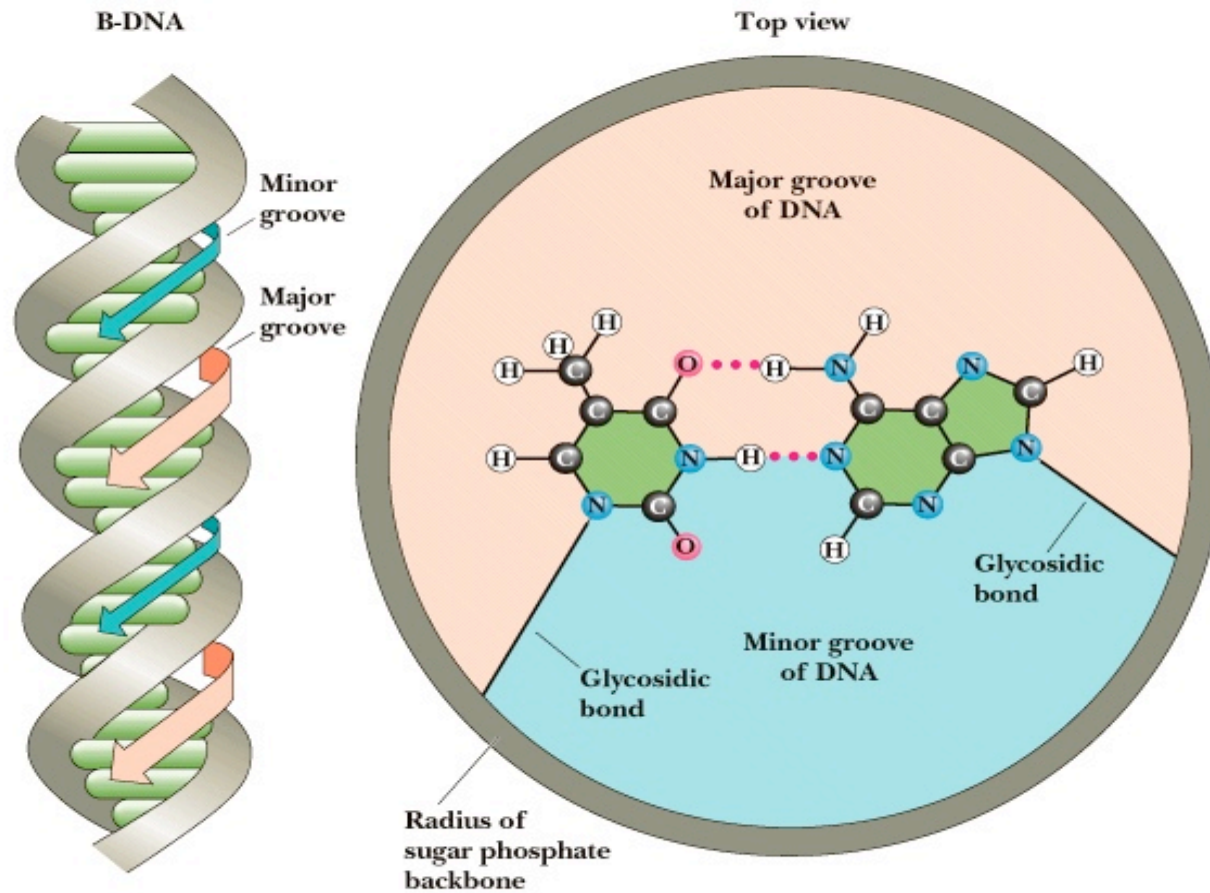


Essentials of protein - nucleic acids interactions and thermodynamics

B-DNA – major & minor grooves

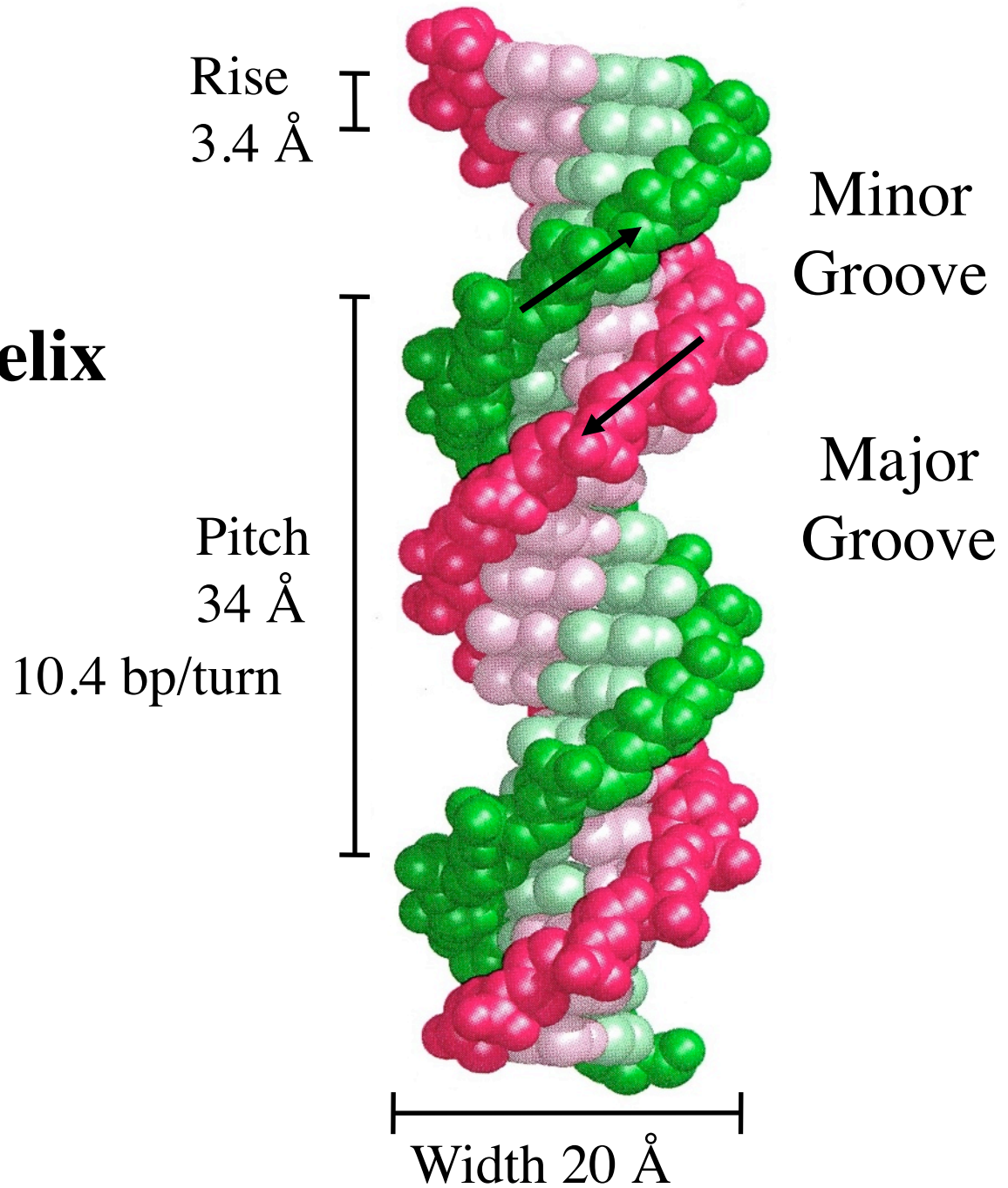
Garrett & Grisham: Biochemistry, 2/e
Figure 12.11



G & G 12.11

Saunders College Publishing

**B-DNA: A right
Handed double helix**

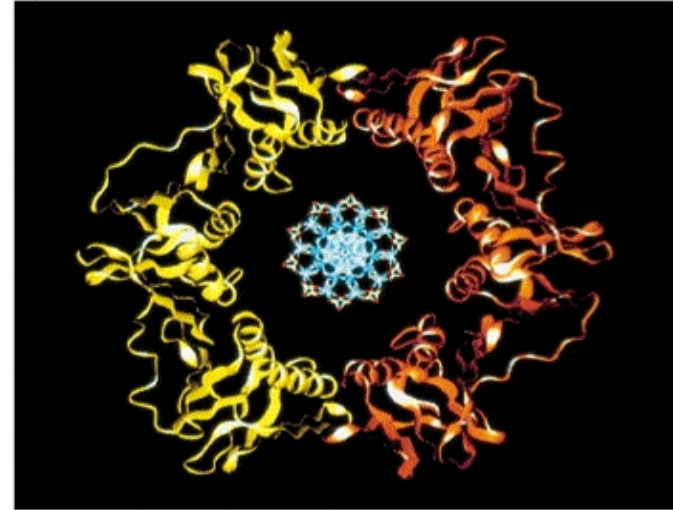


Protein-DNA interaction

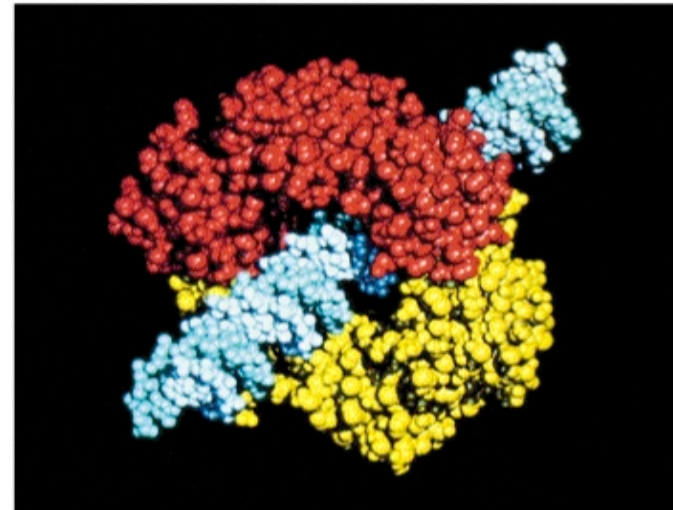
- Sequence independent
 - may interact with the negatively charged sugar-phosphate backbone
- Sequence dependent
 - need to recognize the bases *in the double-helical structure* (don't have access to the atoms involved in base pair H-bonds)

DNA Polymerase III - replication of the E.coli chromosome

- At least 10 different subunits
- α is polymerase, ϵ is 3'-exonuclease, θ unknown function
- β dimer forms a ring around DNA
 - acts as sliding clamp to hold res of PolIII in place
 - sequence independent

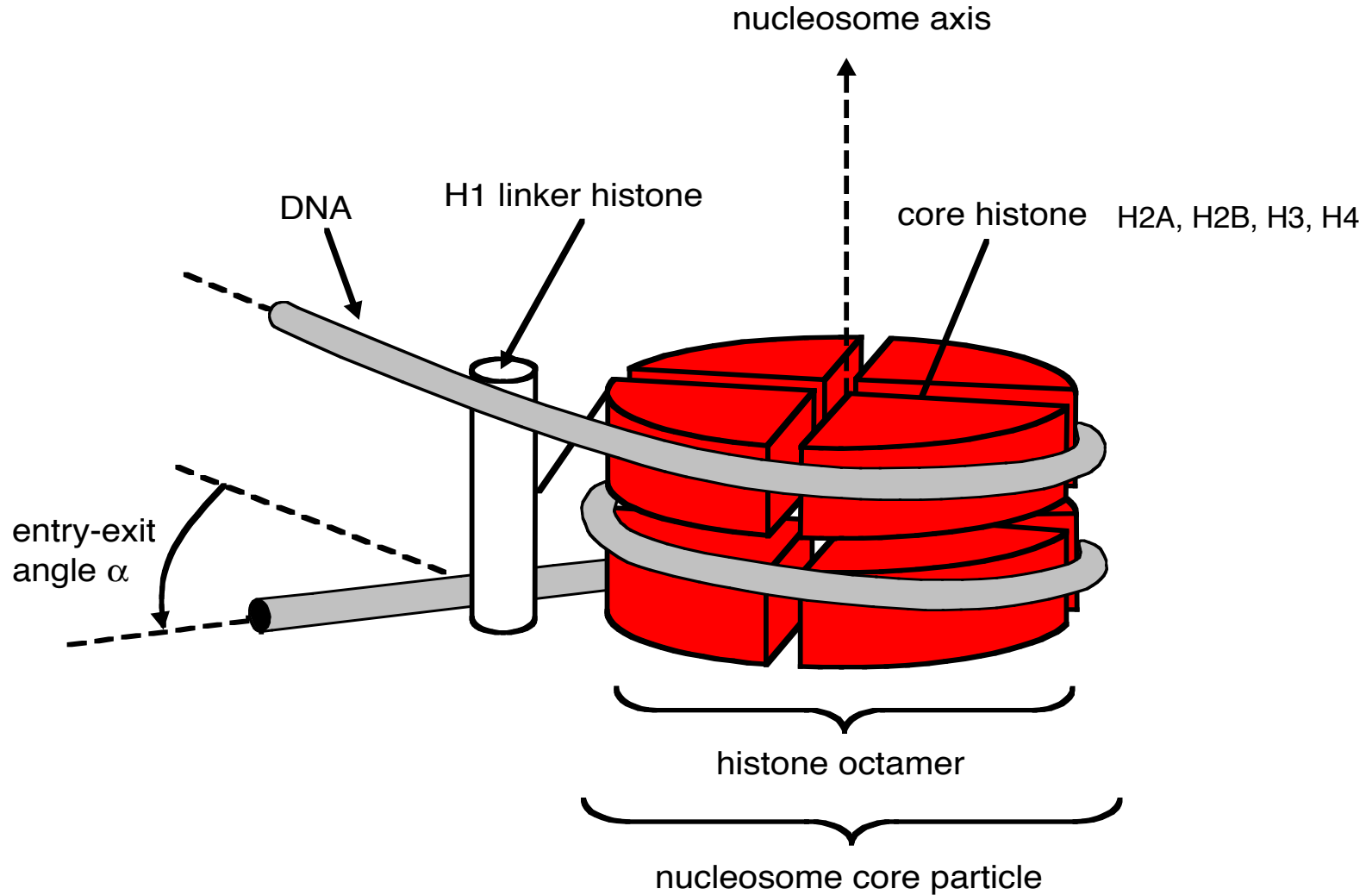


(a)

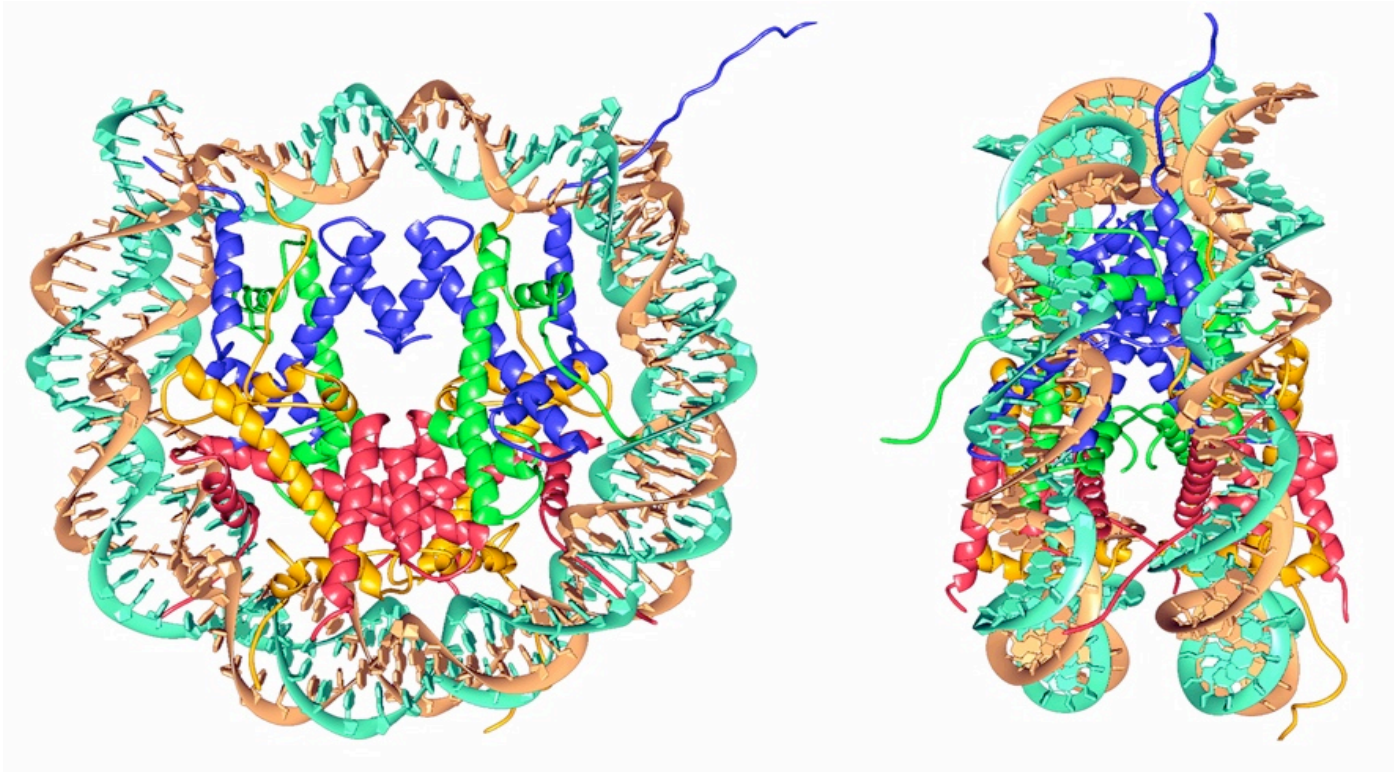


(b)

Histone octamer - Nucleosome

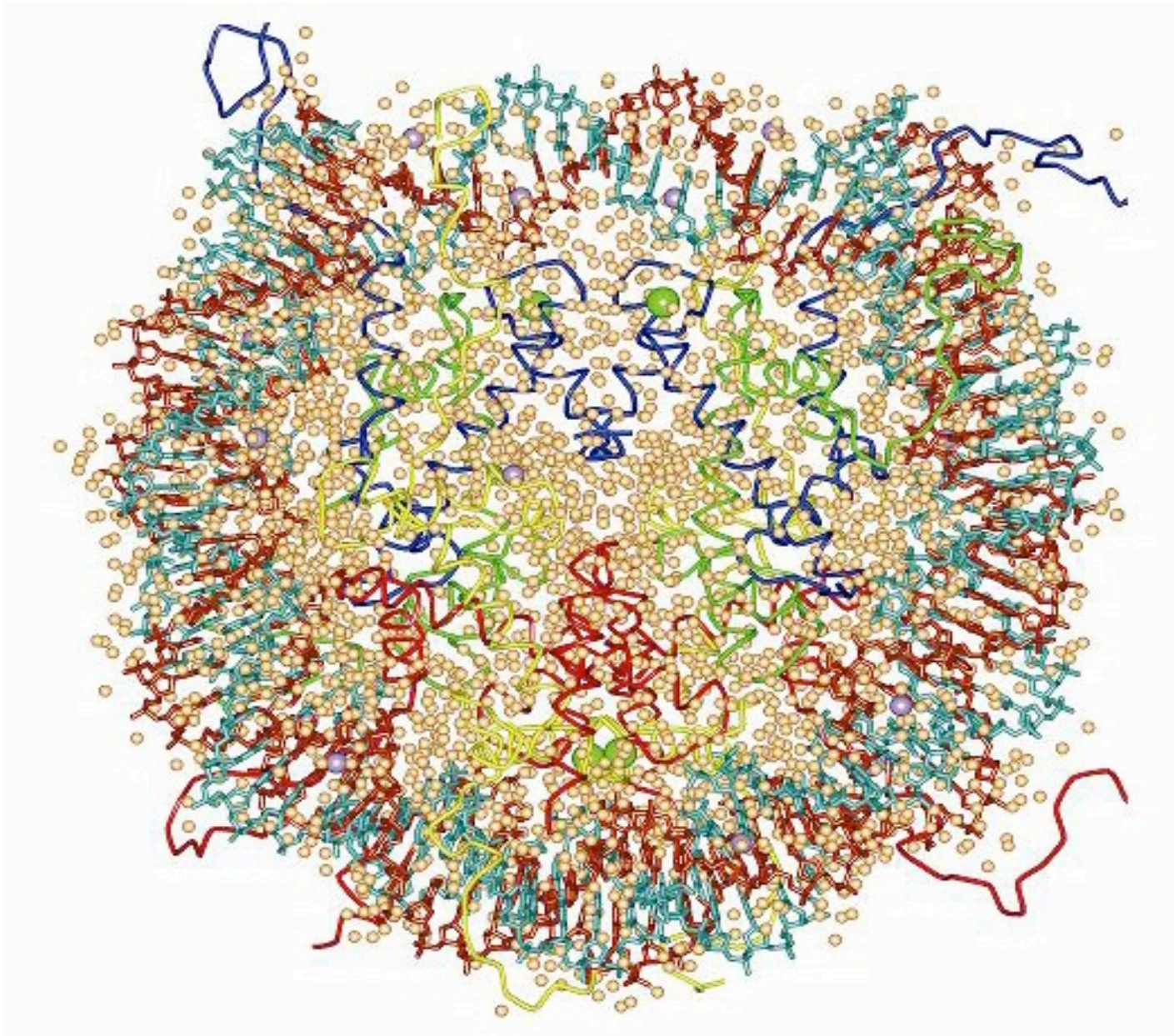


Nucleosome crystal structure



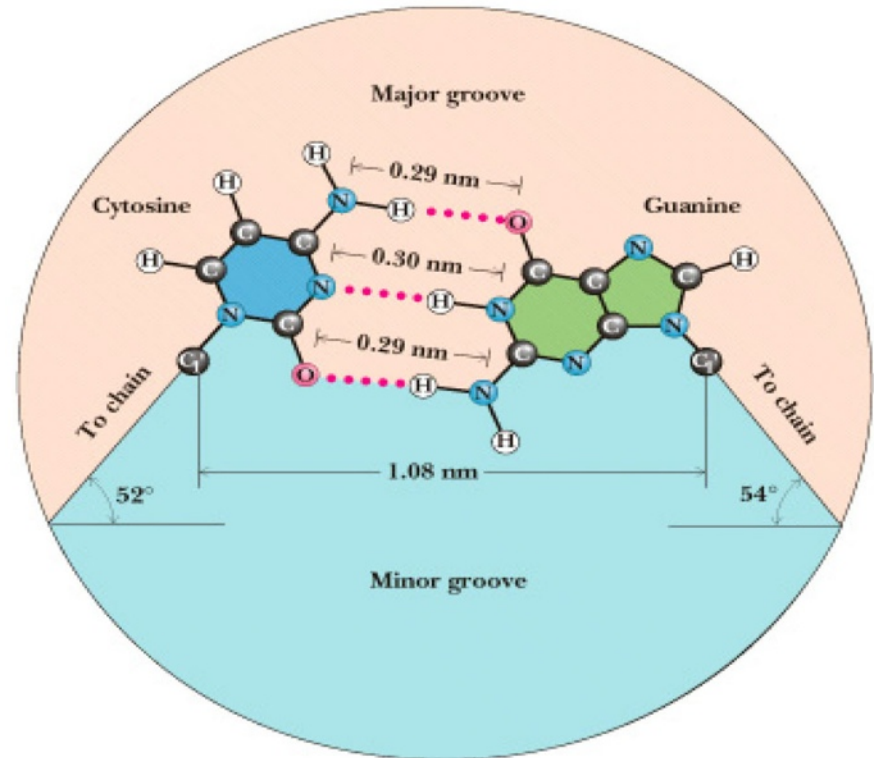
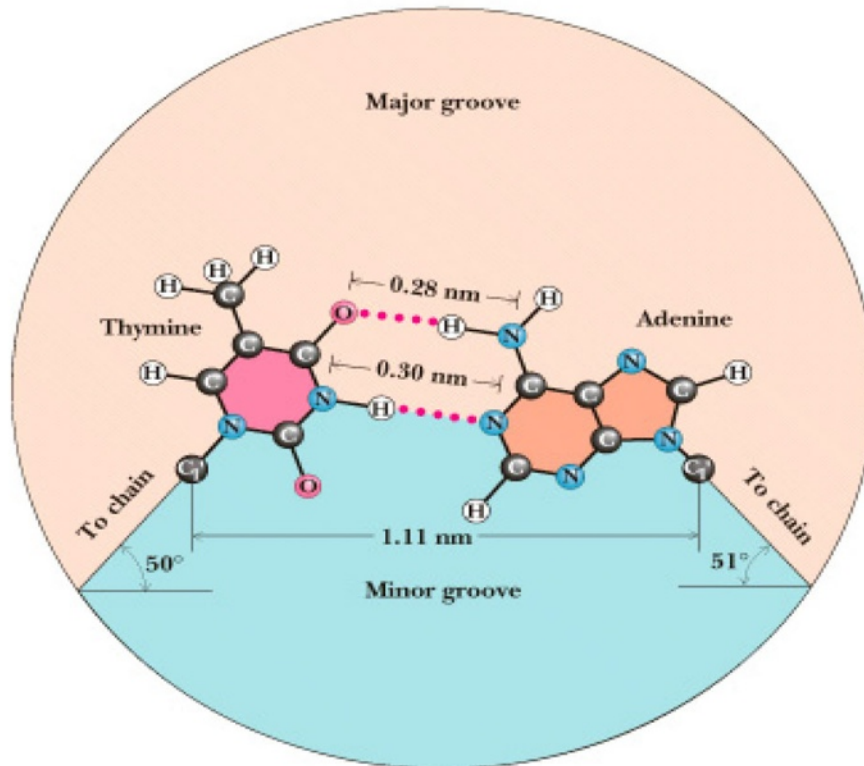
- Histone proteins are Lys & Arg rich – basic
- Histones form octamers
- Basic histones interact with the negatively charged phosphates of the DNA
- The DNA is wrapped around a core formed by 4 different histone polypeptides

Hydration of nucleosome



Specific binding of proteins to DNA

Which groove?



- H-bond donors and acceptors different in major groove for 2 types of base pair but very similar in the minor groove
- Minor groove too small to accommodate large protein probe

Which groove?

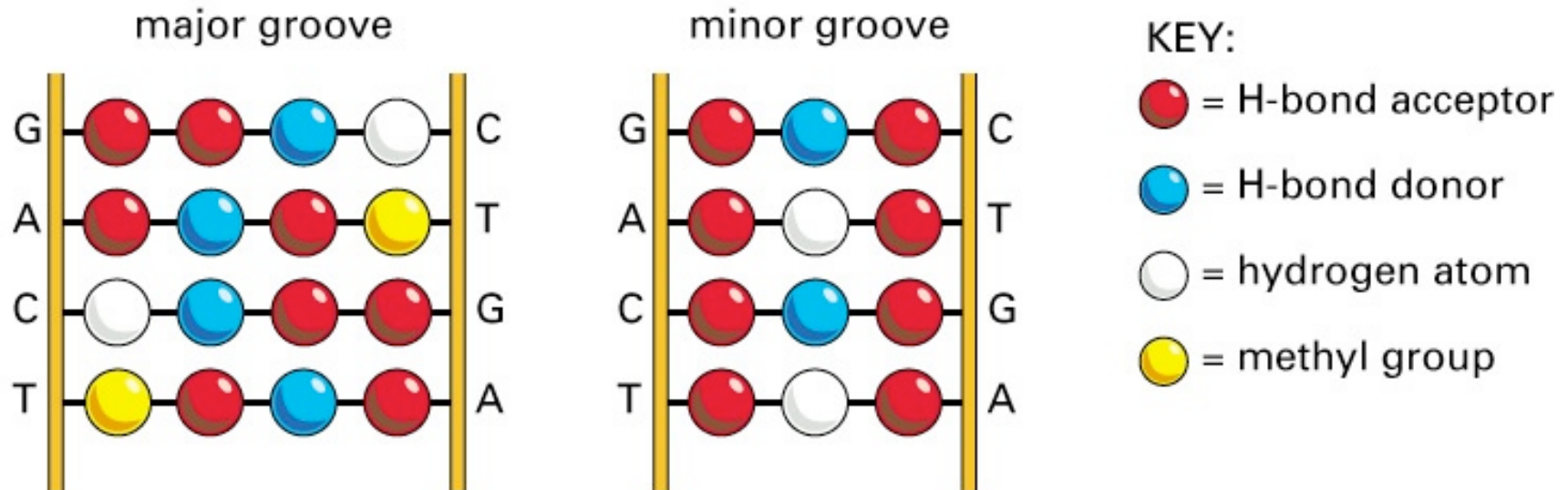
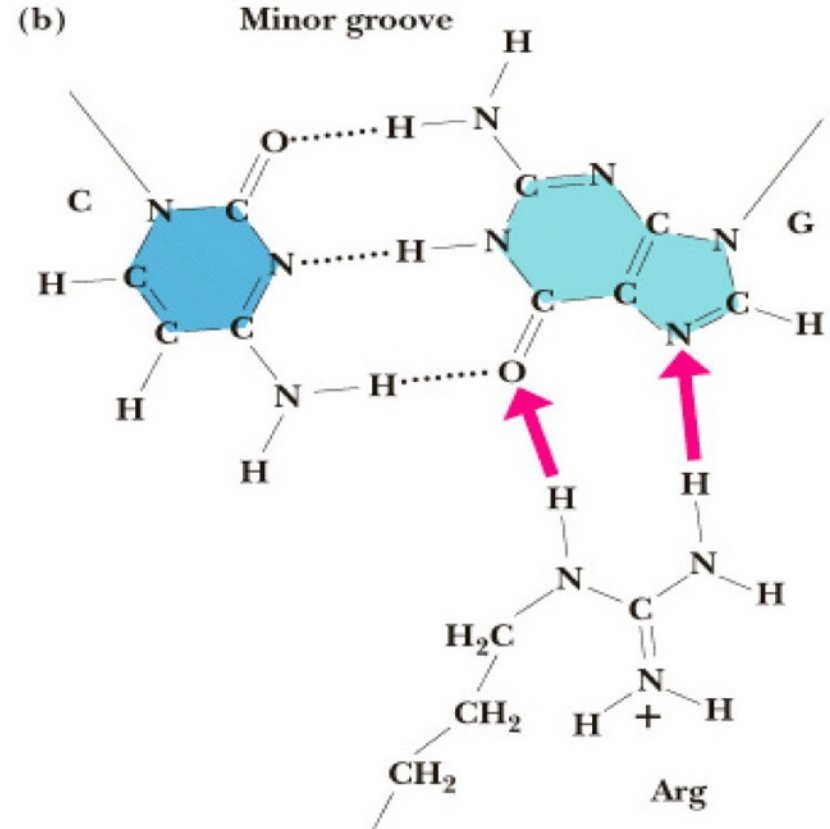
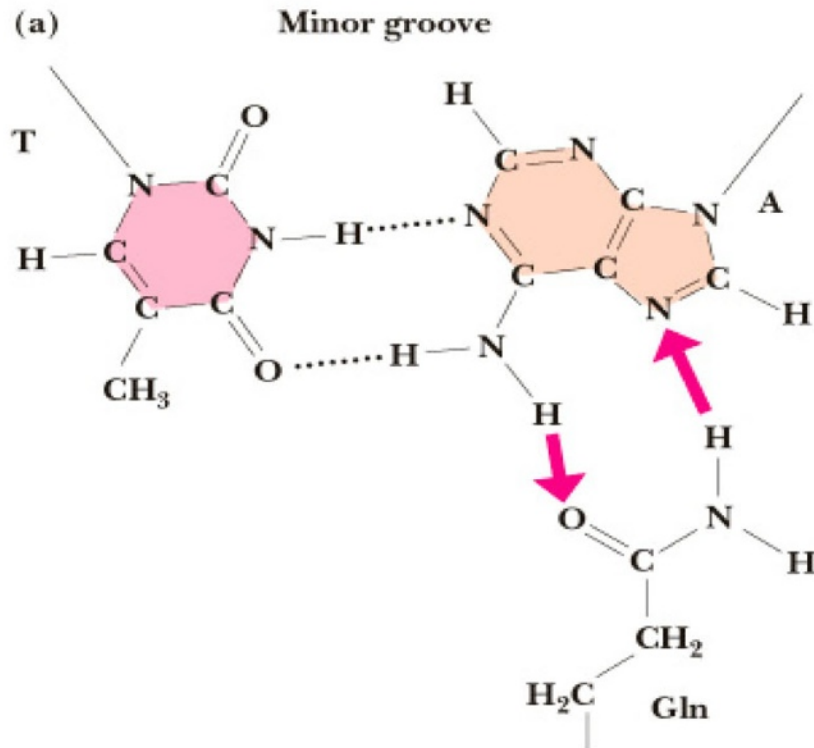


Figure 7-8. Molecular Biology of the Cell, 4th Edition.

- H-bond donors and acceptors different in major groove for 2 types of base pair but very similar in the minor groove
- Minor groove too small to accommodate large protein probe

Which groove?



- H-bond donors and acceptors different in major groove for 2 types of base pair but very similar in the minor groove
- Minor groove too small to accommodate large protein probe

Hydrogen bonding between asparagine and adenine

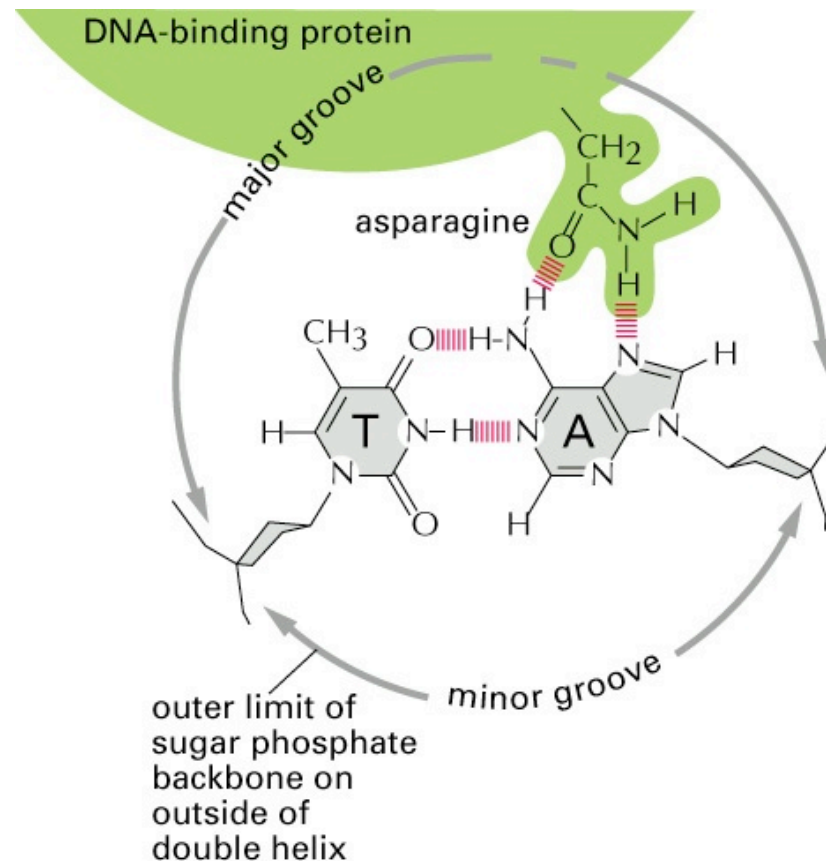


Figure 7-12. Molecular Biology of the Cell, 4th Edition.

Structural motifs in DNA-binding regulatory proteins

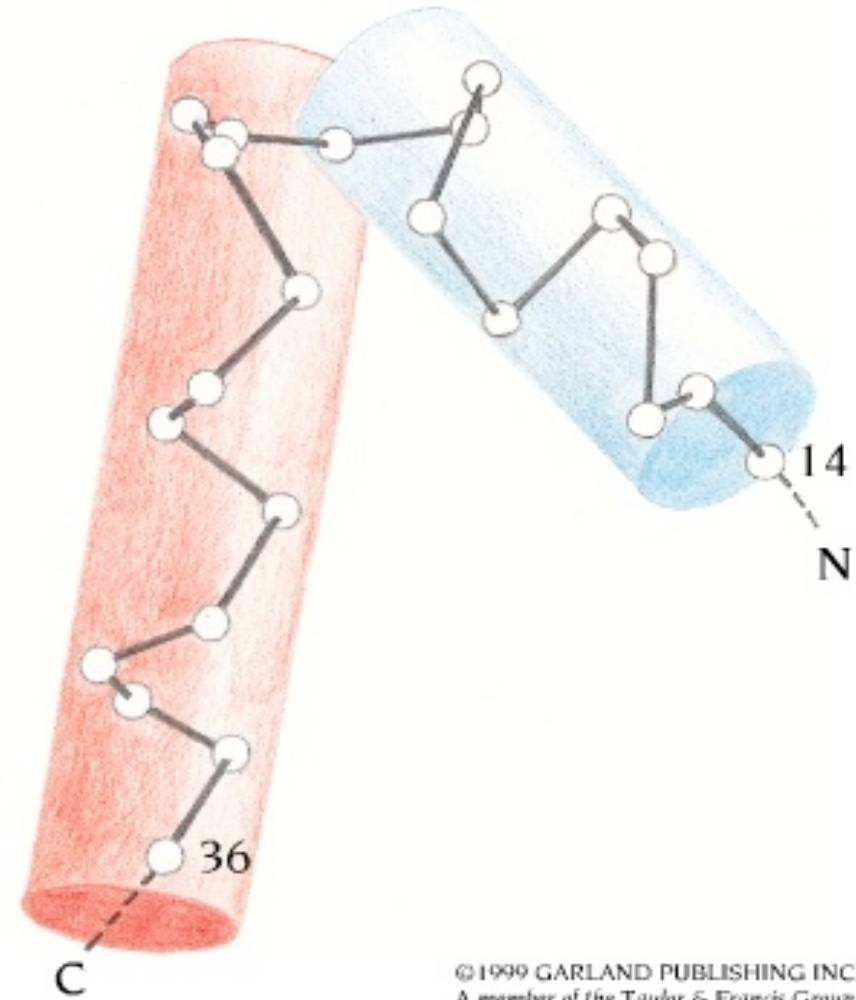
- Crucial feature must be atomic contacts between protein residues and bases and sugar-phosphate backbone of DNA
- Most contacts are in the major groove of DNA
- 80% of regulatory proteins can be assigned to one of three classes:
 - helix-turn-helix (HTH)
 - zinc finger (Zn-finger)
 - leucine zipper (bZIP)
 - helix-loop-helix (HLH)
- In addition to DNA-binding domains, these proteins often possess other domains that interact with other proteins

α Helices and DNA - a perfect fit

- DNA-binding proteins often have an α -helical segments that fit directly into the major groove of B-form DNA
- Diameter of helix is 1.2 nm (12 Angstroms)
 - Major groove of DNA is about 1.2 nm wide and 0.6 to 0.8 nm deep
- Proteins can recognize specific sites (sequences) in DNA

The helix-turn-helix motif

- Generally bind as dimers to dyad-symmetric sites on DNA
- All contain two alpha helices separated by a loop with a beta turn
- The C-terminal helix fits in major groove of DNA
- N-terminal helix stabilised by hydrophobic interactions with C-terminal helix



The helix-turn-helix motif: homeodomain transcription factor

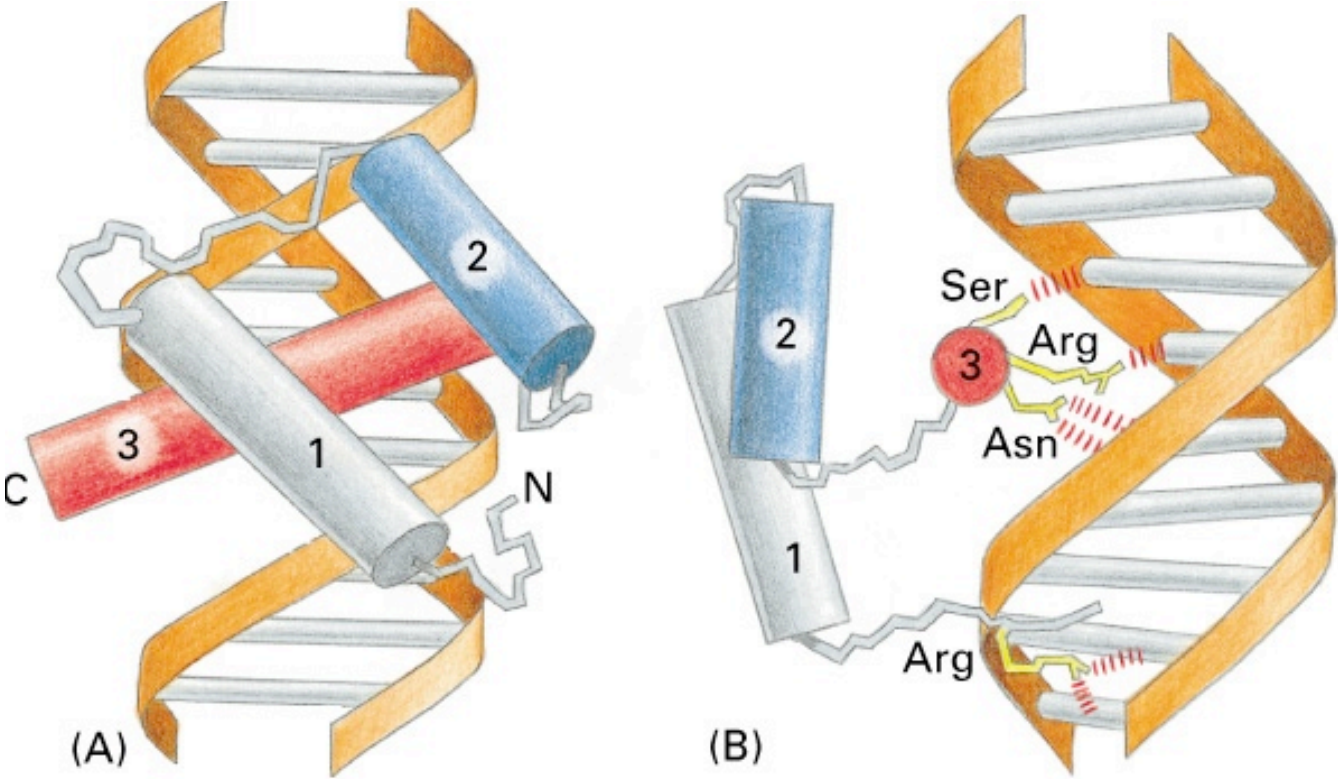


Figure 7-16. Molecular Biology of the Cell, 4th Edition.

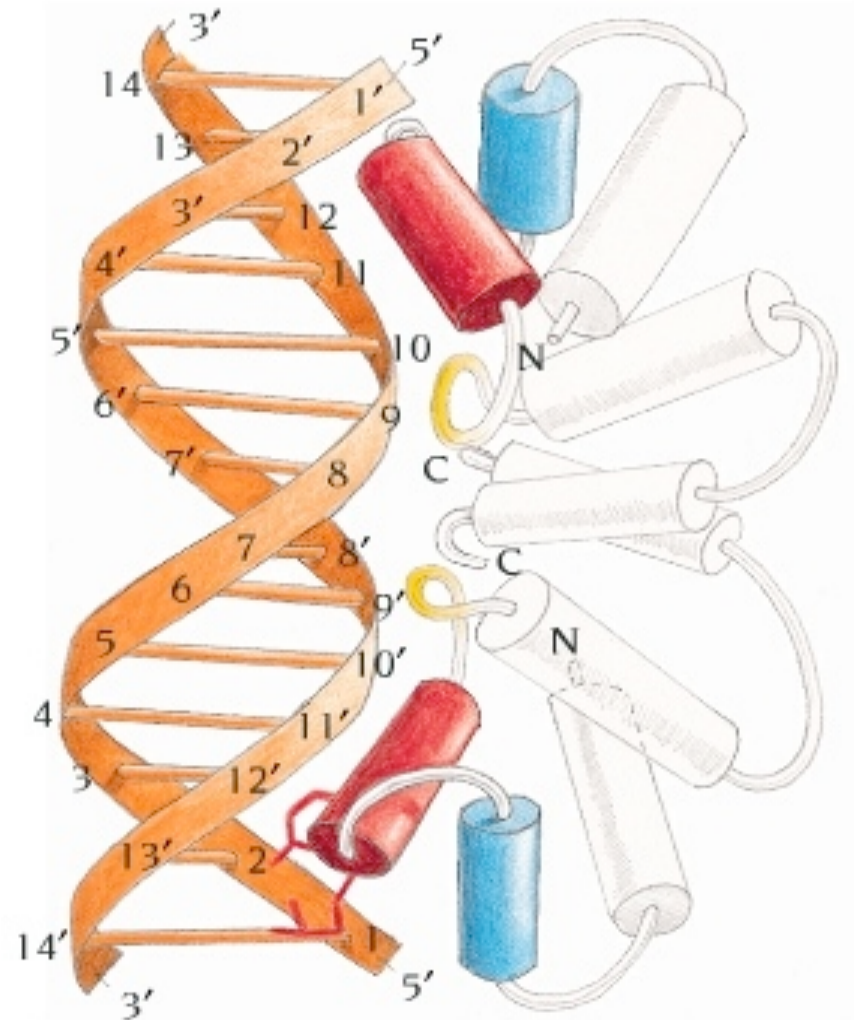
Binding of a protein dimer to a symmetric DNA binding site



Figure 7–15. Molecular Biology of the Cell, 4th Edition.

The helix-turn-helix motif (tryptophan repressor, lambda Cro, lambda repressor, Lac repressor etc)

- Generally bind as dimers to dyad-symmetric sites on DNA
- All contain two alpha helices separated by a loop
- The C-terminal helix fits in major groove of DNA
- N-terminal helix stabilised by hydrophobic interactions with C-terminal helix



Cys₂His₂ zinc finger protein family

largest known
DNA-binding
family in
multicellular
organisms

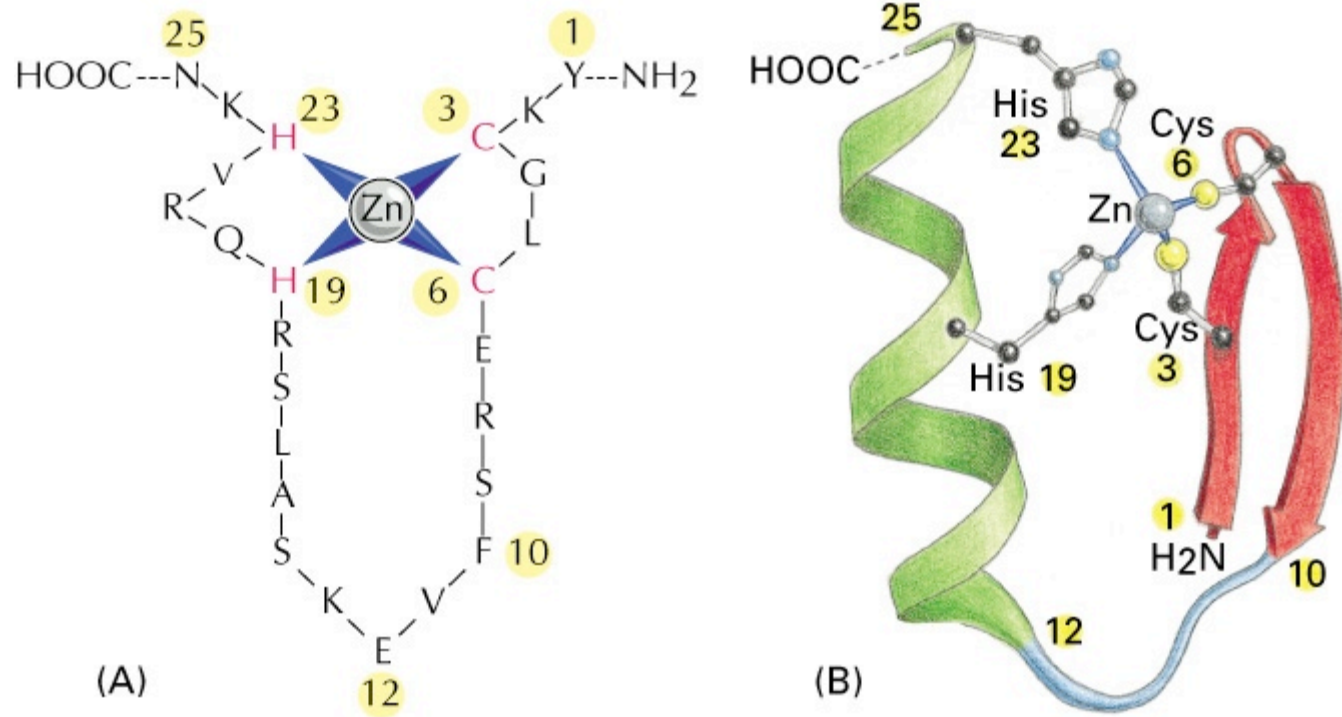


Figure 7-17. Molecular Biology of the Cell, 4th Edition.

Cys₂His₂ zinc finger: DNA binding

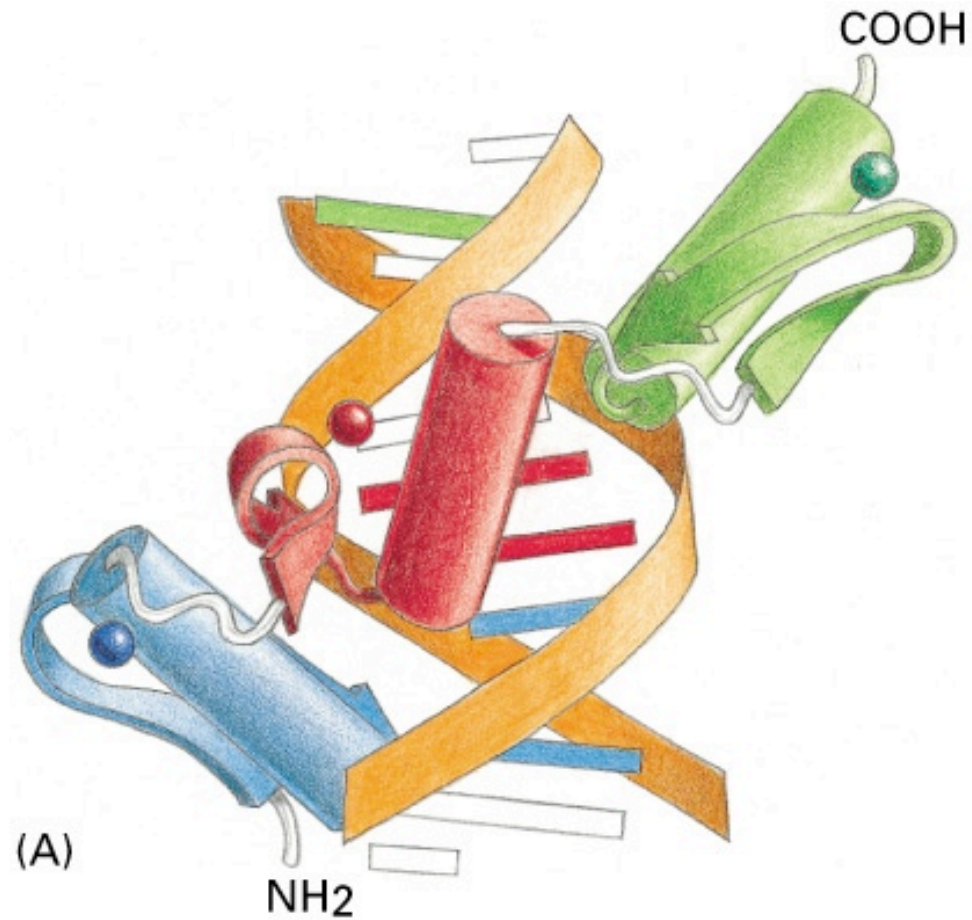


Figure 7-18 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Estrogen receptor binds DNA via zinc fingers

- Homodimer
- 2 Zn fingers interact with the DNA
- 2 additional Zn ions stabilized protein fold

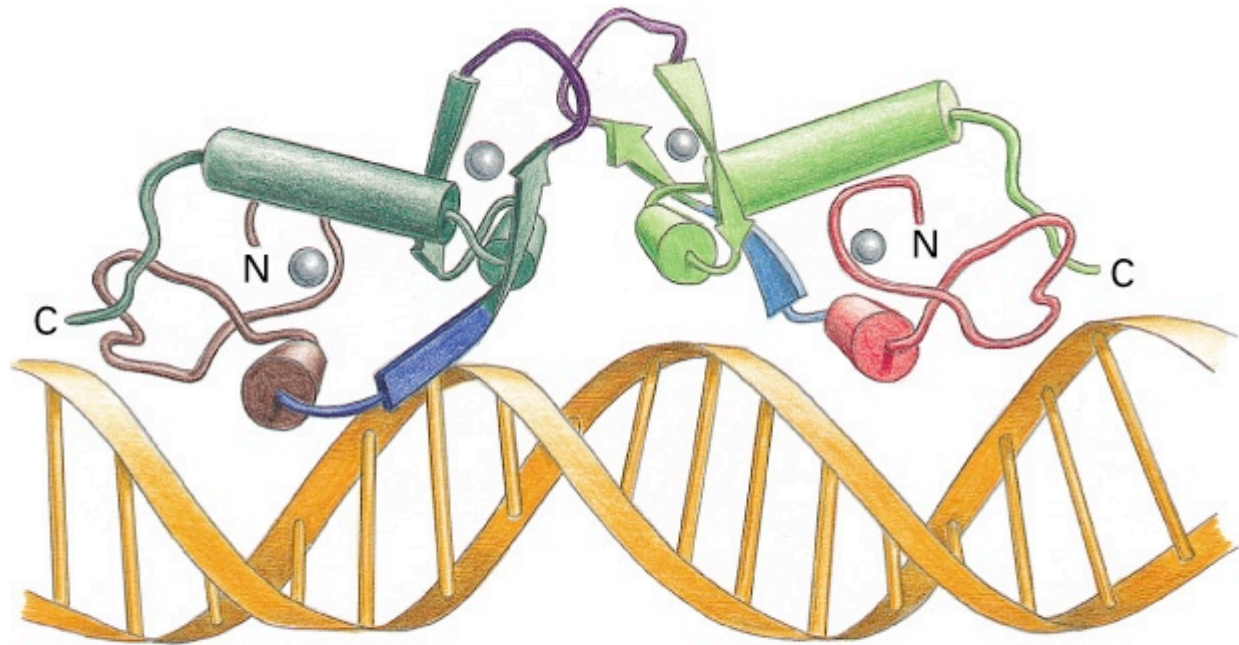


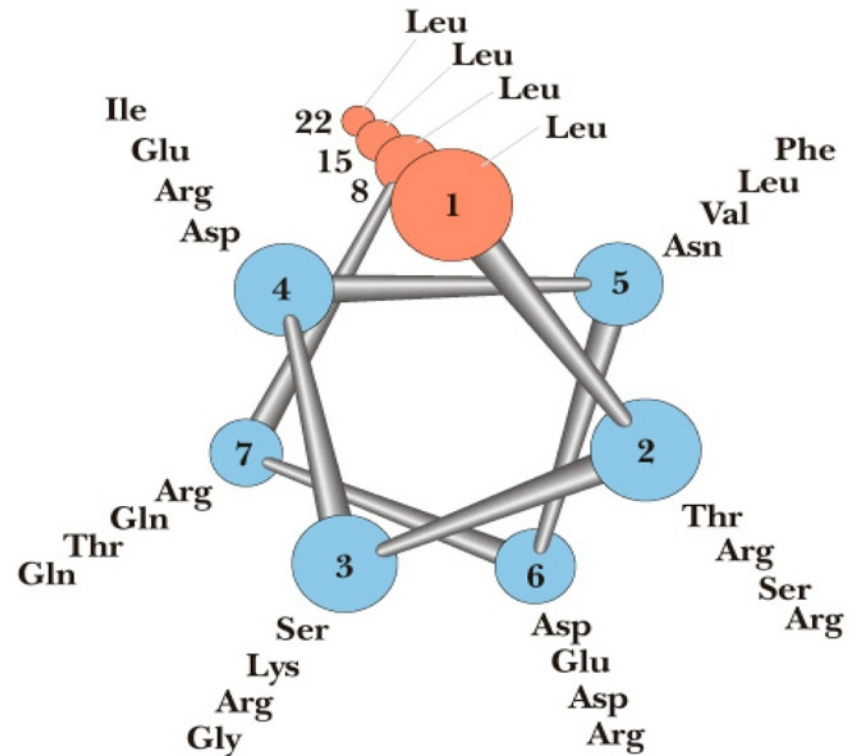
Figure 7-19. Molecular Biology of the Cell, 4th Edition.

The Leucine Zipper Motif

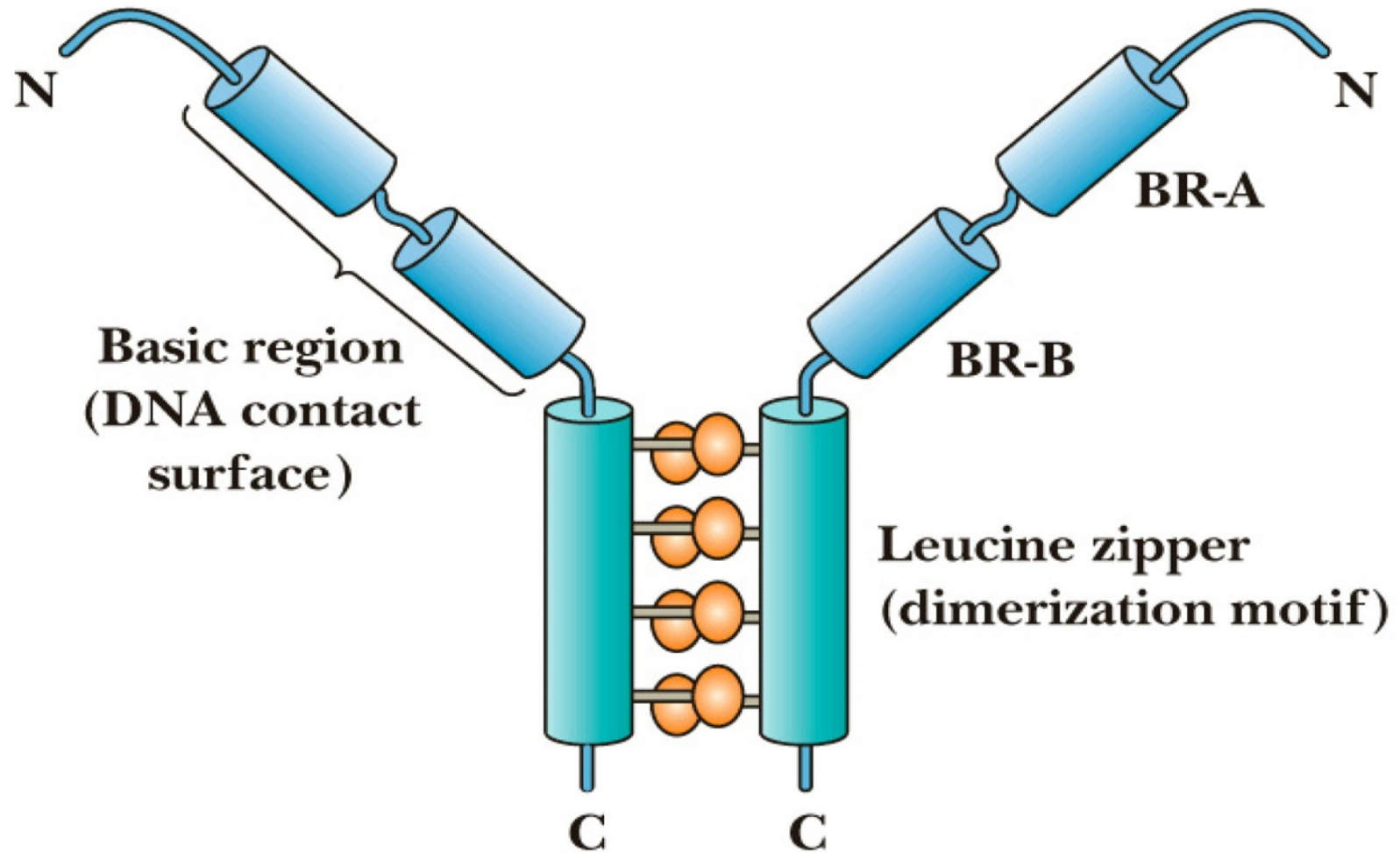
- First found in C/EBP, a DNA-binding protein in rat liver nuclei
- Present in nearly all organisms
- Characteristic features: a 28-residue sequence with Leu every 7th position and a "basic region"
- An amphipathic alpha helix and a coiled-coil dimer (hydrophobic core)

Helical wheel from C/EBP (CAAT enhancer binding protein)

- Structure of α -helix makes every 7th residue lie on one side
 - all the Leu (hydrophobic residues line up)
- When two helices like this coil together the Leu stripes will form a hydrophobic core



bZIP Protein



- Zipper helps for dimer
- Basic regions interact with DNA

Leucine zipper (bZIP, GCN4)

- Zipper helps for dimer
- Basic regions interact with DNA



Figure 7–21. Molecular Biology of the Cell, 4th Edition.

Many proteins recognize a DNA sequence via alpha-helices positioned in the major groove of the DNA

but

there are also very different DNA binding proteins that do not fit into the simple classification of binding motifs

TATA box binding protein at the promoter

