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
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- restriction enzymes are dimers
- enzyme/DNA interaction \( \Rightarrow \) distortion of DNA

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

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

Figure 9-40  Distortion of the recognition site

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Mg$^{2+}$ binding sites

orange: nonspecific DNA
red: cognate DNA

Figure 9-41 Nonspecific and cognate DNA within EcoRV

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

NUCLEOTIDE MONOPHOSPHATE KINASES (NMP kinases)

e.g. adenylate kinase

\[ \text{ATP} + \text{NMP} \rightleftharpoons \text{ADP} + \text{NDP} \]

Main features:

- conserved NTP-binding motif:

  \[ \text{a helix - } \beta\text{-sheets - a helix} \]

  \[ \downarrow \]

  \[ P\text{-loop} \]

  \[ (G\times\times\times\times\times\times\times\times\times\text{GK}) \]

- substrate: \( \text{NTP} + \text{Mg}^{++} \)
- 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 protein
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