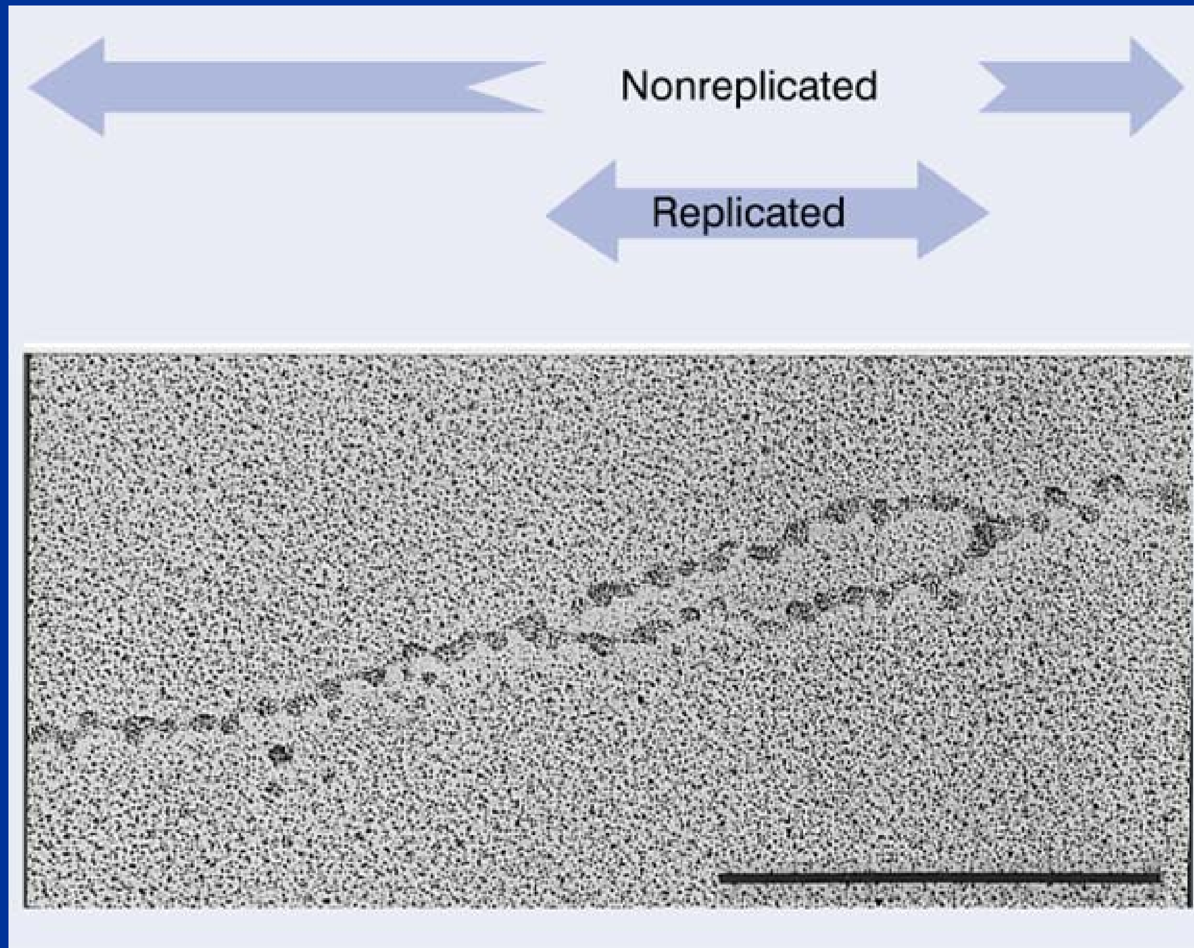
Figure 19.31 Translational positioning describes the linear position of DNA relative to the histone octamer. Displacement of the DNA by 10 bp changes the sequences that are in the more exposed linker regions, but does not alter which face of DNA is protected by the histone surface and which is exposed to the exterior. DNA is really coiled around the nucleosomes, and is shown in linear form only for convenience.

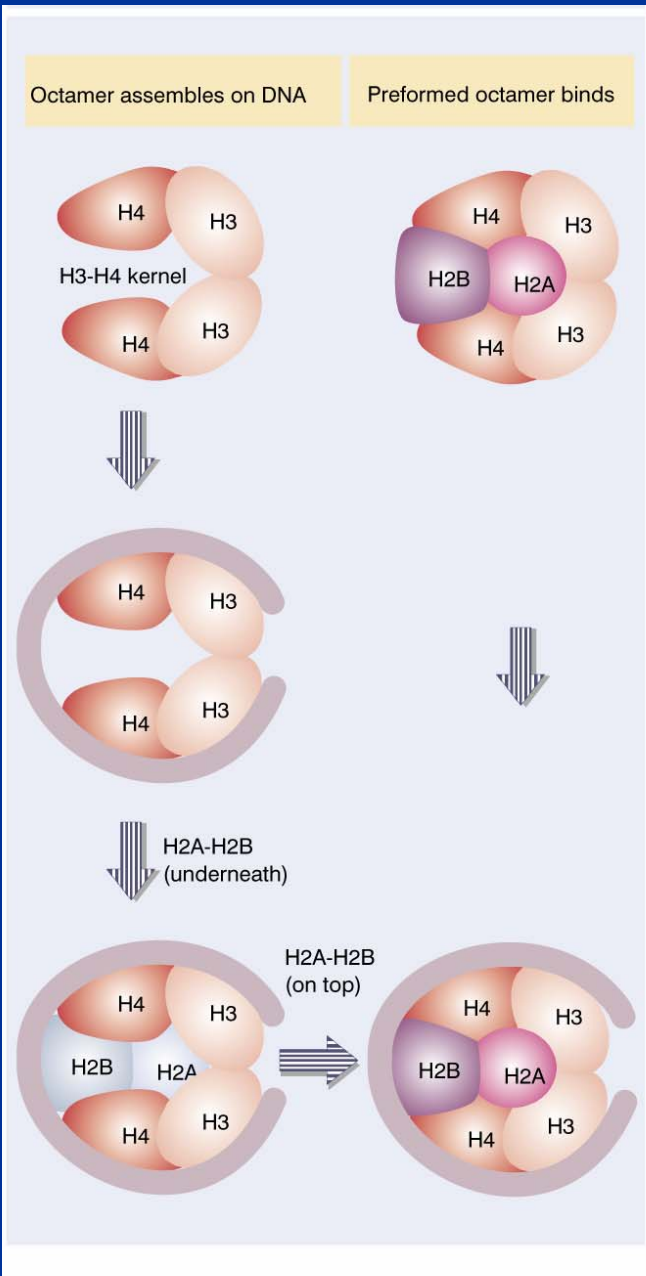


Nucleosome mobility



Nucleosome reproduction during DNA replication





Nucleosome reproduction during DNA replication

Nucleosomes in transcribed genes

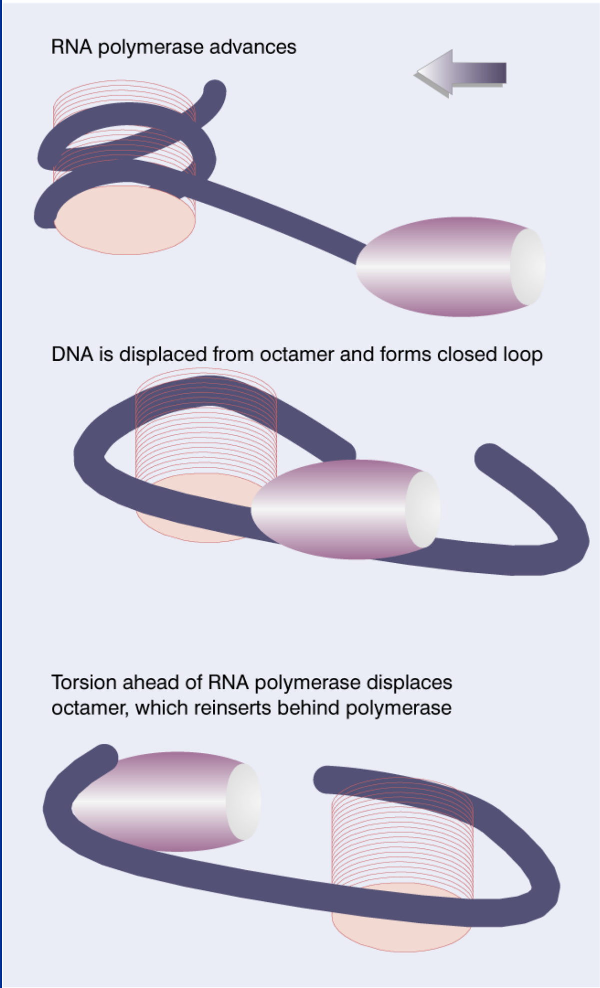


Figure 19.37 RNA polymerase displaces DNA from the histone octamer as it advances. The DNA loops back and attaches (to polymerase or to the octamer) to form a closed loop. As the polymerase proceeds, it generates positive supercoiling ahead. This displaces the octamer, which keeps contact with DNA and/or polymerase, and is inserted behind the RNA polymerase.

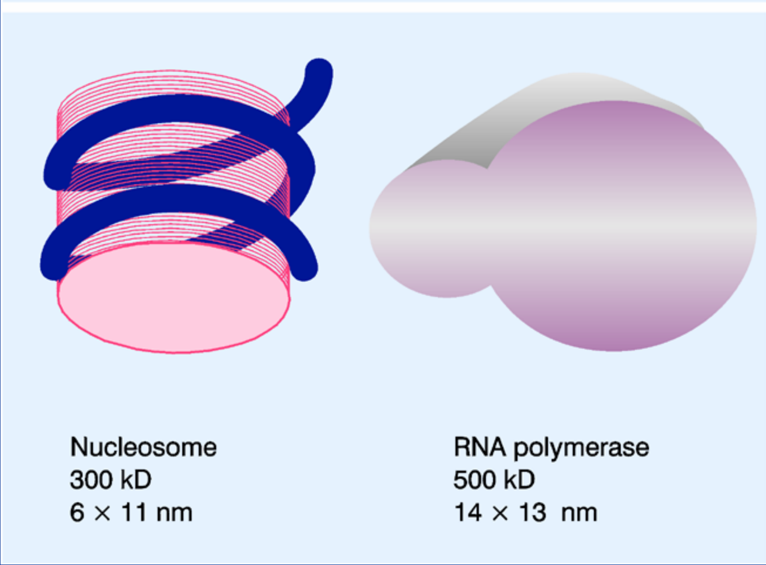
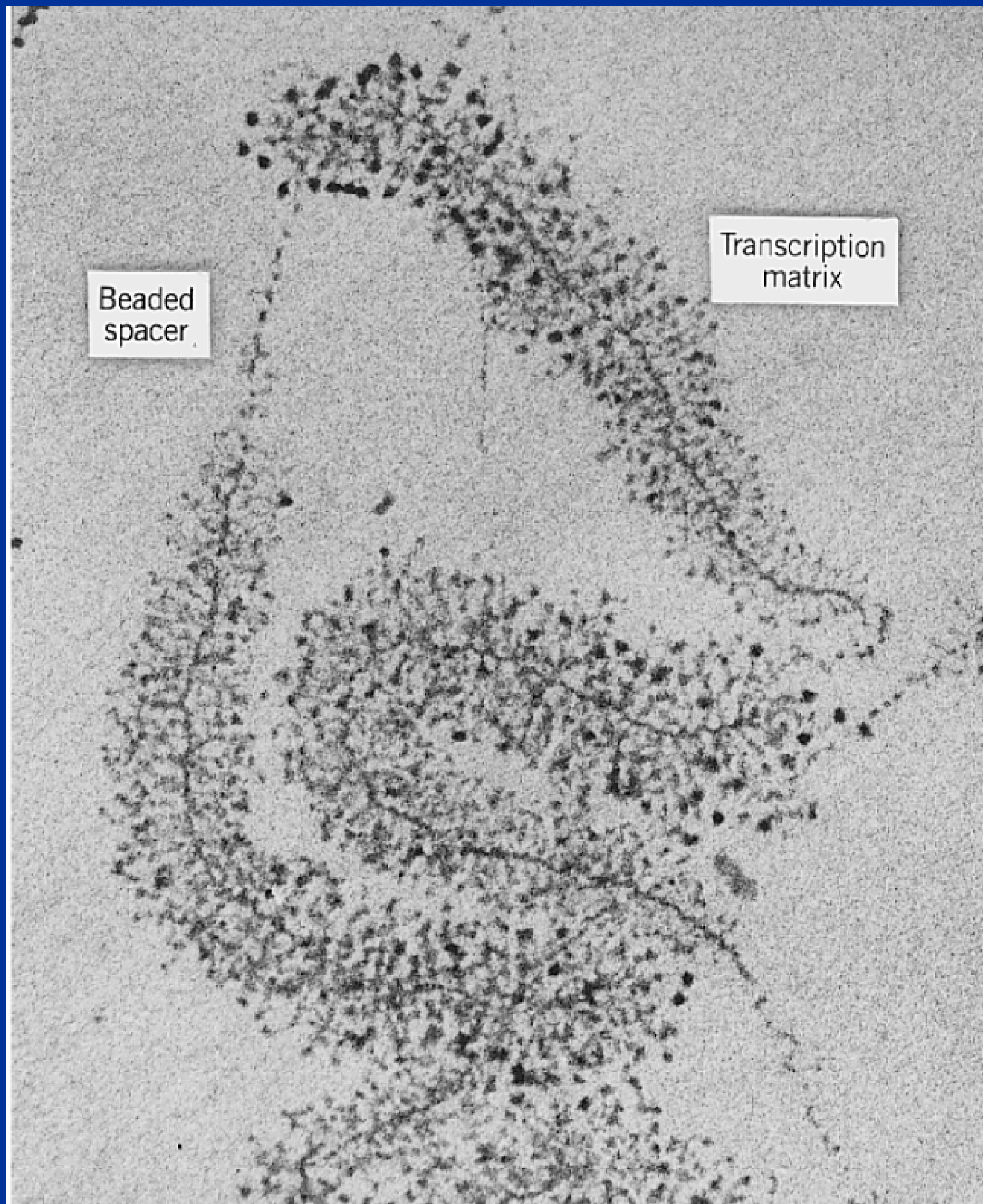


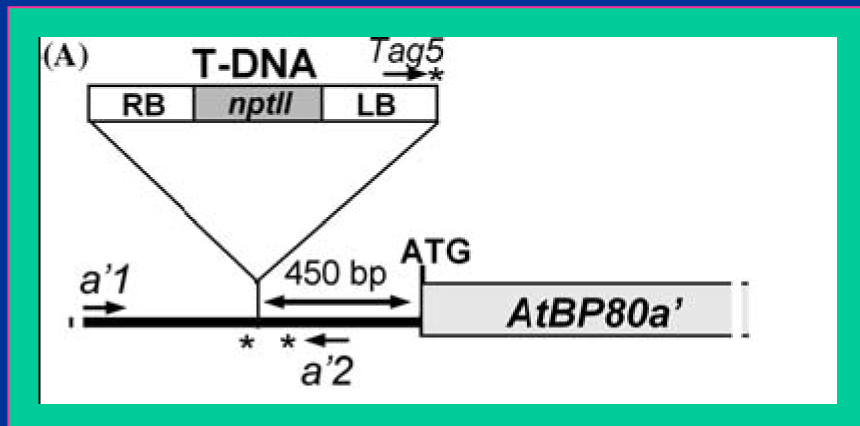
Figure 19.35 RNA polymerase is comparable in size to the nucleosome and might encounter difficulties in following the DNA around the histone octamer.



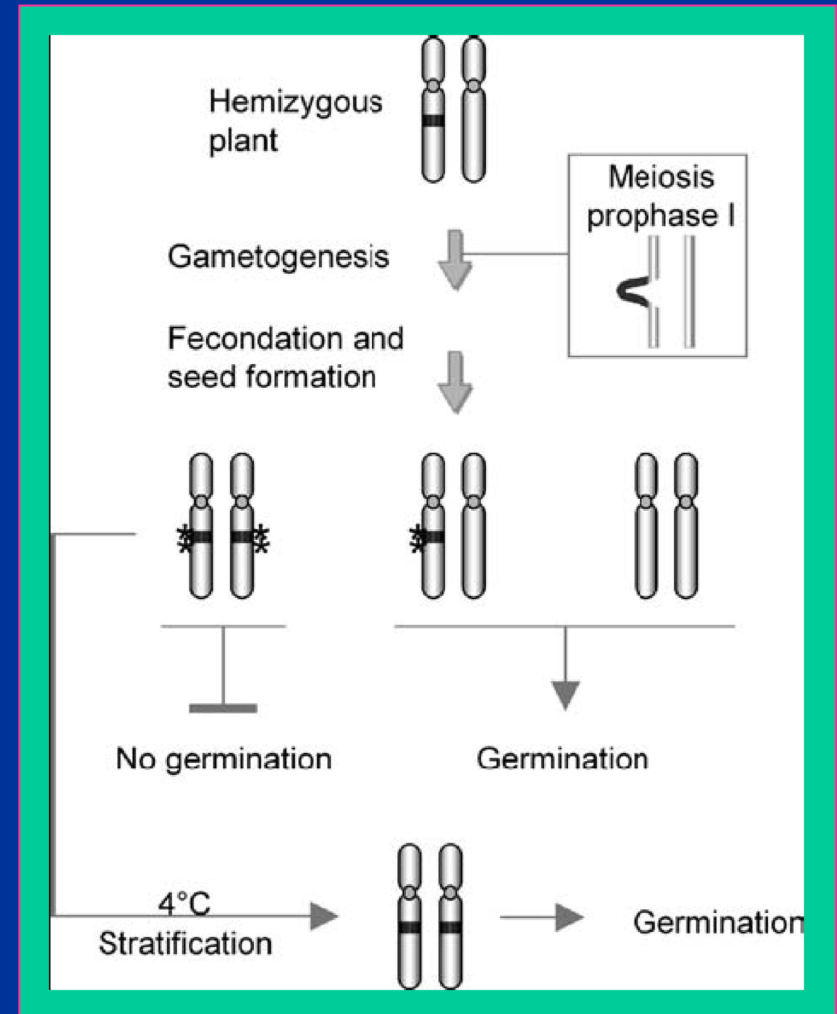
Nucleosomes in transcribed genes

3. DNA methylation

- Methylation of cytosins in C5 position of CpG dinucleotides



Masclaux et al., 2005



complexes involved in chromatin modification

- ATP-dependent (chromatin remodelling) complexes
  - ATP hydrolysis, local disruptions or changes

- histon acetyltransferases and deacetylases (chromatin modifying complexes)
  - the level of histone acetylation regulates transcriptional activity of genes

Functional chromatin domains

Structural domains

loops formed by MARs

not identical to functional domains, but often define regions of transcription

Functional domains

mutually independent domaind of gene expression

structural changes of chromatin occur upon induction of gene expression in the domain

Positional effect

Relocation of an active gene within genome can lead to the inactivation of its expression (incorrect interaction of regulation proteins with promoter, incorrect chromatin structure...)

And the other way round

Boundary chromatin elements

protect transgene from the positional effect

MARs

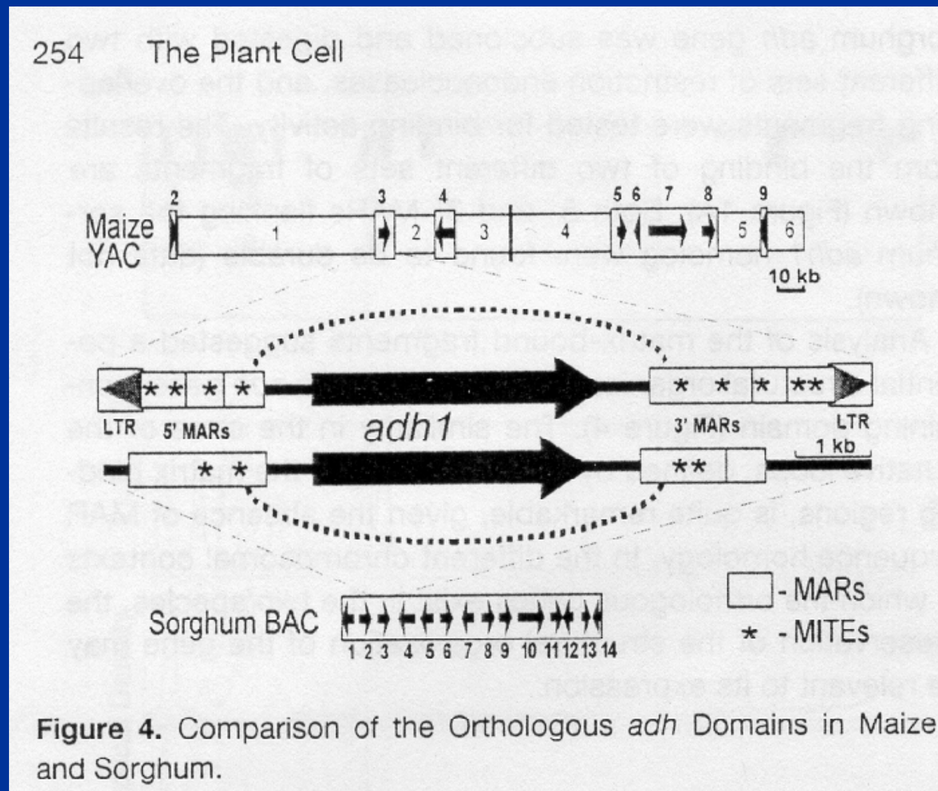


Figure 4. Comparison of the Orthologous *adh* Domains in Maize and Sorghum.

Tikhonov et al., 2000

MARs

adh loci of two genomes

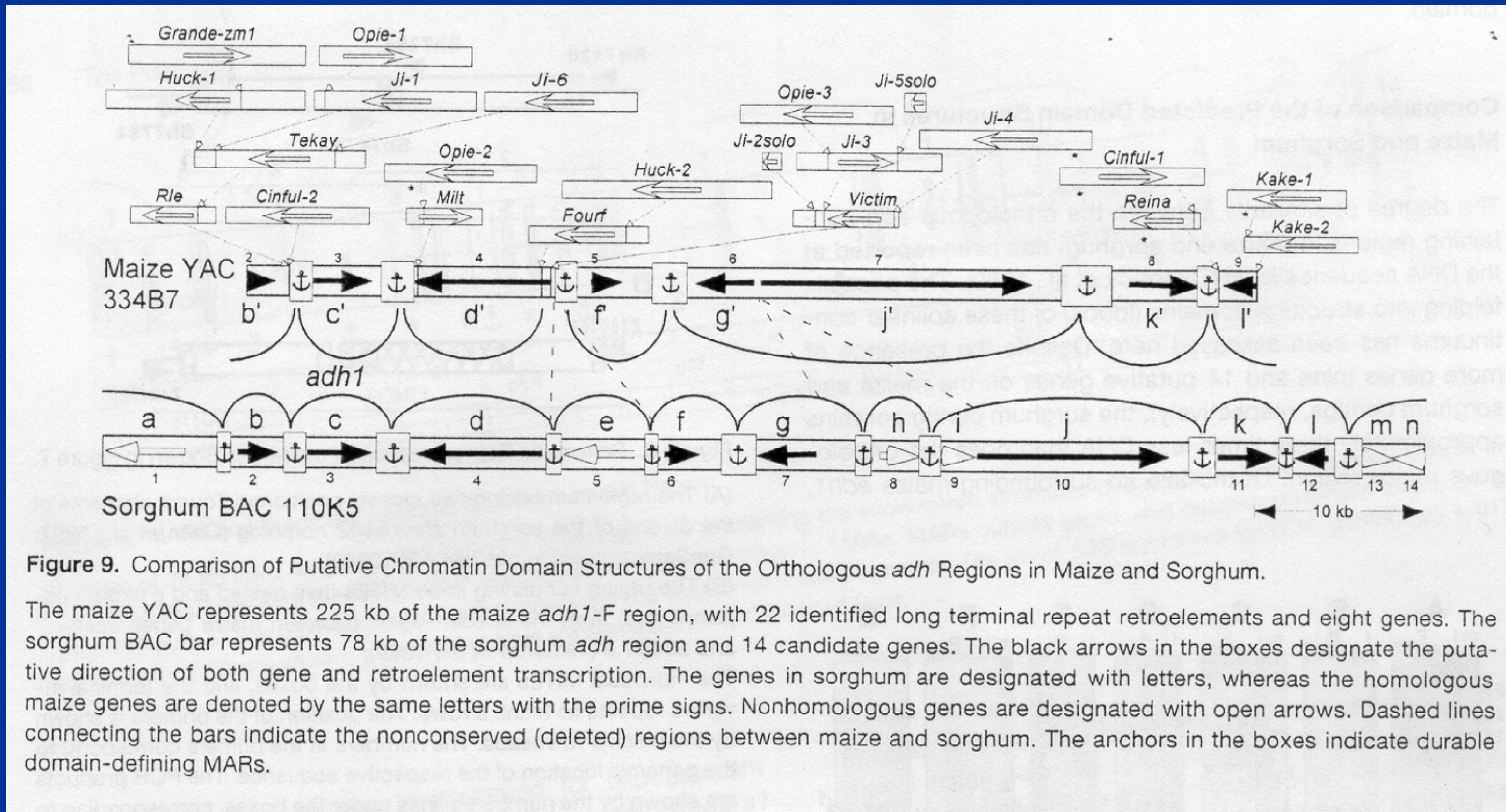


Figure 9. Comparison of Putative Chromatin Domain Structures of the Orthologous *adh* Regions in Maize and Sorghum.

The maize YAC represents 225 kb of the maize *adh1*-F region, with 22 identified long terminal repeat retroelements and eight genes. The sorghum BAC bar represents 78 kb of the sorghum *adh* region and 14 candidate genes. The black arrows in the boxes designate the putative direction of both gene and retroelement transcription. The genes in sorghum are designated with letters, whereas the homologous maize genes are denoted by the same letters with the prime signs. Nonhomologous genes are designated with open arrows. Dashed lines connecting the bars indicate the nonconserved (deleted) regions between maize and sorghum. The anchors in the boxes indicate durable domain-defining MARs.

Tikhonov et al., 2000

Boundary chromatin elements

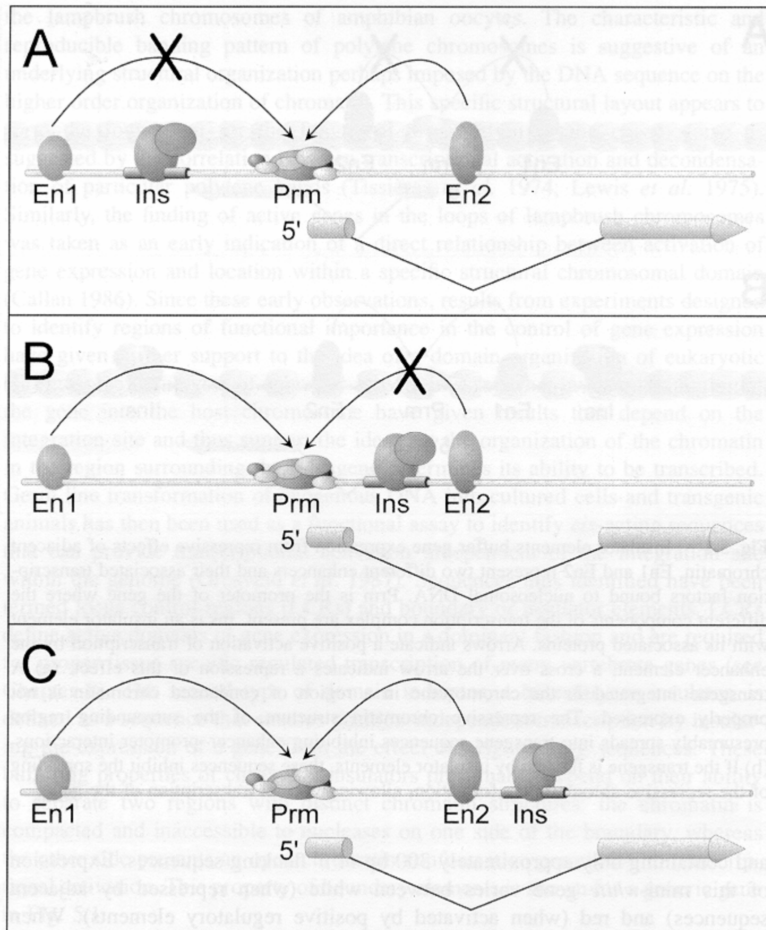


Fig. 5.2 Polar effect of an insulator on enhancer–promoter interactions. Symbols are as in Fig. 5.1. (a) An insulator located in the 5' region of the gene inhibits its transcriptional activation by an upstream enhancer (En1) without affecting the function of a second enhancer (En2) located in the intron of the gene. (b) When the insulator is located in the intron, expression from the downstream enhancer (En2) is blocked, whereas the upstream enhancer (En1) is still active. (c) When the insulator is located in the intron but distal to the En2 enhancer, both enhancers are active and transcription of the gene is normal. This property distinguishes an insulator from a typical repressor.

Insulator

Boundary chromatin elements

LCRs

(locus control regions), abundant in genome

DnaseI-sensitive site and TF-binding motif

enhancer activity – remodelling/opening of chromatin structure in a region of 10-100 kb

insulating function

many genes close to LCRs