RESTRICTION ENDONUCLEASES

- bacterial enzymes that provide protection from viral infections

- type I : recognition site different than cleavage site
  type II : recognition site similar to cleavage site

- features : (1) highly specific

  (2) do not degrade host DNA
  (restriction-modification systems)

  (3) cleavage site : the bond between the 3' oxygen atom and the phosphorus atom is broken

  (4) require Mg^{2+} for catalytic activity

  (5) the nucleophile attack on phosphorus atom is carried by magnesium-activated water

recognition / cleavage site for EcoRV

5′ ...GATATC... 3′
3′ ...CTATAG... 5′

inverted repeat

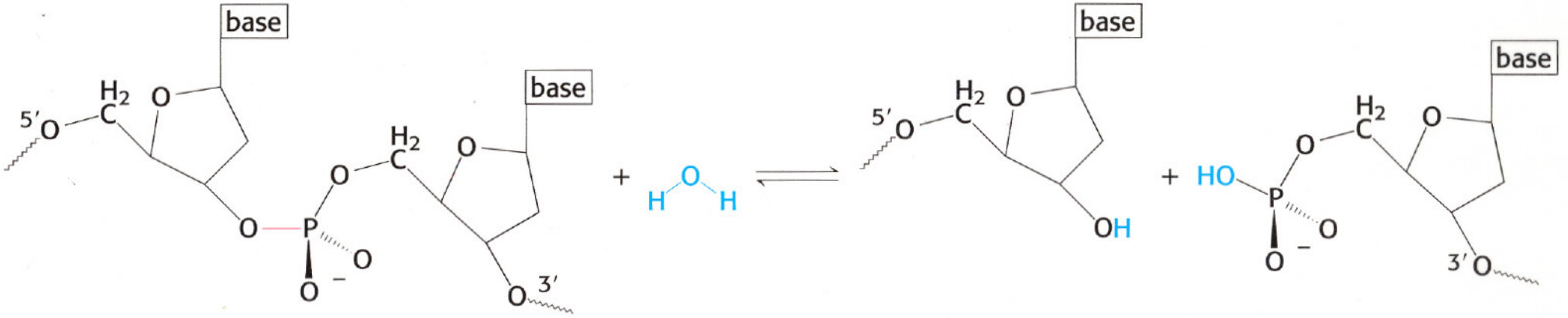

Figure 9-33 Hydrolysis of a phosphodiester bond

- restriction enzymes are dimers
- enzyme / DNA interaction ⇒ distortion of DNA

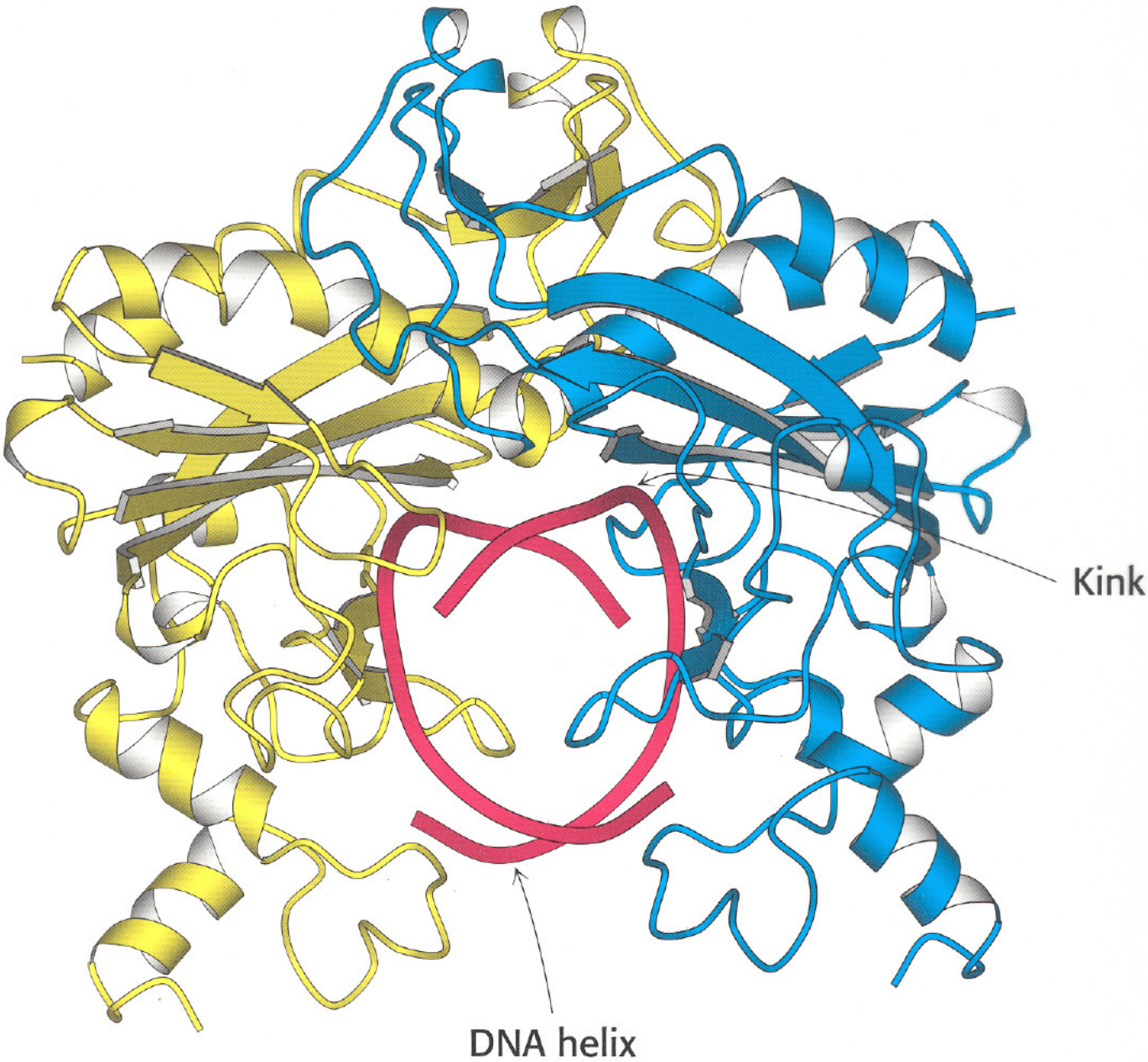

Figure 9-38 Structure of the EcoRV-cognate DNA complex

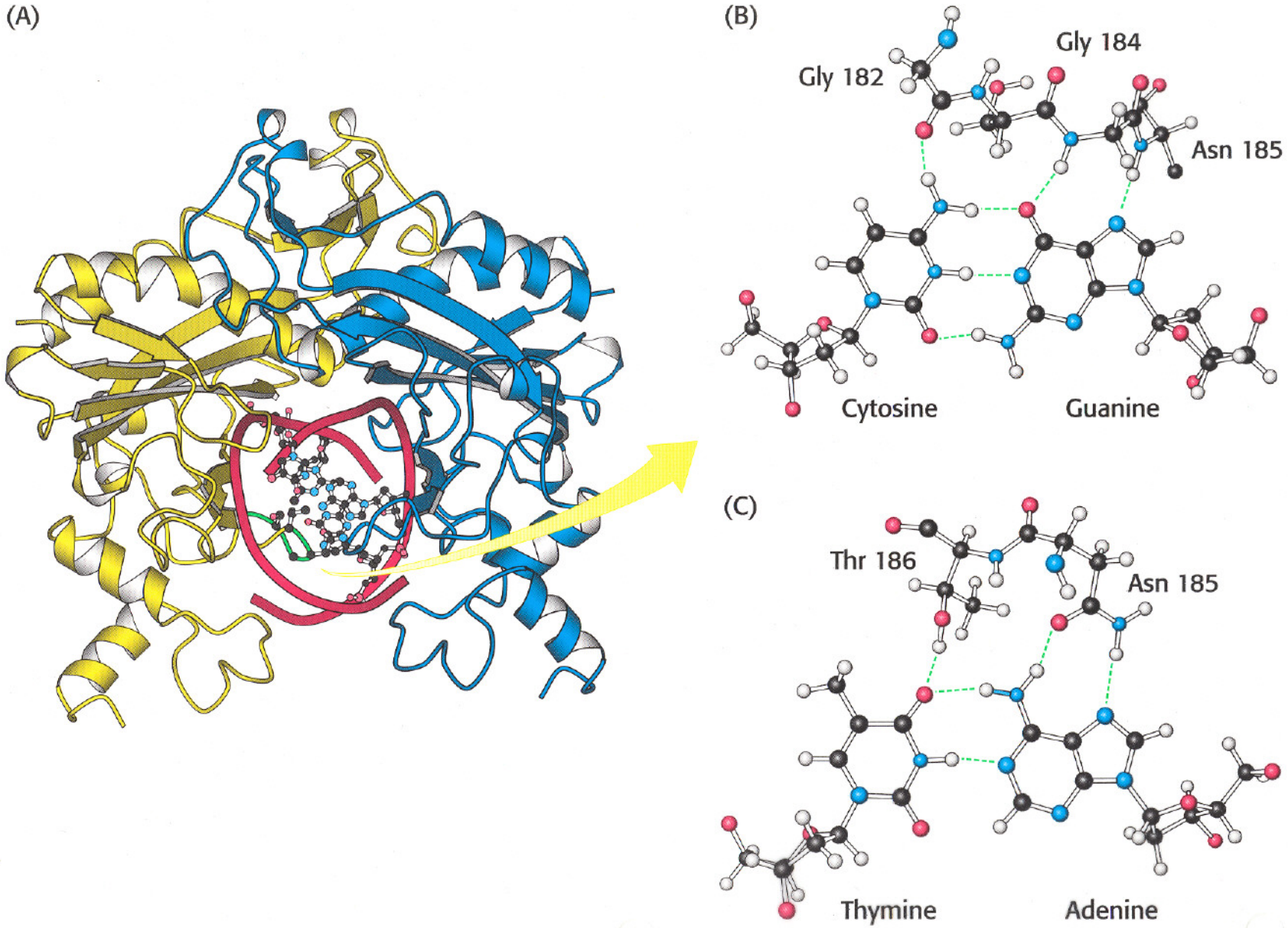

Figure 9-39 Hydrogen bonding interactions between EcoRV and its binding substrate

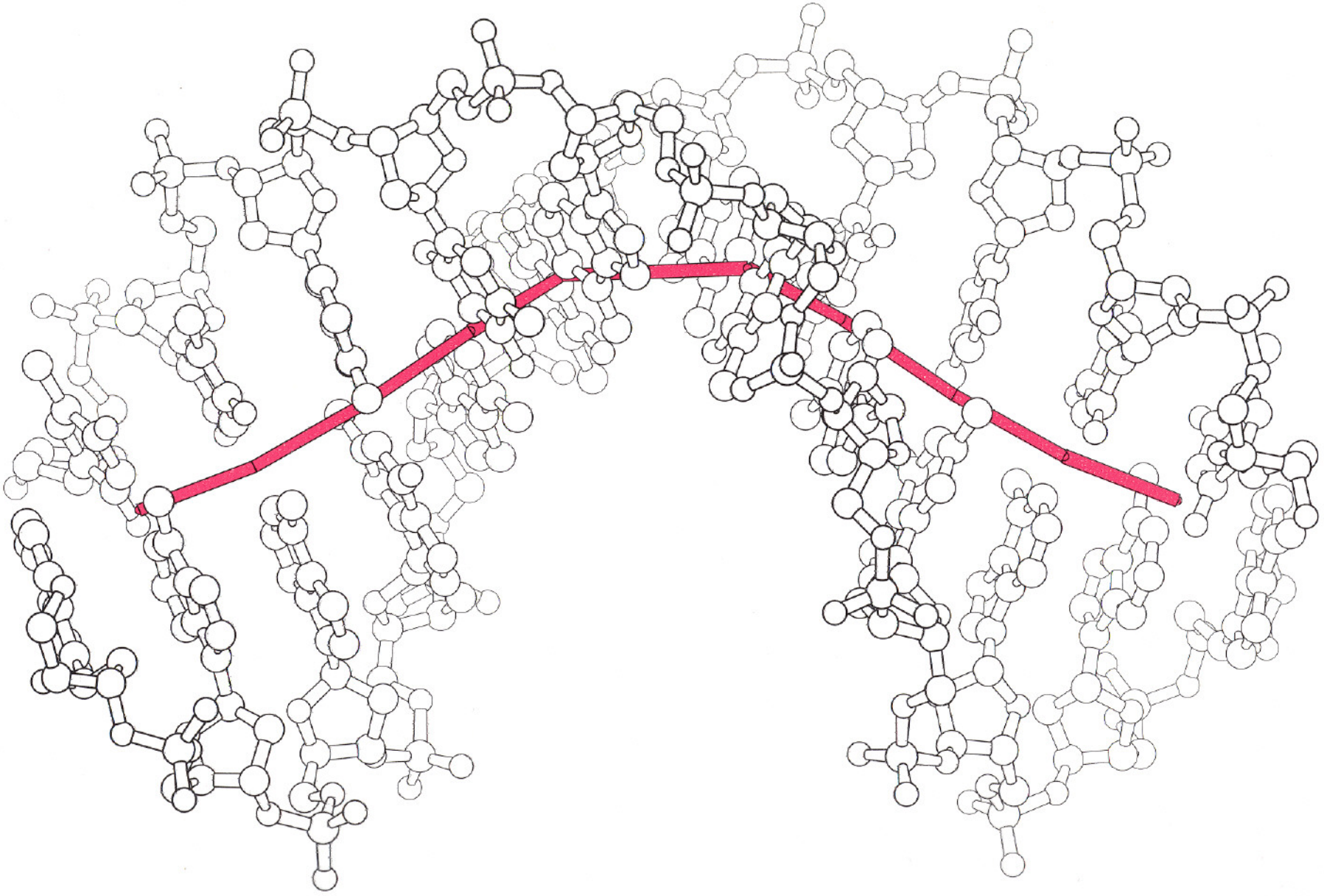

Figure 9-40 Distortion of the recognition site

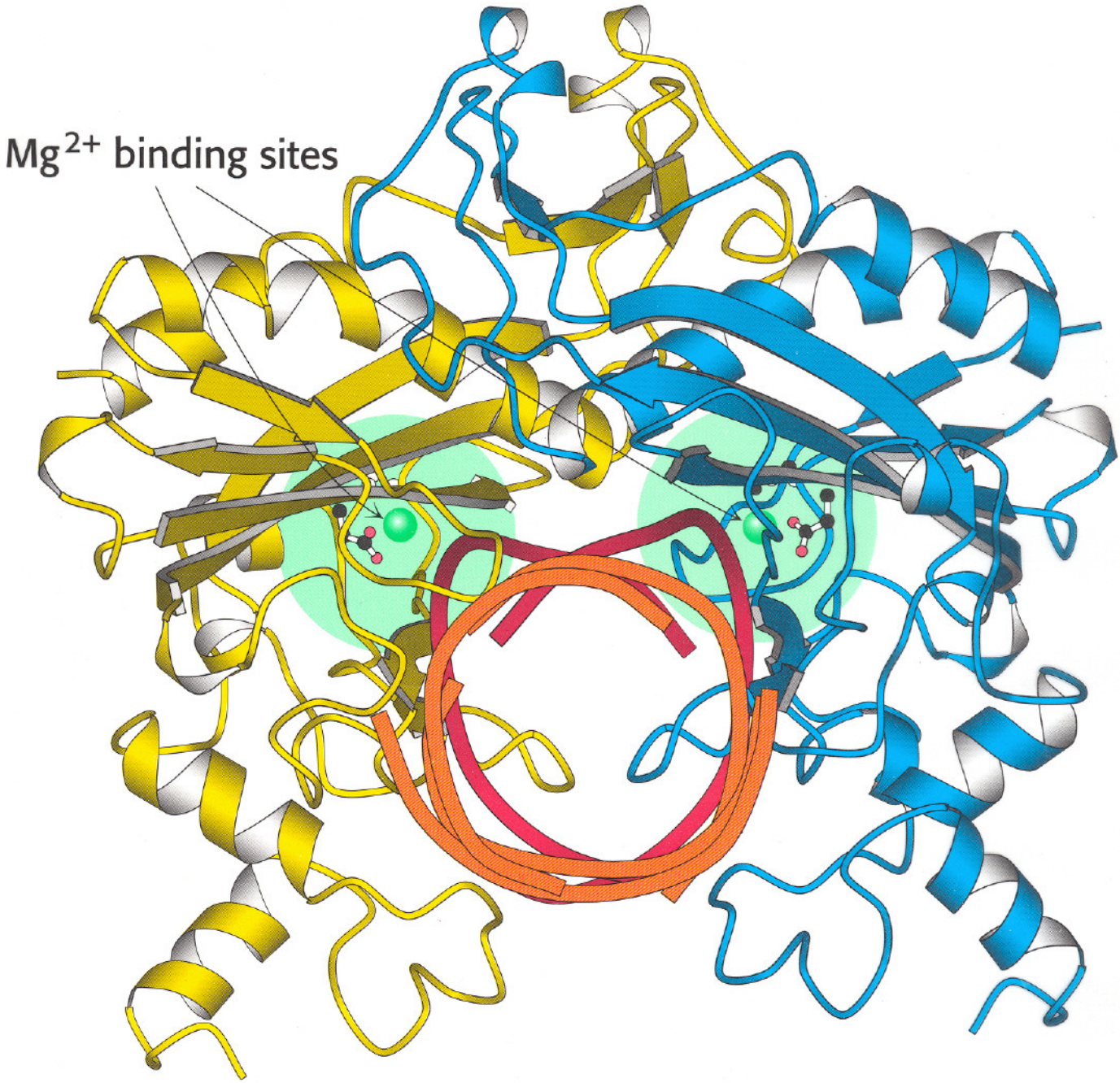

Figure 9-41 Nonspecific and cognate DNA within EcoRV

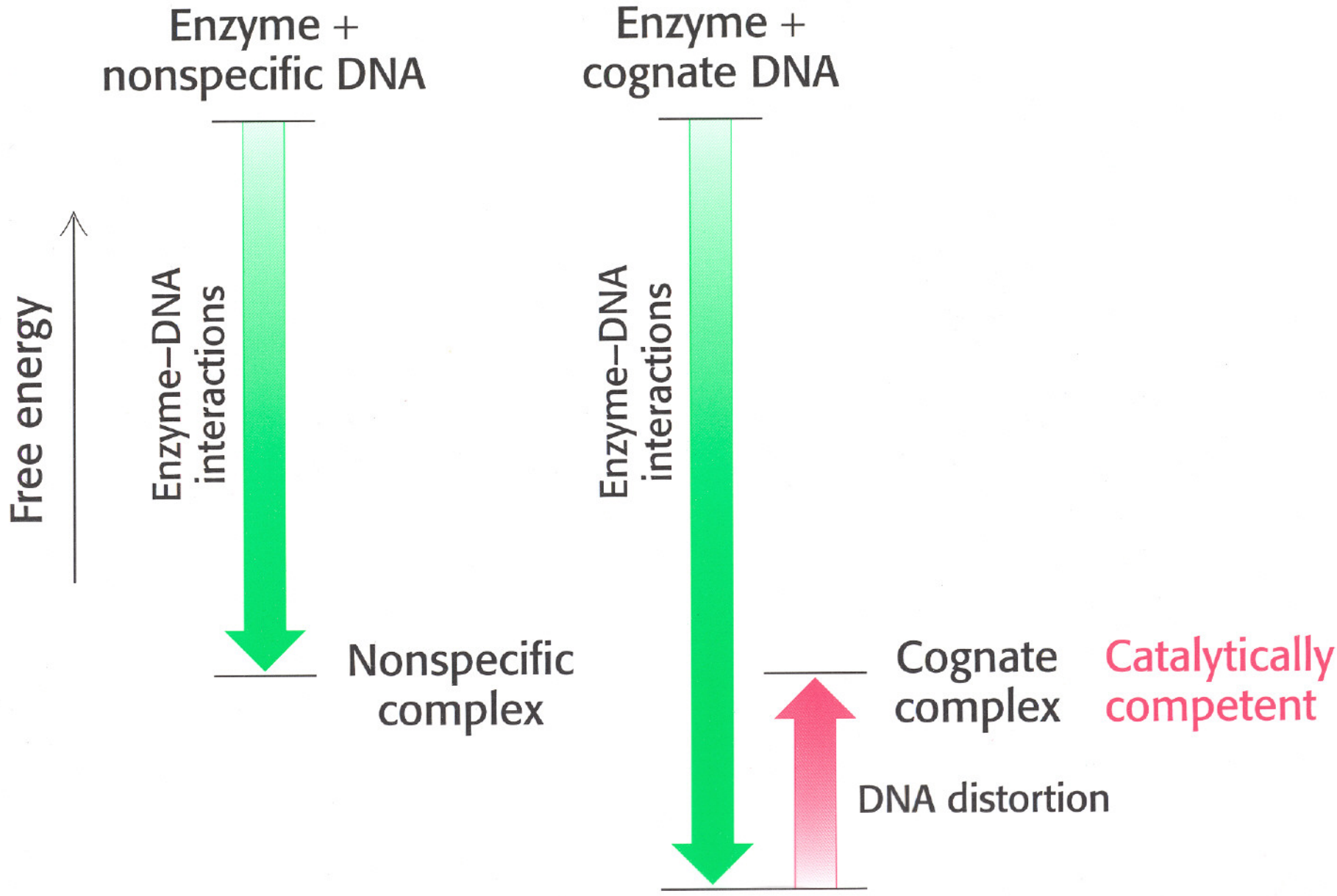

Figure 9-42 Greater binding energy of EcoRV bound to cognate versus noncognate DNA.

NUCLEOTIDE MONOPHOSPHATE KINASES (NMP kinases)

e.g. adenylate kinase

$$ATP + NMP \rightleftharpoons ADP + NDP$$

Main features:

- conserved NTP-binding motif:

  α helix – β-sheets – α helix

  ⋁

  P-loop

  (GXXXXGK)

- substrate: NTP + Mg^{++}

- catalytic mechanism : an example of catalysis by approximation

(1) ATP binding induces large conformational changes on the enzyme

(2) NMP binding induces additional conformational changes

P-loop domains are also found in :

(1) ATP synthase

(2) molecular motor proteins, such as myosin

(3) signal-transduction proteins

(4) proteins essential for translating mRNA into proteins

(5) DNA and RNA unwinding helicases

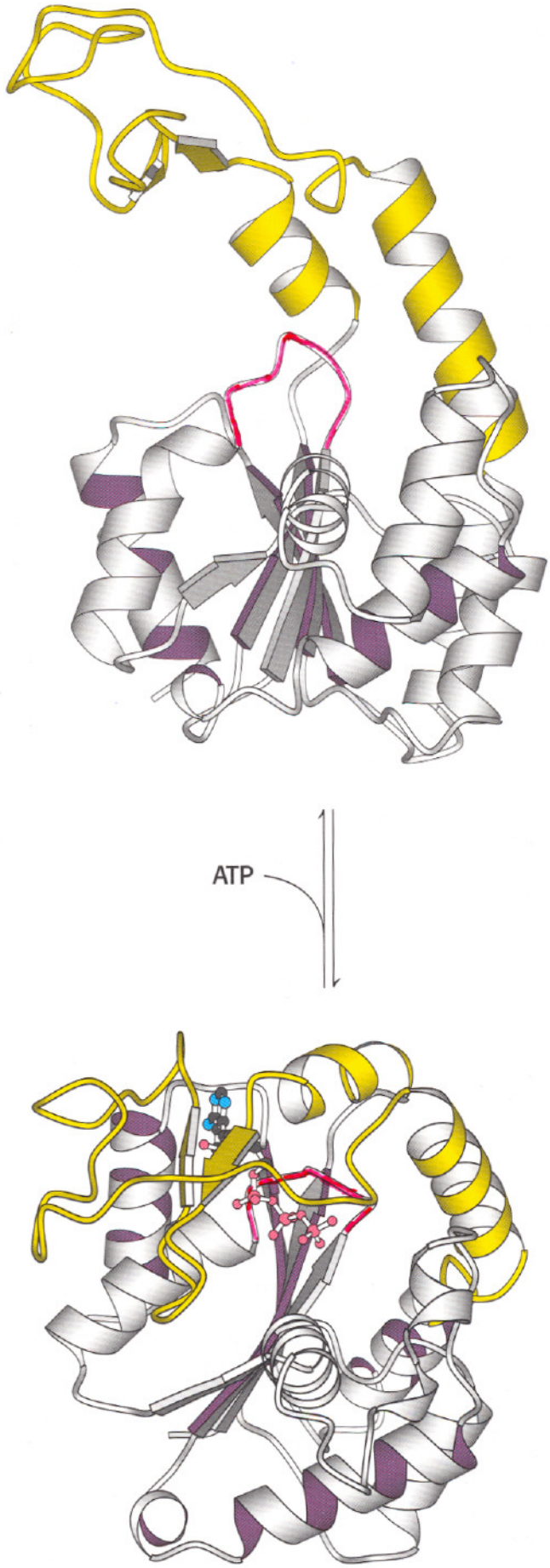

Figure 9-51 Conformational changes in adenylate kinase

Chapter 9

Problems from

(1) Textbook : # 8, 9, 11

(2) Companion : # 13