

BCMB 3100 - Nucleic Acids - Chapter 19

- Discovery of DNA
- Nucleotides, nucleosides & bases
- Polynucleotides
- DNA as genetic material
- Structure of double-stranded DNA
- Chromatin
- RNA
- Nucleases

1

DNA and RNA are made up of nucleotides

_____ : base + sugar + phosphate_(n)
deoxyribonucleotide (sugar = 2-deoxyribose)
ribonucleotide (sugar = ribose)
_____ : base + sugar

_____ of nucleotides: heterocyclic rings
containing nitrogen

Two class of bases: _____ and _____

3

DNA is the genetic component of life

Central Dogma for Biological Information Flow

DNA → RNA → PROTEIN

Friedrich Miescher (1869): discovered DNA (nuclein →
nucleic acid)

↓
C, H, O, N, P

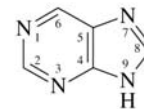
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Purines and Pyrimidines

Figure 19.3



Pyrimidine

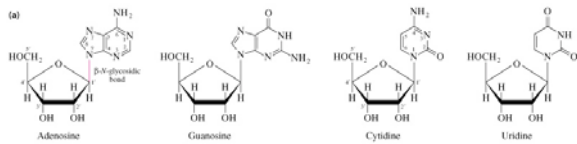


Purine

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Nucleosides

Figure 19.7 (a) Nucleoside structures

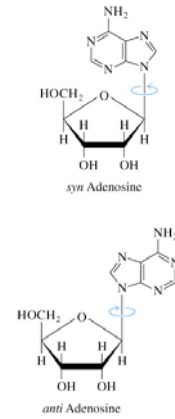


9

Fig 19.8

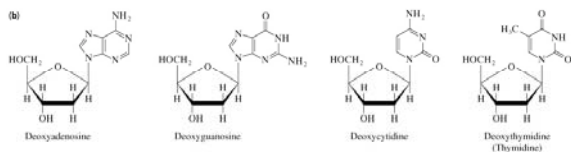
Two conformations of nucleosides & nucleotides are possible due to rotation around the glycosidic bond: **syn** and **anti**

The _____ conformation predominates



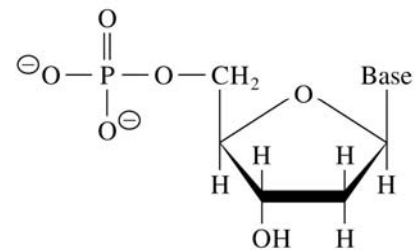
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Figure 19.7 (b)



10

Fig 19.1 Chemical structure of a _____



12

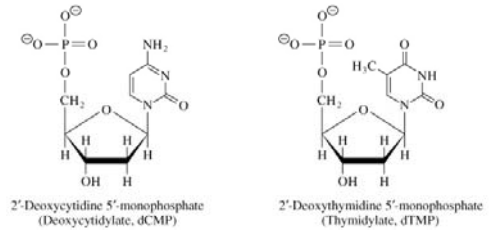
TABLE 19.1 Nomenclature of bases, nucleosides, and nucleotides

Base	Ribonucleoside	Ribonucleotide (5'-monophosphate)
Adenine (A)	Adenosine	Adenosine 5'-monophosphate (AMP); adenylate ^a
Guanine (G)	Guanosine	Guanosine 5'-monophosphate (GMP); guanylate ^a
Cytosine (C)	Cytidine	Cytidine 5'-monophosphate (CMP); cytidylate ^a
Uracil (U)	Uridine	Uridine 5'-monophosphate (UMP); uridylate ^a

Base	Deoxyribonucleoside	Deoxyribonucleotide (5'-monophosphate)
Adenine (A)	Deoxyadenosine	Deoxyadenosine 5'-monophosphate (dAMP); deoxyadenylate ^a
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5'-monophosphate (dGMP); deoxyguanylate ^a
Cytosine (C)	Deoxycytidine	Deoxycytidine 5'-monophosphate (dCMP); deoxycytidylate ^a
Thymine (T)	Deoxythymidine or thymidine	Deoxythymidine 5'-monophosphate (dTMP); deoxythymidylate ^a or thymidylate ^a

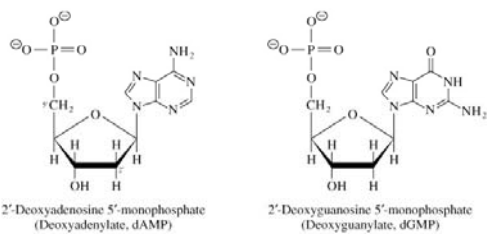
^aAnionic forms of phosphate esters predominant at pH 7.4.

Fig 19.9 (continued)



15

Fig 19.9 Structures of the deoxyribonucleoside-5'-monophosphates



14

In vivo the negatively charged phosphates on nucleotides are complexed with cations or positively charged proteins

16

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Story of DNA as Genetic Material

Discovery of the structure of double stranded DNA, 1953

James Watson, Francis Crick, Rosalind Franklin, Maurice Wilkins

A. Nucleotides joined by 3'-5' phosphodiester linkages

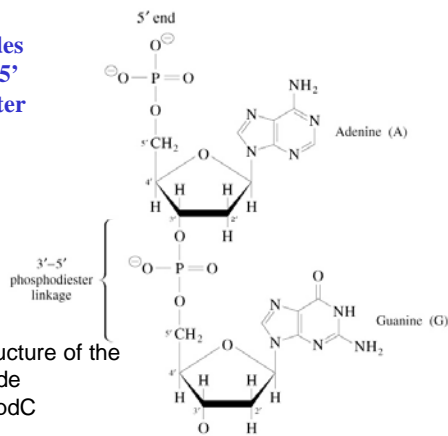


Fig 19.11 Structure of the tetranucleotide pdApdGpdTpdC

S = capsular polysaccharide = death

R = no capsule = live

Isolation & characterization of the transforming principle proved the chemical make-up of the genetic material

HISTORY OF DNA AS GENETIC COMPONENT IN CELLS

Fred Griffith (1928): studied pathogenic S, + capsular polysaccharide) and non-pathogenic (R, no capsular polysaccharide) pneumococci



Neither live R nor dead S caused death in mice. But, a mixture of dead S + live R did cause death! Concluded that non-pathogenic R could be transformed into pathogenic S by dead S due to transfer of transforming principle. R-S also occurred in vitro.

Oswald Avery, Colin MacLeod, Maclyn McCarty (1944): Up to this time it was believed that protein in chromosome was the transforming principle. They isolated transforming principle from heat-killed extract of S pneumococci & found that it was a 'nucleic acid of the deoxyribose type'. It had chemical, optical, ultraviolet, diffraction & electrophoretic properties of DNA. It was not destroyed or lost upon extraction for lipid or protein or treatment with proteases or ribonucleases (RNase). It was destroyed by treatment with DNase! DNA was the genetic material!!!!

Experiments by Roger Hershey (1951) and Alfred Hershey & Martha Chase (1952) demonstrated that it was DNA in the T2 bacteriophage that was transferred to E. coli and led to infection by virus.

Erwin Chargaff (1946) found that in prokaryotes and eukaryotes the molar ratio of dA = dT & dC = dG.

Evidence that DNA is the genetic material in cells

Figures 19-18 Summary of the Hershey-Chase experiment. Two batches of completely labeled bacteriophage particles were prepared. One was labeled with ³²P in the phosphate groups of the DNA, and the other with ³⁵S in the sulfur-containing amino acids of the protein coats. The phages were allowed to infect bacteria. Each suspension of phage-infected cells was agitated in a blender to shear the viral coats from the bacteria. The bacteria and empty virus coats were then separated by centrifugation. The ³²P-labeled DNA was found to have entered the cells, and the ³⁵S-labeled protein coats were found to have remained outside. Proper virus particles were produced in both batches of bacteria. When the viral coats were removed, the genetic message for their replication had been introduced to viral DNA, not by viral protein.

21

Mar 26, 1953

1. Each strand can be a template for the replication of the other
2. Strands must separate (semiconservative)
3. Enzymes may be necessary for polymerization: DNA polymerase
4. Molecular basis for genetic replication; mutation

"We wish to put forward a radically different structure for the salt of deoxythymine nucleic acid. This structure has two helical chains each coiled round the same axis.... Each chain follows right-handed helices.... the bases are on the inside of the helix and the phosphates on the outside.

If it is assumed that the bases only occur in the structure in the most plausible... form... it is found that only specific pairs of bases can bond together.... adenine with thymine, and guanine with cytosine.

(It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

Watson & Crick (1953) Nature 171:737-748

• each strand can be the template for the other

• The DNA structure proposed by Watson & Crick is known as 'B-DNA'

• Most DNA is a helical or subhelical genome

• Other DNA forms: A-DNA, Z-DNA, and other hybrids of B-DNA

File: Fig 20-11

23

"DNA carries genetic information in all prokaryotes and eukaryotes. In some viruses DNA is the genetic material (e.g. TMV, Adenovirus)

"A gene carries biological information in a form that must be precisely copied and transmitted from each cell to all its progeny.

HOW IS DNA REPLICATED?

1958 - James Watson & Francis Crick deduced the 3D structure of DNA and inferred a mechanism of replication in their two papers in April and May of 1953 (Nature 171:737-748 & Nature 171:964-967) (Revised from: P. H. Raven, 1971)

May 30, 1953 NATURE

Major points from Watson & Crick (1953) Original report on structure of DNA and implications for replication

April 25, 1953

1. Double helix
2. Sugar-phosphate backbone, 5'-3' linkage
3. Right-handed
4. Antiparallel
5. Base pairs are hydrogen bonded, A-T, G-C (base on outside)
6. Strands are complementary (base on inside)
7. Copying mechanism
8. Transmission of base-carrying genetic information

22

Fig 19.6

Adjacent nucleotides can hydrogen bond to each other

(Deoxy)Adenosine

(Deoxy)Cytidine

(Deoxy)Guanosine

(Deoxy)Thymidine

24

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25

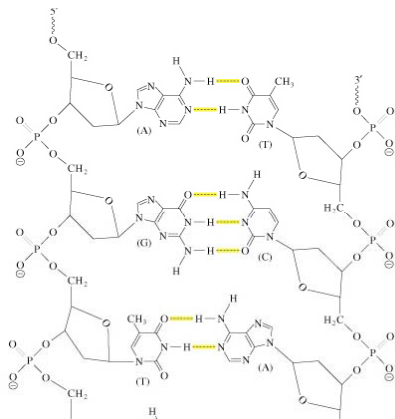
DNA is double-stranded with equal ratios of G:C and of A:T. However, the ratio of (G+C):(A+T) varies in an species specific manner

Table 19.2

TABLE 19.2 Base composition of DNA (mole%) and ratios of bases								
Source	A	G	C	T	A/T ^a	G/C ^a	(G+C)	Purine/pyrimidine ^a
<i>Escherichia coli</i>	26.0	24.9	25.2	23.9	1.09	0.99	50.1	1.04
<i>Mycobacterium tuberculosis</i>	15.1	34.9	35.4	14.6	1.03	0.99	70.3	1.00
Yeast	31.7	18.3	17.4	32.6	0.97	1.05	35.7	1.00
Cow	29.0	21.2	21.2	28.7	1.01	1.00	42.4	1.01
Pig	29.8	20.7	20.7	29.1	1.02	1.00	41.4	1.01
Human	30.4	19.9	19.9	30.1	1.01	1.00	39.8	1.01

27

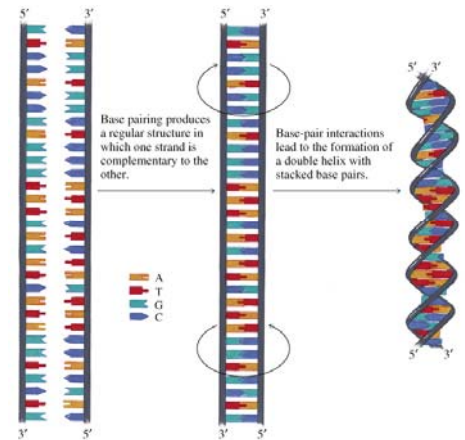
Fig. 19.12



26

Fig 19.13

- Complementary base pairing and stacking in DNA



- Structure of B-DNA
- Sugar phosphate backbone outside
- Stacking creates two unequal grooves (major and minor)
- Hydrophobic attraction between the bases
- Van der Waals contact between bases
- H-bonds between bases
- Electrostatic repulsion between phosphates inhibited by cations (Mg^{++})

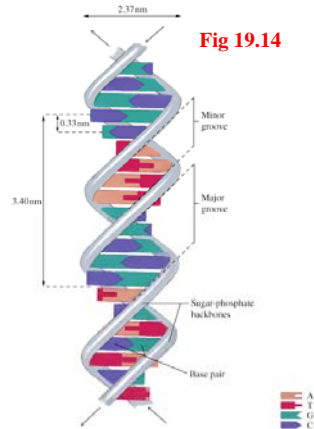


Fig 19.18 Forms of DNA

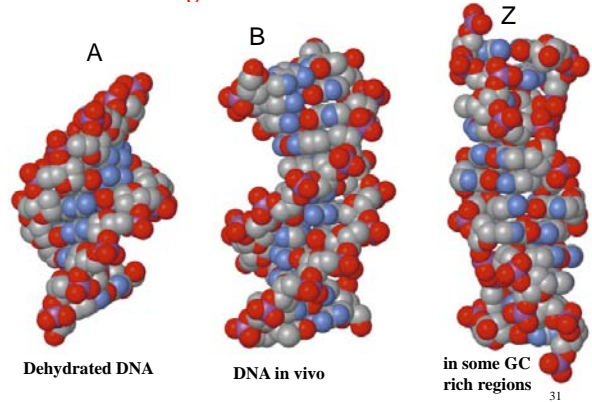
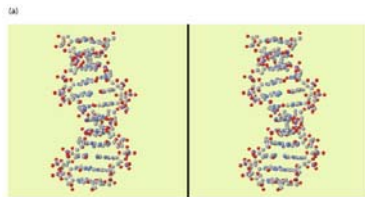
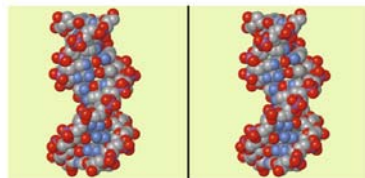


Fig. 19.15

(a) Ball-and-stick model



(b) Space-filling model



30

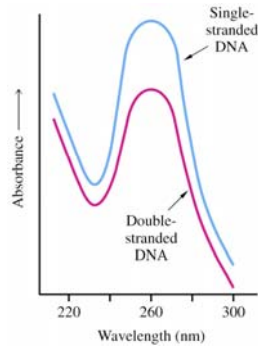
DNA molecules vary greatly in length depending upon the organism and organelle

E. coli	4.2 x 10⁶ bp
fruit fly	62 x 10⁶ bp
mitochondria	0.015 x 10⁶ bp
human	240 x 10⁶ bp

32

Fig 19.16 Absorption spectra of double-stranded and single-stranded DNA

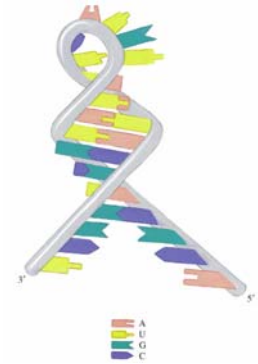
- Double-stranded (ds) DNA absorbance max 260 nm
- _____ absorbs more than ds DNA
- dsDNA can be denatured by heat and chaotropic agents
- Extent of denaturation can be measured by OD_{260}



33

Fig 19.21 Stem-loop structures in RNA

- ssRNA can also have ds regions
- _____ or _____ can form from short regions of complementary base pairs
- Stem: base-paired nucleotides
- Loop: noncomplementary nucleotides

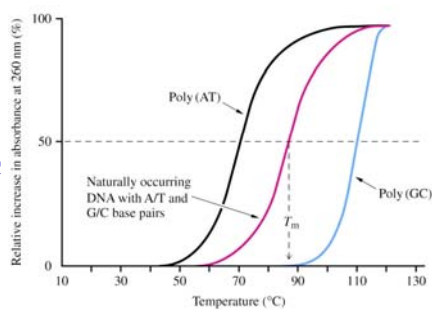


35

Fig 19.17 Melting curve for DNA

Temperature at which amount of dsDNA = ssDNA is T_m (_____)

T_m for poly GC is greater than T_m for poly AT



34

Four Classes of RNA in living organisms

_____ (rRNA) - ~80% of total RNA, part of ribosomes (translation machinery)

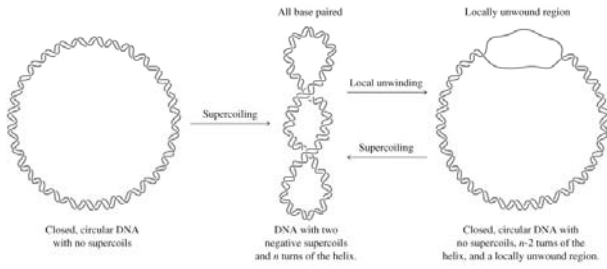
- _____ (tRNA) - ~15% of total RNA, 73-95 nucleotides long, carry activated amino acids to ribosomes during translation

_____ (mRNA) - linear "copies" of DNA that encode genetic information. Encode primary structure of protein. ~1-3% of total RNA, relatively unstable

_____ - may have catalytic activity and/or associate with proteins to enhance activity, some involved with RNA processing in the nucleus

36

Fig 19.19 Structure of supercoiled DNA. Circular B-DNA has 10.4 bases/turn of helix. If DNA is underwound (or overwound), it is supercoiled to restore 10.4 bases/turn. Supercoiling is done by topoisomerases.



37

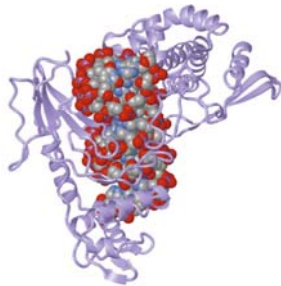
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39

Fig 19.20 Human topoisomerase I bound to DNA

- **Topoisomerases** can add or remove supercoils in DNA
- Cleave one or both DNA strands, unwind or overwind by rotating cleaved ends, then rejoin ends



38

- In the nucleus DNA is found as _____
- **Chromatin:** an association of DNA with proteins (mostly **histones**) → compact & manageable packing. Chromatin looks like long threads of 30 nm diameter.
- **Histones** - the major proteins of chromatin
- **Eukaryotes** contain five small, basic **histone** proteins containing many lysines and arginines: **H1, H2A, H2B, H3, and H4**
- **Positively charged histones** bind to **negatively-charged sugar-phosphates** of DNA

40

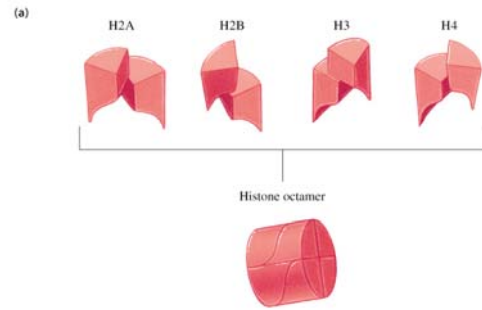
Table 19.3

TABLE 19.3 Basic and acidic residues in mammalian histones

Type	Molecular weight	Number of residues	Number of basic residues	Number of acidic residues
Rabbit thymus H1	21 000	213	65	10
Calf thymus H2A	14 000	129	30	9
Calf thymus H2B	13 800	125	31	10
Calf thymus H3	15 300	135	33	11
Calf thymus H4	11 300	102	27	7

41

Fig 19.23 Histone octamer



43

A structural unit in chromatin is the _____
Nucleosome: a ~200 bp DNA strand wound around a histone core.

Chromatin treated with a low salt solution extends into a “beads on a string” structure. Beads are the nucleosomes; the string is DNA.

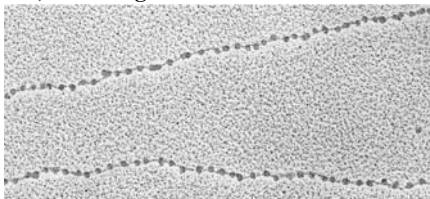
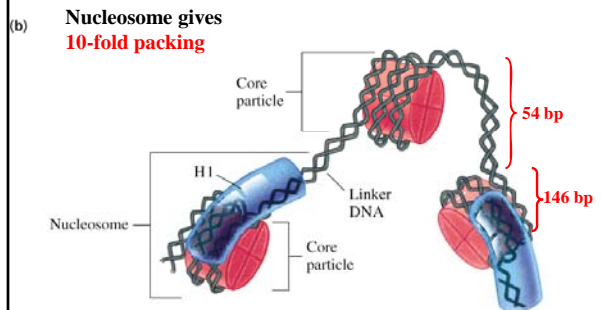


Fig 19.22 Electron micrograph of chromatin

42

Fig. 19.23 (b) Nucleosome



44

Fig 19.25

Solenoid: a higher level of chromatin structure in which adjacent nucleosome associate via histone H1



Solenoid give further **4-fold packing**

45

Final chromosome is 1/8000 of length of B-DNA.

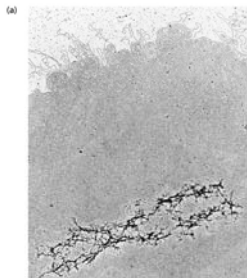
This allows DNA to be packaged into cells. For example, the largest human chromosome is 2.4×10^8 bp.

This chromosome would be 8.2 cm long if it were not packaged as chromatin (as opposed to $1 \mu\text{m}$)!!

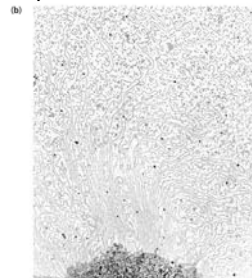
47

Fig 19.26 Histone-depleted chromosome scaffold. Attachment of DNA to RNA-protein scaffold gives further **200-fold packing**

Protein scaffold



Loops attached to scaffold



46

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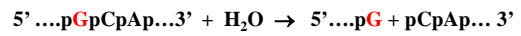
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Nucleases and Hydrolysis of Nucleic Acids

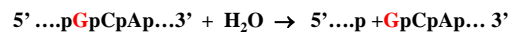
- **Nucleases** - hydrolyze phosphodiester bonds
 - RNases** (RNA substrates)
 - DNases** (DNA substrates)
- May cleave either the 3'- or the 5'- ester bond of a 3'-5' phosphodiester linkage
- **Exonucleases** start at the end of a chain
- **Endonucleases** hydrolyze sites within a chain

49

Cleavage of 3' ester of Guanylate



Cleavage of 5' ester of Guanylate



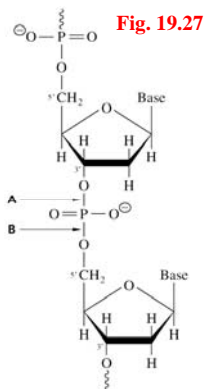
51

• Nuclease cleavage sites

- Cleavage at bond A generates a 5'-phosphate and a 3' OH terminus
- Cleavage at bond B generates a 3'-phosphate and a 5'-hydroxyl terminus

A = cleavage of 3'- ester bond

B = cleavage of 5'- ester bond



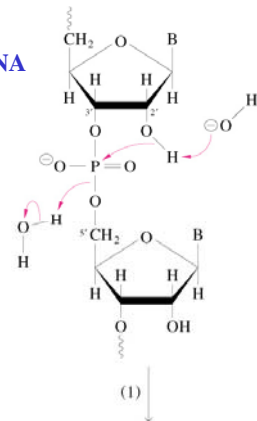
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Alkaline Hydrolysis of RNA

DNA is stable in basic solution

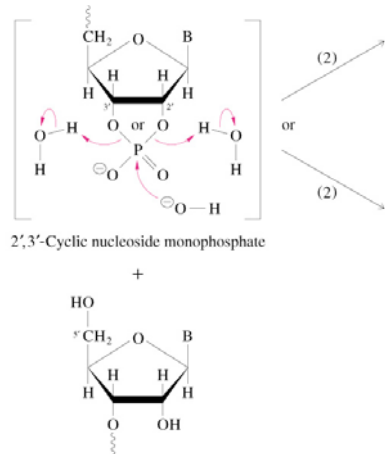
RNA is unstable in base

Fig 19.28



52

**Fig 19.28
(cont)**



53

Ribonuclease-Catalyzed Hydrolysis of RNA
RNase A cleaves 5' ester to right of
pyrimidines

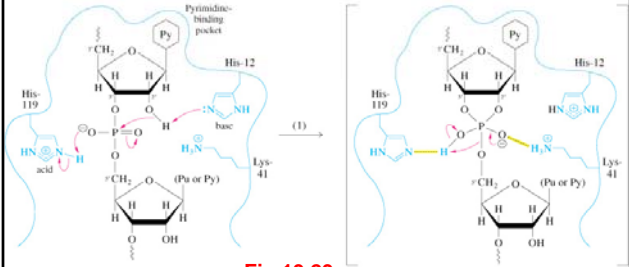
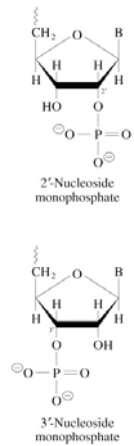


Fig 19.29

55

**Fig 19.28
(cont)**

(From previous page)



54

**Fig 19.29
(cont)**

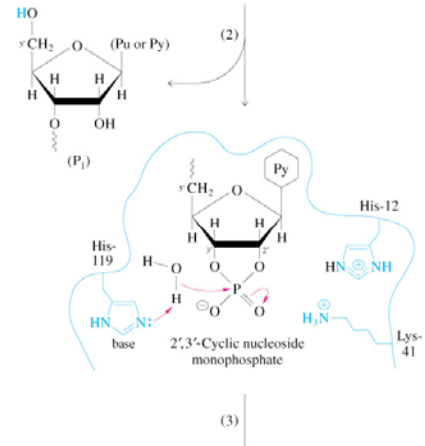
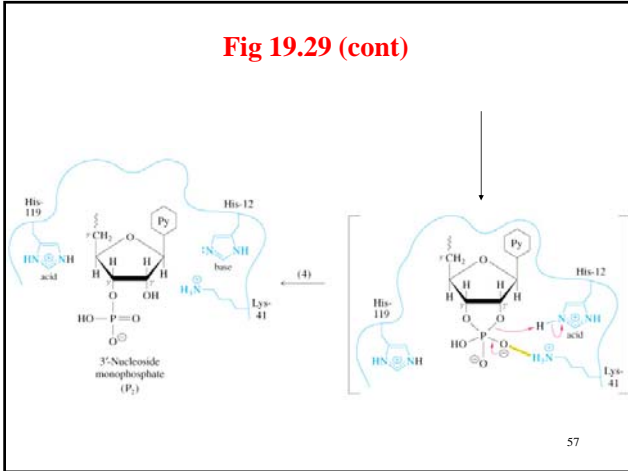


Fig 19.29 (cont)



57

Most restriction enzymes recognize **palindromes**: inverted sequences with two-fold symmetry over two strands



59

_____: site-specific endodeoxyribonucleases causing cleavage of both strands of DNA at points within or near the specific site recognized by the enzymes; important tools in genetic engineering

_____: catalyze both methylation of host DNA and cleavage of non-methylated DNA at recognition site

_____: cleave non-methylated DNA at recognition site

58

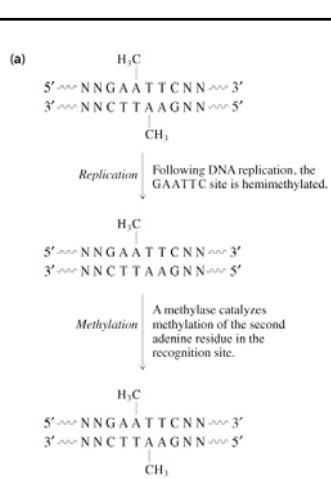
TABLE 19.4 Specificities of some common restriction endonucleases

Source	Enzyme ^a	Recognition sequence ^b
<i>Acetobacter pasteurianus</i>	<i>Apa</i> I	GGGCC [↓] C
<i>Bacillus amyloliquefaciens</i> H	<i>Bam</i> HI	G [↓] GATCC
<i>Escherichia coli</i> R Y13	<i>Eco</i> RI	G [↓] AATTC
<i>Escherichia coli</i> R245	<i>Eco</i> RII	↓CC [↓] TGG
<i>Haemophilus aegyptius</i>	<i>Hae</i> III	GG [↓] CC
<i>Haemophilus influenzae</i> R ₁₃	<i>Hin</i> dIII	A [↓] AGCTT
<i>Haemophilus parainfluenzae</i>	<i>Hpa</i> II	C [↓] CGG
<i>Klebsiella pneumoniae</i>	<i>Kpn</i> I	GGTAC [↓] C
<i>Nocardia otitidis-caviarum</i>	<i>Not</i> I	GC [↓] GGCCG
<i>Providencia stuartii</i> 164	<i>Pst</i> I	CTGCA [↓] G
<i>Serratia marcescens</i> S ₈	<i>Sma</i> I	CCC [↓] GGG
<i>Xanthomonas badrii</i>	<i>Xba</i> I	T [↓] CTAGA
<i>Xanthomonas holcicola</i>	<i>Xho</i> I	C [↓] G

60

Fig 19.30

- Methylation and restriction at the *EcoR1* site



61

D. *EcoR1* Binds Tightly to DNA

- *EcoR1* has 2 identical subunits (purple and yellow)
- Bound to a fragment of DNA (strands blue and green)

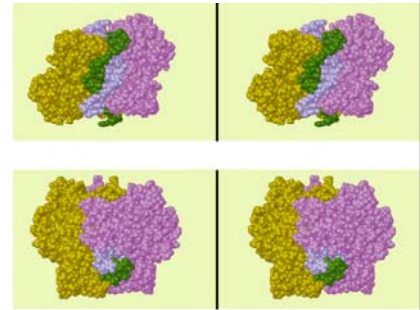


Fig. 19.31
(like)

63

Fig 19.30 (continued)

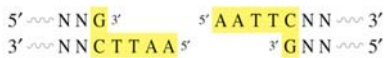
(b)



Restriction

↓

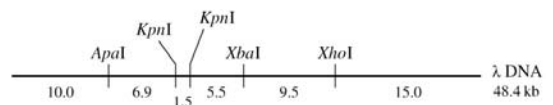
The endonuclease recognizes the GAATTC sequence and cleaves both strands of the foreign DNA to produce fragments with staggered ends.



62

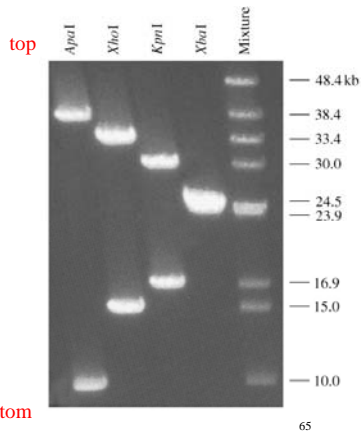
Fig. 19.32 Uses of Restriction Endonucleases

- Developing restriction maps (indicates specific cleavage sites in a DNA fragment)
- Map of bacteriophage λ showing cleavage sites of some restriction enzymes



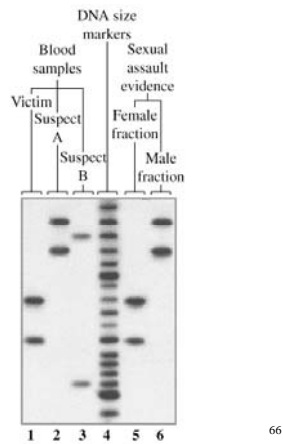
64

Fig 19.33



- Restriction digest of bacteriophage λ
- Four restriction enzymes used
- Sizing gel separates fragments (smallest move fastest)

Fig 19.34



- DNA Fingerprinting