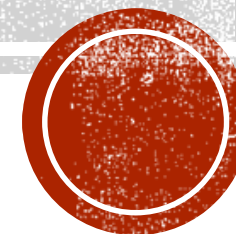


# METAL COMPLEXES OF DNA AND RNA AND ITS TRANSCRIPTION AND REPLICATION



Prof. Ramesh Chandra  
Department of Chemistry  
University of Delhi

# Introduction

The design of new pharmaceuticals like cisplatin requires a detailed understanding of how platinum and other metal ions interact with nucleic acids and nucleic-acid processing. Furthermore, we are finding that metal complexes can be uniquely useful in developing spectroscopic and reactive probes of nucleic acids, and hence may become valuable in developing new diagnostic agents. Nature itself takes advantage of metal/nucleic acid chemistry, from the biosynthesis of natural products such as bleomycin, which chelates redox-active metal ions to target and damage foreign DNA, to the development of basic structural motifs for eukaryotic regulatory proteins, the zinc-finger proteins, which bind to DNA and regulate transcription. In all these endeavors, we need first to develop an understanding of how transition-metal ions and complexes interact with nucleic acids and how this chemistry may best be exploited.



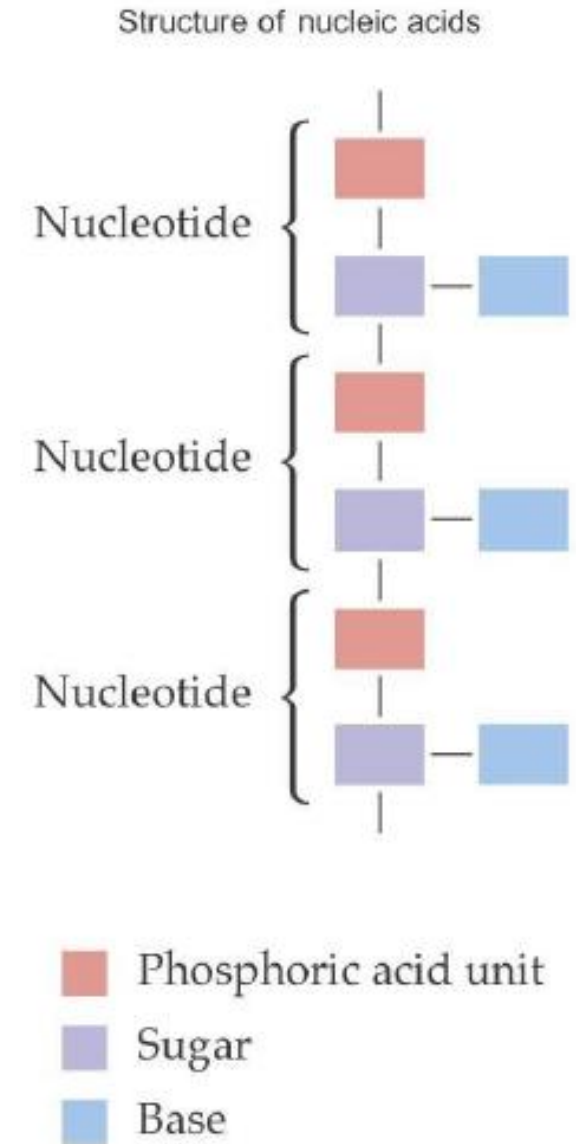
# Nucleic Acids

- There are two kinds of nucleic acids in cells:
  - 1) ribonucleic acids (RNA)
  - 2) deoxyribonucleic acids (DNA)
- Both RNA and DNA are polymers built from monomers called nucleotides.
- A nucleotide is composed of:
  - a base, a monosaccharide, and a phosphate.



# Nucleic Acids

- made up of nucleotides
- found in all living cells except RBC
- deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
- DNA is in the nucleus
- RNA is in the cytoplasm
- function in the storage and transmission of genetic material
- And control and direct all protein synthesis



# Nucleic-Acid Structures

Figure displays a single deoxyribonucleotide and the four different nucleic acid bases. As may be evident, each mononucleotide along a nucleic-acid polymer contains a variety of sites for interactions with metal ions, from electrostatic interactions with the anionic phosphate backbone to soft nucleophilic interactions with the purine heterocycles. The different nucleic-acid bases furthermore offer a range of steric and electronic factors to exploit. Coordination of a metal complex to the N7 nitrogen atom of a purine, for example, would position other coordinated ligands on the metal center for close hydrogen bonding to the O6 oxygen atom of guanine, but would lead to clashes with the amine hydrogen atoms of adenine.

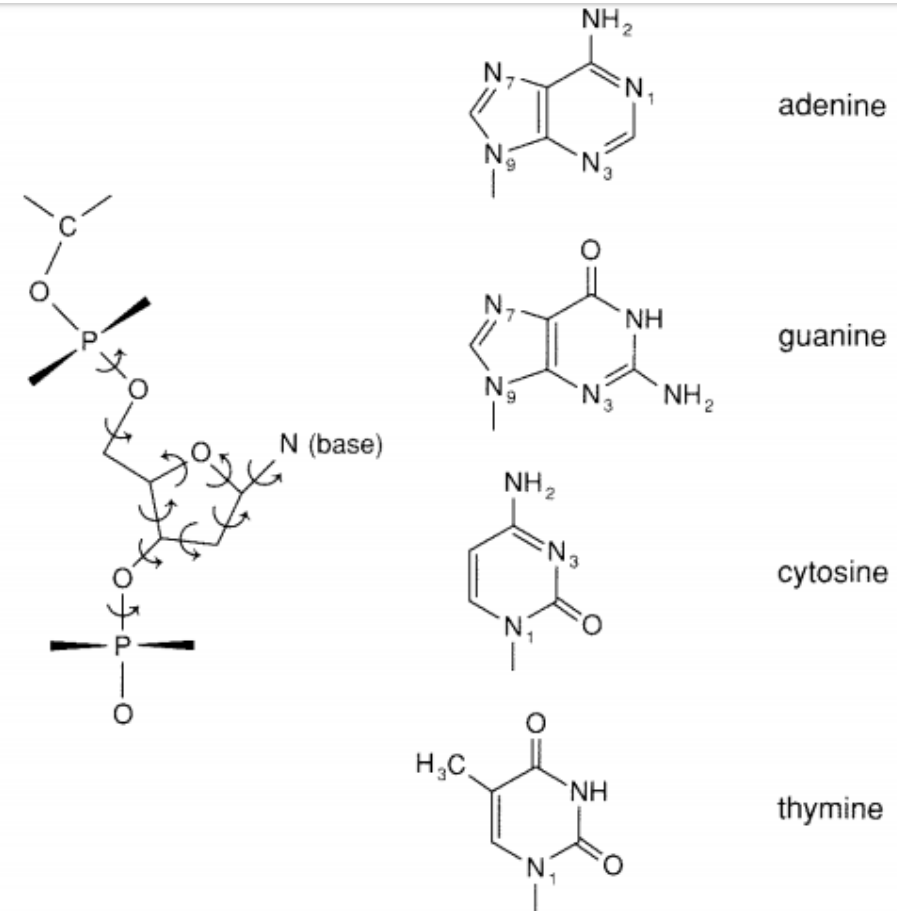


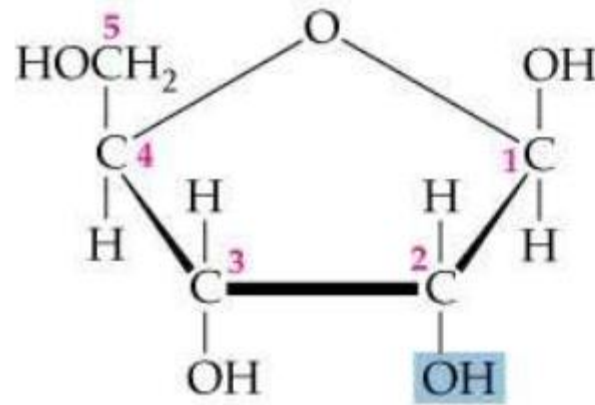
Illustration of a mononucleotide unit. Arrows indicate the various torsional angles within each unit that together generate the wide range of conformations available in the polymer. Also shown are the individual bases as well as the commonly employed numbering scheme.



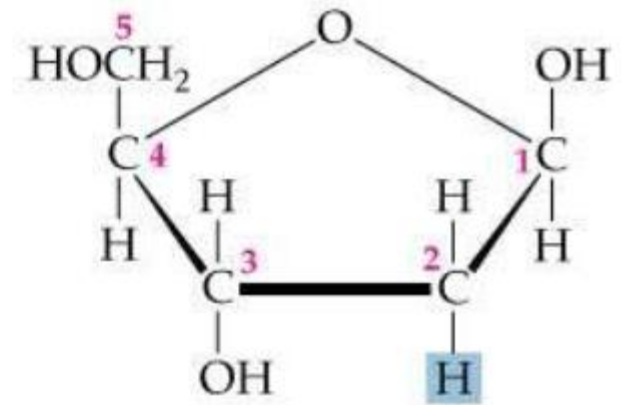
-each nucleotide  
contains

- 1) a sugar
- 2) a base
- 3) phosphoric acid  
unit

## 1) Ribose and Deoxyribose



ribose  
RNA



2-deoxyribose  
DNA

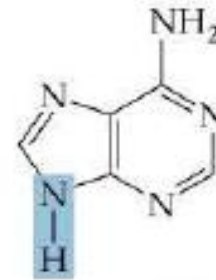


# Bases in Nucleic Acids

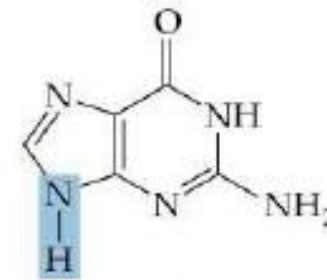
Bases for DNA:  
A, G, C, T

Bases for RNA:  
A, G, C, U

purines

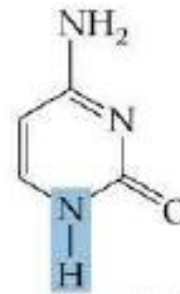


adenine (A)  
DNA  
RNA

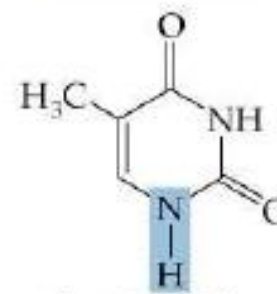


guanine (G)  
DNA  
RNA

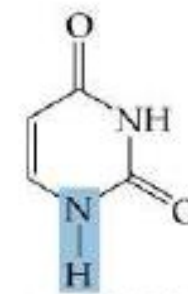
pyrimidines



cytosine (C)  
DNA  
RNA



thymine (T)  
DNA



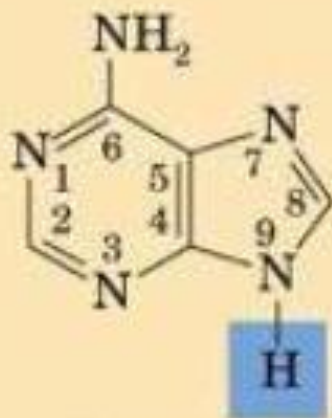
uracil (U)  
RNA



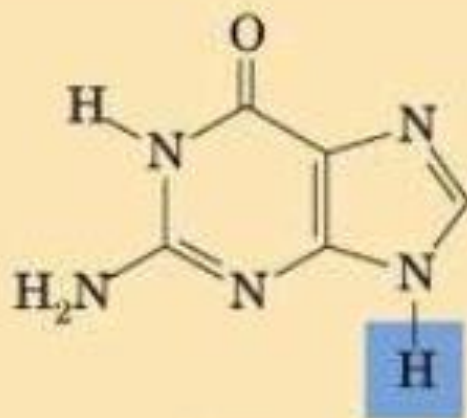


# Pyrimidine/Purine Bases

## Purines



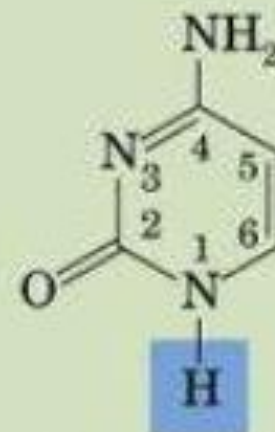
Adenine (A)



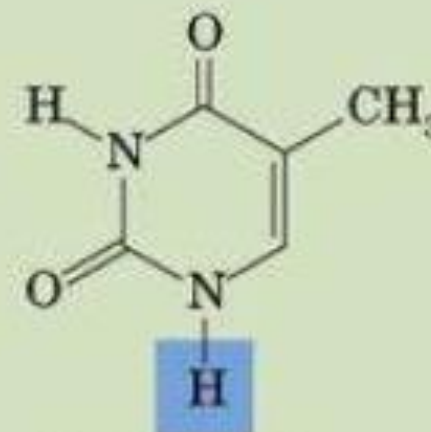
Guanine (G)

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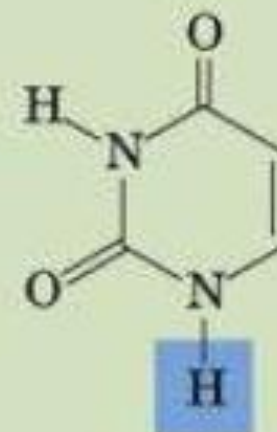
## Pyrimidines



Cytosine (C)



Thymine (T)  
(DNA only)



Uracil (U)  
(RNA only)





## DNA - 2° Structure

- Secondary structure: the ordered arrangement of nucleic acid strands.
  - the double helix model of DNA 2° structure was proposed by James Watson and Francis Crick in 1953.
- Double helix: a type of 2° structure of DNA in which two polynucleotide strands are coiled around each other in a screw-like fashion.

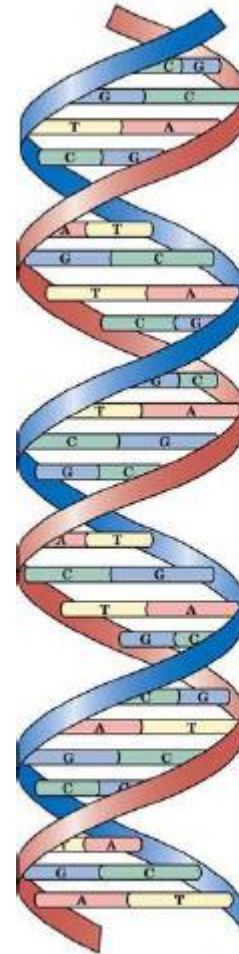


# Watson and Crick

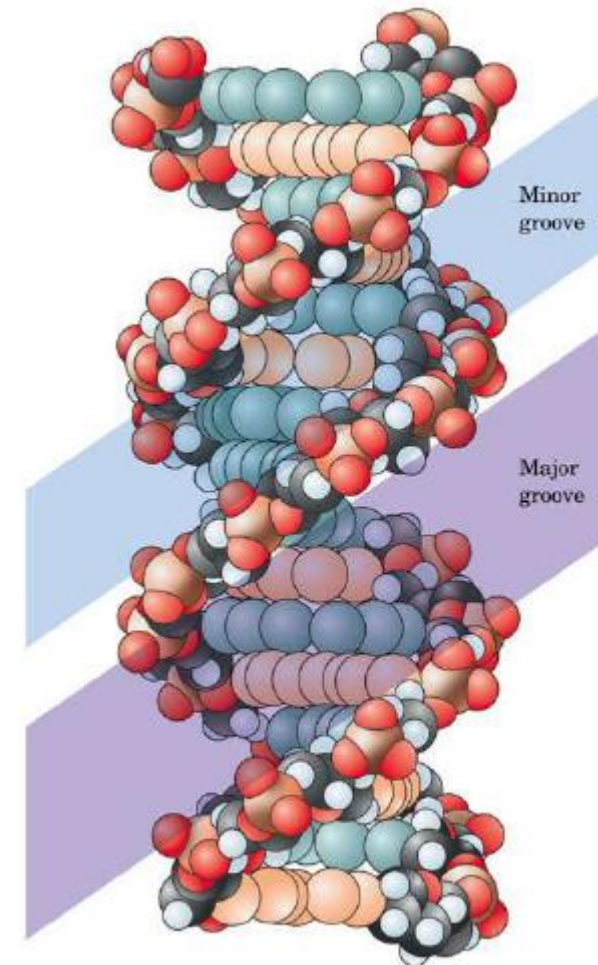


# The DNA Double Helix

Three dimensional structure of a DNA double helix.



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# DNA Double Helix

Like a spiral staircase:

-the phosphate sugar backbone represents the hand rail, the bases represent the steps

- Hydrogen bonding occurs between the bases.....

For DNA:

A bonds with T

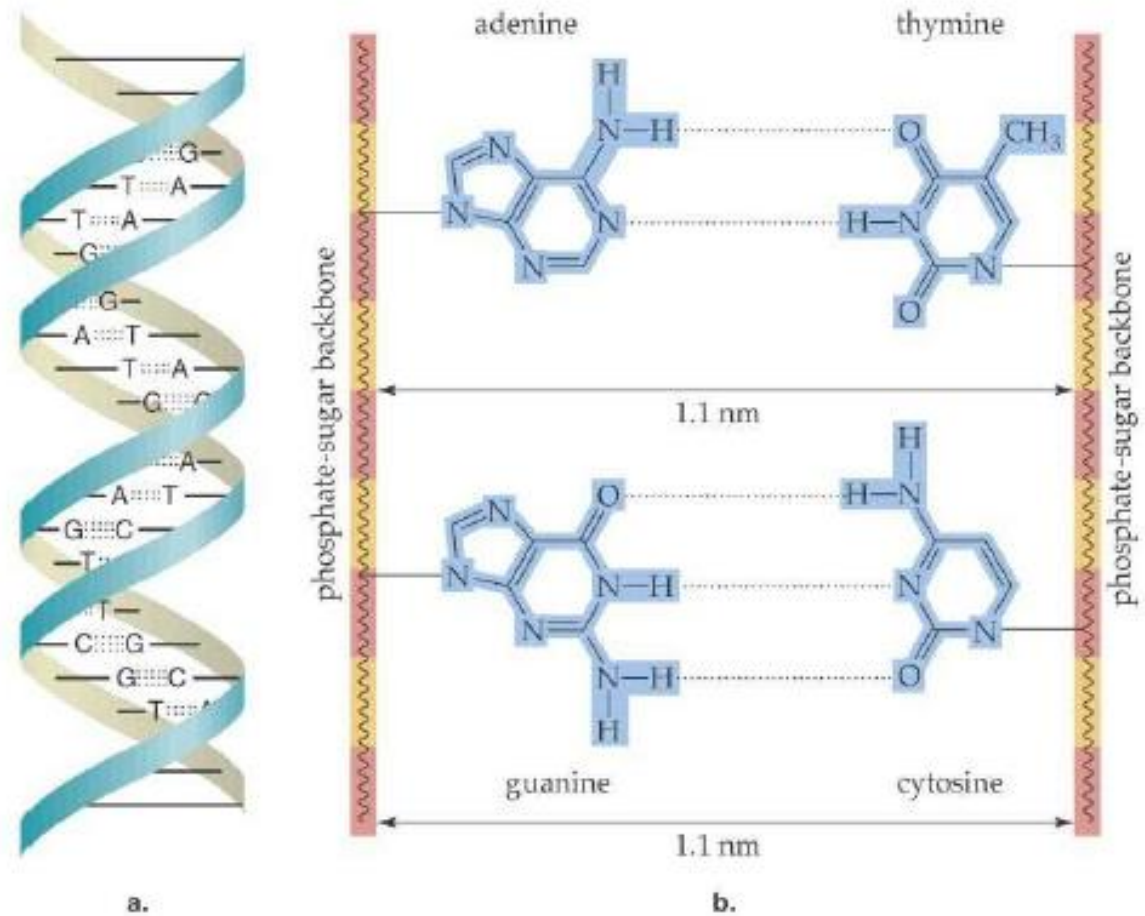
C bonds with G

For RNA:

A bonds with U

C bonds with G

(a) Schematic diagram of double helix of DNA (b) Base pairings in DNA



# DNA and RNA

- The three differences in structure between DNA and RNA are:
  - DNA bases are A, G, C, and T; the RNA bases are A, G, C, and U.
  - The sugar in DNA is deoxyribose; in RNA it is ribose.
  - DNA is always double stranded; there are several kinds of RNA, most of which are single-stranded.



# Higher Structure of DNA

- DNA is coiled around proteins called histones.
- Histones are rich in the basic amino acids Lys and Arg, whose side chains have a positive charge.
- The negatively-charged DNA molecules and positively-charged histones attract each other and form units called nucleosomes.
- Nucleosome: a core of eight histone molecules around which the DNA helix is wrapped.
- Nucleosomes are further condensed into chromatin.
- Chromatin fibers are organized into loops, and the loops into the bands that provide the superstructure of chromosomes.





# Interaction of Metal Ions with DNA and RNA

Metal ions coordination to the nucleic acids (DNA and RNA) is critical for their structural properties and function.

A number of factors play critical roles in controlling the particular effects on structure and function-

1. including the nature of the metal ion,
2. its charge and concentration,
3. nucleic acid concentration,
4. length and type of nucleic acid sequence,
5. temperature,
6. polarity of given solvent and buffer, and
7. ionic strength.

Metal ions stabilize a particular nucleic acid structure and can lead to the denaturation of the native conformation, forming other structural motifs such as triple-strand formation, nucleic acid aggregation and condensation.



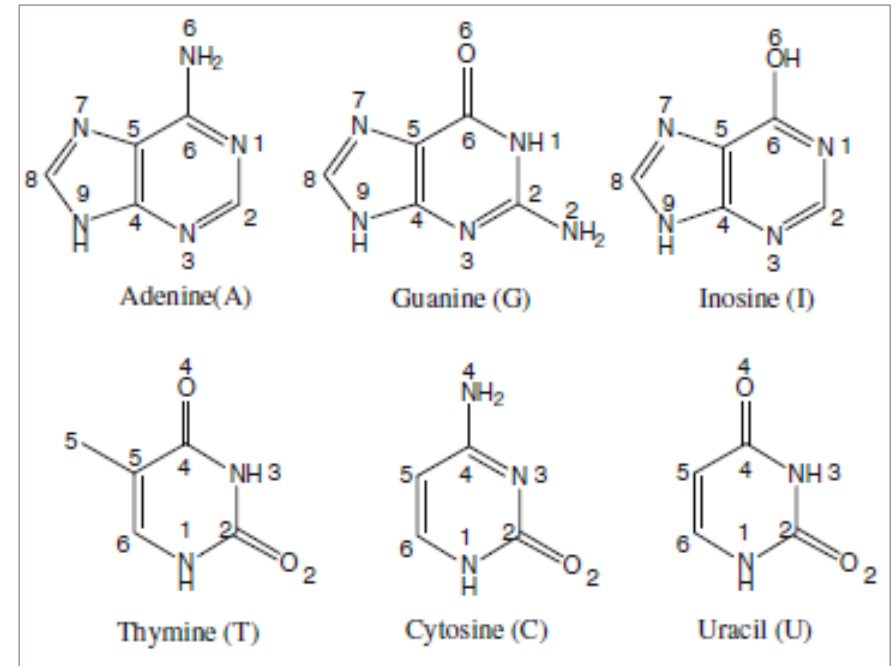


# Structural Properties of Nucleic Acids

Deoxyribonucleic acid (DNA) is a biopolymer composed of nucleotide monomers. A nucleotide monomer is formed by a combination of three basic building blocks:

1. a planar aromatic derivative of pyrimidine or purine base,
2. a deoxyribose sugar and
3. a phosphate group.

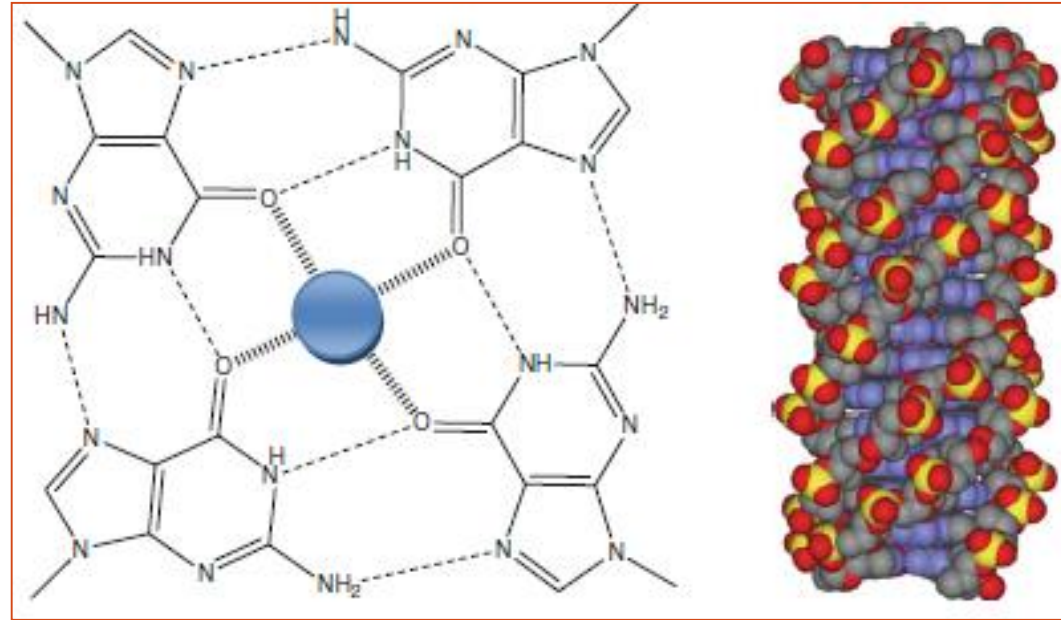
There are two purine bases, adenine (A) and guanine (G) and two pyrimidine bases, thymine (T) and cytosine (C), involved in DNA structure. Uracil (U), lacking the C5 methyl group, replaces thymine in RNA(ribonucleic acid). Inosine (I), a deaminated guanine analogue found in tRNA, can pair with C, U and A nucleobases in mRNA.



- Divalent cations are required for the replication, transcription and translation of the genetic code.
- Metal ions are involved in the stabilization of the DNA structure by coordination to the phosphodiester backbone of DNA.
- In the absence of any cations, the native double-helical B-conformation of DNA cannot be formed and, thus DNA is unable to perform its functions.
- Small, mobile, multivalent cations can bring about structural change in DNA, such as bending.
- Base pairing of nucleobases within the strand (e.g. RNA) or between two different strands (e.g. DNA) in conjunction with metal coordination (usually  $Mg^{2+}$ ) leads to distinct structural patterns and structure of higher order, for instance DNA triple helices, G-quadruplex, and helical junctions.



- In 1962 Davis proposed that guanine rich sequences of DNA can assume very unusual structures, in which the guanines could form planar H-bonded arrangements called guanine quartets.
- These arrangements are stabilized by metal ion coordination. G-quartets (also known as quadruplexes, tetraplexes or G4- structures) play an important biological role in telomeres, which protect the ends of chromosomes and can be an effective drug target.



- a). The arrangement of guanine bases in the G-quartet, shown together with a centrally placed metal ion. Hydrogen bonds are shown as dotted lines.
- b). Space-filled model of G-quadruplex DNA, which is poly(dG) four-fold, right handed helix.



Two types of interaction between the DNA and metal ions,

(a) ligand-mediated interactions and

(b) direct metal ion bonding with DNA.

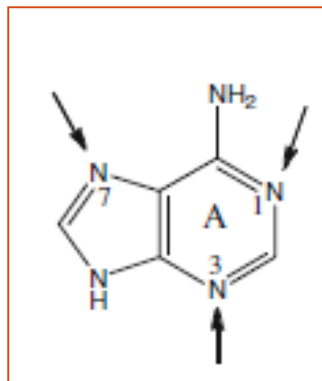
- Ligand-mediated interactions occur via H-bond, p-p interactions between a ligand of a metal complex, such as  $\text{Ru(phen)}_2\text{Cl}_2$ ,  $[\text{Ru(phen)}_3]^{2+}$ ,  $[\text{Zn(phen)}_3]^{2+}$ , and the heterocyclic nucleobases by intercalation or shape-selective binding to the grooves employing weak forces such as van der Waals interactions.
- Direct bonding involves the interaction between the filled orbital of the ligand atom of a nucleobase and a suitable, empty orbital of the metal.
- Metal ion-phosphate interaction is an important interaction contributing to the stability of B-DNA and involves the coordination of positively charged metal ion and the negatively charged phosphate backbone and is characterized by an approximate metal-phosphate distance [7 Å].
- Sodium and potassium ions serve as bulk electrolytes in this mode of binding. However, divalent and trivalent metal cations bind more tightly due to greater charge density.
- $\text{Mg}^{2+}$  serves second to  $\text{K}^+$  in intracellular concentration as counter ion for the phosphate groups of nucleic acids in cell.



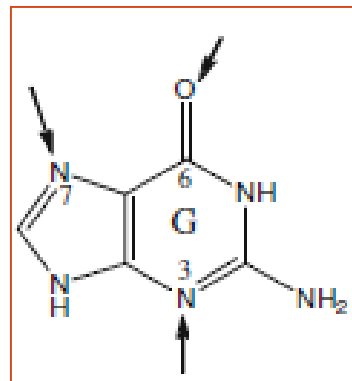
The unprotonated endocyclic N-atoms and exocyclic carbonyl O-atoms of nucleobases in their preferred amino and keto tautomeric forms are metal binding sites.

These include-

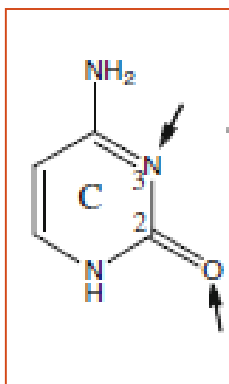
N1, N3 and N7 sites  
in adenine



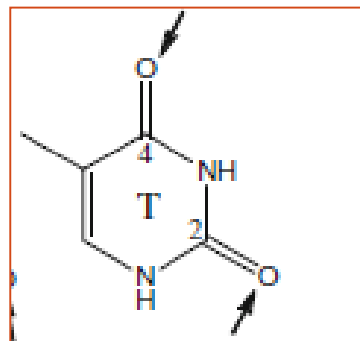
N3, N7 and O6 sites in guanine



N3 and O2 in cytosine



O2 and O4 sites in thymine



The exocyclic amino groups, having a lone pair on N-atom, are not usually a useful metal binding sites due to the delocalization of the lone pair into heterocyclic ring, which leads to very low basicity.

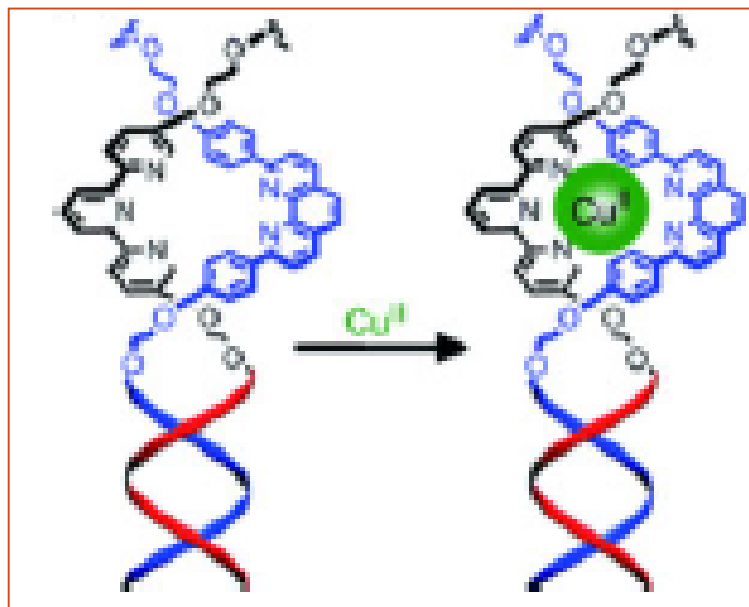


# Applications of DNA-Metal Ion Interactions

## 1). DNA-Metal Nanostructures

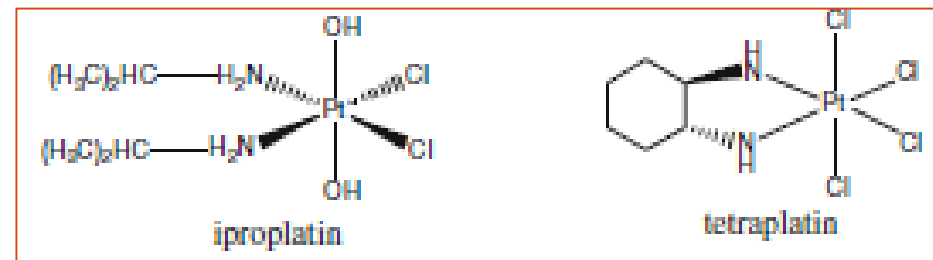
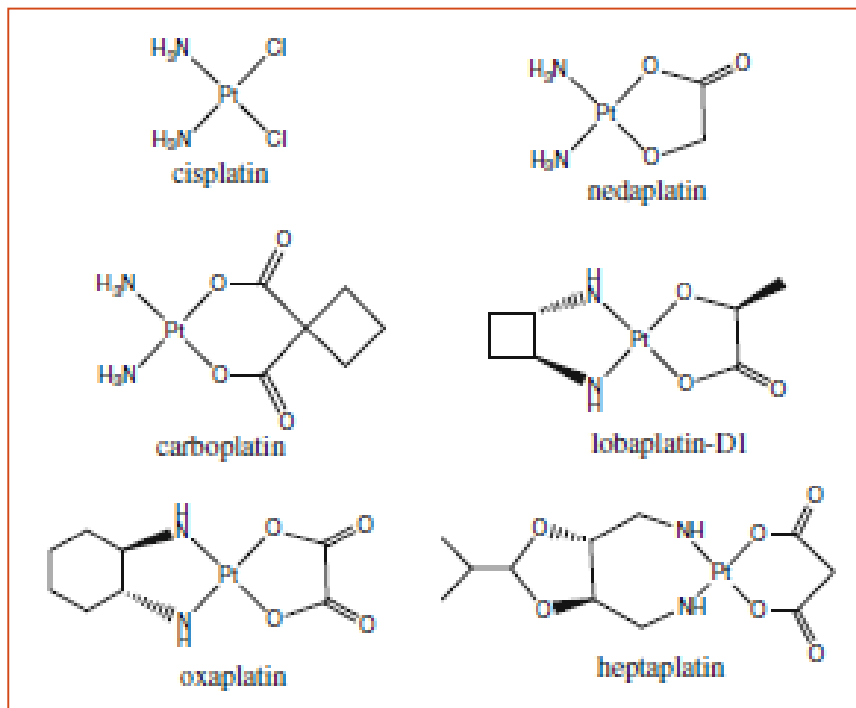
Metal ions or metallo-ligand attached to DNA can be used to promote DNA interactions. This has led to the use of DNA as a building-block for the assembly of nanostructures.

Using  $(dpp)_2$ -metal-DNA junctions, which provide to a 120 deg. angular coordinative building block allows the construction of triangles and prisms. This approach has also led to the development of structures that enable to make the transition from the nanoscopic into the macro-level, which shows the tremendous potential of this approach for the construction of molecularly designed macroscopic objects.



## 2). Medicinal Applications of Metal Complexes

Pt complexes are approved for anticancer treatment worldwide including cisplatin, carboplatin, oxaplatin, nedaplatin, lobaplatin, heptaplatin.



Pt(IV) complexes such as iproplatin and tetraplatin, in contrast to Pt(II) cisplatin and its analogues, are potentially promising drug since Pt(IV) complexes are octahedral and less susceptible to substitution reactions. This in turn lowers their toxicity and may increase activity.





### 3). Biosensor for DNA Mismatch Detection

- Metal ions can recognize the specific types of mismatches, such as  $\text{Hg}^{2+}$  and  $\text{Ag}^{+}$ , which have a strong affinity for T–T and C–C mismatches, respectively. Such recognition changes structural conformation which may not be ideal for sensing multiple events.
- X. Wang et al have successfully developed a sensitive, convenient, low-cost fluorescence strategy for pesticide detection based on AChE(acetylcholinesterase)-catalyzed hydrolysis triggered  $\text{Hg}^{2+}$  release-induced DNA conformational change coupled with subsequent nicking enzyme assisted signal amplification.
- $\text{Zn}^{2+}$  was reported to be a useful for signal amplification without causing structural deformation.
- Sensitive, selective, rapid, and cost-effective analysis of nucleic acids plays a critical role in medical diagnostics, genetic and environmental monitoring, drug discovery and food safety. Based on specific adsorption properties towards nucleic acids, transition metal nano sheets are widely employed for nucleic acid detection.



# DNA Stability

The structure of DNA is stabilized by internal hydrogen bonds between purines and pyrimidines. Externally, electronegative oxygen atoms have the potential to form hydrogen bonds with surrounding molecules. The positioning of the phosphate groups prevents them from having a significant effect on each other; however, they do still have some slight repulsion in-between. Their positioning on the exterior of the DNA molecule allows them to interact with cations, which are able to lessen the repulsion between the phosphate groups. The result is a more stable DNA molecule with a higher overall melting temperature when it is in a solution containing cations. In other words, the two strands of DNA are both negatively charged, and, hence, are repelled by one another due to the large charge density. The addition of positively charged ions can reduce the charge density by surrounding and interacting with the negative charges, thereby stabilizing the molecule.



# DNA Stability

Slight variations in the DNA sequence can have profound implications on the stability of the DNA duplex. For example, mutations in the base sequence that result from errors that occur during DNA replication can result in mismatches that lead to relatively unstable duplexes. This instability is exploited by proofreading enzymes which recognize the mutation and replace it with the correct nucleotide (see Mutagenesis and DNA repair).

To gain an insight into DNA duplex stability, and how it is affected by changes in primary structure, scientists have studied the structure and thermodynamic stability of a variety of DNA duplexes by using a combination of physical methods including X-ray crystallography, ultraviolet (UV) melting and NMR.



# FACTORS INFLUENCING DNA DUPLEX STABILITY

DNA duplex stability is determined primarily by hydrogen bonding, but base stacking also plays an important role.

## Hydrogen bonding

The heterocyclic bases of single-stranded DNA have polar amido, amidino, guanidino and carbonyl groups that form a complex network of hydrogen bonds with the surrounding water molecules. Some of these bonds must be broken during duplex formation as the inter-base hydrogen bonds are formed. The overall process is one of "hydrogen bond exchange" and the net change in enthalpy upon duplex formation is partly due to  $\Delta H(\text{H-bonds formed}) - \Delta H(\text{H-bonds broken})$ . For duplexes of any significant length this is an exothermic process at ambient temperature. Not surprisingly the coming together of two large oligomeric molecules is entropically unfavourable ( $\Delta S$  is negative).

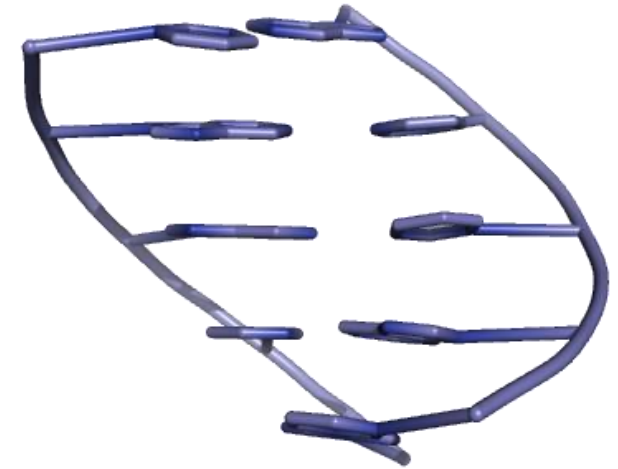


# Base stacking

Inter-strand hydrogen bonding is clearly important in driving the formation of DNA duplexes, but it is by no means the only contributing factor. The individual bases form strong stacking interactions which are major contributors to duplex stability, as base stacking is much more prevalent in duplexes than in single strands (Figure in right). Base-stacking interactions are hydrophobic and electrostatic in nature, and depend on the aromaticity of the bases and their dipole moments. Base-stacking interactions in nucleic acid duplexes are partly inter-strand and partly intra-strand in nature. However, it is probably more informative to consider base pairs rather than individual bases as discrete units in order to visualize the stabilizing effects of base stacking.

The degree of stabilization afforded by base stacking depends on the DNA sequence. Some combinations of base pairs form more stable interactions than others, so nearest neighbor base-stacking interactions are important determinants of duplex stability.

Base-stacking interactions increase with increasing salt concentration, as high salt concentrations mask the destabilizing charge repulsion between the two negatively charged phosphodiester backbones. DNA duplex stability therefore increases with increasing salt concentration. Divalent cations such as  $Mg^{2+}$  are more stabilizing than  $Na^{+}$  ions, and some metal ions bind to specific loci on the DNA duplex.



Cartoon  
representation of base  
stacking in a DNA  
double helix



# Replication of DNA

The DNA in the chromosomes carries out two functions:

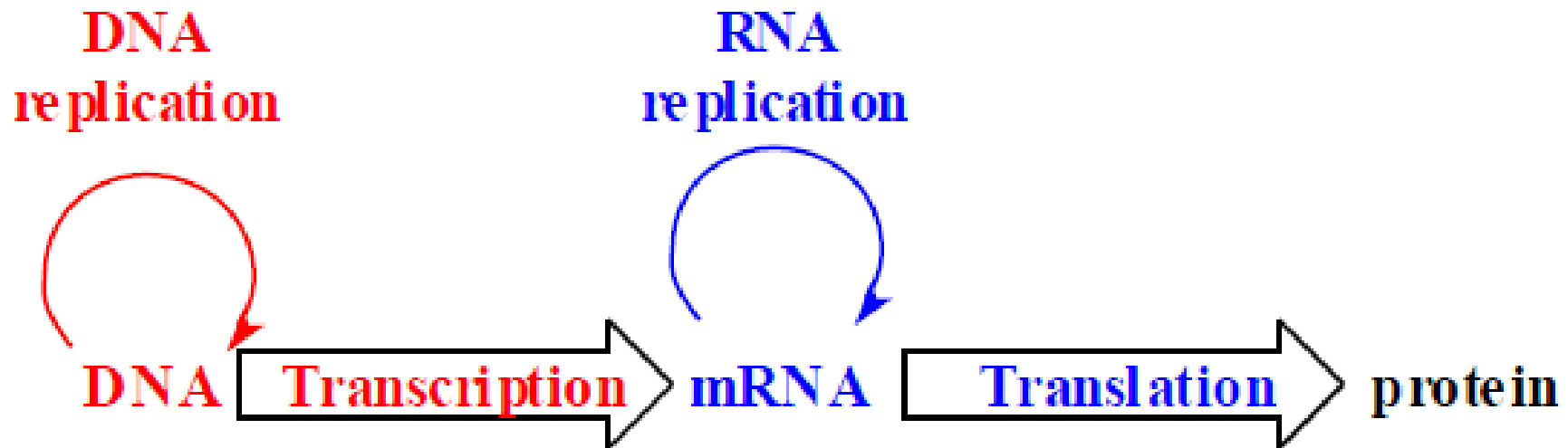
- (1) It reproduces itself. This process is called replication.
- (2) It supplies the information necessary to make all the RNA and proteins in the body, including enzymes.

Replication begins at a point in the DNA called the origin of replication or a replication fork.



## The central dogma of molecular biology:

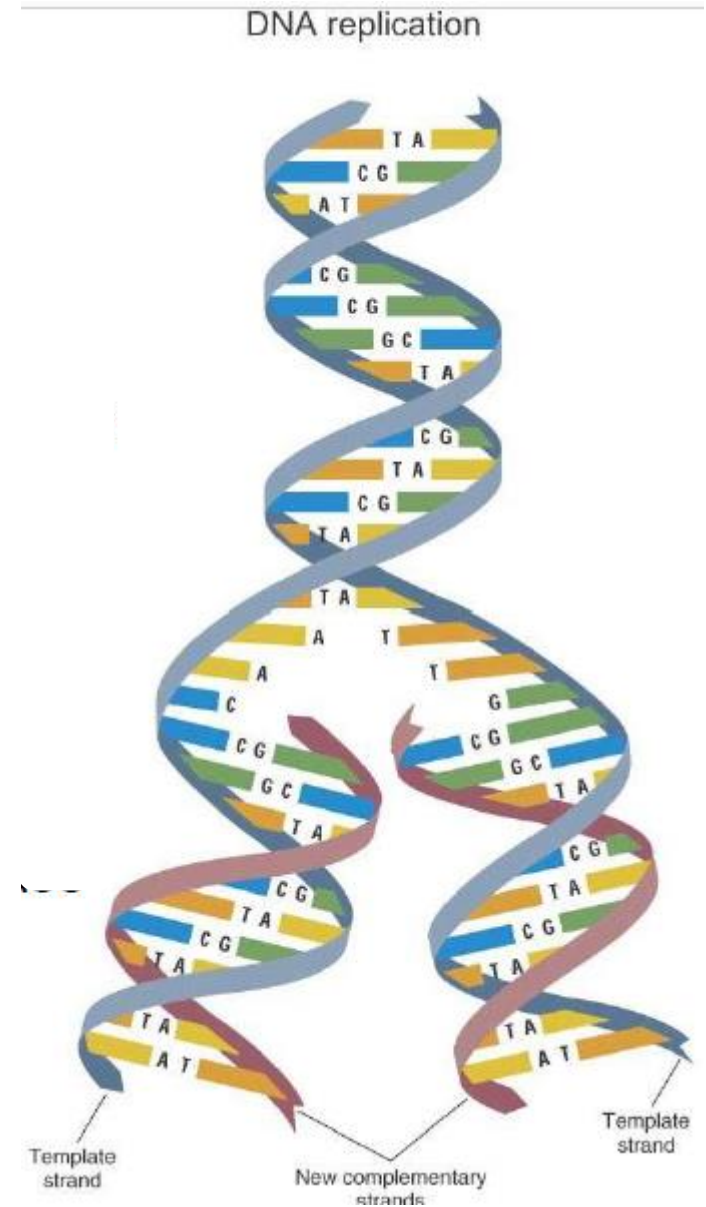
- Information contained in DNA molecules is expressed in the structure of proteins.
- Gene expression is the turning on or activation of a gene.





# DNA Replication

- The two strands of DNA in the helix are complementary
- When ready to replicate the two strands unwind
- Bases in the cell will migrate and bind with their complementary base to form an exact replica of the original



# DNA Replication

- Replication involves separation of the two original strands and synthesis of two new daughter strands using the original strands as templates.
- DNA double helix unwinds at a specific point called an origin of replication.
- Polynucleotide chains are synthesized in both directions from the origin of replication; that is, DNA replication is bidirectional.



# DNA Replication

- Unwinding the DNA double helix.
  - Replication of DNA starts with unwinding of the double helix.
  - Unwinding can occur at either end or in the middle.
  - Unwinding proteins called helicases attach themselves to one DNA strand and cause separation of the double helix.

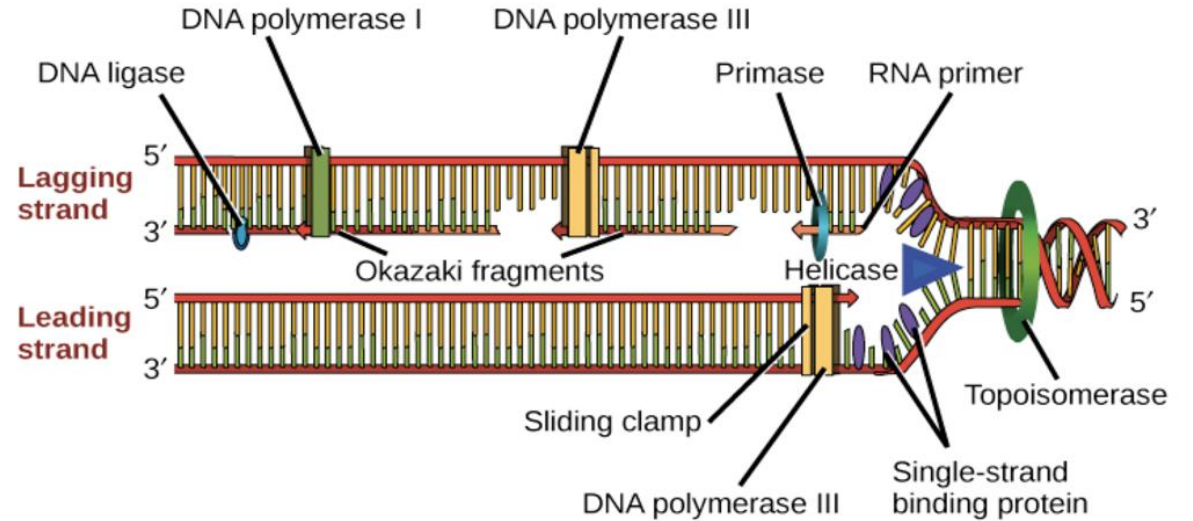


# Basic rules of replication

- Semi-conservative
- Starts at the 'origin' of replication
- Synthesis always in the 5'-3' direction
- Can be uni or bidirectional
- Semi-discontinuous

- RNA primers required

The reaction requires a template-primer complex, four deoxynucleotide substrates, DNA polymerase, other enzymes and a divalent cation activator such as  $Mg^{2+}$  or  $Mn^{2+}$



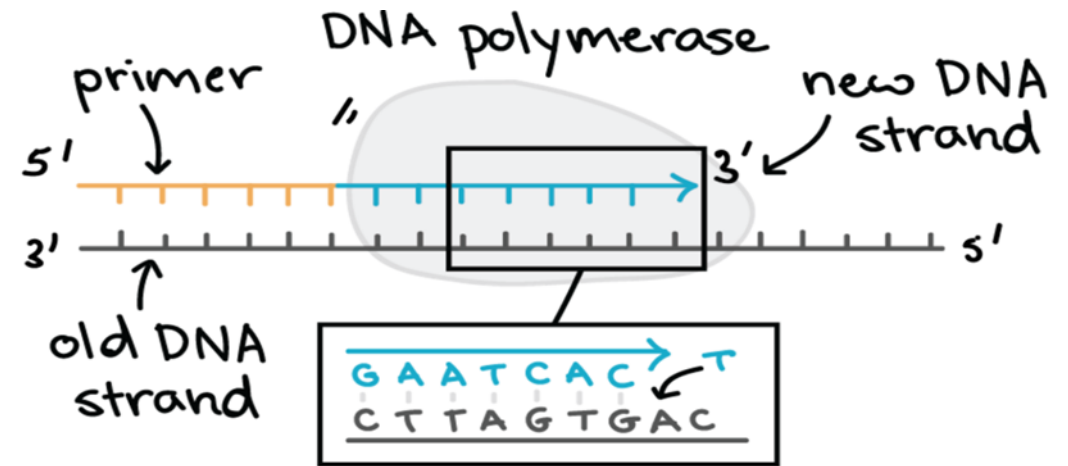
# DNA polymerase

One of the key molecules in DNA replication.

Responsible for synthesizing DNA: they add nucleotides one by one to the growing DNA chain, incorporating only those that are complementary to the template.

Some key features of DNA polymerases:

- 1) They always need a template
- 2) They can only add nucleotides to the 3' end of a DNA strand
- 3) They can't start making a DNA chain from scratch, but require a pre-existing chain or short stretch of nucleotides called a primer
- 4) They proofread, or check their work, removing the vast majority of "wrong" nucleotides that are accidentally added to the chain



# Role of bound $Mg^{2+}$

Examination of the structures of DNA polymerases, with bound substrates and substrate analogs, reveals the presence of two metal ions in the active site.

One metal ion binds both the deoxynucleoside triphosphate (dNTP) and the 3'-hydroxyl group of the primer, whereas the other interacts only with the 3'-hydroxyl group.

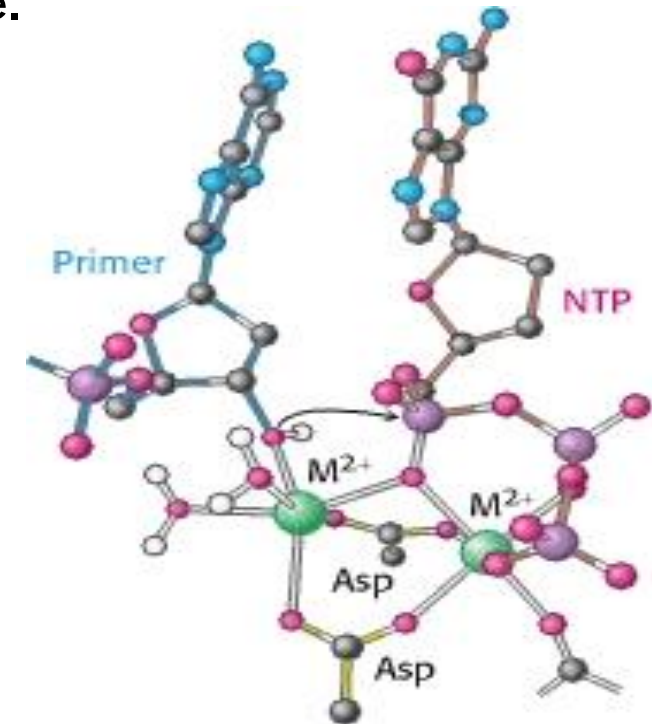
The two metal ions are bridged by the carboxylate groups of two aspartate residues in the palm domain of the polymerase.

These side chains hold the metal ions in the proper position and orientation.

The metal ion bound to the primer activates the 3'-hydroxyl group of the primer, facilitating its attack on the  $\alpha$ -phosphate group of the dNTP substrate in the active site.

The two metal ions together help stabilize the negative charge that accumulates on the pentacoordinate transition state.

The metal ion initially bound to dNTP stabilizes the negative charge on the pyrophosphate product.



# Presence and Role of bound $\text{Zn}^{2+}$

- DNA polymerases from animal, bacterial, and viral source have been found to contain stoichiometric quantities of tightly bound  $\text{Zn}^{2+}$
- Essential role for Zn in all polymerases has been suggested, based on their inhibition by the chelating agent, o-phenanthroline, and little or no inhibition by its nonchelating analog, m-phenanthroline. The inhibition by o-phenanthroline is not due to chelation of the added divalent cation,  $\text{Mg}^{2+}$
- Bound  $\text{Zn}^{2+}$  in DNA polymerase interacts with the DNA template-primer complex

Source	Zinc (g-atom/mol)	o-Phenanthroline for 50% inhibition (mM)
DNA Polymerases		
Bacteria		
<i>E. coli</i> DNA Pol I	1.8	0.04
<i>E. coli</i> DNA Pol I	$1.0 \pm 0.15$	—
Bacteriophage		
T <sub>4</sub> phage	1.0	—
Eucaryotes		
Sea urchin	4.2	0.4
Human lymphocytes	ND*	1.0
Human placenta- $\alpha$	ND	0.5
Human placenta- $\beta$	ND	0.5
RNA tumor viruses		
Avian myeloblastosis	1.3	0.4
Avian myeloblastosis	1.8—2.0	0.1
Murine leukemia	1.4	0.01
Woolly monkey	1.0	0.01
RNA polymerases		
<i>E. coli</i> RNA polymerase	2	0.04
<i>B. subtilis</i>	2.0	1.2
<i>E. gracilis</i> (I)	2.2	0.01
<i>E. gracilis</i> (II)	2.2	0.3
Yeast (I)	2.4	0.3
Yeast (B)	$1.0 \pm 0.4$	0.6
Rat liver RNA polymerase	ND	0.025
T <sub>7</sub> RNA polymerase	2—4	—





# Requirement for Added Divalent Metal Cations

- Added metal ions serve as metal activators, and include  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$
- Maximal rate of nucleotide incorporation with  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  is 153, 57, and 4% of that achieved with  $\text{Mg}^{2+}$ .

## Effects of Added Monovalent Cations

- Many DNA polymerases are stimulated as much as 3- to 5-fold by monovalent cations, particularly  $\text{K}^+$  and  $\text{NH}_4^+$  at concentrations up to 50 mM.
- At higher concentrations of monovalent cations, most DNA polymerases are inhibited. For example, it has been reported that calf-thymus DNA polymerase- $\alpha$  is inhibited 90% by 100 mM  $\text{LiCl}$ ,  $\text{NaCl}$ ,  $\text{KCl}$ , or  $\text{NH}_4\text{Cl}$ .
- Inhibition by  $\text{Na}^+$  or  $\text{K}^+$  of DNA polymerases from human KB cells, HeLa cells, rabbit and mouse testis, has been studied.
- Inhibition by monovalent cations has been used to distinguish between DNA polymerase- $\alpha$  and - $\beta$  from eukaryotic cells since the latter enzyme is not inhibited by concentrations as great as 300 mM.
- Also, a DNA polymerase coded for by Herpes virus is uniquely stimulated by both  $\text{Na}^+$  and  $\text{K}^+$



# RNA

- RNA molecules are classified according to their structure and function.

## Roles of different kinds of RNA

RNA Type	Size	Function
Transfer RNA	Small	Transports amino acids to site of protein synthesis
Ribosomal RNA	Several kinds—variable in size	Combines with proteins to form ribosomes, the site of protein synthesis
Messenger RNA	Variable	Directs amino acid sequence of proteins
Small nuclear RNA	Small	Processes initial mRNA to its mature form in eukaryotes
Micro RNA	Small	Affects gene expression; important in growth and development
Small interfering RNA	Small	Affects gene expression; used by scientists to knock out a gene being studied



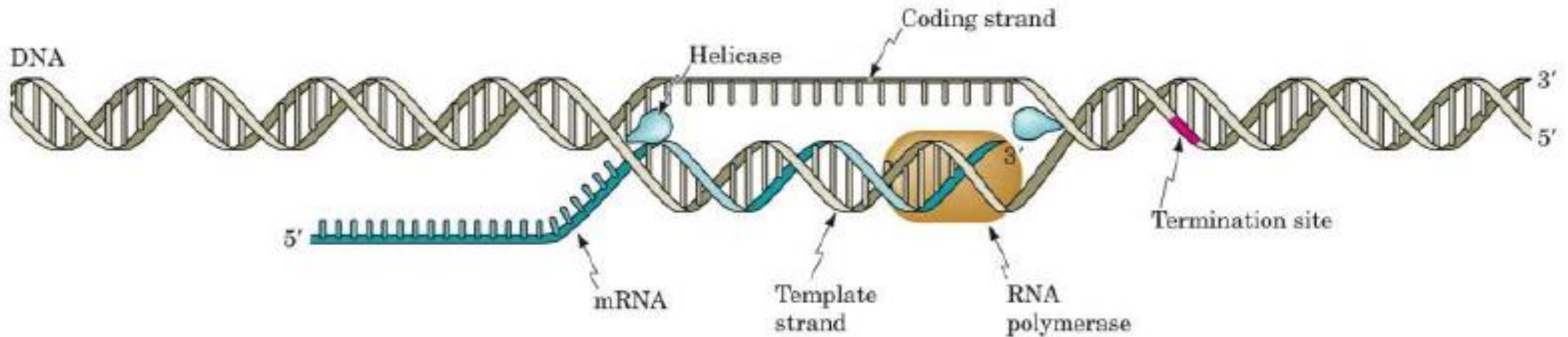
# Transcription

- Transcription: the process by which information encoded in a DNA molecule is copied into an mRNA molecule.
  - Takes place in the nucleus
  - Transcription starts when the DNA double helix begins to unwind near the gene to be transcribed.
  - Only one strand of the DNA is transcribed.
  - Ribonucleotides assemble along the unwound DNA strand in a complementary sequence.
  - Enzymes called polymerases (poly) catalyze transcription



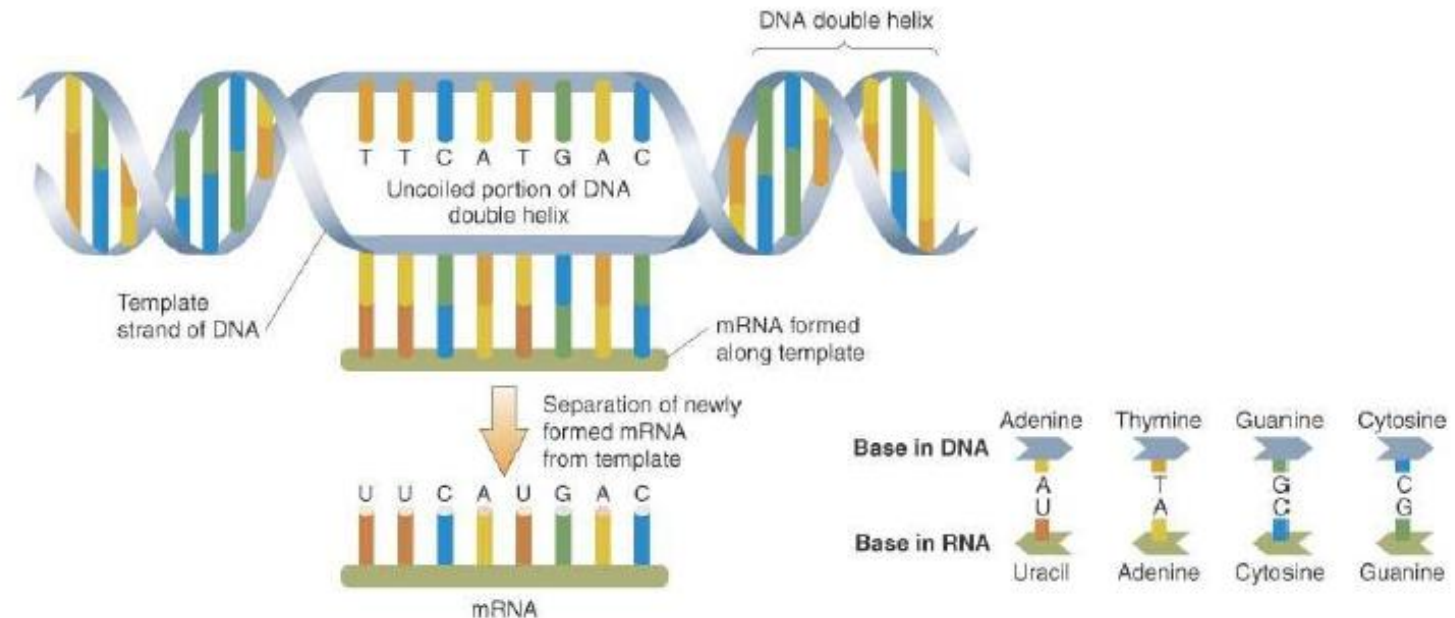
# Transcription

The information in one DNA strand is transcribed to a strand of RNA. The termination site is the locus of termination of transcription.



# Transcription by RNA

- First step in protein synthesis
- The segment of DNA that contains the necessary information, unwinds, to expose the bases
- The exposed bases, provide the template for messenger RNA (mRNA) synthesis



# Basic rules of transcription

Transcription is the synthesis of a single stranded RNA molecule using the DNA template (1 strand of DNA is transcribed).

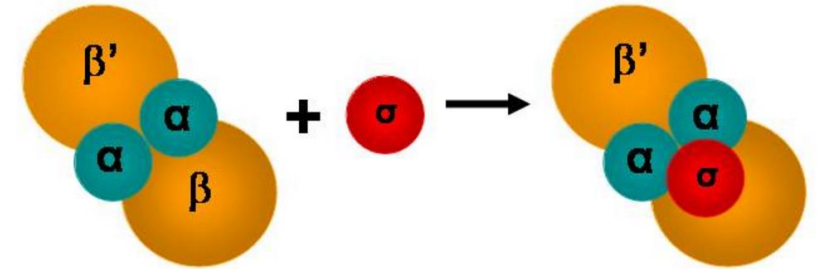
How is an RNA strand synthesized?

1. Regulated by gene regulatory elements within each gene.
2. DNA unwinds next to a gene.
3. RNA is transcribed 5' to 3' from the template (3' to 5').
4. Similar to DNA synthesis, except:
  - ✓ NTPs instead of dNTPs (no deoxy-)
  - ✓ No primer
  - ✓ No proofreading
  - ✓ Adds Uracil (U) instead of thymine (T)
  - ✓ RNA polymerase



# RNA polymerase

RNA polymerase from *E. coli* is known to consist of at least 5 subunits  $\beta'\beta\alpha_2\omega$  with a globular arrangement. The total molecular weight is 3- to 5-fold greater than those of DNA polymerases



Four complex sub-steps are present in the RNA-polymerase reaction, namely template binding, RNA chain initiation, RNA chain elongation, and RNA chain termination, and release.

## The Role of Bound Zinc in RNA Polymerases

- $\text{Zn}^{2+}$  has been found in RNA polymerases from a virus, yeast, and from *Bacillus subtilis*.
- While the presence of  $\text{Zn}^{2+}$  in RNA polymerases is widespread, the essentiality of it for enzyme activity has not been rigorously established by removal and replacement experiments, with the possible exception of phage- $\text{T}_7$  RNA polymerases.
- Many RNA polymerases are irreversibly inactivated upon the removal of Zn.

Ex. The phage- $\text{T}_7$  RNA polymerase enzyme was inhibited in a time-dependent manner by a variety of metal complexing agents such as EDTA, Chelex,  $\text{CN}^-$ , azide, sulfide, and o-phenanthroline.





# The Role of Bound Zinc in RNA Polymerases

- Unlike DNA polymerases which appear to contain only one  $\text{Zn}^{2+}$ /mol, many RNA polymerases contain multiple  $\text{Zn}^{2+}$  ions, despite single initiation and elongation sites. This suggests multiple roles for  $\text{Zn}^{2+}$ , including purely structural ones.
- The two  $\text{Zn}^{2+}$  ions in the enzyme from *B. subtilis* appear to have different affinities for the enzyme.
- A catalytic role of  $\text{Zn}^{2+}$  has also been suggested, as interacting with the template and/or the initiator. Interaction with the template might facilitate promoter site selection.
- Interaction with the initiator might facilitate priming in a manner analogous to the role proposed for  $\text{Zn}^{2+}$  in DNA polymerase.
- Zn is located predominantly on the  $\beta'$  subunit of the enzyme from *E. coli* which binds DNA, and on the analogous subunit from *B. subtilis*. However, a significant amount of  $\text{Zn}^{2+}$  is also located on the  $\beta$  subunit of the *E. coli* enzyme on which the initiation and elongation nucleotide- binding sites are located.
- The biosynthetic replacement of  $\text{Zn}^{2+}$  by  $\text{Co}^{2+}$  in the enzyme from *E. coli* produced relatively few and small kinetic changes, but these changes were in template binding and in initiation.





# The Role of the Added Divalent Cations in RNA Polymerase

- All RNA polymerases require a divalent cation such as  $Mg^{2+}$  or  $Mn^{2+}$  for activity.
- A direct  $Mn^{2+}$ -bindings study by measurements of water proton-relaxation rates revealed the presence of one tight  $Mn^{2+}$  binding site per molecule of *E. coli* RNA polymerase with a dissociation constant less than 10  $\mu M$ , and approximately six weaker  $Mn^{2+}$  binding sites with dissociation constants 100-fold greater.
- Although the role of the six weaker sites is not clear, four lines of evidence indicate that the one tight  $Mn^{2+}$  binding site functions as the active site for RNA chain elongation.



# Effect of Divalent Cations on Fidelity

- The cations,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$  can substitute for  $\text{Mg}^{2+}$  in activating DNA polymerases from diverse organisms, with two- to threefold diminutions in the fidelity of DNA synthesis, at concentrations which produce maximal enzyme
- Interestingly, substitution of  $\text{Mn}^{2+}$  for  $\text{Mg}^{2+}$  increases the accuracy of RNA synthesis with E. coli RNA polymerase.
- With DNA polymerases, the effects of substitution of  $\text{Mn}^{2+}$  for  $\text{Mg}^{2+}$  have been most extensively studied.
- $\text{Be}^{2+}$ , a nonactivating, slowly exchanging cation, forms a stable complex with AMV DNA polymerase. This results in a 20-fold enhancement of the rate of misincorporation.
- Hence, certain metal ions can serve as agents of mutagenesis and carcinogenesis.

