DNA

Created by Naomi Boxall
Concept by John Tweedie
Institute of Molecular Biosciences
Massey University, New Zealand

http://www.massey.ac.nz/~wwbioch/DNAprot/tutehome/tutetext.htm

Chapter 8.
DNA recognition in prokaryotes by helix-turn-helix motifs

1. ***Helix-turn-helix proteins***
2. *Zinc finger proteins*
3. *Leucine zipper proteins*
4. *Beta-scaffold factors*
5. *Others*

Repressor and Cro proteins operate a prokaryotic genetic switch region

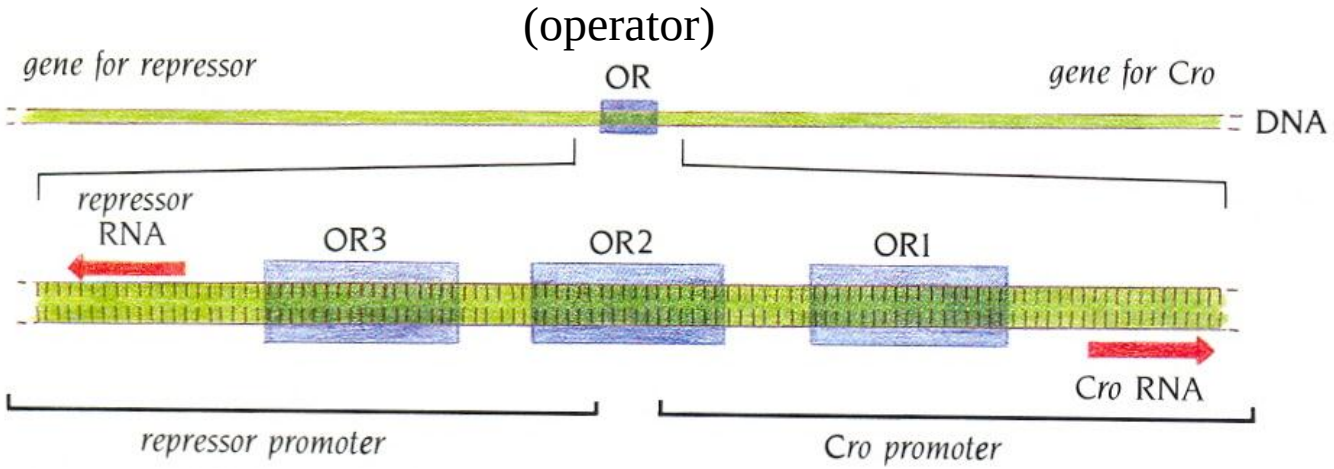
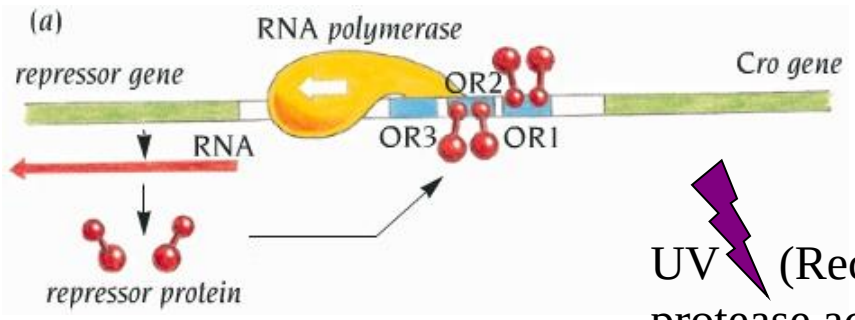
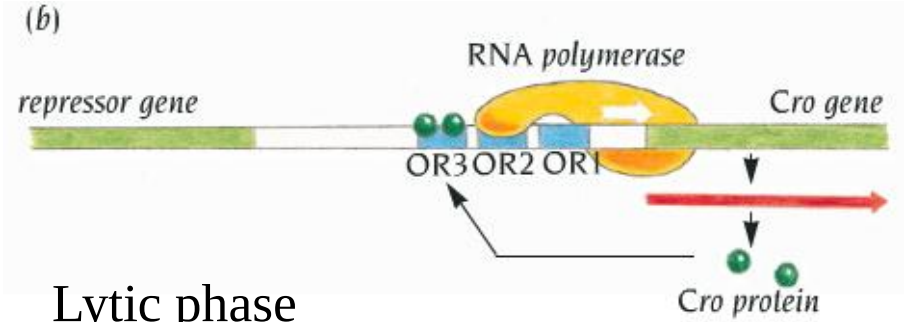


Figure 7.1 A region of DNA in the related bacteriophages lambda, 434, and P22 that controls the switch for synthesis of new phage particles. Two structural genes are involved in this switch; one coding for a repressor protein and one coding for the Cro protein. Between these genes there is an operator region (OR) that contain three protein binding sites—OR1, OR2, and OR3.



UV (RecA → protease activation) → Lytic phase

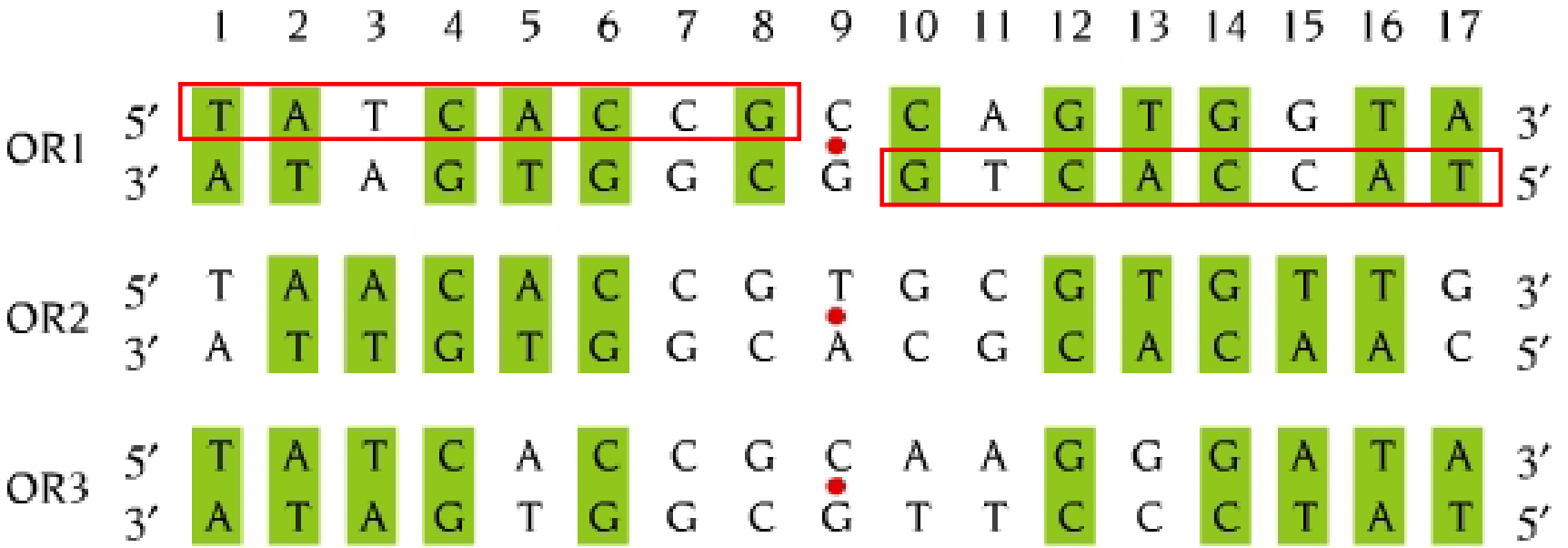


"Turning off syn of Cro"
or "activator for its own syn"

"Turning off syn of repressor"

Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.130.

Table 8.1 The nucleotide sequences of the three protein-binding regions OR1, OR2, and OR3 of the operator of bacteriophage lambda

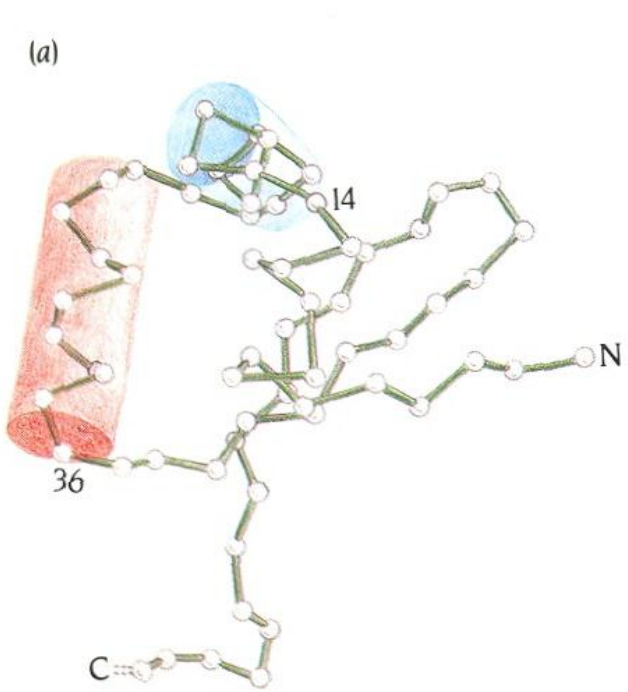


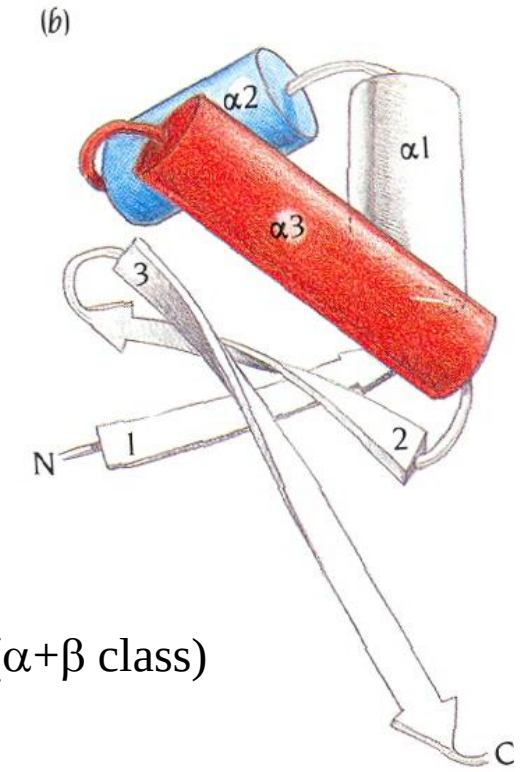
Palindromic base pairs that are most frequent at the two ends are green, and the pseudo-twofold symmetry axis is indicated by a red dot.

Partly palindromic sequences: 8 bp per a monomer
Sequence information shows a pseudo 2-fold symmetry

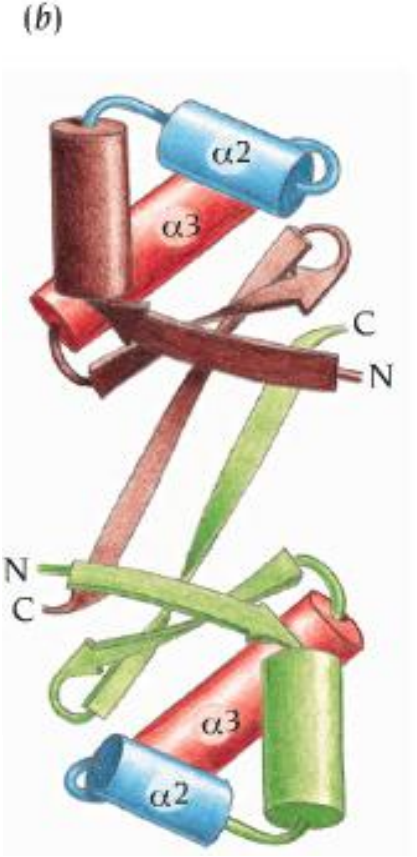
Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.131.

Dimeric structure of lambda Cro





(α+β class)



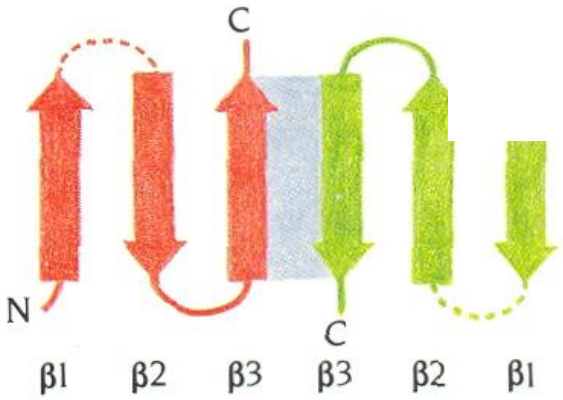


Figure 7.4 The DNA-binding protein Cro from bacteriophage lambda contains amino acid residues that fold into three α helices and three β strands. (a) A plot of t C_α positions of the first 62 residues of the polypeptide chain. The four C-terminal residues are not visible in the electron density map. (Adapted from Anderson et al., *Nature* 290: 755, 1981.) (b) A schematic diagram of the subunit structure. α helices 2 and 3 that form the helix-turn-helix motif are colored blue and red, respectively. Tl view is different from that in (a). (Adapted from D. Ohlendorf et al., *J. Mol. Biol.* 16 757, 1983.)

Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.132.

Dimeric structure of N-terminal domain of lambda repressor

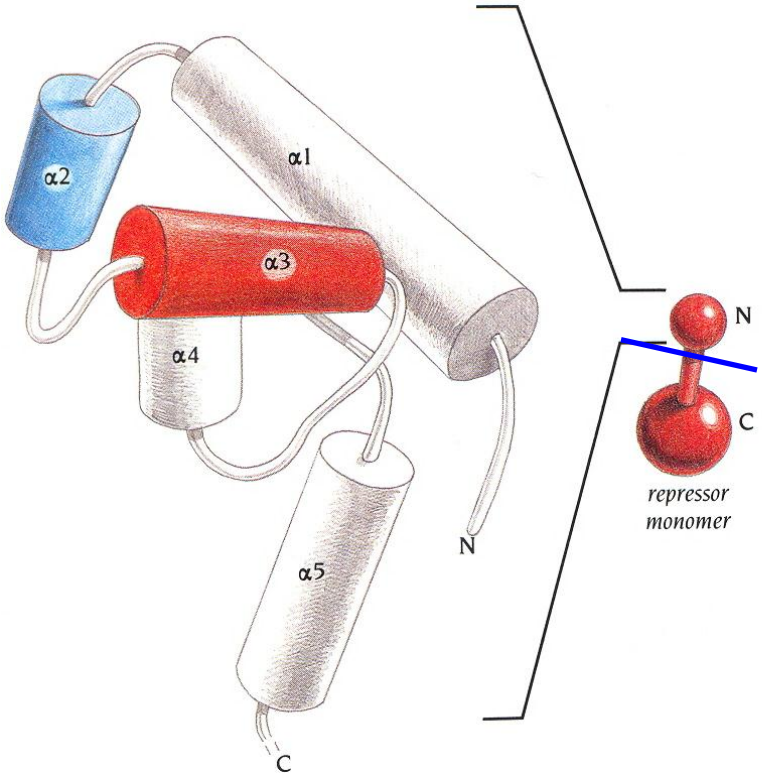
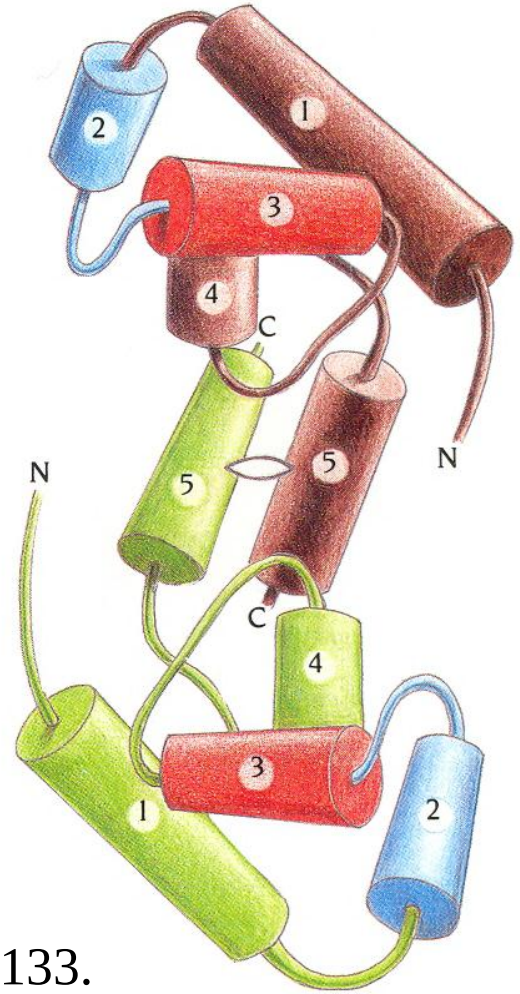


Figure 7.7 The N-terminal domain of lambda repressor, which binds DNA, contains 92 amino acid residues folded into five α helices. Two of these, α2 (blue) and α3 (red) form a helix-turn-helix motif with a very similar structure to that of lambda Cro shown in Figure 7.5. The complete repressor monomer contains in addition a larger C-terminal domain. (Adapted from C. Pabo and M. Lewis, *Nature* 298: 445, 1982.)

DNA binding domain

dimerization domain

Figure 7.8 The N-terminal domains of lambda repressor form dimers, in spite of the absence of the C-terminal domains, which are mainly responsible for dimer formation in the intact repressor. The dimers are formed by interactions between α helix 5 from each subunit. The different subunits are colored green and brown, except the helix-turn-helix motif, which is colored blue and red as in Figure 7.5. (Adapted from C. Pabo and M. Lewis, *Nature* 298: 446, 1982.)



Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.133.

Both lambda Cro and repressor proteins have a specific DNA-binding motif

"& also Catabolite-gene Activating Protein"

(a) (stabilizing helix) (recognition helix)

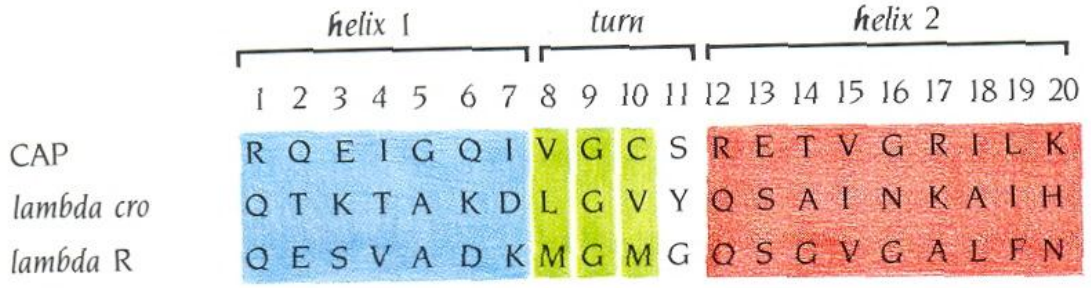
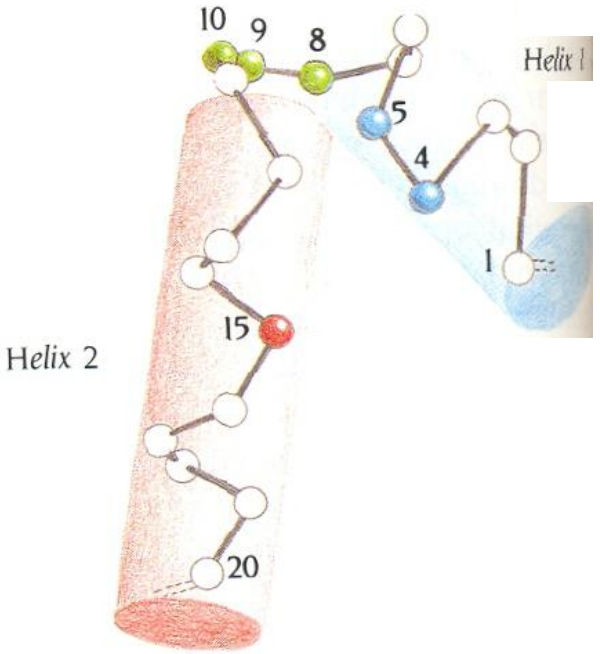


Figure 7.21 (a) Amino acid sequences for the helix-turn-helix motif of CAP, lambda Cro, and lambda repressor. (b) Schematic diagram of the helix-turn-helix motif. The positions subject to constraints in the amino acid sequence are colored. The C_α positions of each residue have been projected onto a plane, and the helical regions are outlined.

(b)

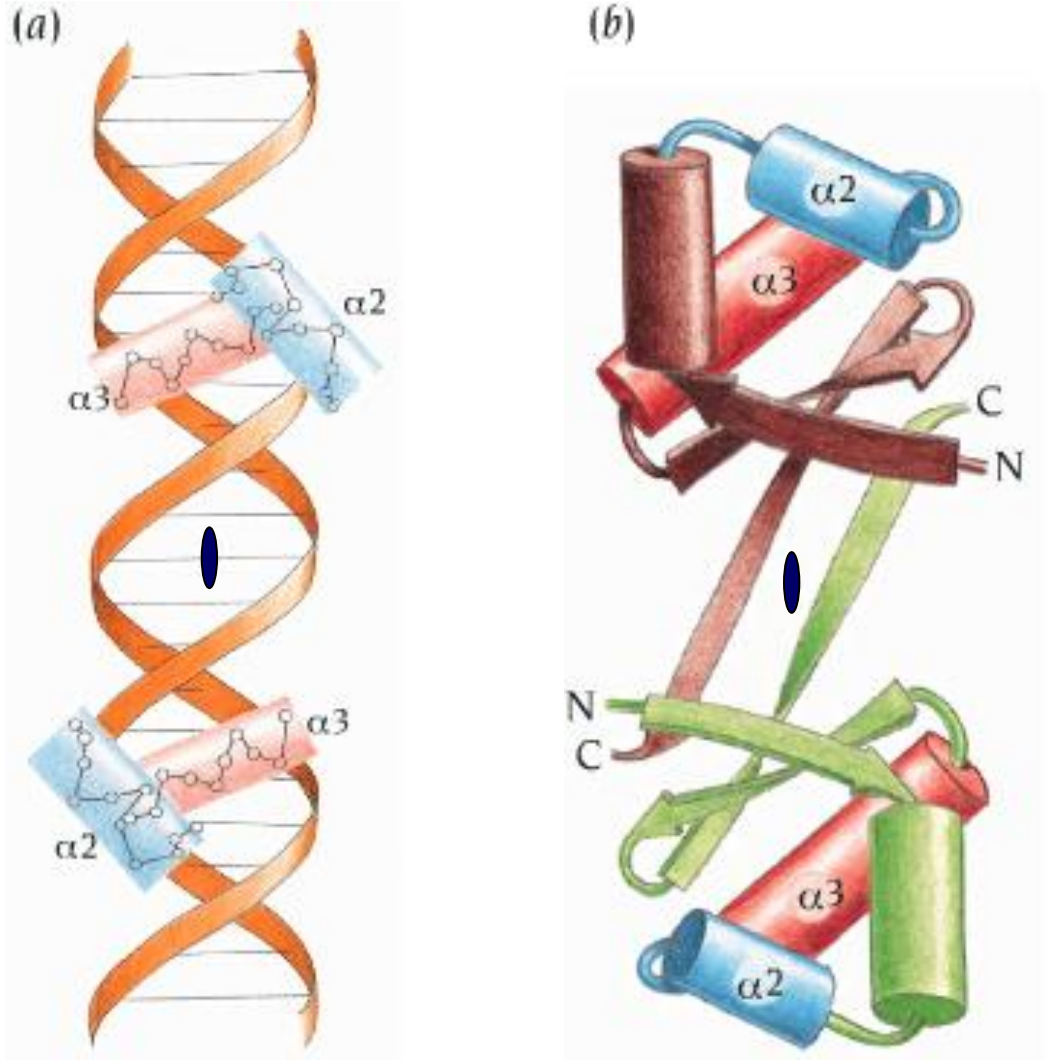


Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.134.

Model building predicts Cro-DNA complex

34 Å apart between two α3 helices in a dimer → 10 bp apart DNA (1-turn)

recognition helix (α3) / stabilizing helix (α2)

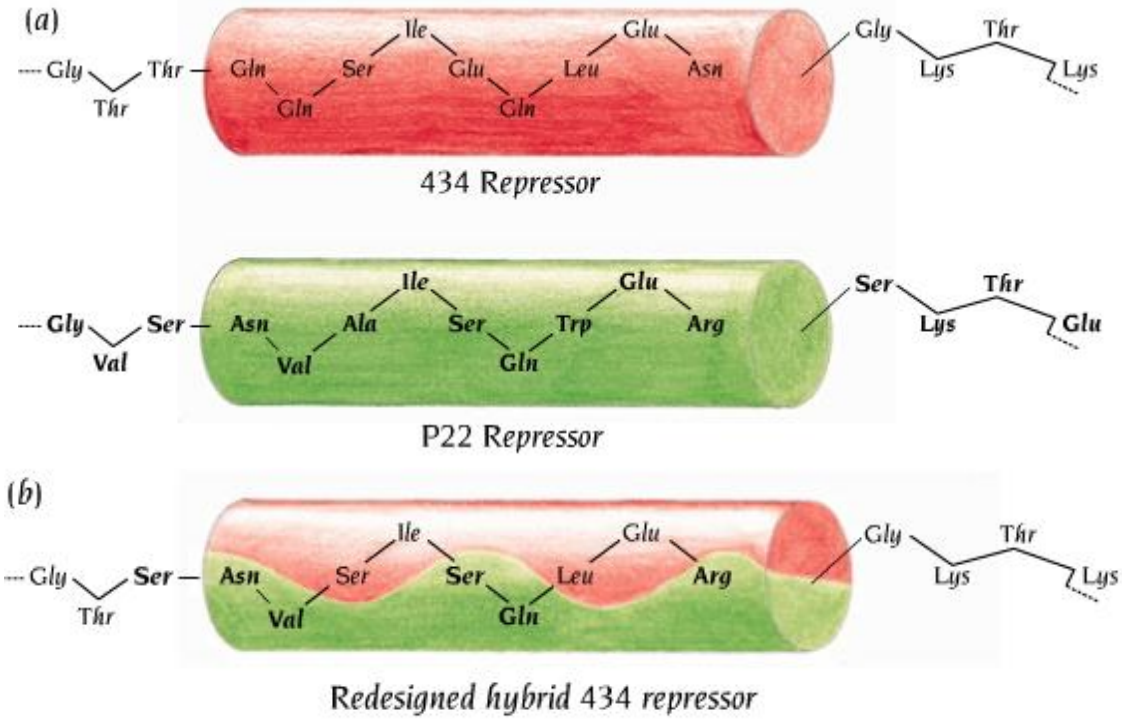


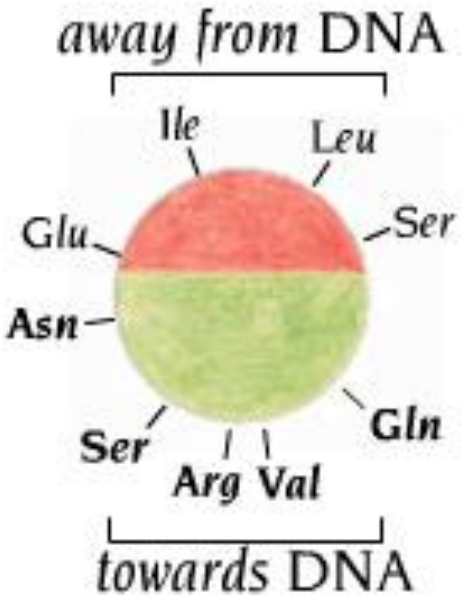
Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.134.

Genetic studies agree with the structural model

Altered DNA binding specificity by redesigning recognition helix (by Mark Ptashne's group at Harvard)

- Recognition helix swap between repressor and Cro → changes in the binding affinity
- Changes in amino acids of repressor (between 434 and P22 phage) → engineered 434 repressor binds to P22 operator, not to 434 operator





Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.136.

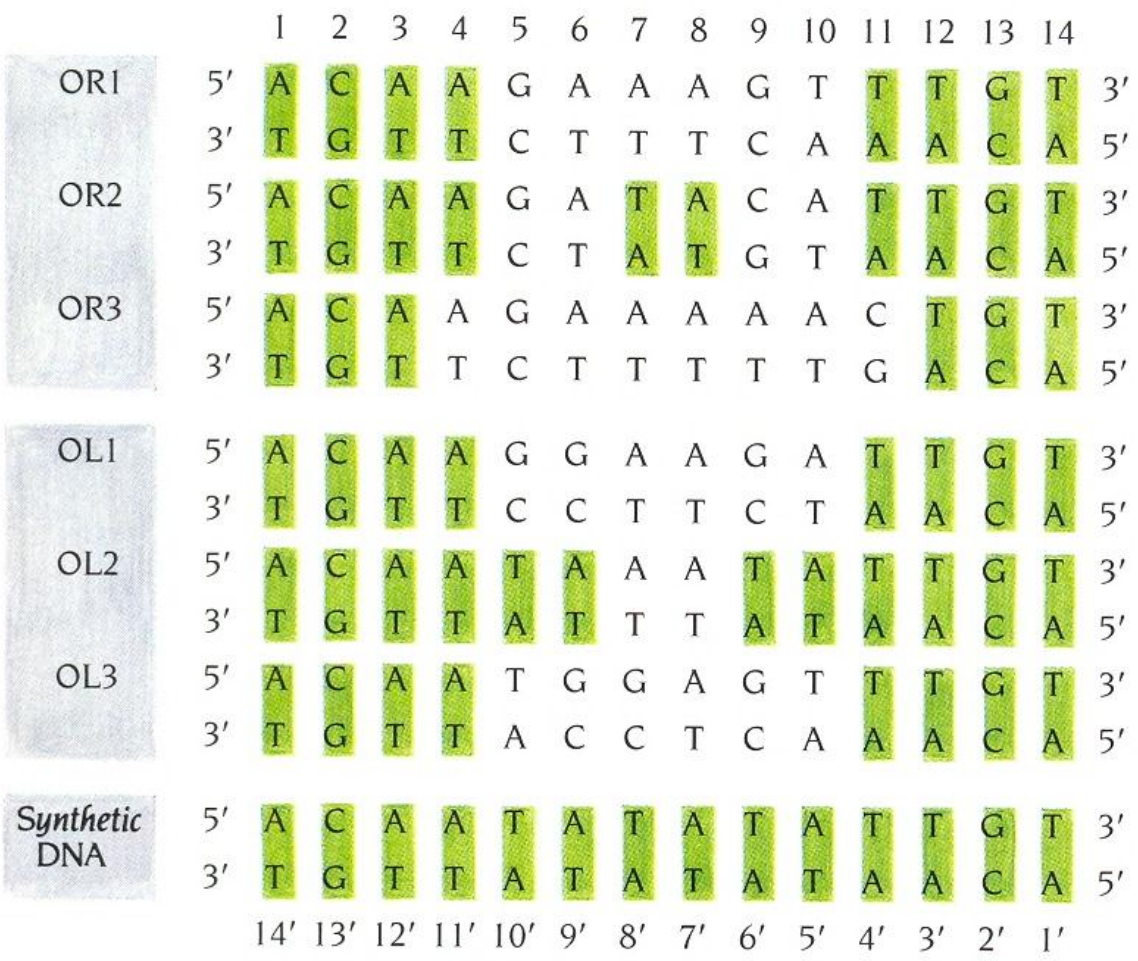


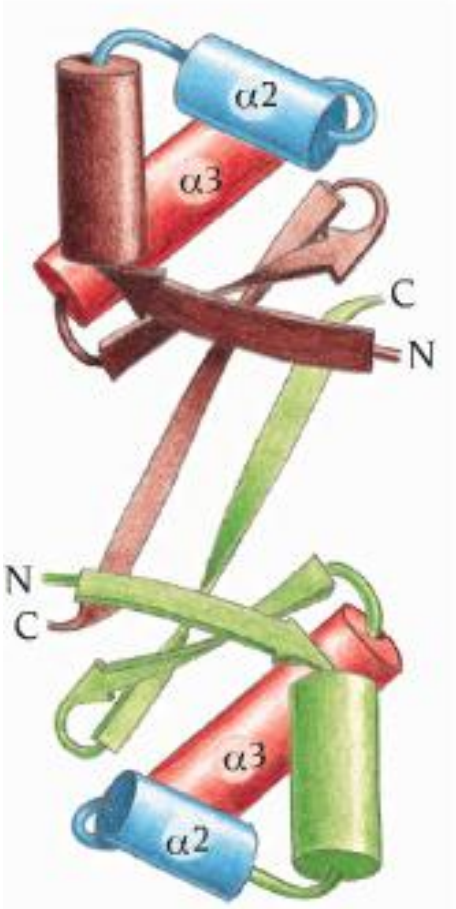
Figure 7.12 There are six operator regions (OR1–OR3 and OL1–OL3), each of 14 base pairs in bacteriophage 434. The palindromic base pairs of these regions are marked in green. Crystal structures have been determined of complexes between both 434 Cro and the repressor fragment with synthetic DNA fragments—one 14 base pairs long (a 14 mer), which is completely palindromic, and one 20 base pairs long (a 20 mer), which contains the sequence of OR1 in its middle region.

Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.137.

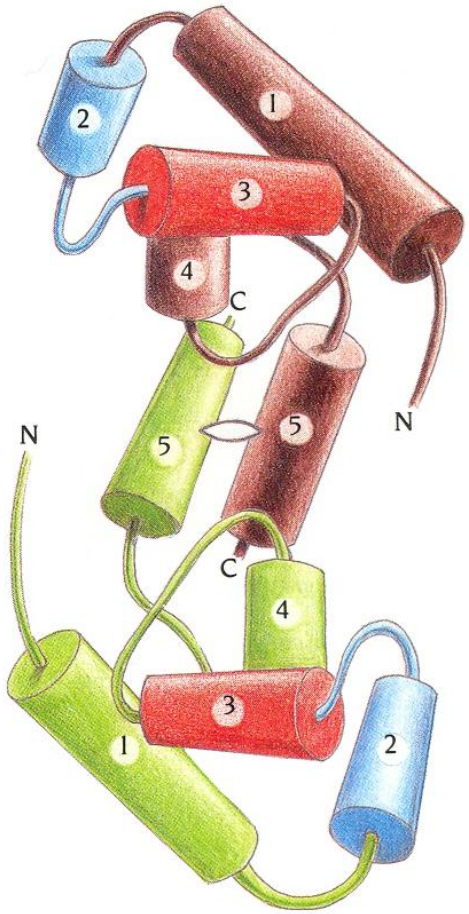
The structures of DNA-binding domain are very similar
(434 Cro *vs* 434 repressor: 48% sequence identity)
(434 Cro *vs* lambda repressor: 26% sequence identity)

(*b*)

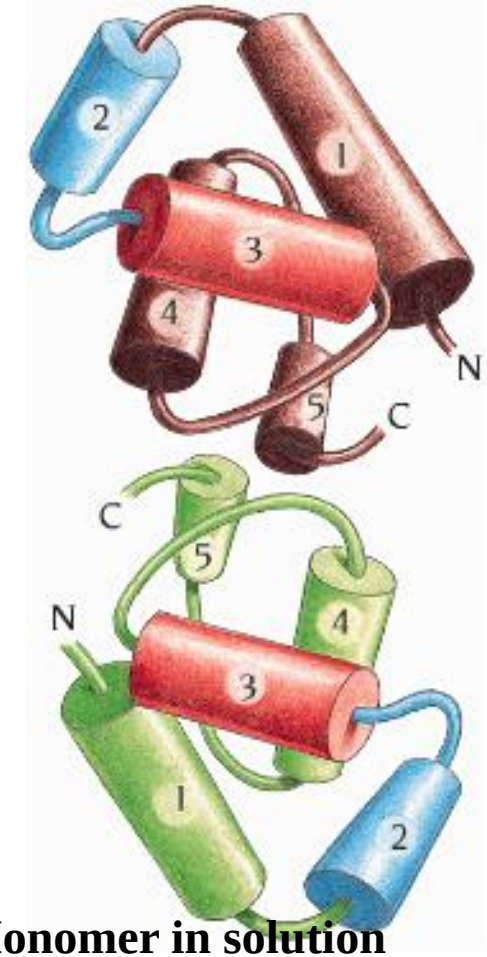
434 Cro



lambda repressor



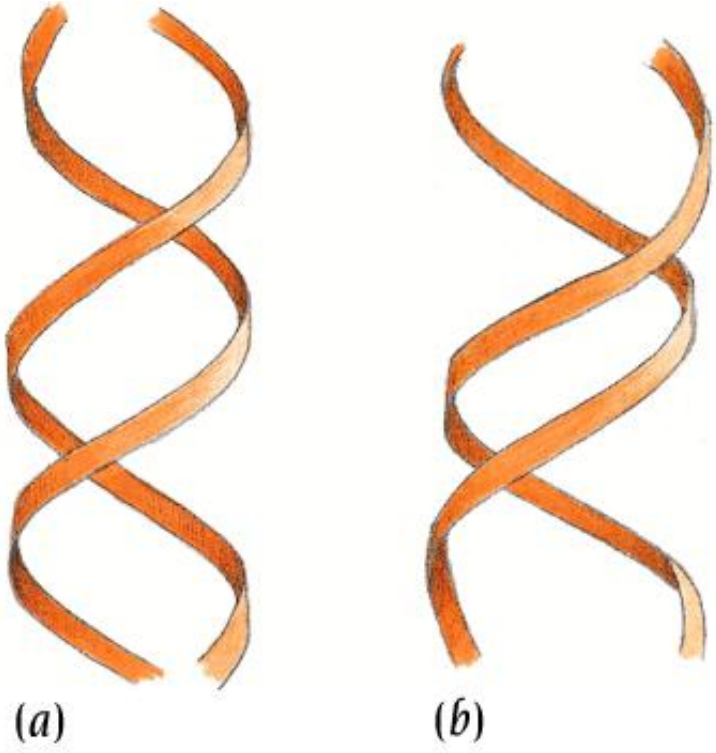
434 repressor



Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.134, 137.

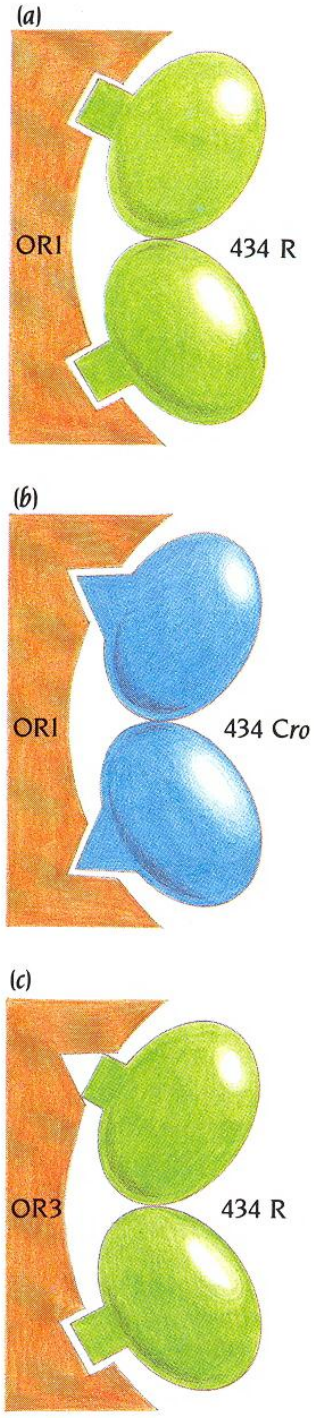
Monomer in solution
Dimer complexed with DNA

The protein impose precise distortions on the B-DNA in the complexes (DNA helical axis bent toward a repressor)



Normal B-DNA

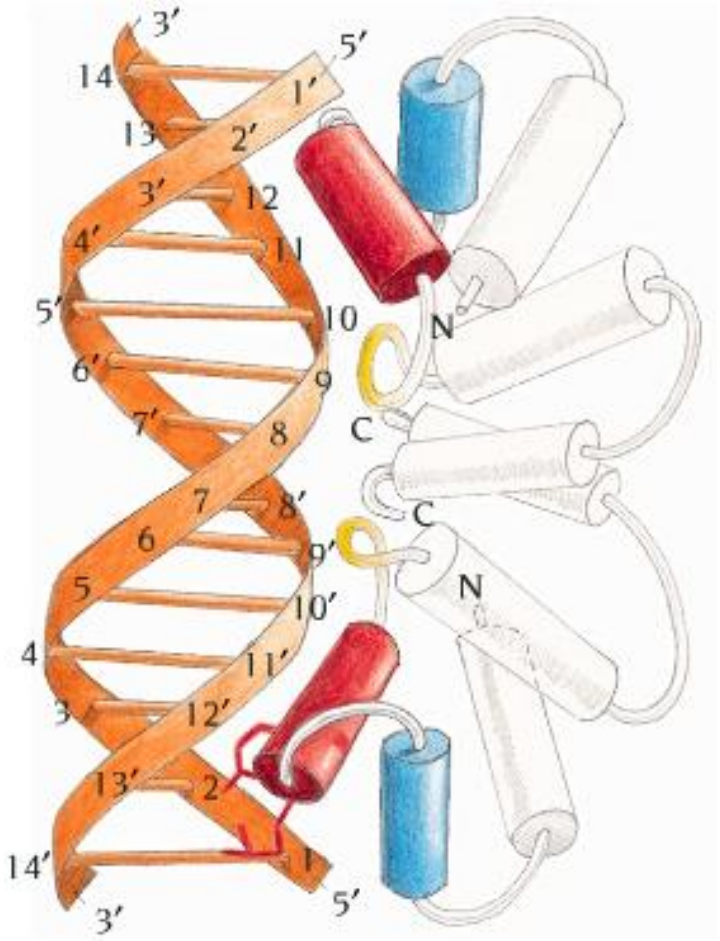
Distorted DNA complexed with 434 Cro & repressor

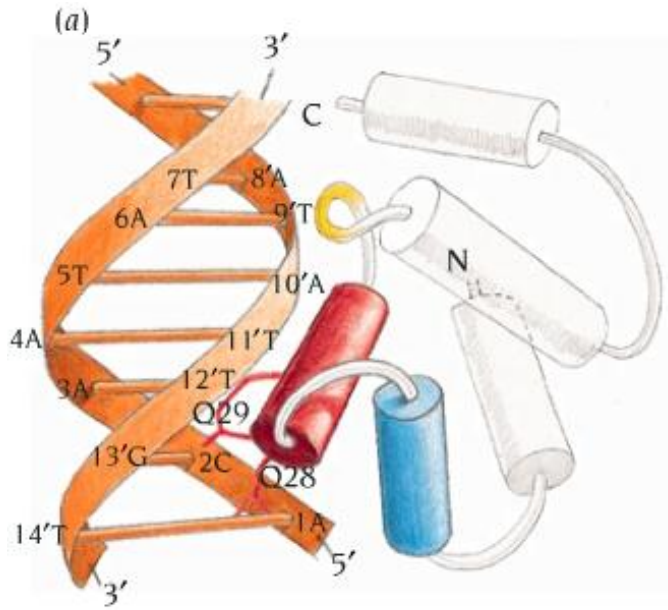


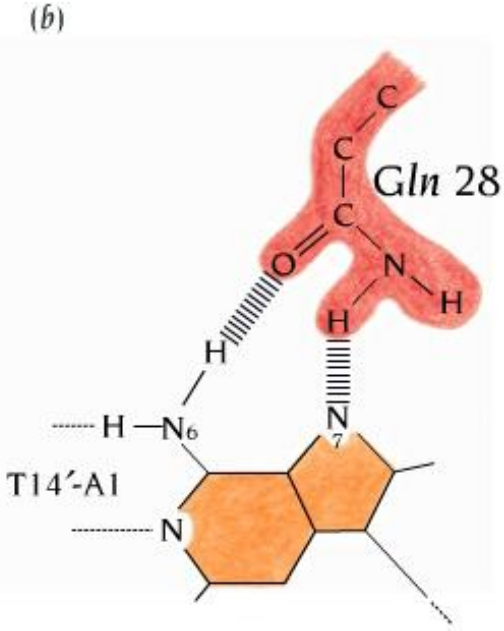
Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.138.

Sequence specific protein-DNA interactions

- Recognition helix ↔ A major groove
- H-bonding / hydrophobic interaction
  (Q28 & Q29 form H-bonds to A1-C2) / (methyl-group on T)

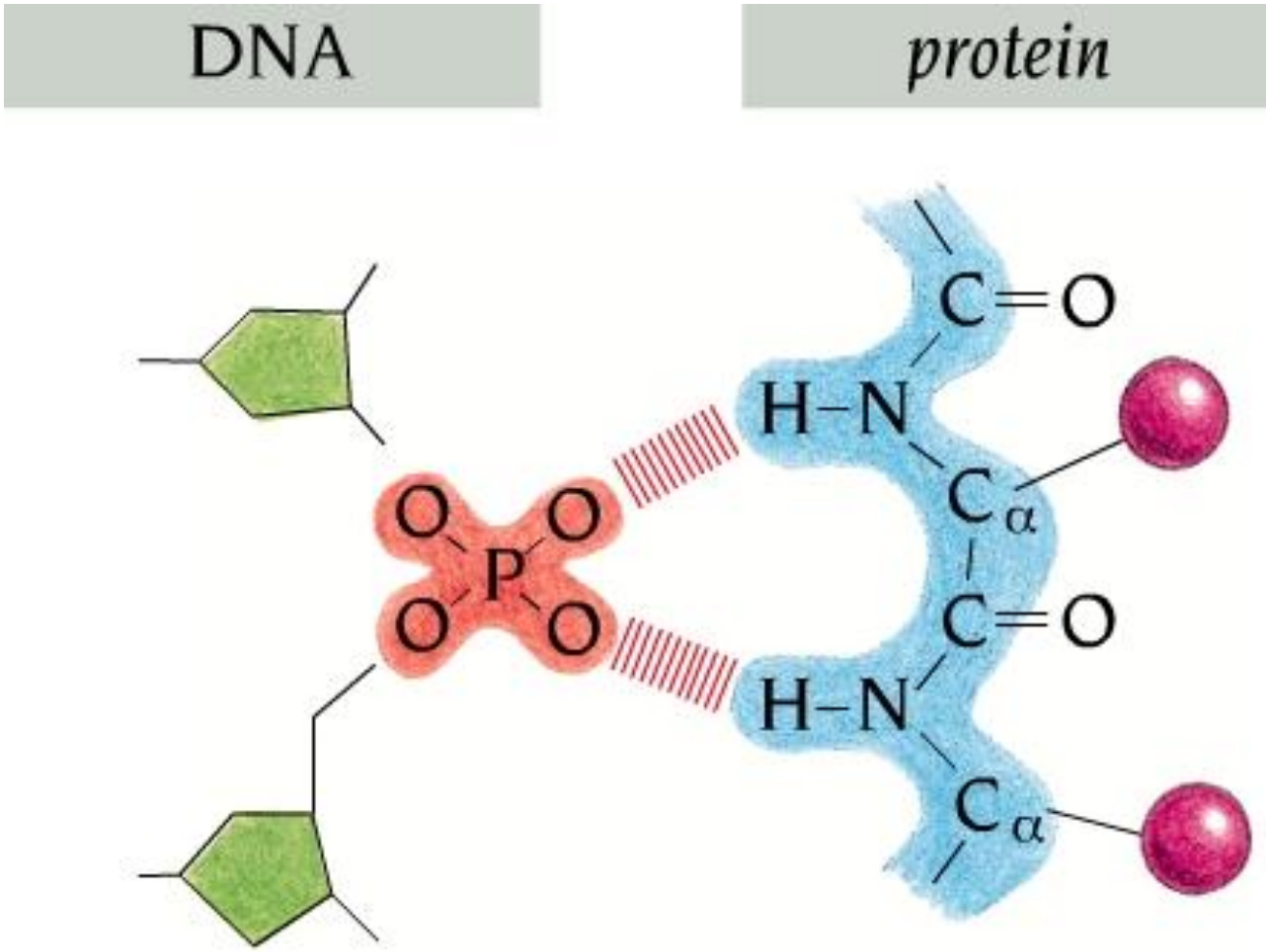






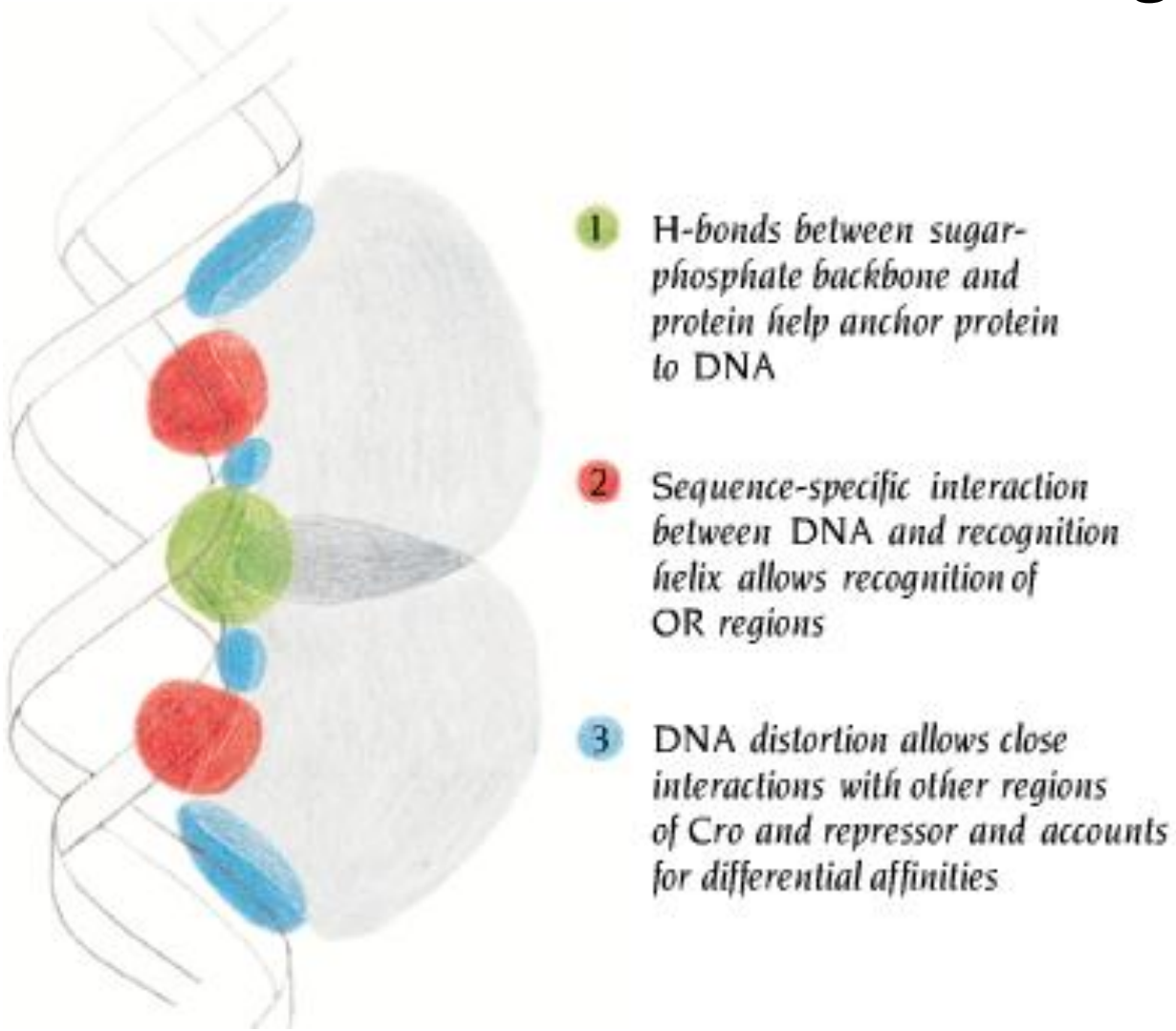
Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.139.

Protein-DNA backbone interactions determined DNA conformation



Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.140.

Main features of the interactions between DNA and the helix-turn-helix motif in DNA binding protein

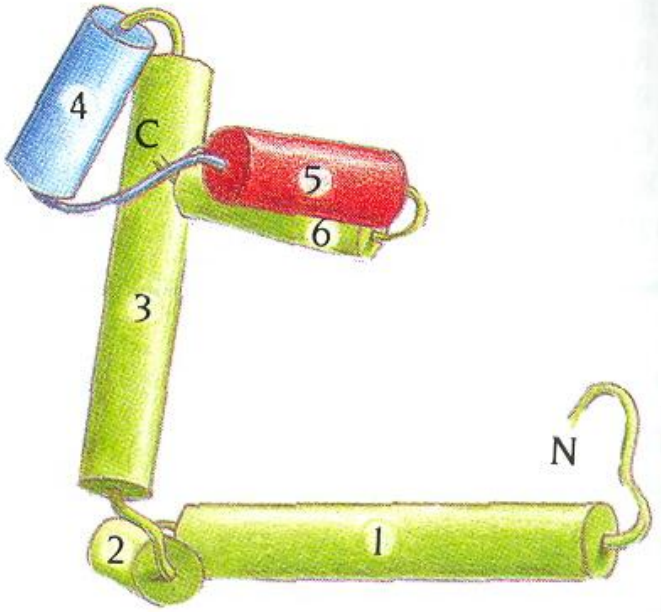


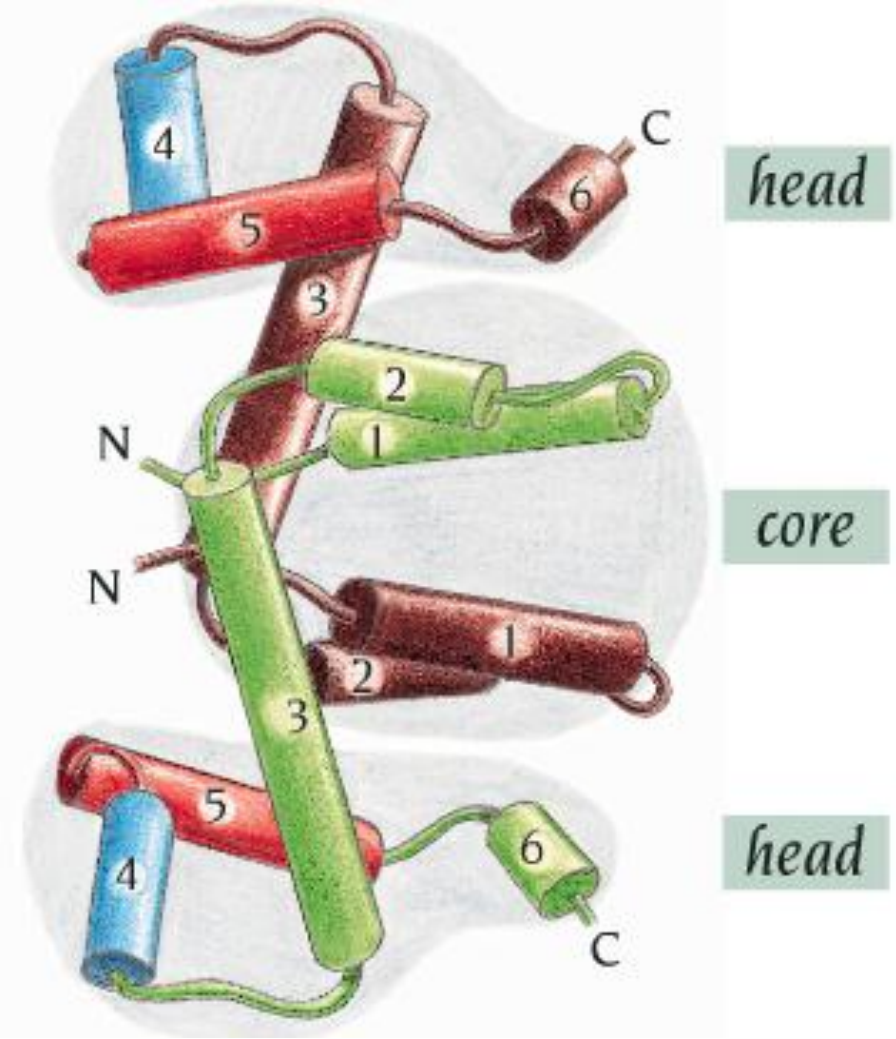
Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.141.

DNA binding is regulated by allosteric control

Binding of the small molecules (i.e., allosteric effectors) to the sites quite different from the functional binding sites of repressor or activator causes the conformational changes

Figure 7.22 The subunit of the *trp* repressor. The subunit contains 107 amino acid residues that are folded into six α helices. Helices 4 (blue) and 5 (red) form the DNA-binding helix turn-helix motif. (Adapted from R. Schevitz et al., *Nature* 317: 782, 1985.)

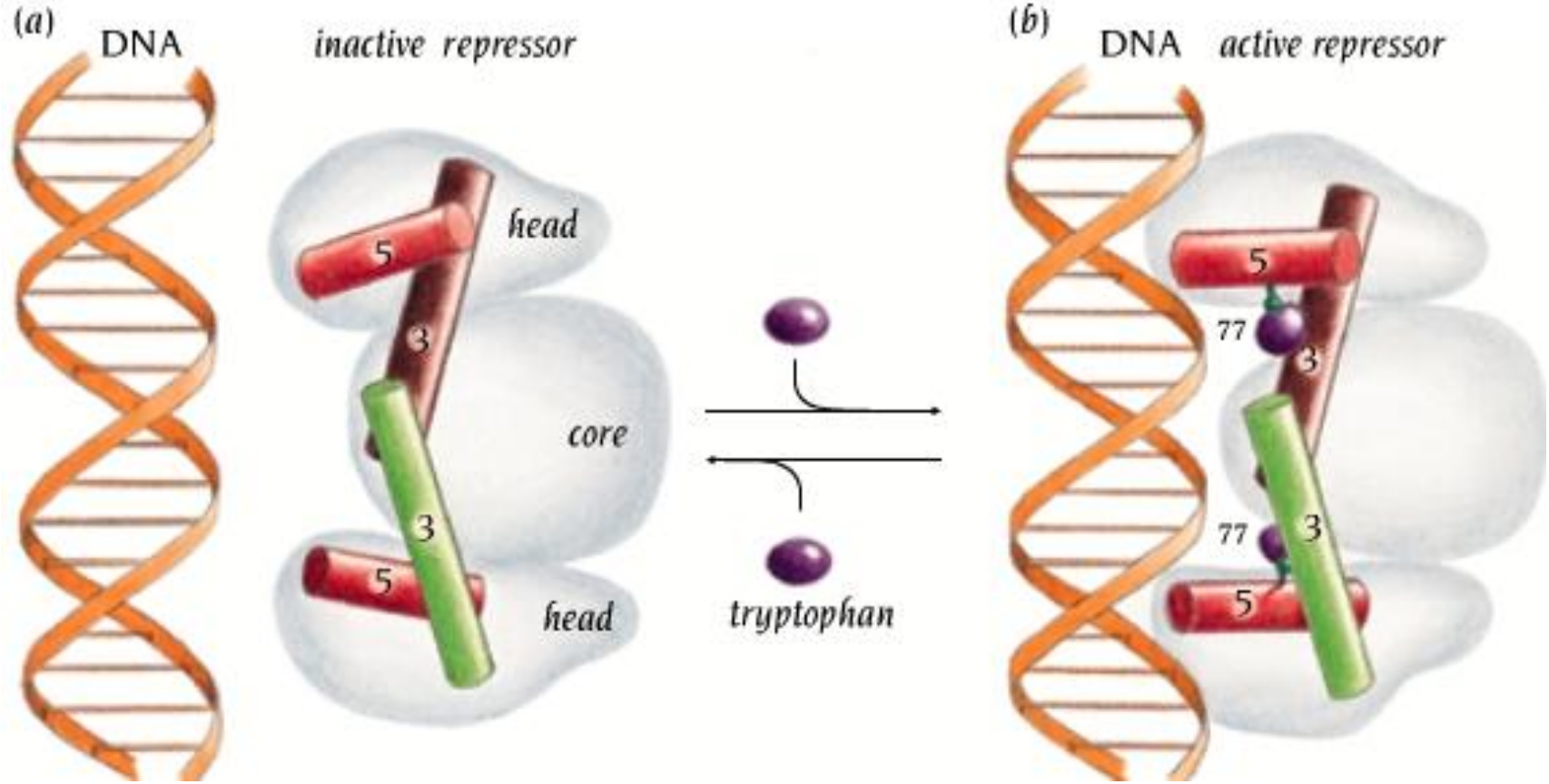




Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.142.

w/o Trp, repressor is inactive
→ turn on operon for the synthesis of Trp

w Trp, repressor is active
→ turn off operon



Major groove binding of α5 via water-mediated interactions

Structural changes by the binding of Trp into a pocket

Inactive (28-29 Å between two α5 helices) → active (34 Å)

Binding of Trp to the cavity alters the orientation of α5 helices

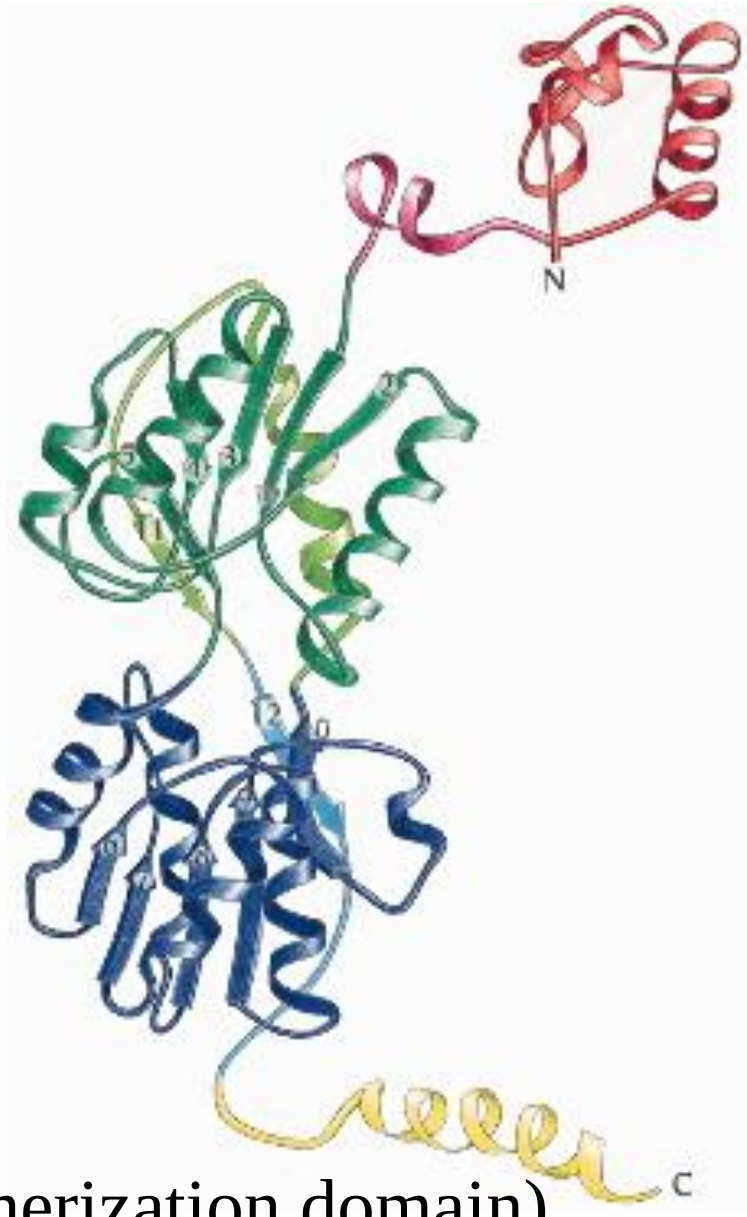
Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.143.

Lac repressor

(*LacI* : 360 residues negative regulator)

→ In the absence of lactose,
Lac repressor binds to an OR site
[RNAP can't bind to the site,
hence operon is off]

→ In the presence of inducer (lactose or IPTG), repressor-effector complex no longer binds to the OR site
[Operon is on]



Four domains in monomer
(HTH - hinge helix - core domains -tetramerization domain)

Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.144.

Tetrameric Lac repressor binds to both the major and the minor grooves inducing a sharp bend in the DNA

Each dimer binds to the separated palindromic DNA sequence

Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.145.

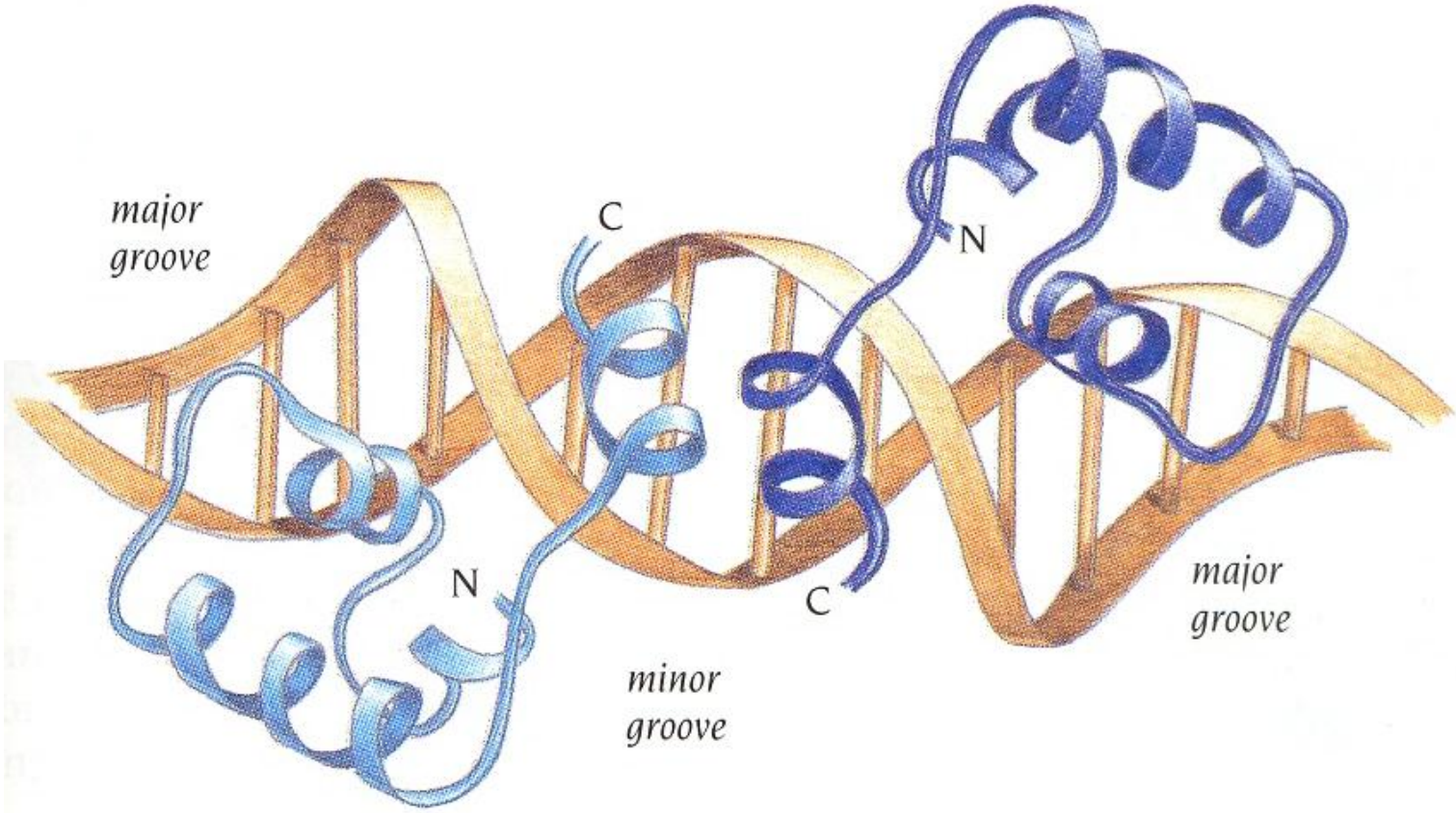


Figure 8.23 The helix
subunits of both the Pu
subunits bind to the m
with the N-terminus of
recognition helix, poin
The two hinge helices
V-shaped tetramer binc
in the minor groove of
and shallow due to dist
structure. (Adapted fro
et al., *Science* 266: 763–

- Major (HTH) & minor (hinge helix) groove binding (seq. specific)
- HTH binding as were seen in Cro and repressor in a phage
- Hinge helix: Leu opens up the minor groove and DNA is bent away from the protein

Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.145.

CAP-induced DNA bending could activate transcription

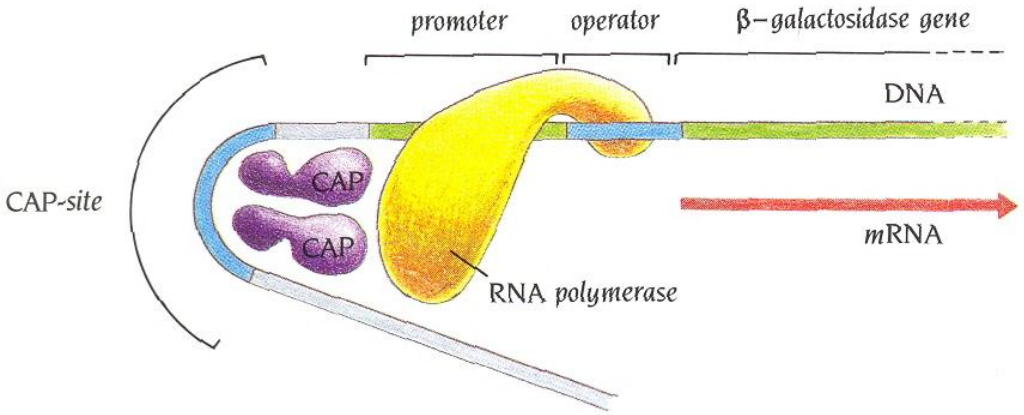
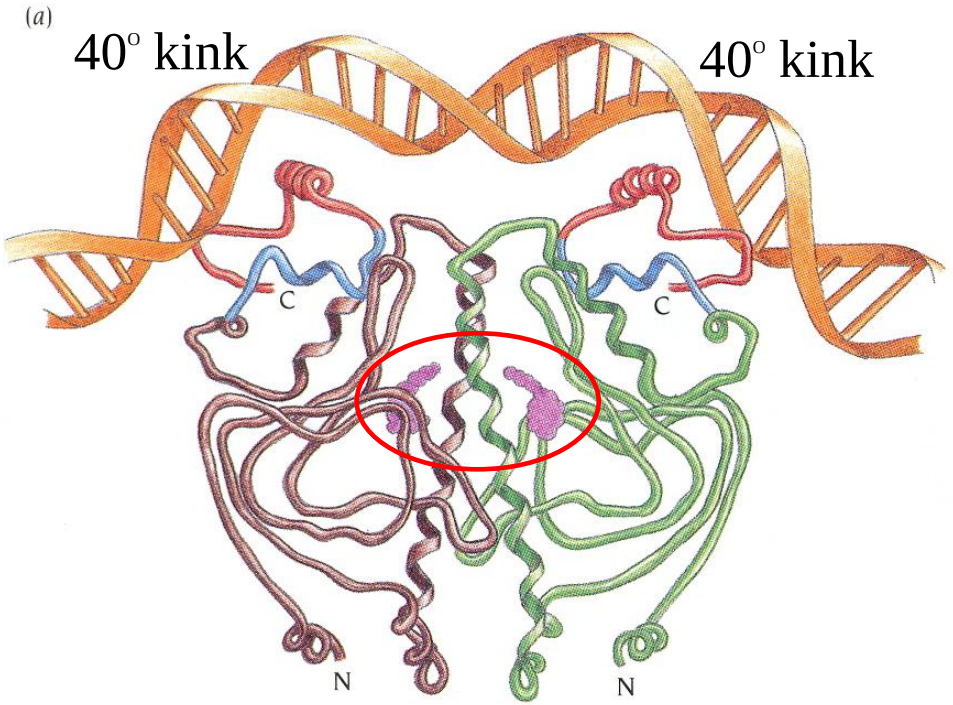
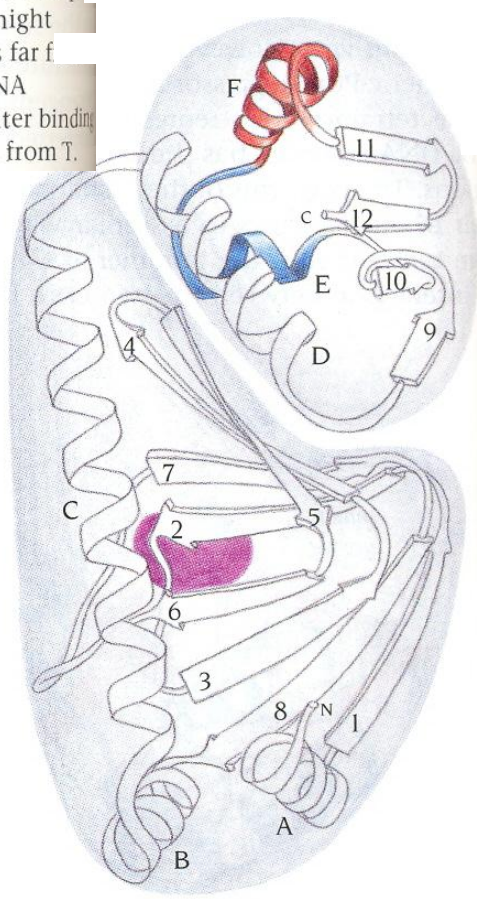


Figure 7.25 Catabolite gene activating protein, CAP, is a DNA-binding protein that assists RNA polymerase to bind more effectively to certain promoters and thereby CAP enhances the rate of initiation of RNA synthesis. A preliminary x-ray structure determination in the laboratory of Tom Steitz, Yale University, of a complex between CAP and a DNA fragment has shown that the CAP dimer induces sharp bends in DNA. *In vivo* CAP binding might induce a loop in DNA so that regions far f the operator site can interact with RNA polymerase. This might result in tighter bindin of the enzyme to the DNA. (Adapted from T.





Branden & Tooze (1998), Introduction to protein structure, 2nd ed., p.146.

DNA & CAP

Created by Naomi Boxall
Concept by John Tweedie
Institute of Molecular Biosciences
Massey University, New Zealand

http://www.massey.ac.nz/~wwbioch/DNAprot/CAP/framset.htm