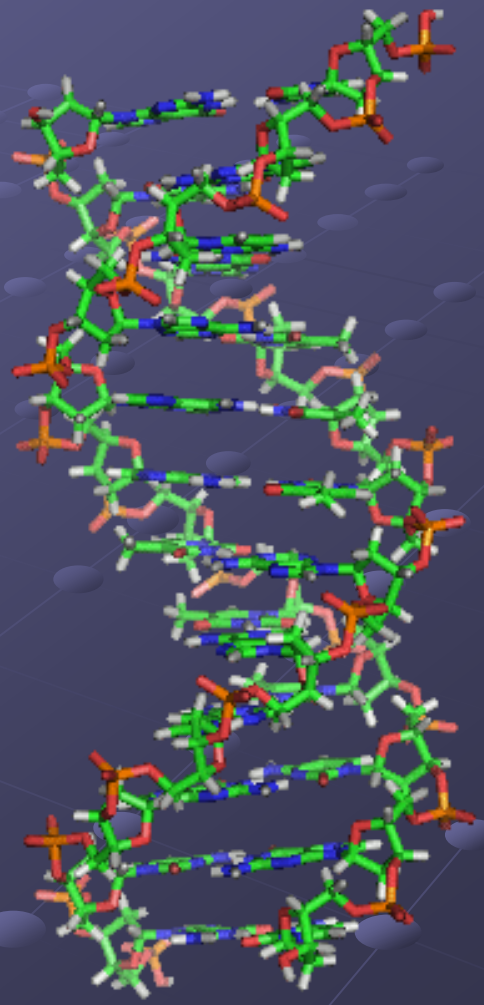
Nucleic acids

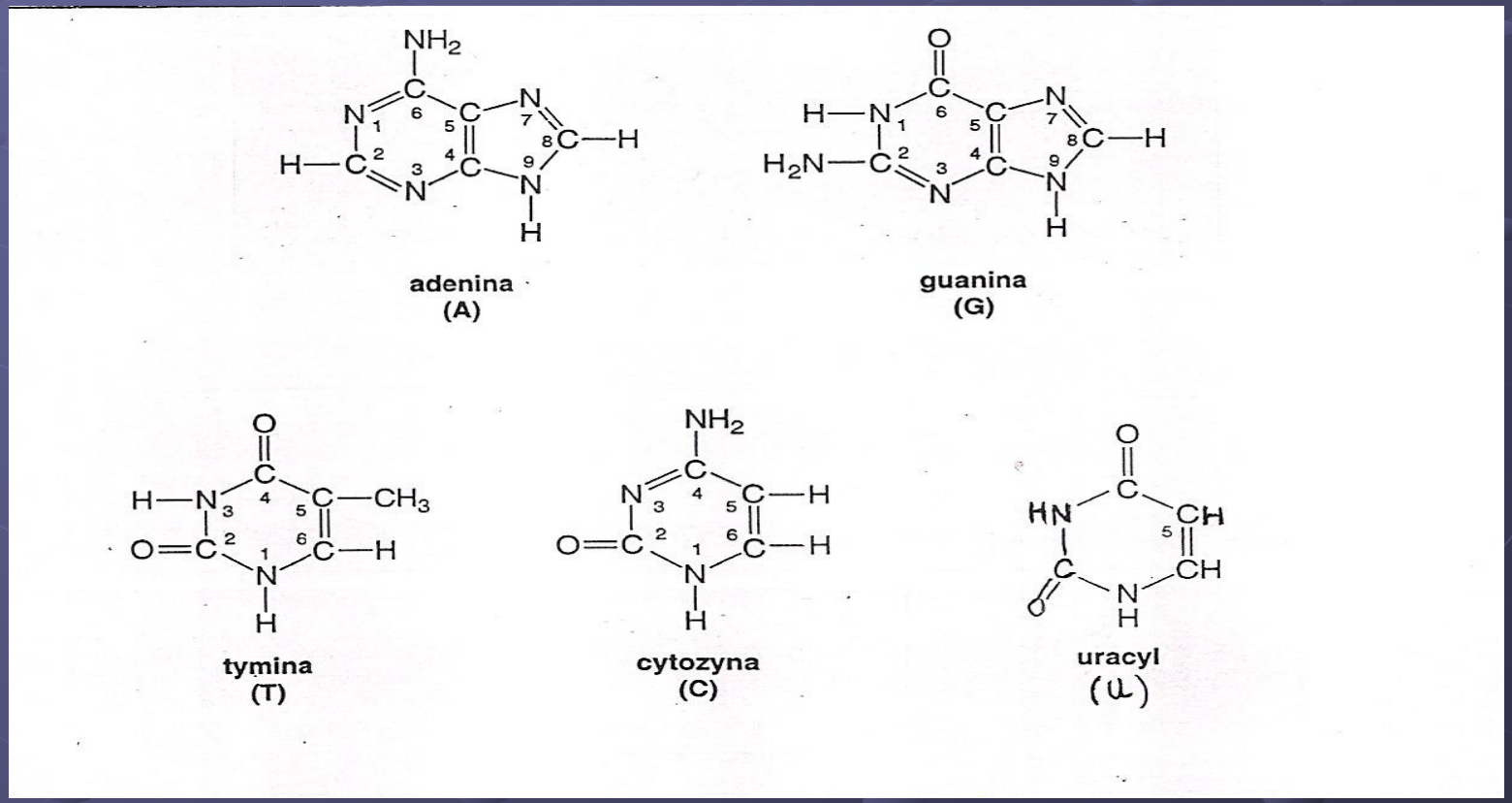


The aim of the labs:

extraction of RNA from yeast cells and detection of its compounds

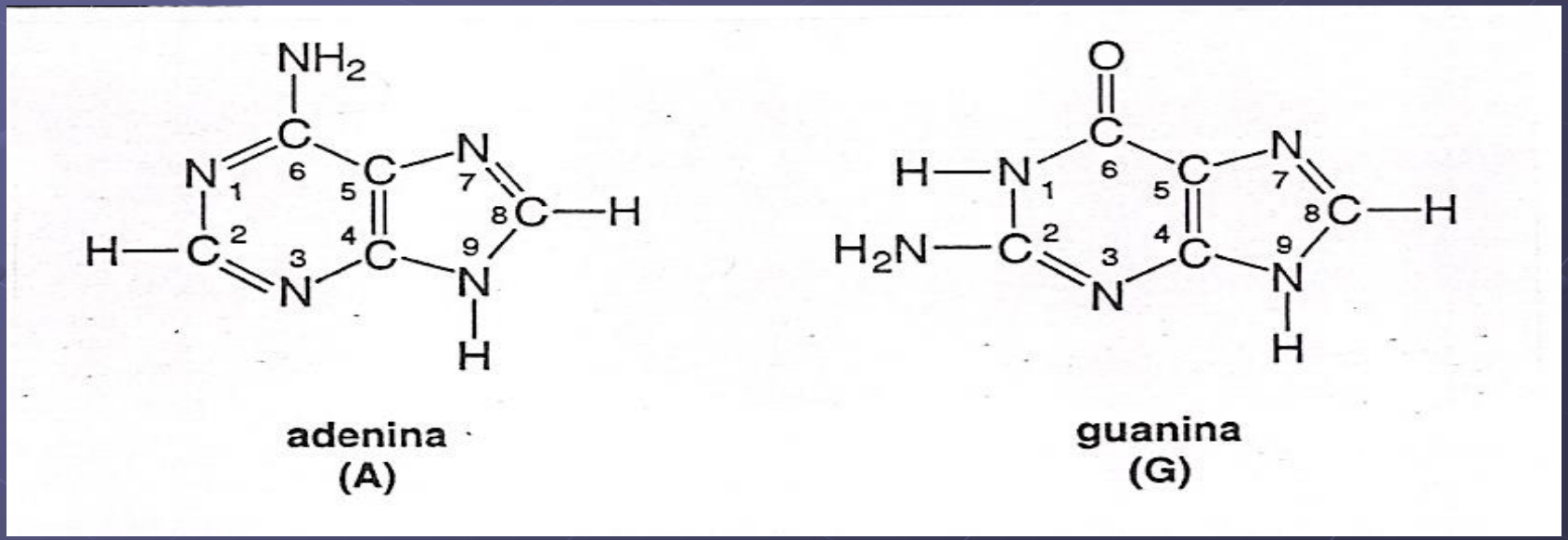
(Nitrogen bases)

- Purines – adenine (A), guanine (G)
- Pyrimidines – cytosine (C), thymine (T), uracil (U)



Nitrogen bases

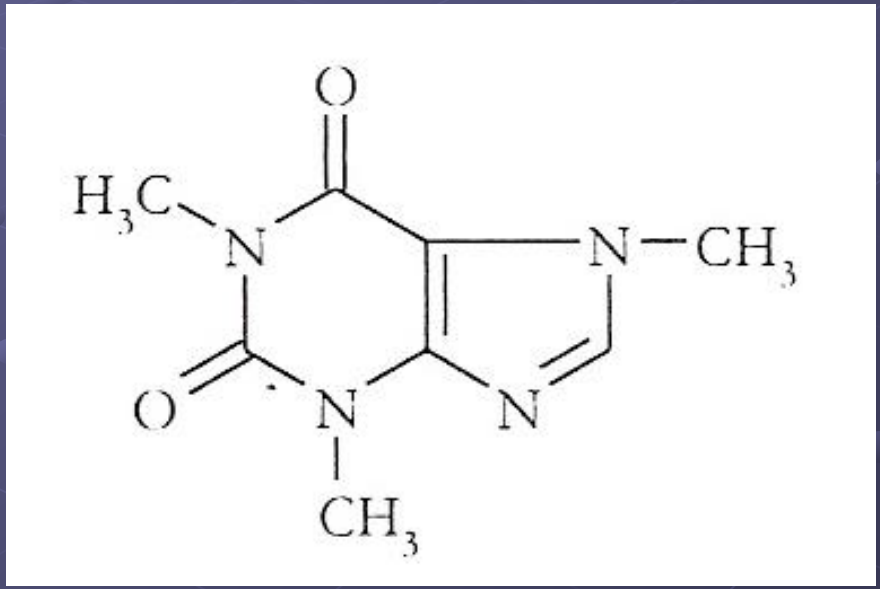
- Adenine – 6-aminopurine
- Guanine –2-amino-6-hydroxypurine



Other nitrogen bases

- Methyl derivatives of nitrogen bases:

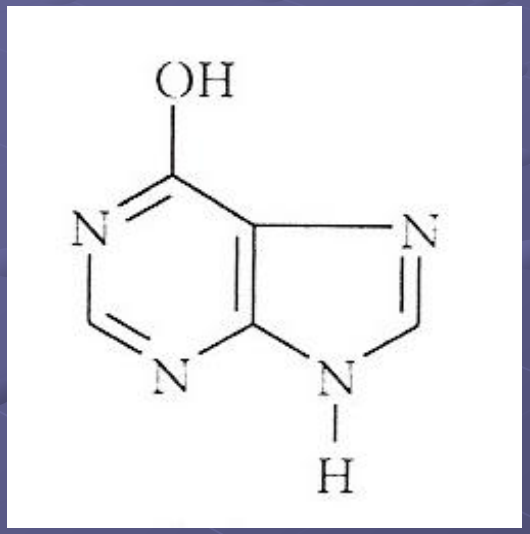
  Caffeine – 1,3,7-trimethylxanthine

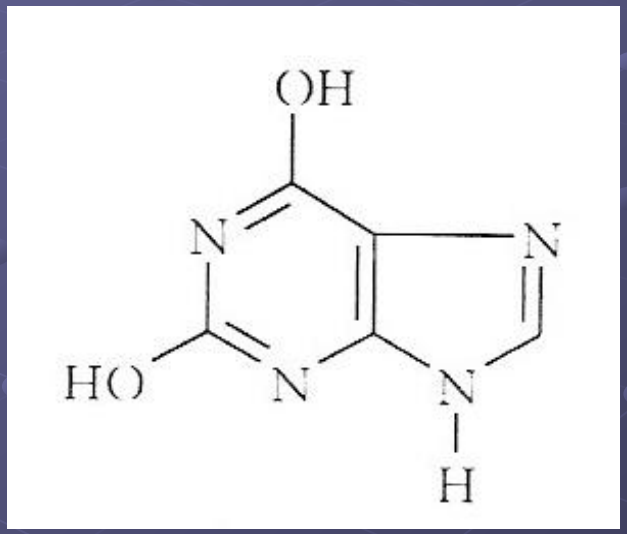


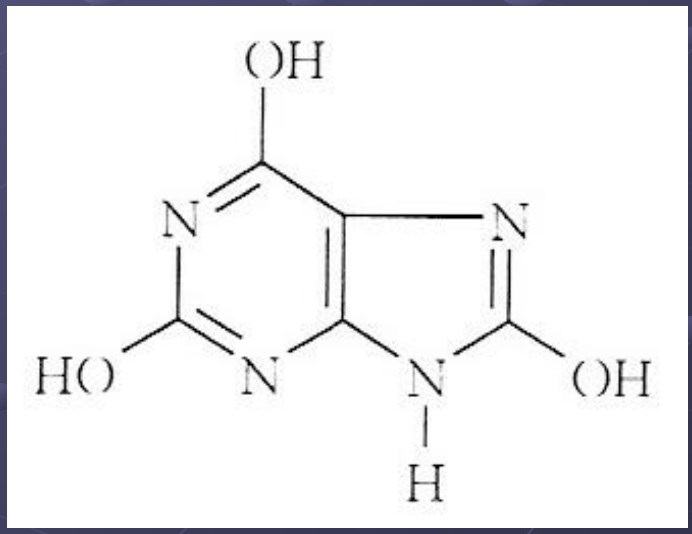
Other nitrogen bases

- Hydroxyl derivatives of nitrogen bases:

Hypoxanthine, xanthine and uric acid



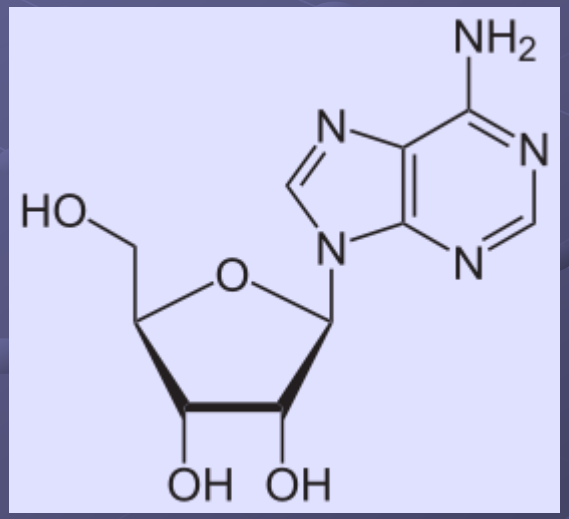




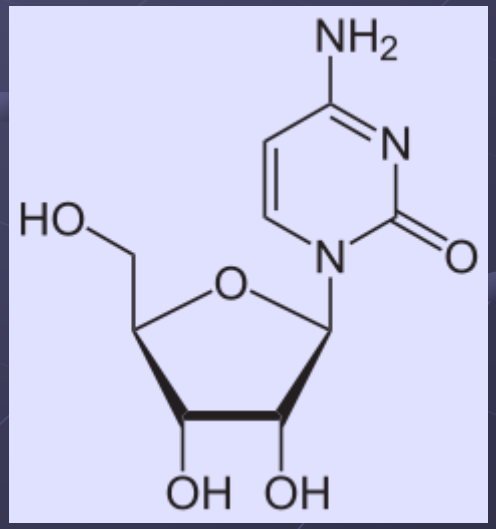
Nucleosides

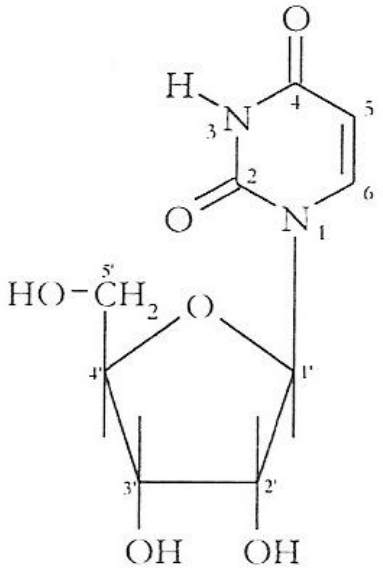
Nucleosides are glycosylamines including purine or pyrimidine base and five-carbon sugar (either ribose or deoxyribose). The glycosidic bond occours between carbon atom 1 in pentose and nitrogen atom 1 in pyrimidine or N9 in purine

Adenosine

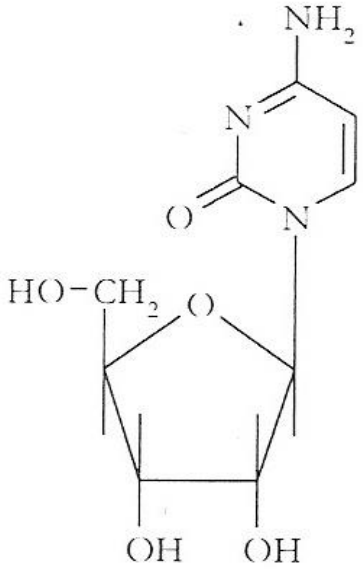


Cytidine

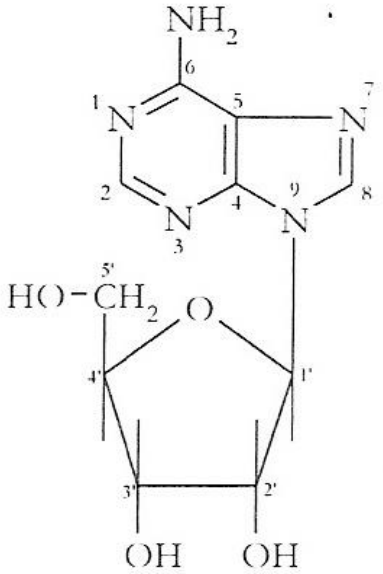




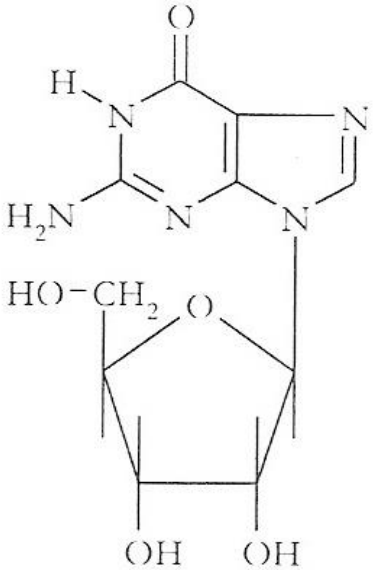
urydyna (1-β-D-rybofuranozylouracyl)



cytydyna (1-β-D-rybofuranozylocytozyna)



adenozyna (9-β-D-rybofuranozyloadenina)

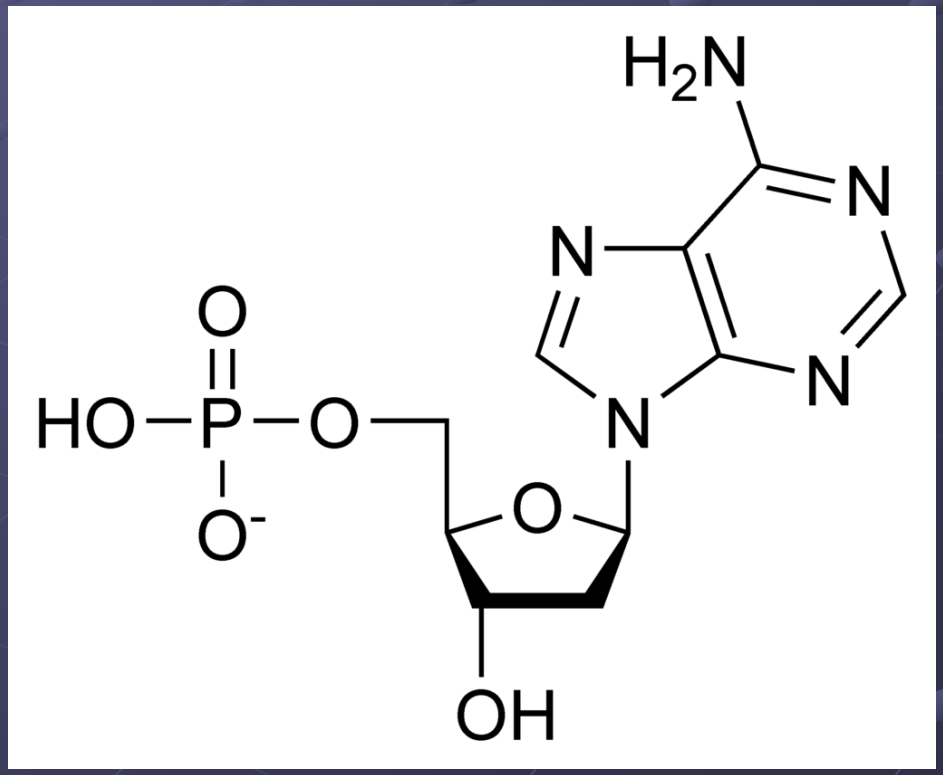


guanozyna (9-β-D-rybofuranozyloguanina)

Nucleotides

Nucleotides are phosphate esters of nucleosides, in which phosphate group is bonded to one of hydroxyl group of pentose:

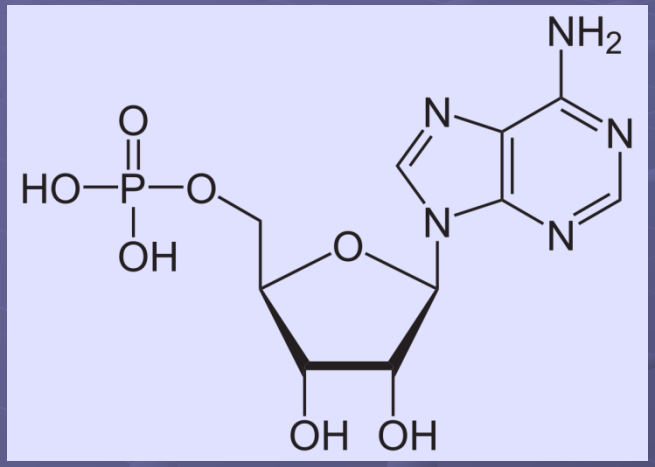
- in deoxyribose: 3`and 5`,
- in ribose: 2`, 3`, 5`



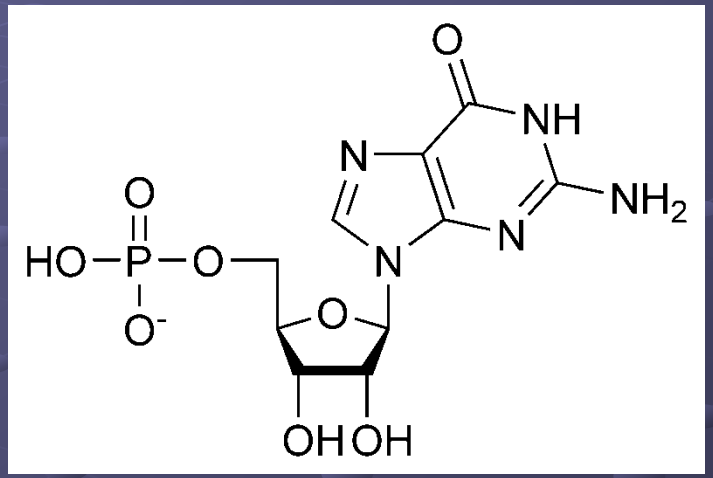
Deoxyadenosine monophosphate

Nucleotide structures

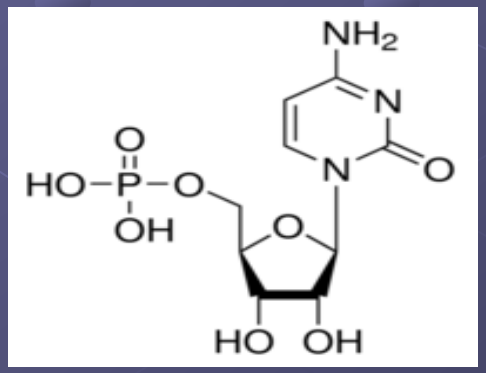
Adenosine 5’-monophosphate



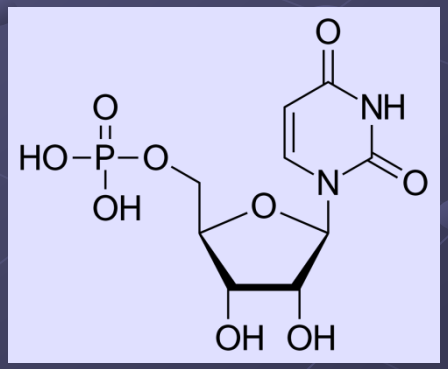
Guanosine 5’-monophosphate



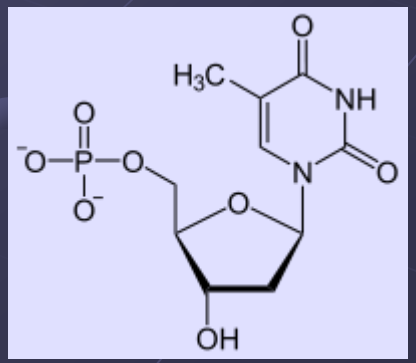
Cytidine 5’-monophosphate



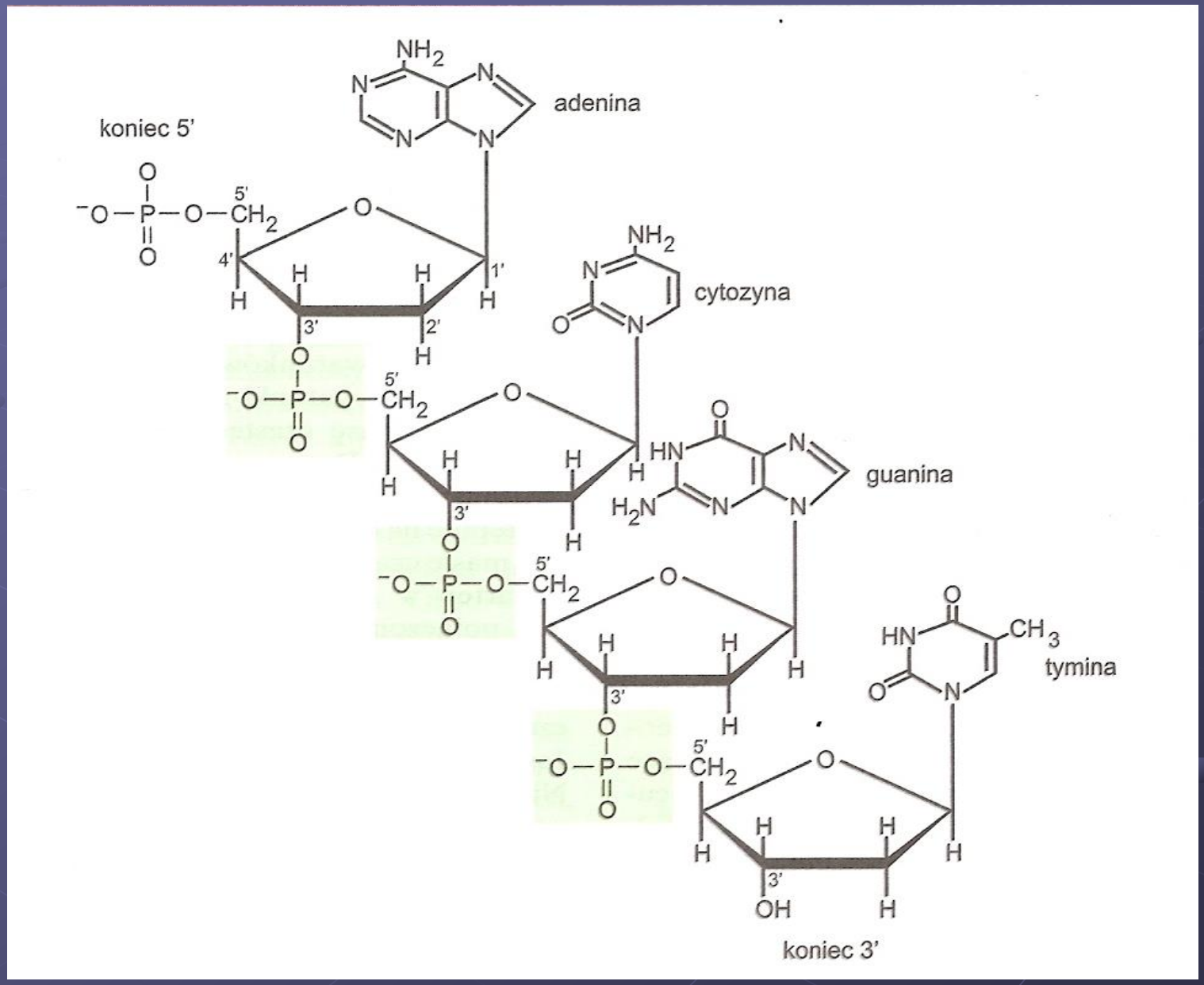
Uridine 5’-monophosphate



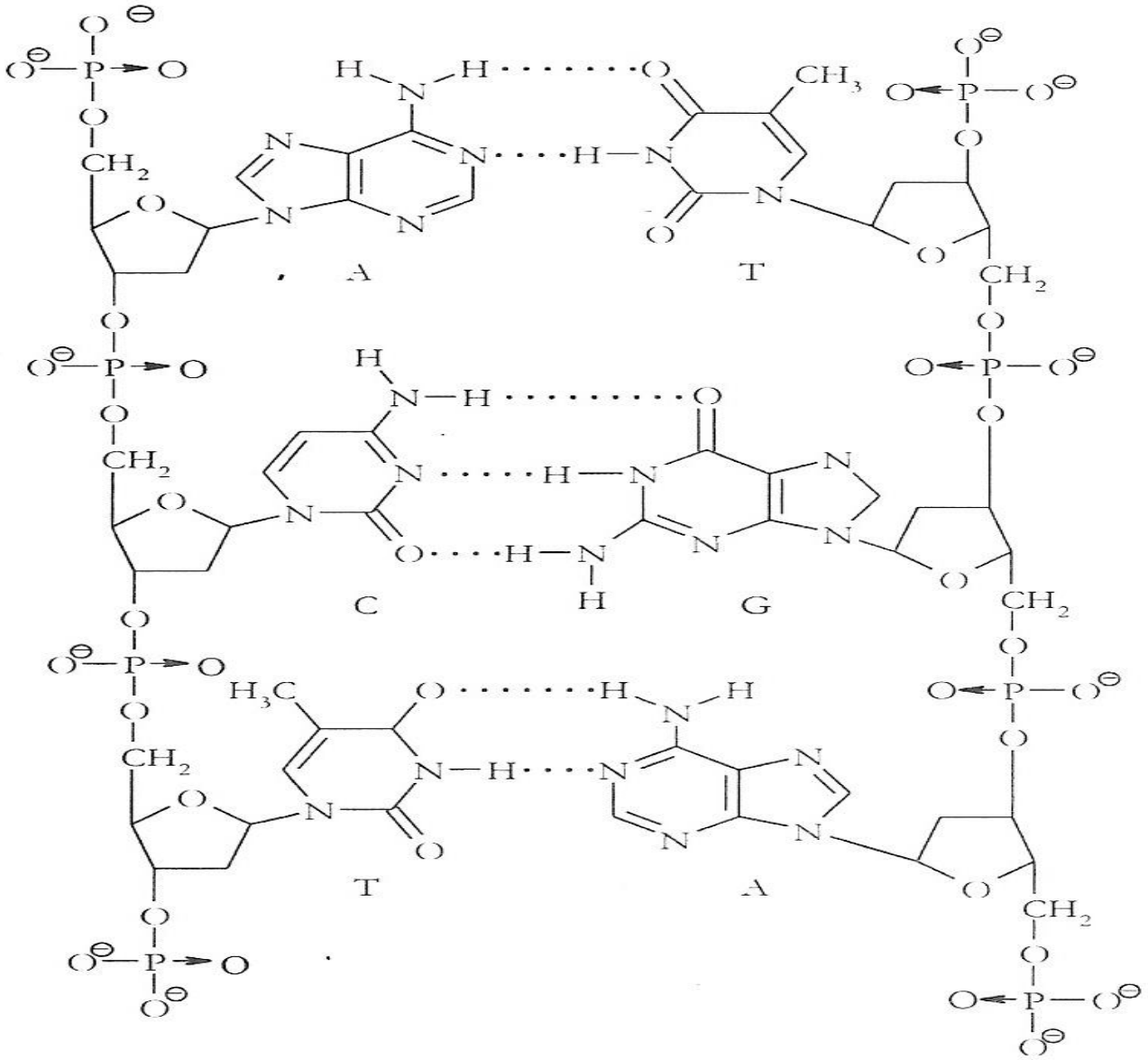
Tymidine 5’-monophosphate

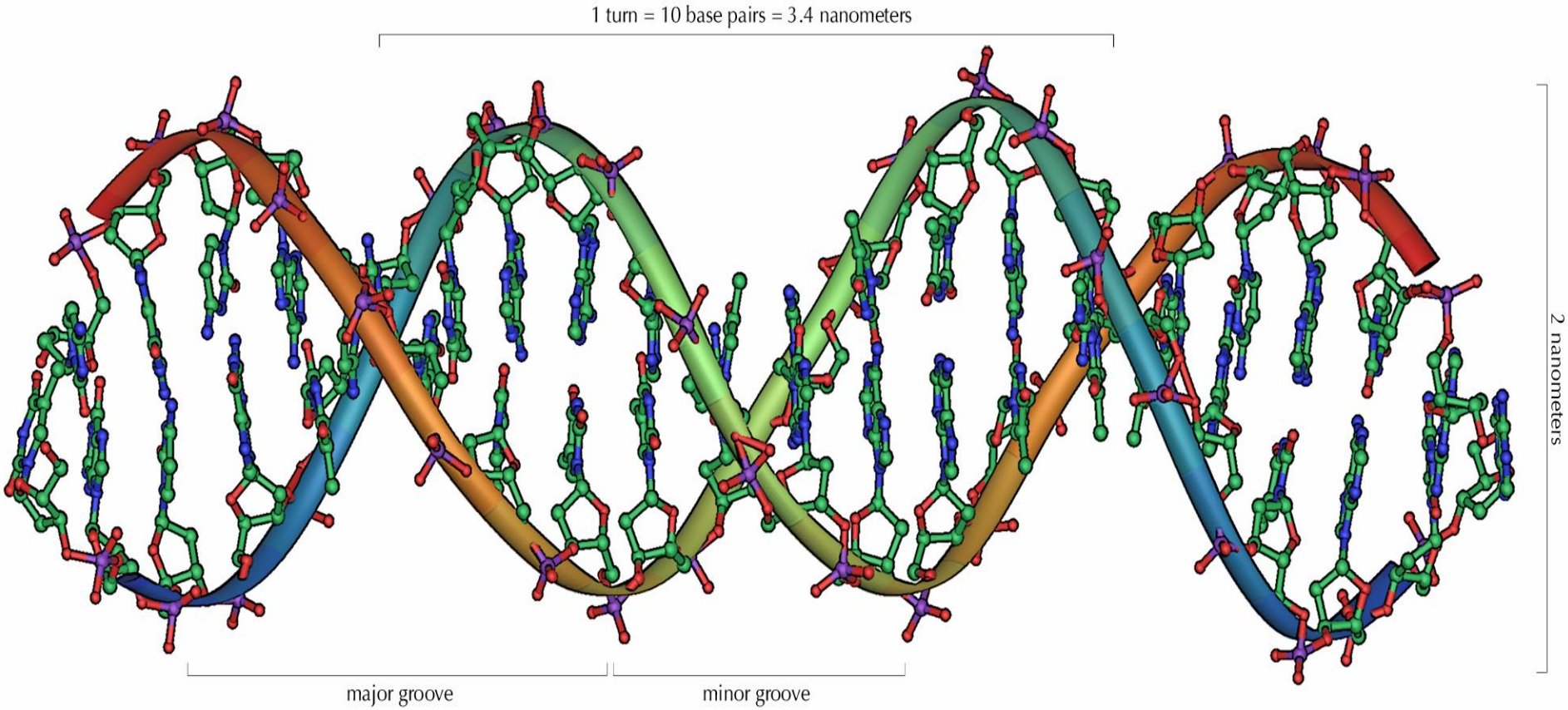


Structure of one strand of DNA



Double-stranded DNA structure




1 turn = 10 base pairs = 3.4 nanometers
2 nanometers
major groove
minor groove

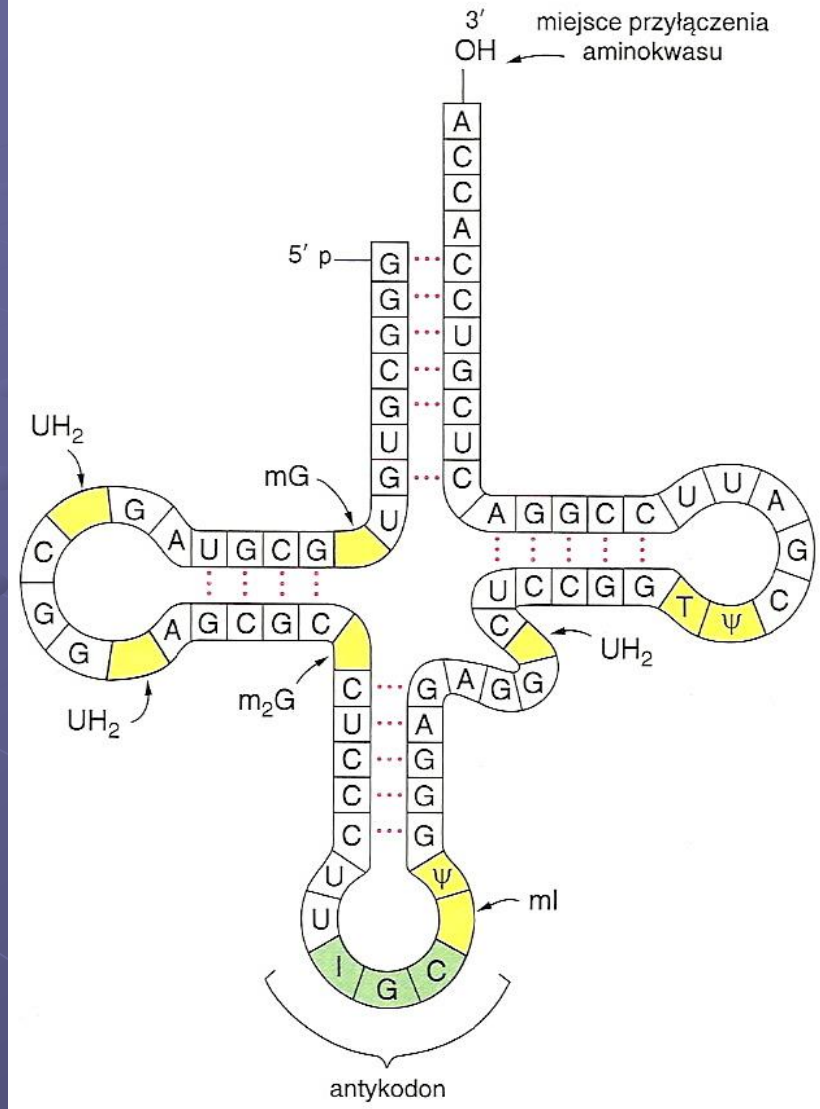
Complementary base pairing

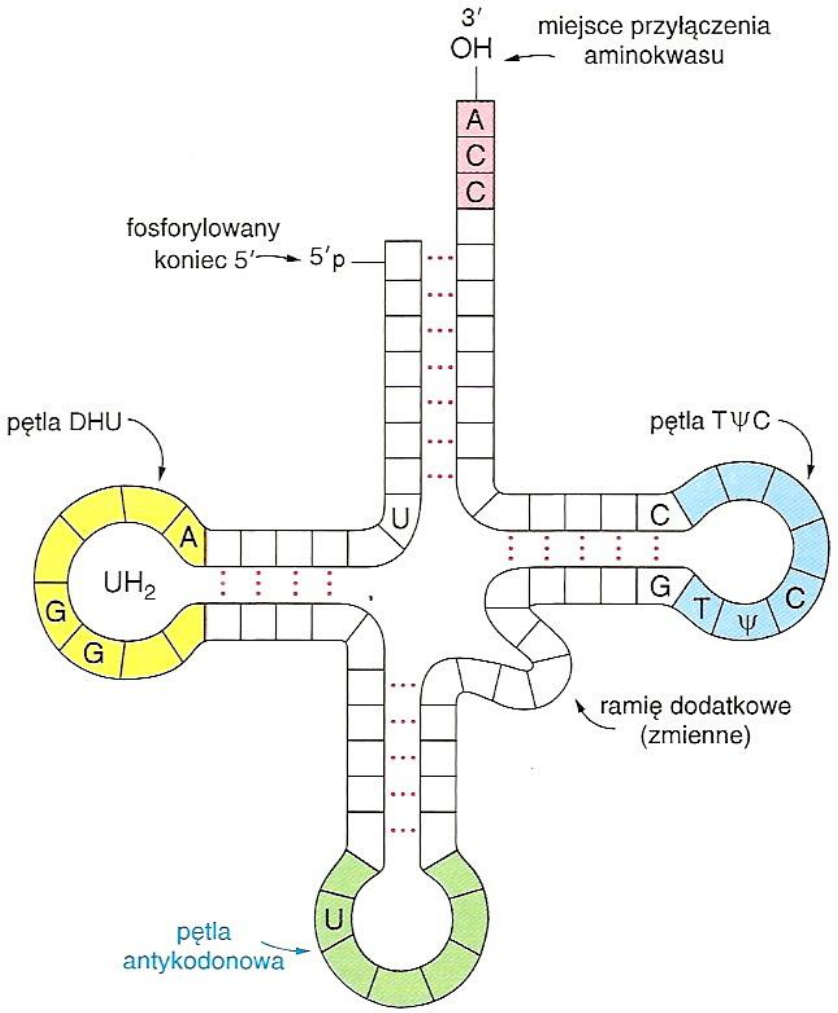
- Adenine = Thymine
  (two hydrogen bonds)
- Guanine ≡ Cytosine
  (three hydrogen bonds)

Where base complementarity can be applicable?

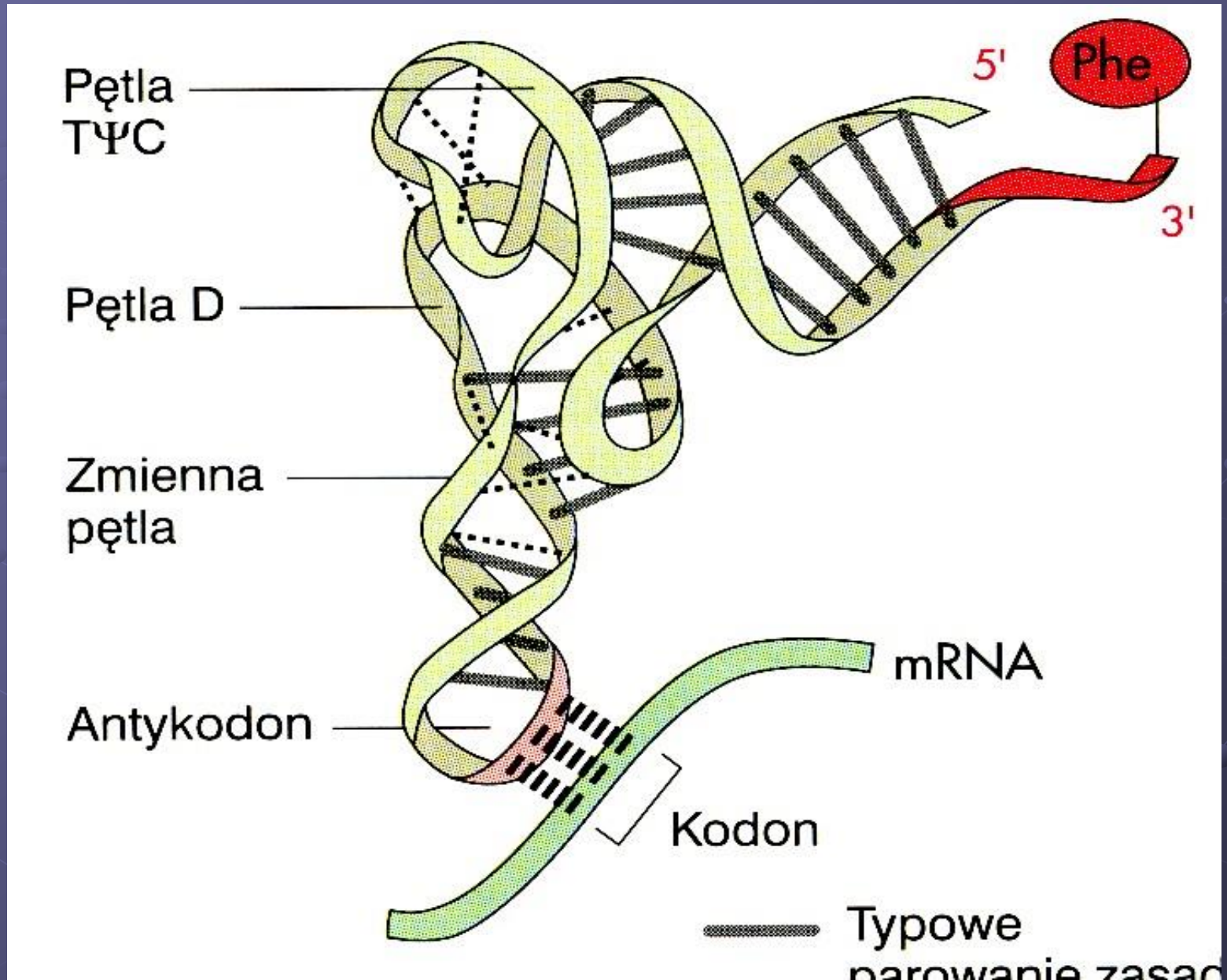
- Double-stranded DNA structure
- Replication
- Transcription
- Translation
- The structure of tRNA

tRNA structure

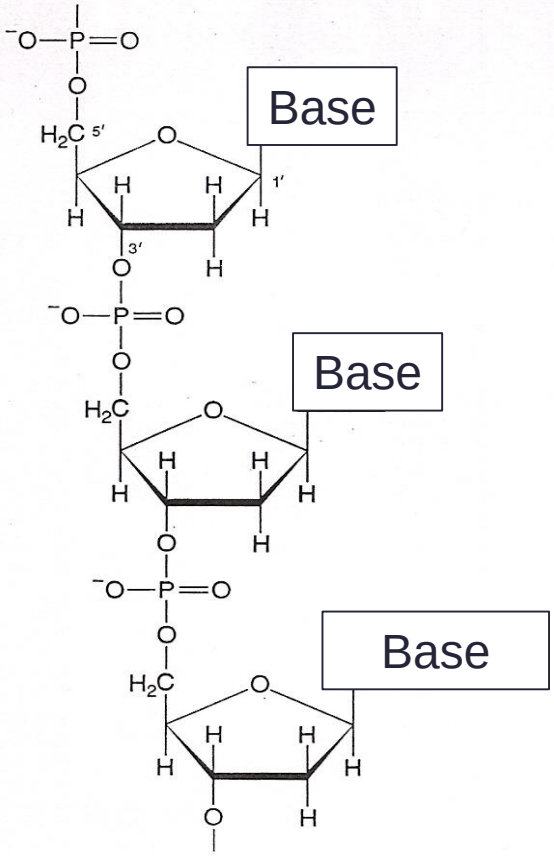




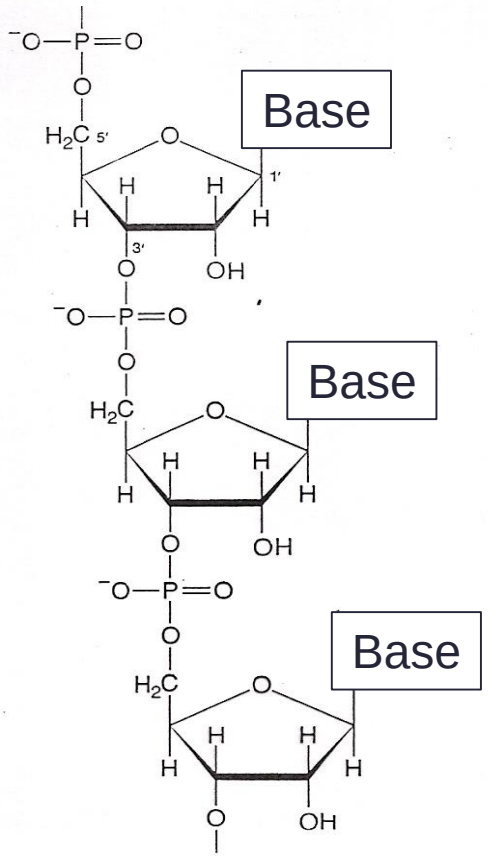
tRNA


Pętla TΨC
Pętla D
Zmienna pętla
Antykodon
5'
Phe
3'
mRNA
Kodon
Typowe

Comparison of DNA and RNA



Rys. 4-2. Struktura fragmentu łańcucha DNA



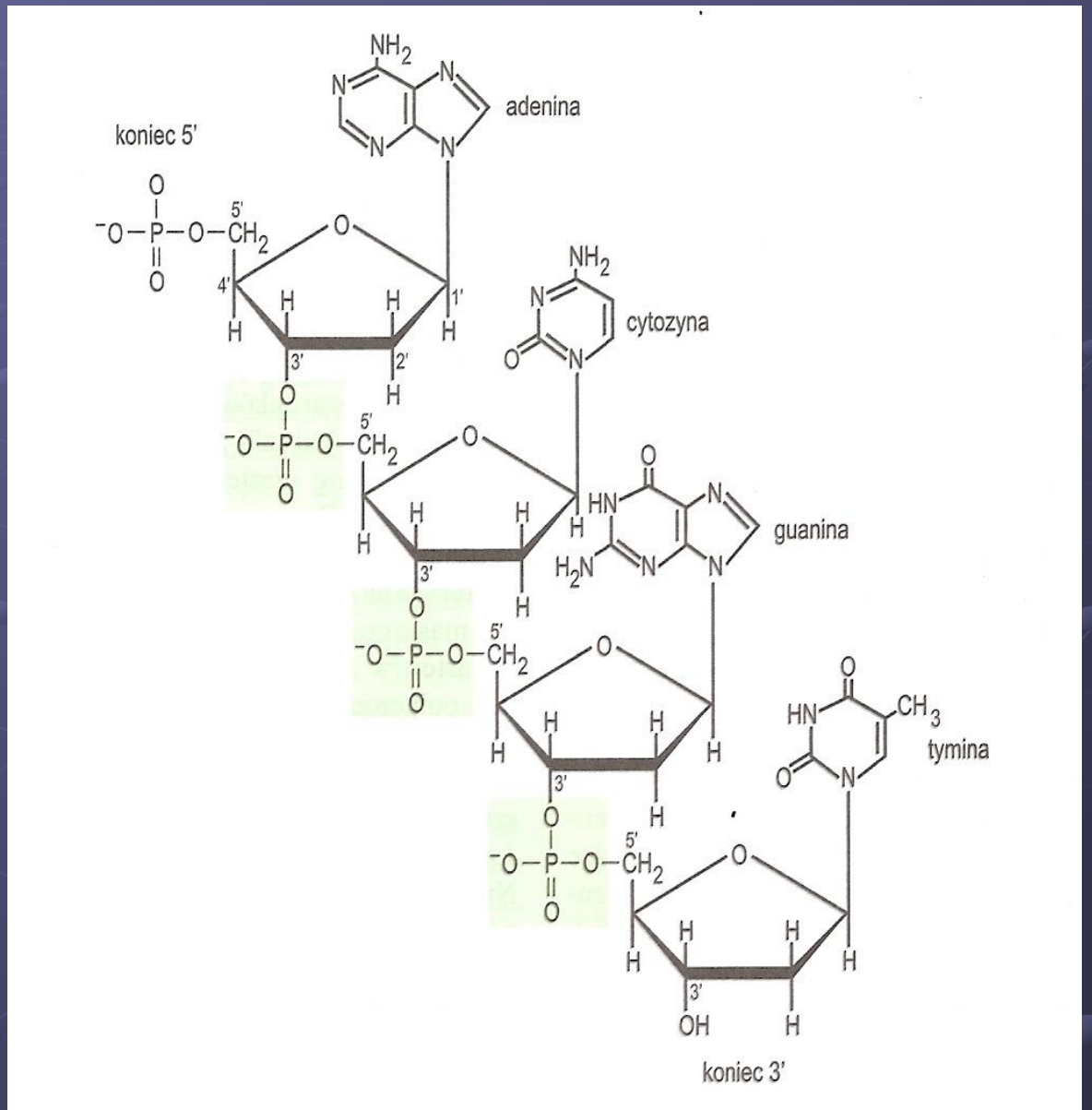
Rys. 4-24. Struktura fragmentu łańcucha RNA

DNA *versus* RNA

- Nitrogen bases
- Sugar
- Strand
- Types

Features of the genetic code

- three-letter (triplet)
- comma less
- unambiguous
- non-overlapping
- „degenerate”
- universal



Genetic code is non-ambiguous and „degenerate”

UUU Phe	UCU Ser	UAU Tyr	UGU Cys
UUC	UCC	UAC	UGC
UUA Leu	UCA	UAA Stop	UGA Stop
UUG	UCG	UAG	UGG Trp
CUU Leu	CCU Pro	CAU His	CGU Arg
CUC	CCC	CAC	CGC
CUA	CCA	CAA Gln	CGA
CUG	CCG	CAG	CGG
AUU Ile	ACU Thr	AAU Asn	AGU Ser
AUC	ACC	AAC	AGC
AUA	ACA	AAA Lys	AGA Arg
AUG Met	ACG	AAG	AGG
GUU Val	GCU Ala	GAU Asp	GGU Gly
GUC	GCC	GAC	GGC
GUA	GCA	GAA Glu	GGA
GUG	GCG	GAG	GGG

Task 1. Extraction of RNA from yeast

The aim of the experiment is to extract RNA from yeast cells and the hydrolysis of extracted RNA to obtain each component of nucleotide.

Yeast homogenization and RNA extraction. Weigh 5 grams of yeast on piece of paper or aluminium foil, put it into a mortar. Add about half a teaspoon of glass sand and grind the mixture using pestle set about 5 minutes. Next start to add drop by drop 2.5 cm^3 of 2M NaCl, still continuing grinding during next 3 minutes.

Put the obtained mixture into long glass tube and shake briskly during 10 minutes.

Now, insert the tube with homogenic mixture into boiling water bath for 7 minutes. To prevent cracking the tube, mix the mixture using glass baguette. Next, cool the tube under the stream of tap water and transfer the mixture to a plastic tube.

Centrifuge the tube using the force 2000 x g during 10 min.

Transfer the obtained supernatant to new and clean plastic tube, add to it drop by drop 3 cm^3 of chloric acid, and wait 10 minutes for the formation of precipitate.

Next, centrifuge this tube using the force 2000 x g during 10 min. After it, remove the supernatant, add to the tube 6 cm^3 of distilled water and dissolve the precipitate by mixing it thoroughly. Transfer this solution to new long, glass tube.

Hydrolysis of obtained RNA. Add to the glass tube including RNA solution 3 cm^3 of 10M sulfuric acid. Wait 30 minutes. Next, make the tests for the detection of pentose, purine base and phosphate residues.

.

Task 2. Detection of each component of nucleotide

The aim of this part of experiment is to detect each component of nucleotide to confirm its composition. Note the results in the table below.

Detection of pentose. Take 1 cm^3 of the acidic hydrolysate achieved in task 1 to new glass tube and add 1 cm^3 of Bial reagent. Heat the tube carefully over the fire of burner.

Now, to make remaining tests, the acidic hydrolysate must be neutralized by adding 2 cm^3 of 2M NH_3.aq.

Detection of purine bases. Take 2 cm^3 of neutralized hydrolysate to new glass tube, add 5 drops of 0.1 M $AgNO_3$ still mixing. Next, add 1 cm^3 of 2 M NH_3.aq. Purine bases form silver salts in ammonium solution, and then precipitate.

Detection of phosphate residues. Take 1 cm^3 of neutralized hydrolysate to new glass tube, and add 0.5 cm^3 of concentrated solution of HNO_3 and 1 cm^3 of ammonium molybdate. Heat the tube carefully over the fire of burner and boil the content of the tube about 3 minutes.

Test	Result
Detection of pentose	
Detection of purine bases	
Detection of phosphate residues	

Thank you for your attention