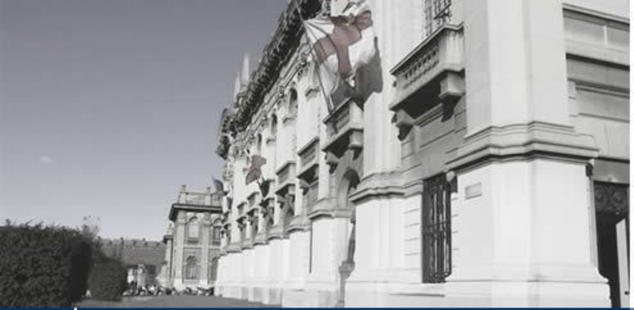


POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry



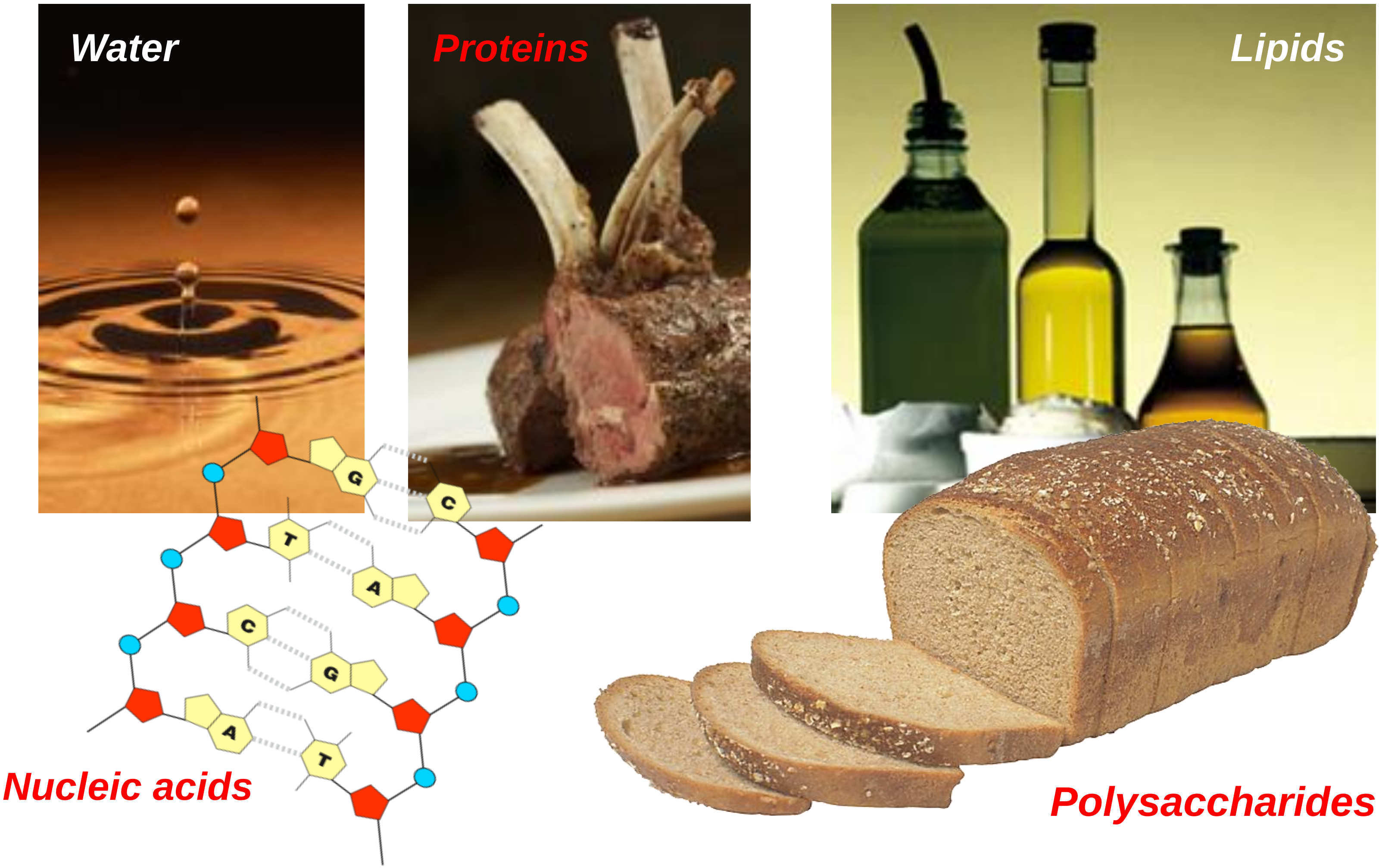


Natural Polymers and Biopolymers – DNA and RNA.

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/

Types of Biological Molecules.



Natural Polymers and Biopolymers.

Bio(Natural)-Polymers are polymeric macromolecules produced by living organisms. **Bio-based polymers** are macromolecules synthetized by human starting from biological raw materials. **Synthetic polymers** are made from oil.

Since they are polymers, biopolymers contain monomeric units that are covalently bonded to form larger structures.

There are three main classes of biopolymers based on the differing monomeric units used and the structure of the biopolymer formed:

1. **polynucleotides**, which are long polymers composed of 13 or more nucleotide monomers;
2. **polypeptides**, which are short polymers of amino acids; and
3. **polysaccharides**, which are often linear bonded polymeric carbohydrate structures.
   - Cellulose most abundant natural biopolymer
   - Chitin next most abundant natural biopolymer
   - DNA fundamental for reproduction
   - Proteins essential for living cell control.

Examples of bio-polymers (natural)

Synthetic Polymers – Bio(derived)Polymers.

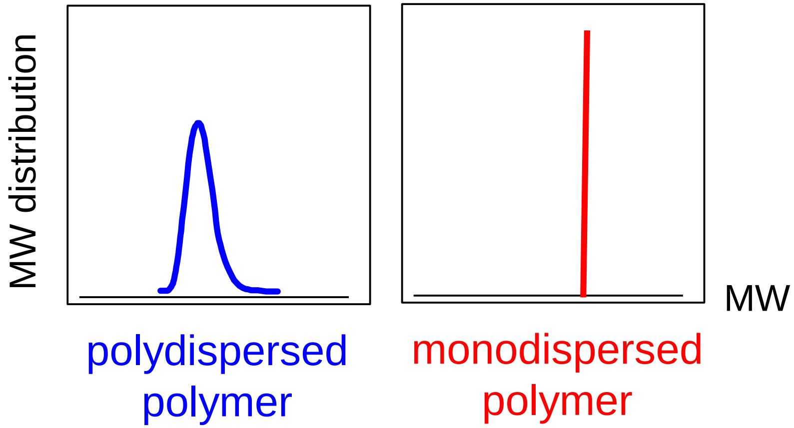
Synthetic polymers are polymer macromolecules made from man and not existing in nature. When these synthetic products are made from natural building blocks, they are called Bio(derived)-Polymers.

Bio(derived)-Polymers are much simpler and with a random molecular mass than natural polymers. This fact leads to a molecular mass distribution that is missing in bio(natural)-polymers.

All natural biopolymers of a type (say one specific protein) are all alike: they all contain the similar sequences and numbers of monomers and thus all have the same mass.

This phenomenon is called monodispersity in contrast to the polydispersity encountered in synthetic polymers.

As a result biopolymers have frequently a polydispersity index of 1.

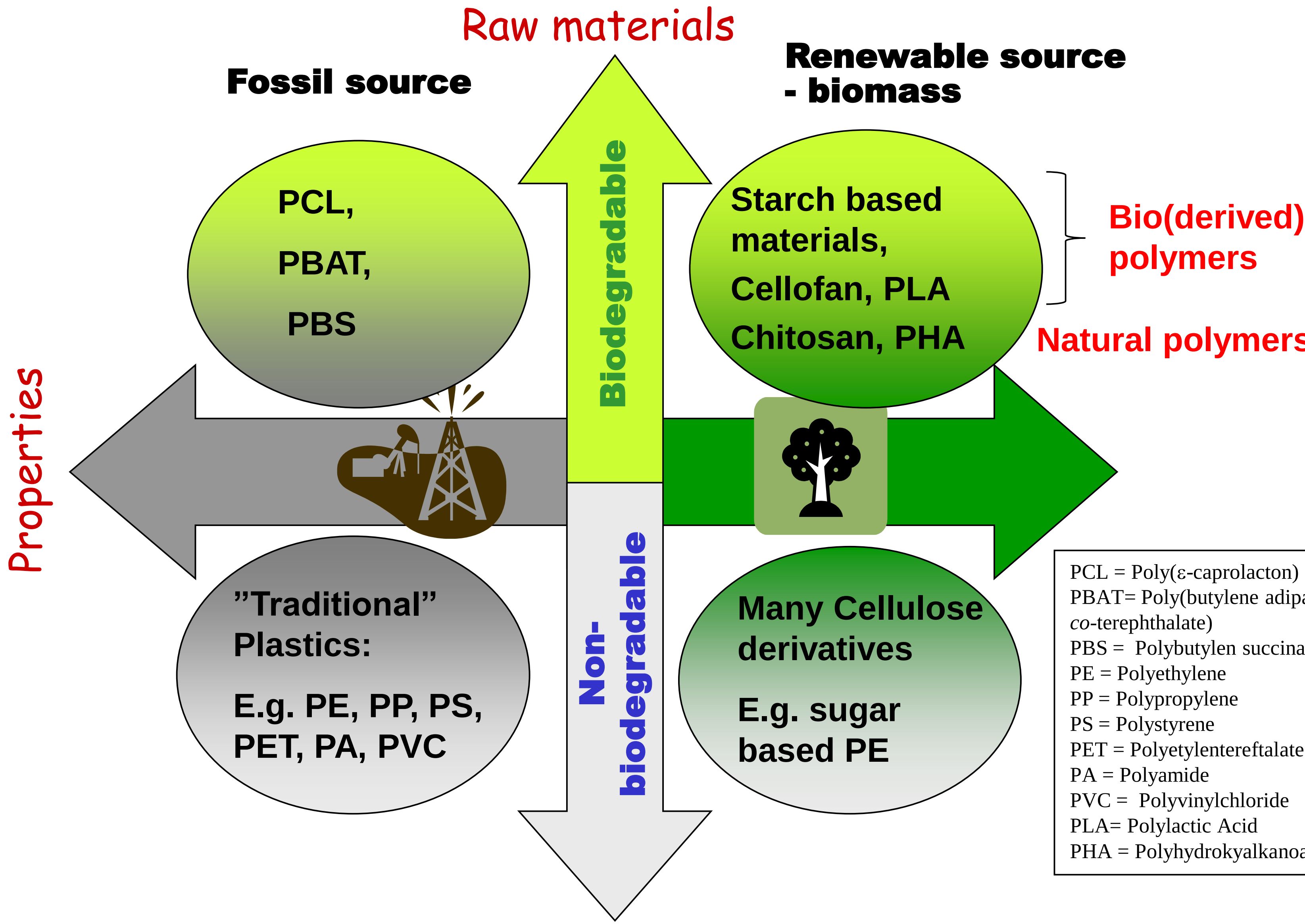


Biodegradability of Organic Chemicals and Polymers.

Degradation of organic chemicals in the environment influences exposure and, hence, it is a key parameter for estimating the risk of long-term adverse effects on biota. Degradation rates, or half-lives, are preferably determined in simulation biodegradation tests conducted under conditions that are realistic for the particular environmental compartment (e.g. STP, surface water, sediment or soil). Simulation tests aim at mimicking actual environmental conditions such as redox potential, pH, T, P, microbial community, concentration of substance and occurrence/concentration of other substrates.

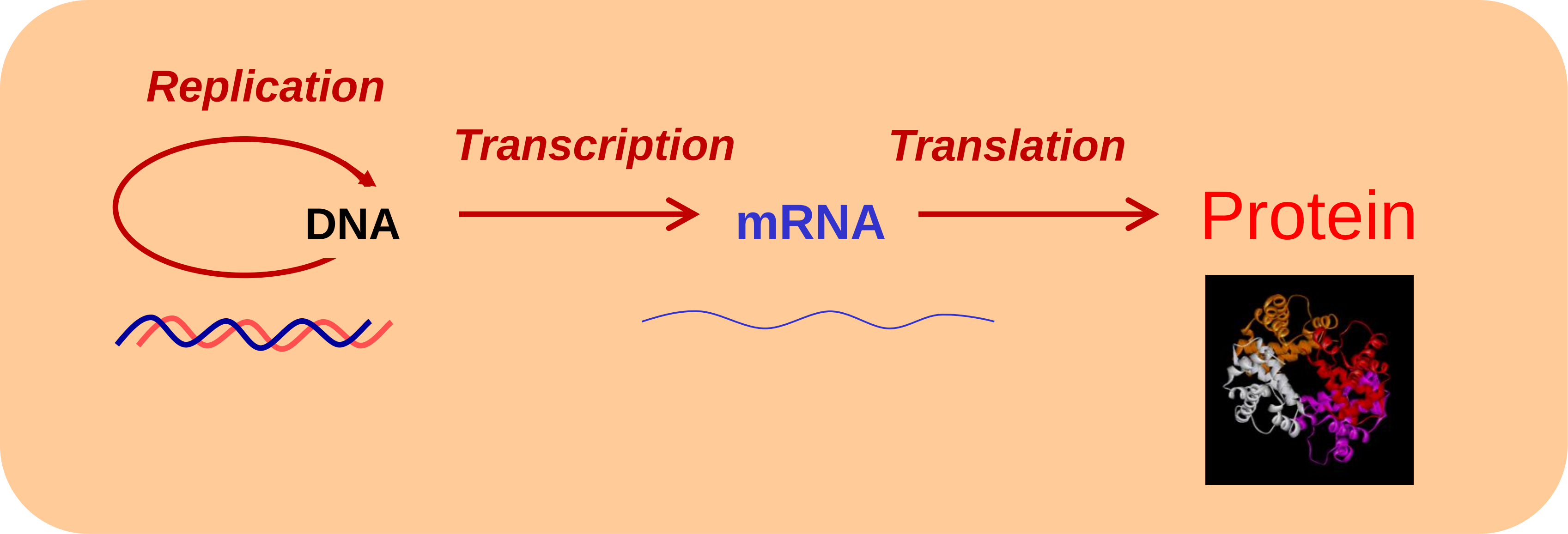
- aerobic biodegradability should be examined in a screening test for ready biodegradability
- If negative result in the previous test, biodegradation of the chemical may be examined in a simulation test to obtain data to be used for assessing the biodegradation rate (DT_{50}) in the environment or in a biological STP
- a screening test for inherent biodegradability may be conducted for describing the potential biodegradability under optimized aerobic conditions,
- In addition, potential biodegradability under anoxic conditions may be examined in a screening test for anaerobic biodegradability.

“Biodegradable” Macromolecules from Fossil and Bio Sources (Synthetic vs. Natural).



PCL = Poly(ε-caprolacton)
PBAT= Poly(butylene adipate-*co*-terephthalate)
PBS = Polybutylen succinate
PE = Polyethylene
PP = Polypropylene
PS = Polystyrene
PET = Polyetylentereftalate
PA = Polyamide
PVC = Polyvinylchloride
PLA= Polylactic Acid
PHA = Polyhydrokyalkanoate

The Central Dogma



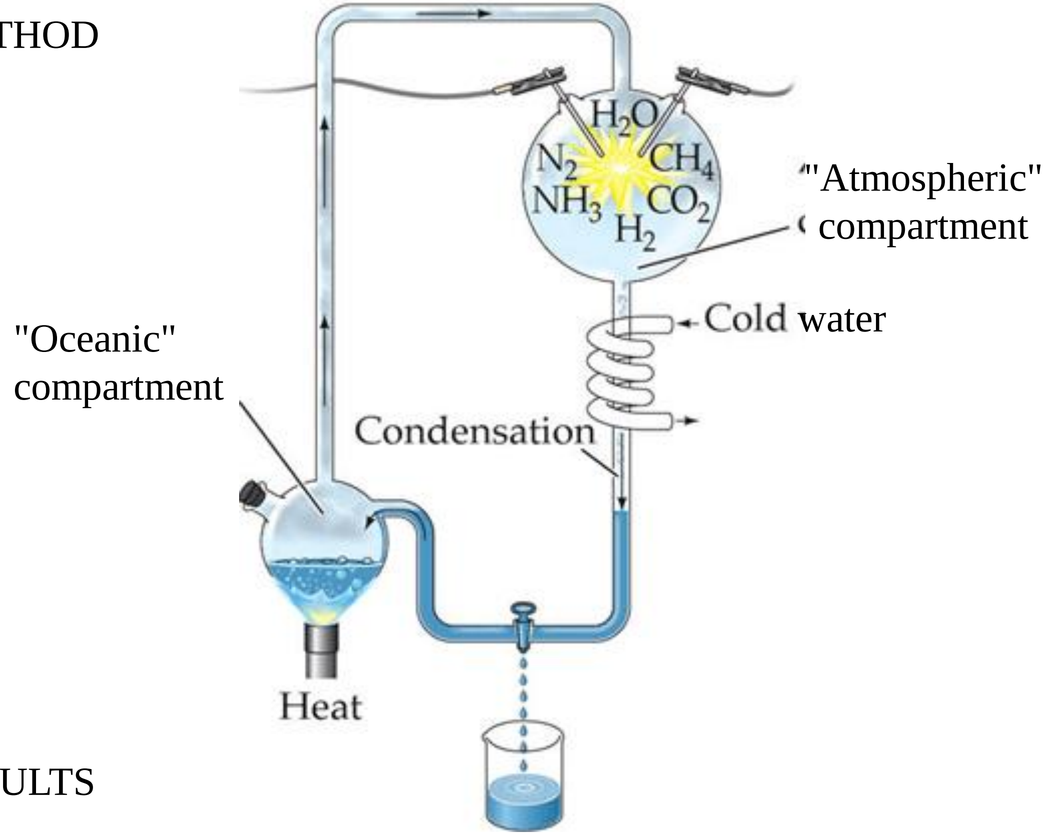
Protein, a linear sequence of amino acids codified by RNA through DNA, a linear sequence of nucleotides.

Origin of Life on Earth?

EXPERIMENT

Question: Can organic chemical compounds be generated under conditions similar to those that existed on primeval Earth?

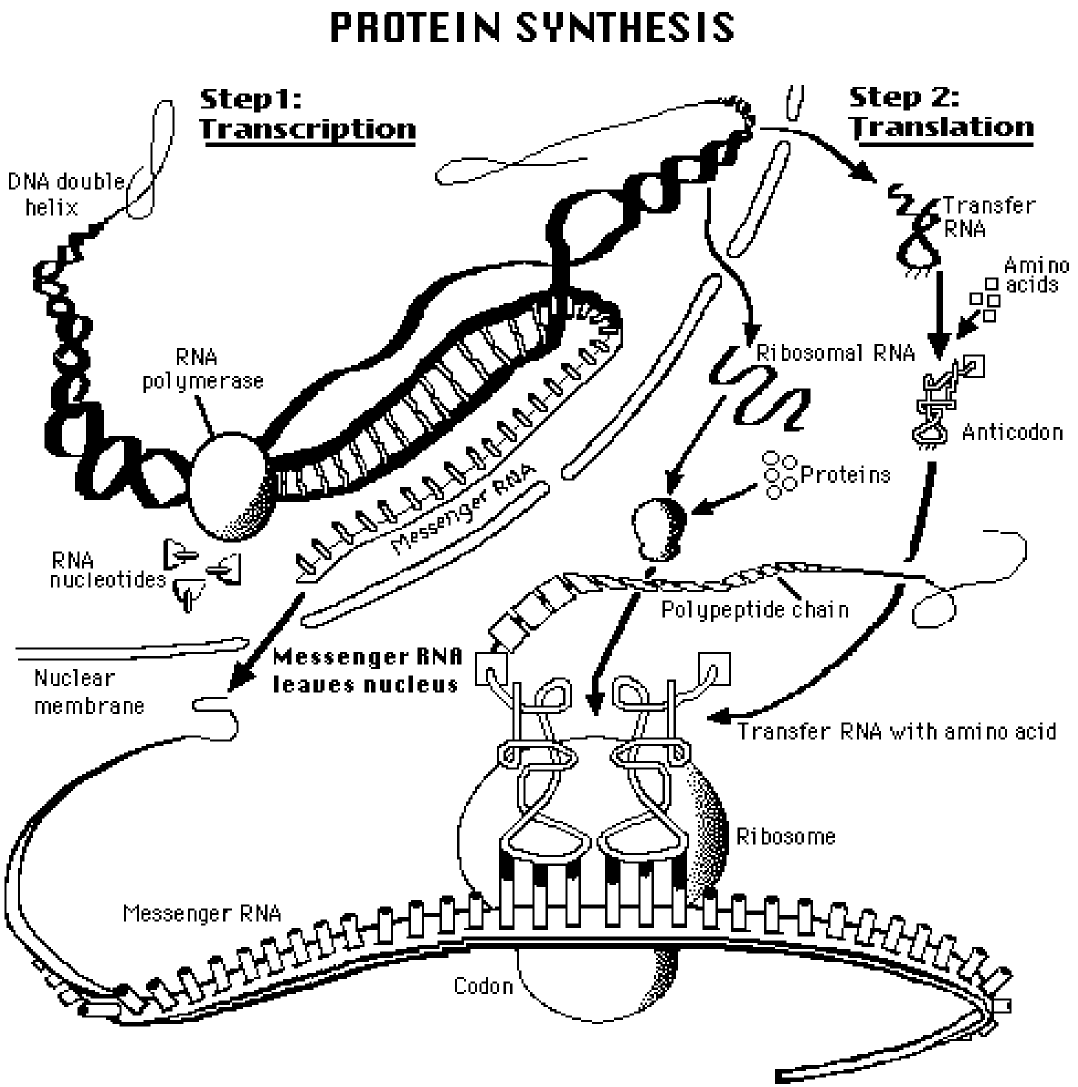
METHOD



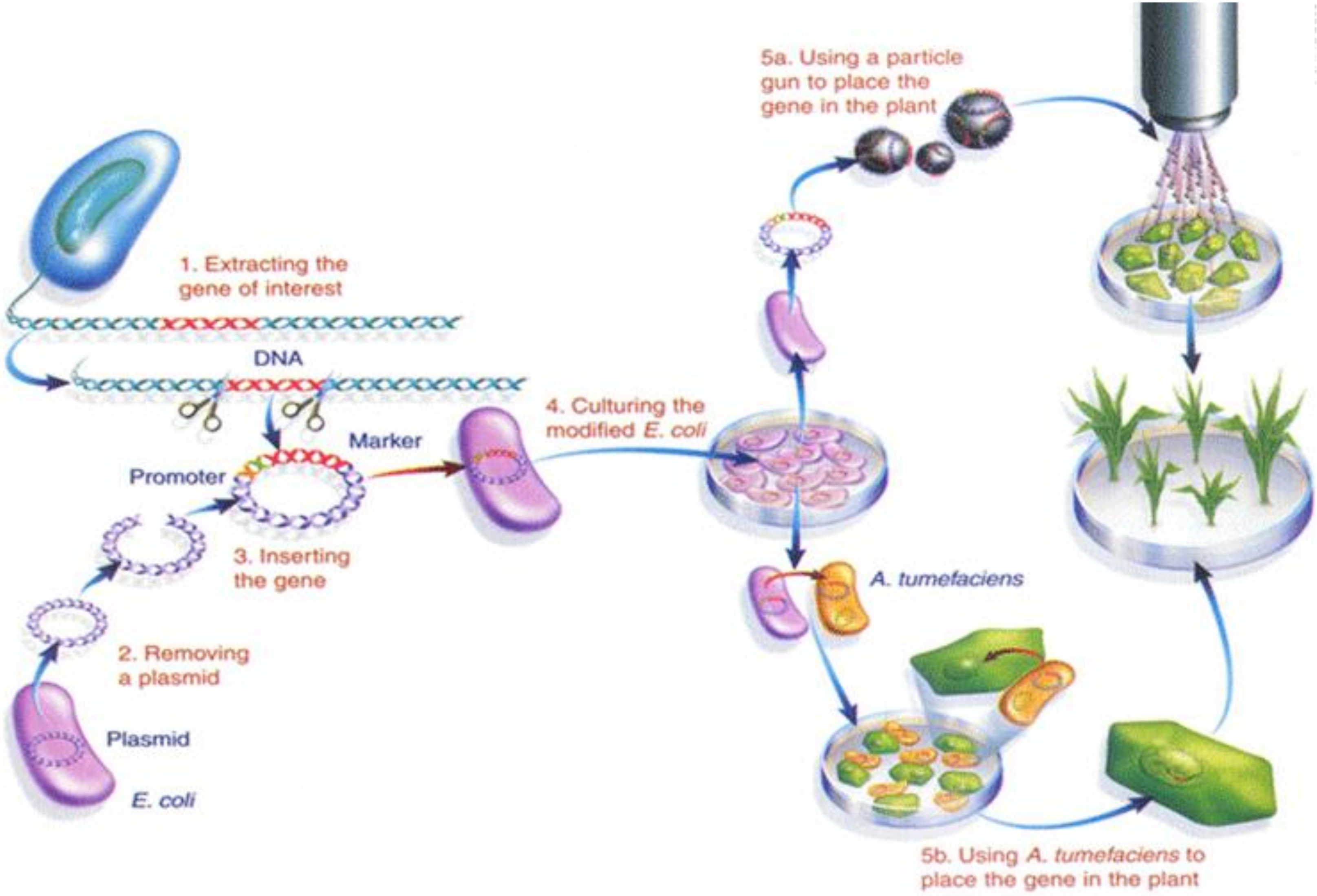
RESULTS

Conclusion: The chemical building blocks of life could have been generated in the probable atmosphere of early Earth.

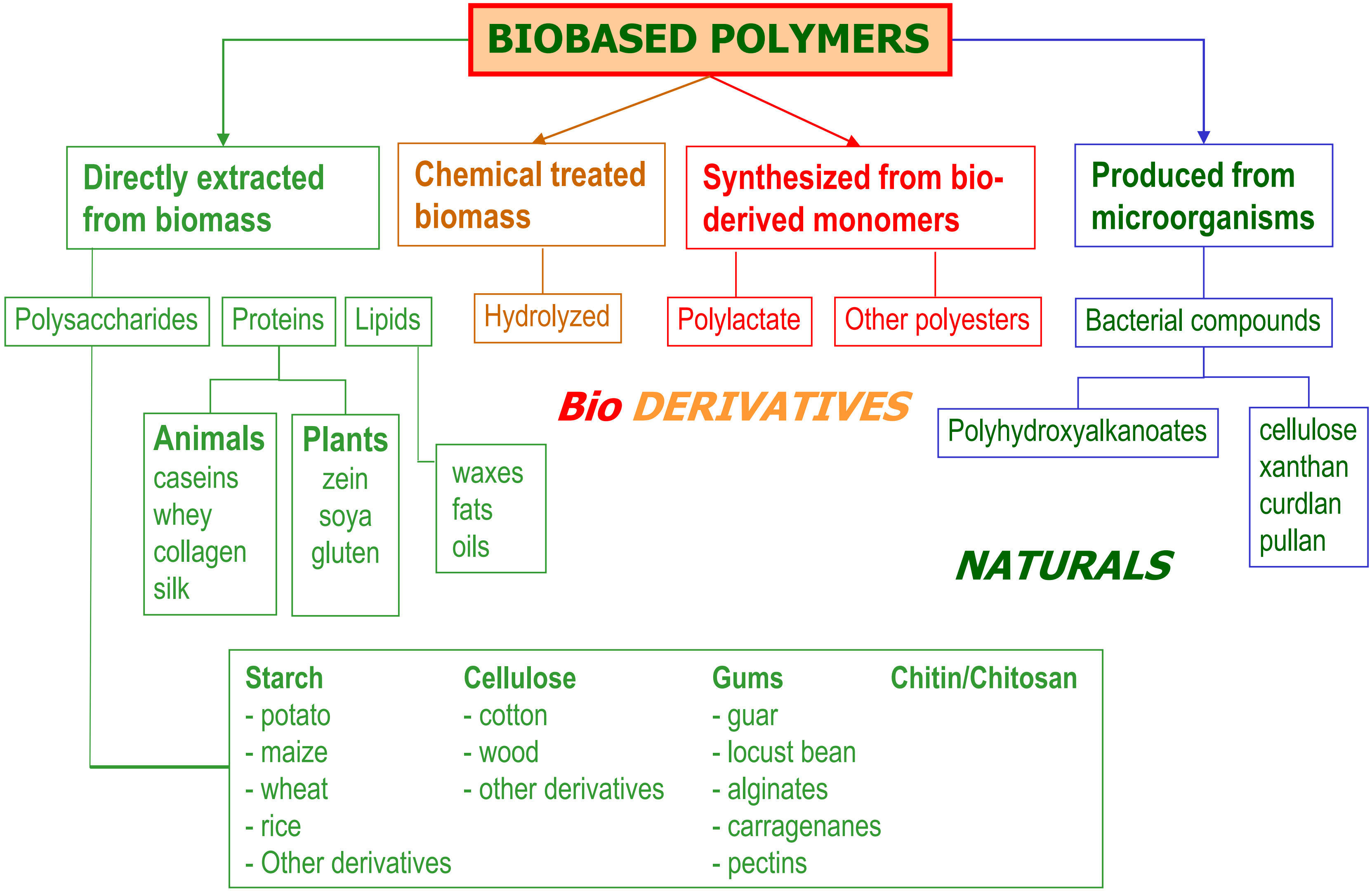
Two Main Information Pathways in the Cell: Transcription and Translation.



Genetic Modified Organisms.



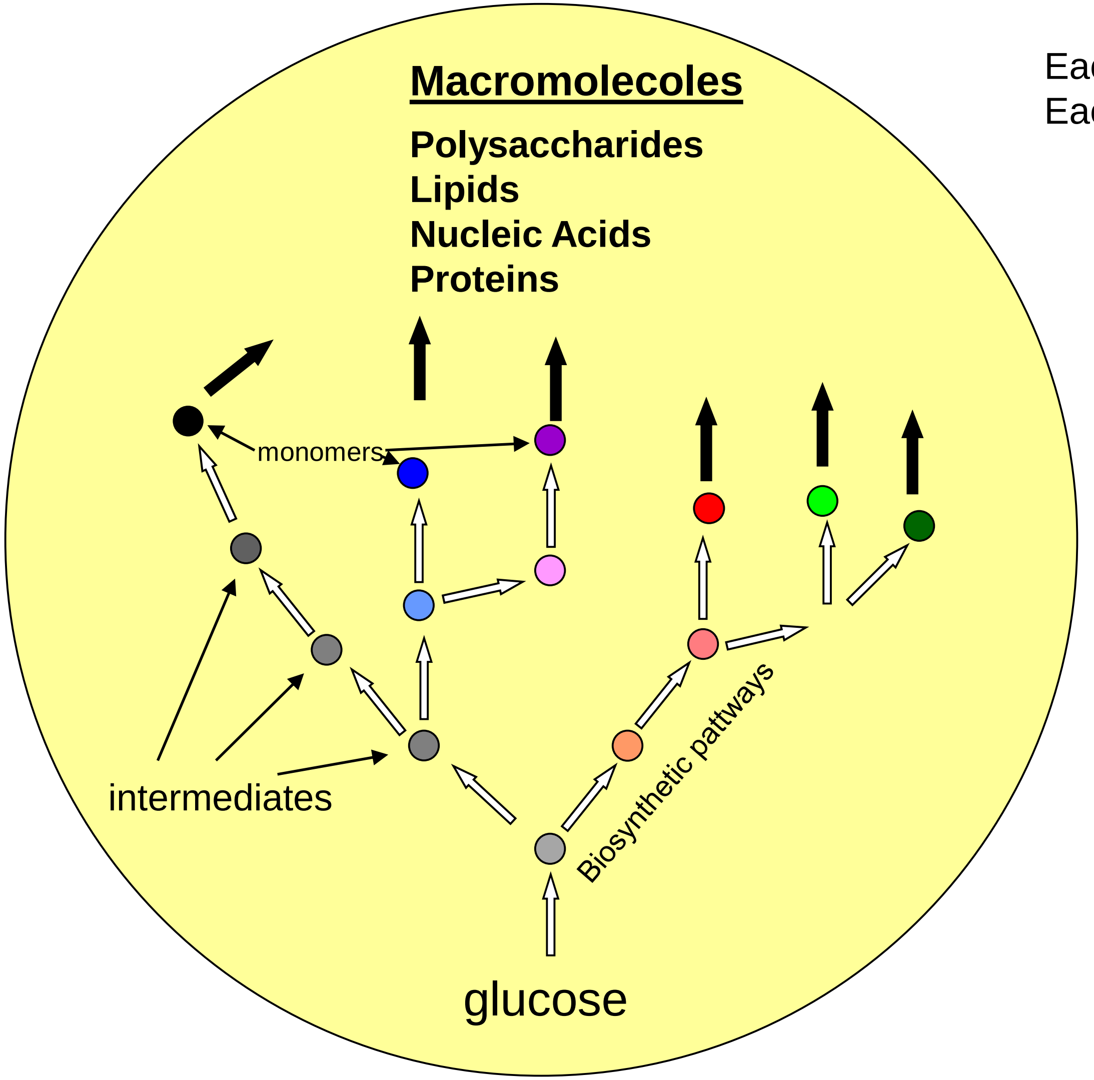
Bio-Degradable (Natural and Synthetic) Polymeric Materials.



Natural Bio-polymers from Renewable Sources – Classification by Structure.

Polysaccharides	Proteins	Polyesters	Lignin	Natural Rubber
Cellulose	Silk	DNA/RNA	poly-	poly-
Starch	Wool	Polylactic Acid	phenols	isoprene
Chitin/	Soy derivatives	PHA		
Chitosan	Polyglutamic Acid			
Alginate				
Emulsan	Biosynthetic			
Pectin	polypeptides			

Flow of Glucose in *E. Coli.*

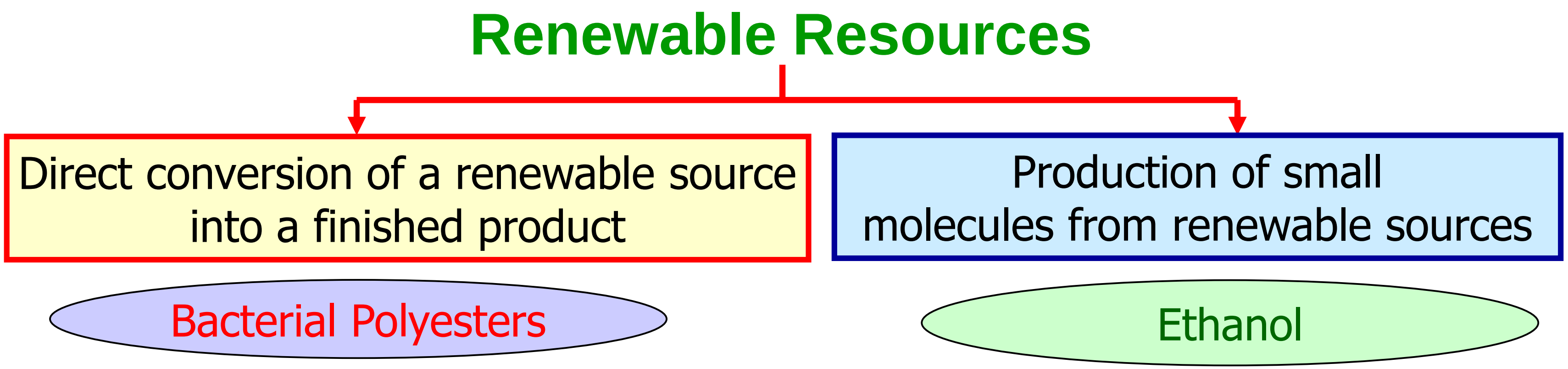


Each arrow = a specific reaction
Each arrow = a different enzyme (protein)

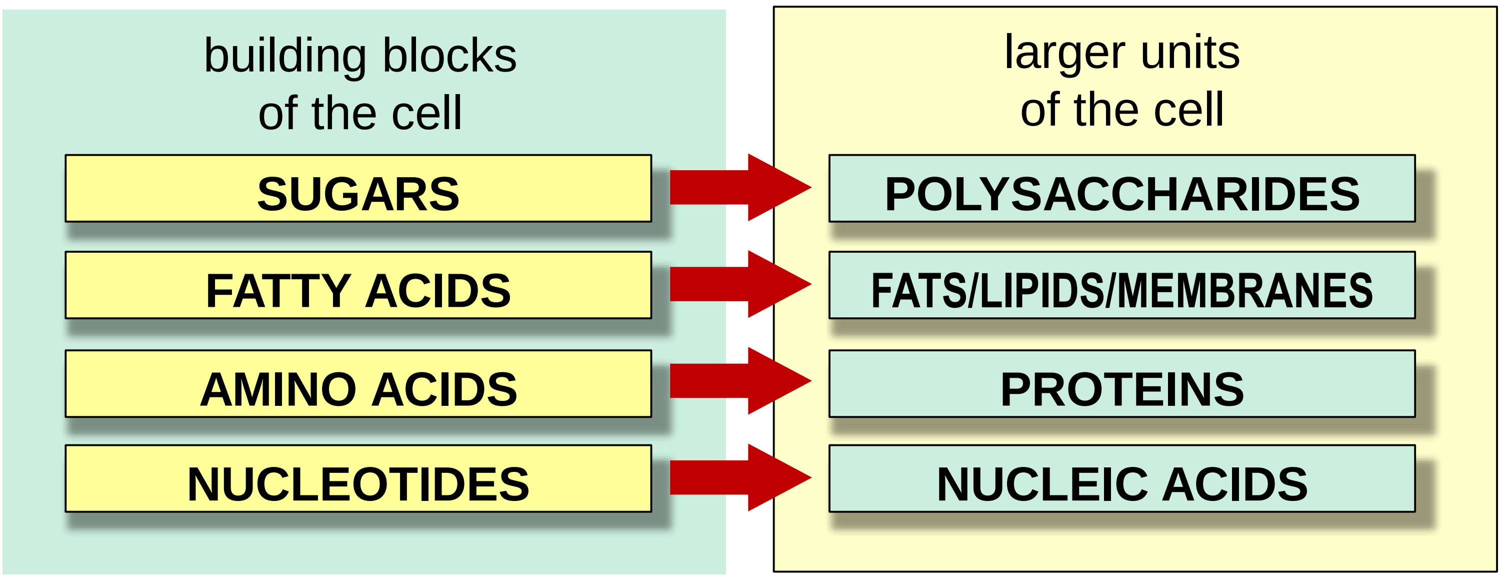
Based on agricultural and forest products, and marine resources

Different routes to produce biopolymers

- Directly by extraction from natural occurring biopolymers in plants:
  - E.g. lipids, proteins, polysaccharides (e.g. starch)
- Chemical processes:
  - E.g. hydrolysis of biomass where bio-monomers is produced, which in turn is the building blocks in the biopolymer like polyesters and polylactate
- Polymers produced by organisms, polymerisation by microorganism:
  - E.g. bacterial cellulose and polyhydroxyalkanoates



Monomeric building blocks of natural macromolecules



….account for most of cell's mass

Approximate Chemical Composition of a Bacterial Cell.

TYPES	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	>6000

Approximate Chemical Composition of the *E. Coli* Bacterium and a Typical Mammalian Cell.

COMPONENT	PERCENT OF TOTAL CELL WEIGHT	
	E. Coli Bacterium	Mammalian Cell
Water	70	70
Inorganic ions	1	1
Miscellaneous small metabolites	3	3
Proteins	15	18
RNA	6	1.1
DNA	1	0.25
Phospholipids	2	3
Other lipids	-	2
Polysaccharides	2	2
Total cell volume	2×10^{-12} cm^3	4×10^{-9} cm^3
Relative cell volume	1	2000

If there is a natural process to make it, there is also a process to degrade it.

→ **Natural Balance of nature**

Inherent Biodegradability

Polymerase

Building block ⇄ Polymer

Depolymerase

↑

Enzymes

Benefits of Use of Biopolymers.

- Environmental compatibility
- Use does not pose environmental burden
- Renewable resources
  - ✓ Superior over petroleum-based (finite) resources
  - ✓ Boost for agricultural industry
- Potential biocompatibility
- Tailoring of structure by genetic manipulation

Mol. weight, stereochemistry sequence, chemical reactivity

May interfere with biodegradability/biocompatibility

Can have higher costs than synthetic polymers

Limits in the Use of Biopolymers.

- Premature degradation
- Unfavorable economic evaluation
- High production costs
- Medical/pharmaceutical use
- Environmental consequences
- Soil fertility
- Use of water to grow crops
- ……

Function of Bio(Natural) Polymers.

Perform functions in their natural setting

Example: polysaccharides

• **Cell wall structure**	structural function
• **Extra-skeleton**	structural function
• **Starch granules**	storage function
• **Heparin**	regulative function
• **Emulsan**	emulsifying function

Protein Functions.

Vital activity	Example of proteins	Functions
Nutrition	Digestive enzymes e.g. trypsin, amylase lipase	• Catalyzes the hydrolysis of proteins to polypeptides • Catalyzes the hydrolysis of starch to maltose • Catalyzes the hydrolysis of fats to fatty acids and glycerol

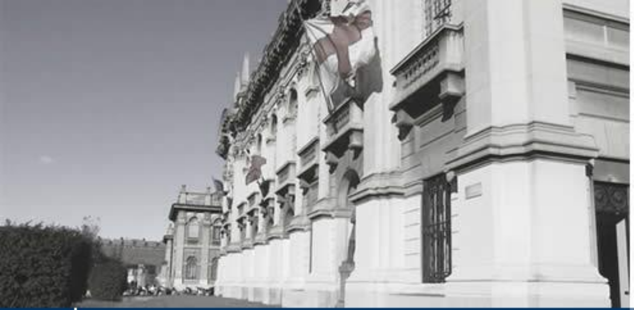
Protein Functions (2).

Vital activity	Example of proteins	Functions
Support and movement	Actin and myosin	• Responsible for muscle contraction
	Collagen	• Gives strength with flexibility in tendons and cartilage
Sensitivity and coordination	Hormones (e.g. insulin)	• Controls blood sugar level

Vital activity	Example of proteins	Functions
Respiration and transport	Haemoglobin	• Responsible for the transport of O_2/CO_2 throughout body
Immune response	Antibodies	• Essential to the defense of body (e.g. against bacterial)
Growth	Hormones (e.g. tyrosine)	• Controls growth and metabolism

Sources for Biopolymers – Organisms.

Plants	Animals	Fungi	Bacteria
Poly-saccharides Proteins Lignin Nat. rubber	Proteins Poly-saccharides chitin (glycogen)	Poly-saccharides (pullulan, chitin)	Poly-saccharides (dextran) PHA PLA



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DNA and RNA.

Prof. Attilio Citterio
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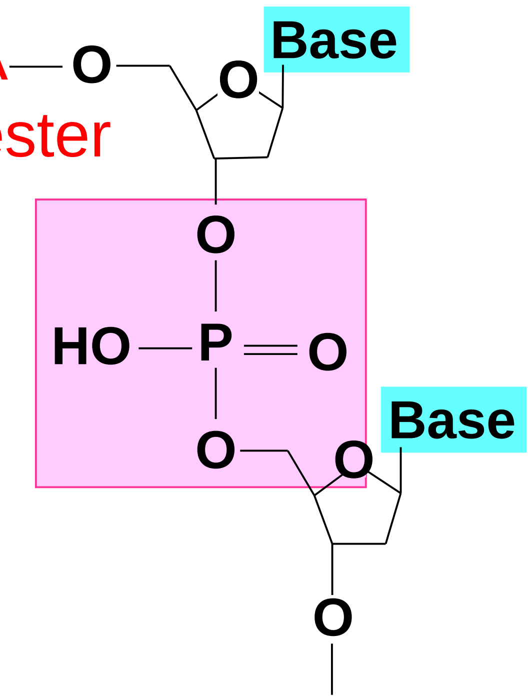
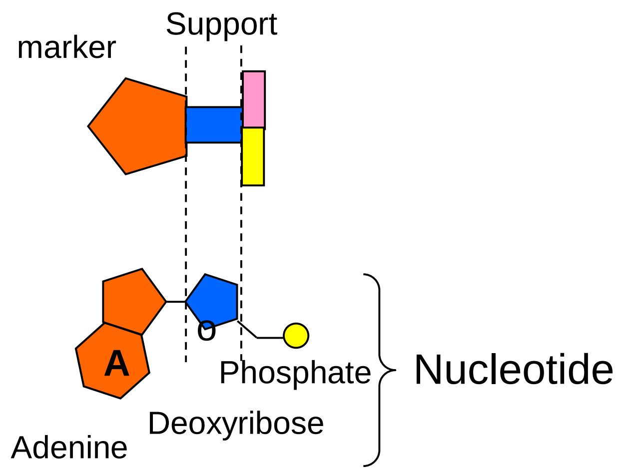
https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/

DNA: A Complex Polyester of Phosphoric Acid.

RNA and DNA
a phosphate ester



Building block



Future: "Lab on the chip"

No material application yet!

Where to Begin?

Information Storage, Retrieval & Use

Nucleic Acids

↑

Repeat Units

Nucleotides

↗ ↖

Information Content

nitrogenous bases

purine

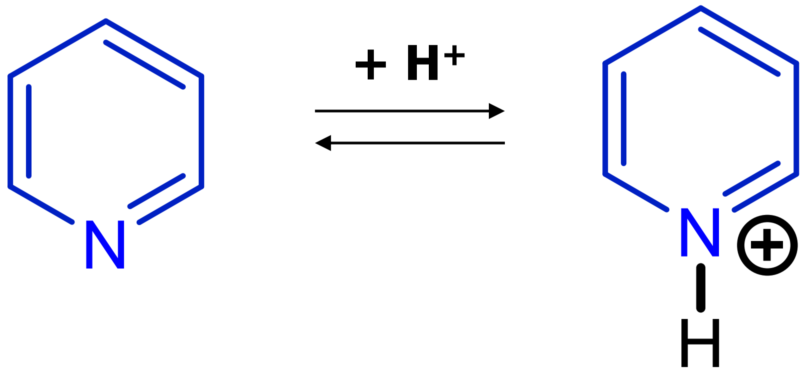
pyrimidine

Scaffolding

ribose + phosphate

Nitrogenous Bases: a Definition.

- Aromatic rings incorporating one or more atoms of nitrogen
- Essentially flat, or nearly so
- Nitrogen imparts a weakly basic character to the ring
- Pyridine is a simple, non-biochemical example:

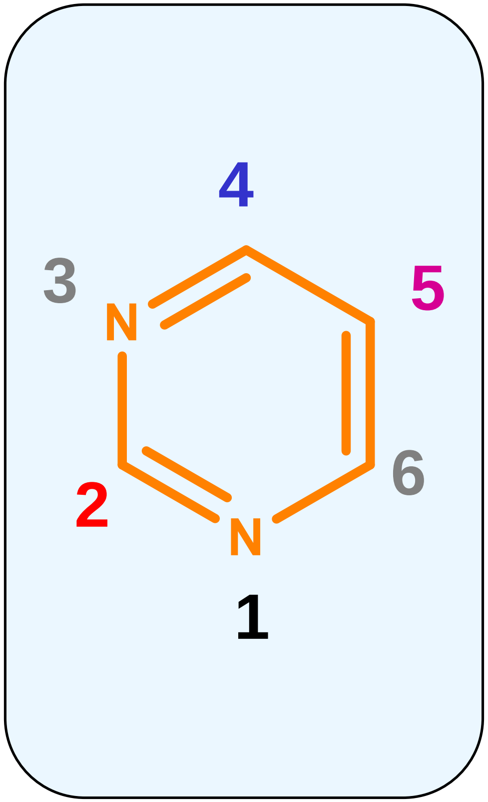


Pyridine

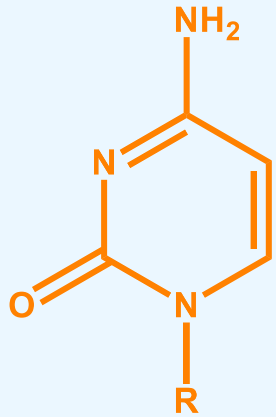
Pyridinium Ion

The Pyrimidine Bases.

- All are built on the pyrimidine platform
- Ring is numbered to assign the lowest possible numbers to the two nitrogens
- Connection to the ribose sugar is via a glycosidic bond from **position 1**
- All have an oxygen bonded to **position 2**
- (*i.e.* all are 2-oxo- substituted pyrimidines)
- **Position 4** will bear an oxo or amino group
- **Position 5** is methyl-substituted in one case

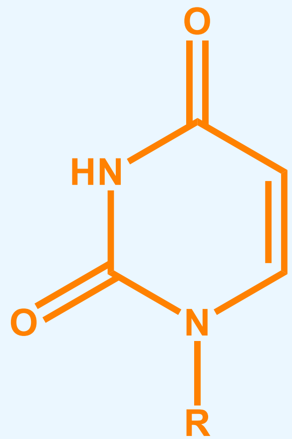


Pyrimidine Bases (2).

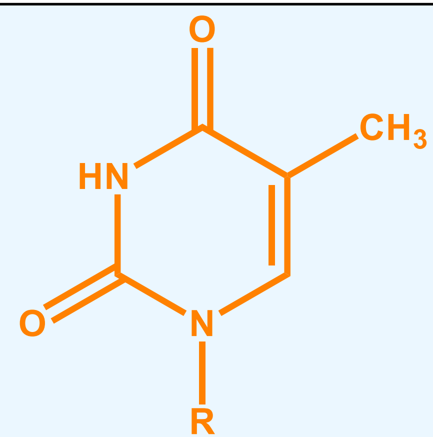


(Root underlined)

Cytosine
2-oxy-4-amino pyrimidine
Used in both DNA & RNA



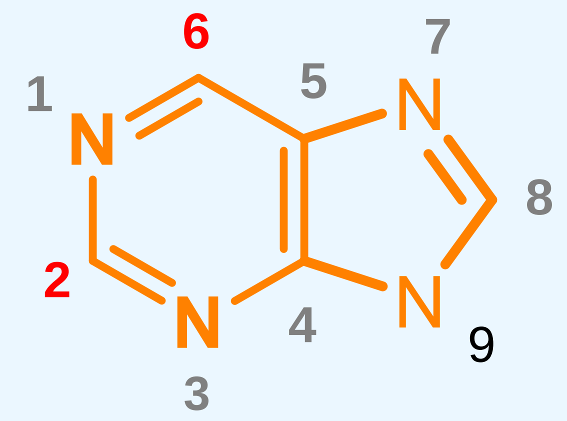
Uracil
2,4-dioxy pyrimidine
(RNA only)



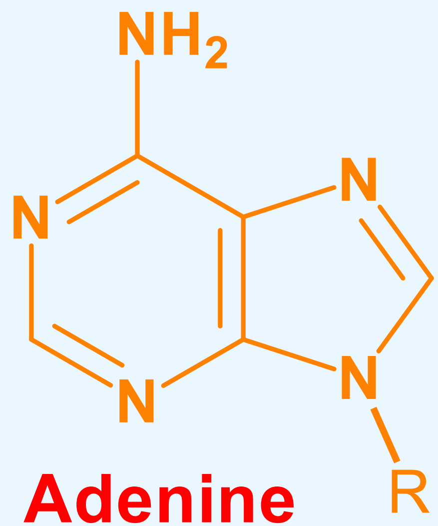
Thymine
2,4-dioxy-5-methyl pyrimidine
(DNA only)

The Purine Bases.

- All are built on the purine platform
- Ring is numbered to assign the lowest possible numbers to the four nitrogens
- Connection to the ribose sugar is via a glycosidic bond from **position 9**
- 6-membered ring will be oxo or amino-substituted at **positions 2 or 6**

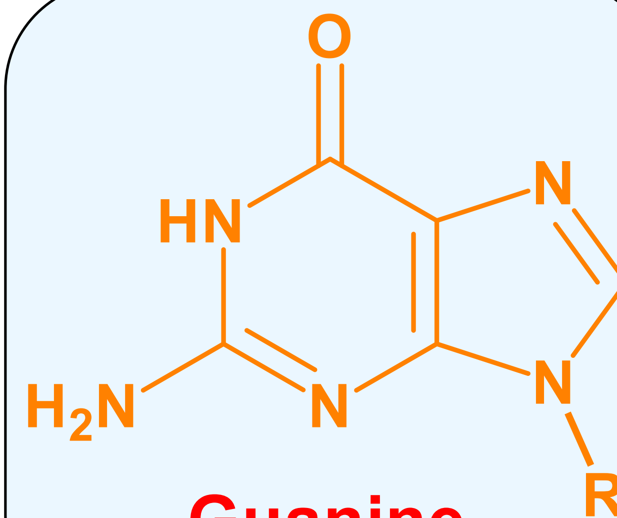


The Purine Bases (2).



Adenine

6-aminopurine



Guanine

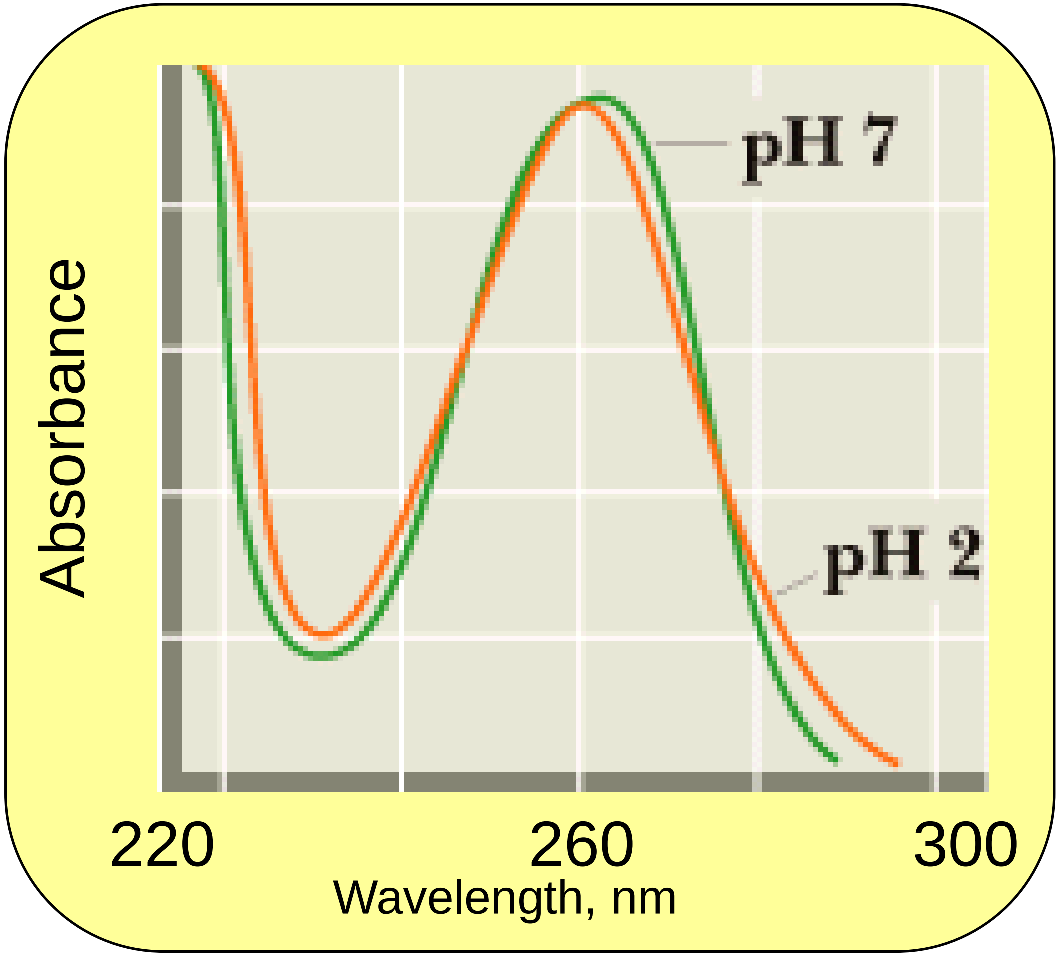
2-amino-6-oxypurine

Found in both DNA and RNA (Root underlined)

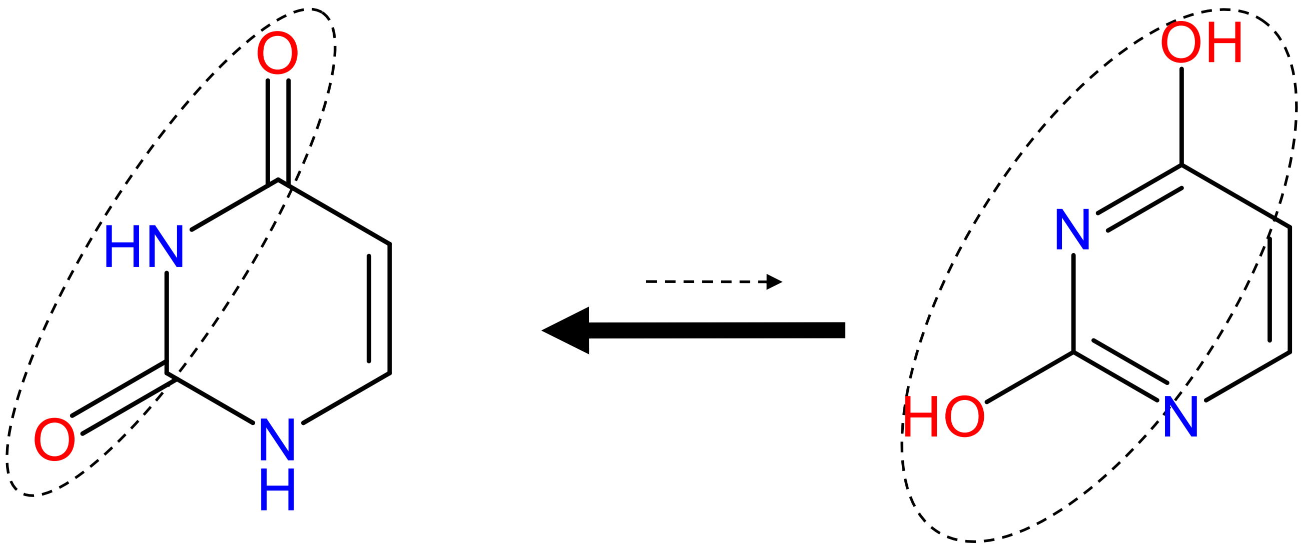
Bases Absorb in the UV-Region.

Aromatic, λ_{max}~ 260 nm useful for:

- quantifying nucleic acids
- assessing purity
- monitoring structural changes (e.g. melting of double-stranded DNA)



Keto-enol tautomerism, e.g. of uracil or thymine

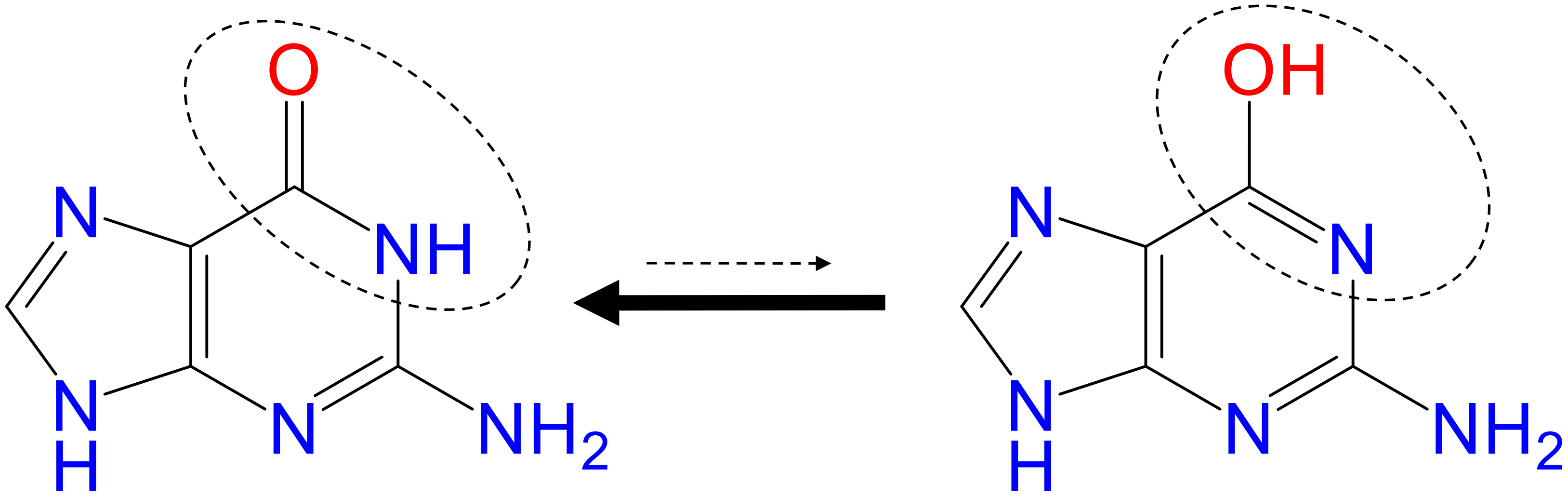


keto-form
(strongly preferred)

enol-form

Importance: altered base pairing preference of the two tautomers

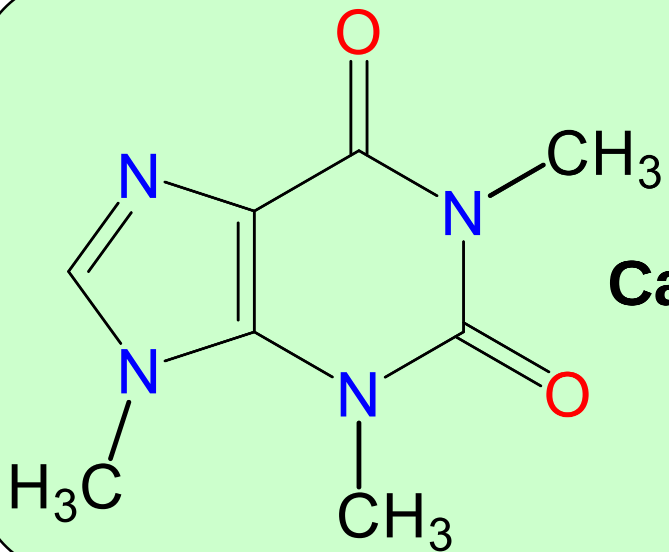
Keto-Enol Tautomerism in Guanine.



Keto form
(strongly favoured)

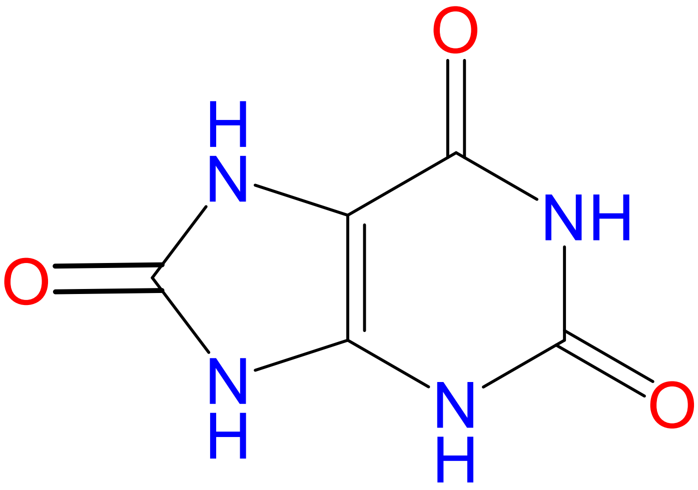
Enol form

Other Bases (not found in nucleic acids).

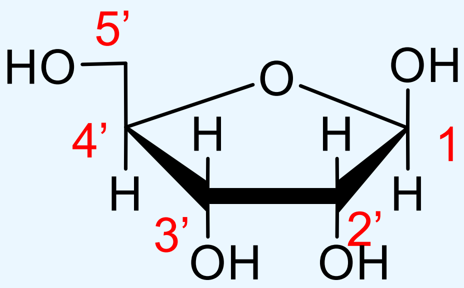


Caffeine

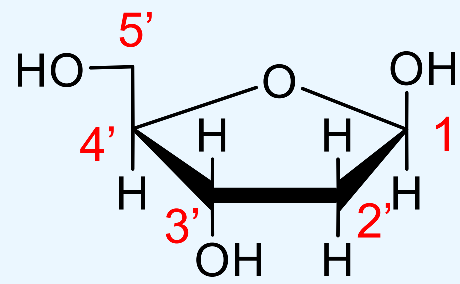
Uric acid: final breakdown product in purine catabolism excreted in urine



Pentoses of Nucleotides.



D-ribose (in RNA)



2-deoxy-D-ribose (in DNA)

Riboses are components of the scaffolding for nucleic acids

The difference: 2'-OH vs. 2'-H

This difference influences:

- the secondary structure of RNA & DNA
- the stability of RNA and DNA

Nucleosides = Base + Pentose.

Base is linked *via* a glycosidic bond

Named by adding:

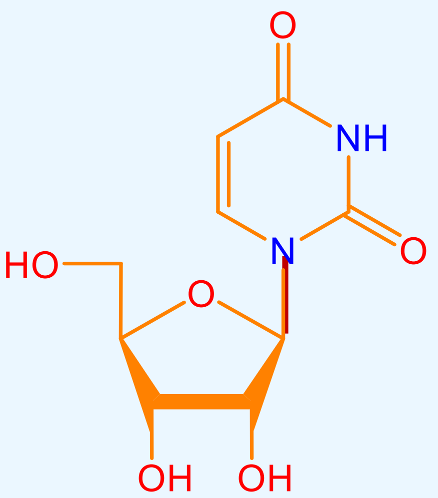
-idine to the root name of a pyrimidine

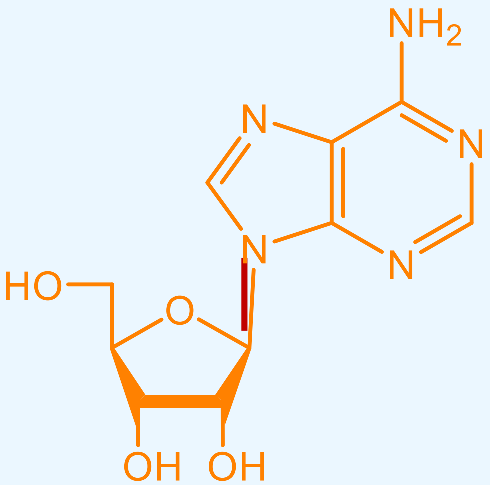
-osine to the root name of a purine

Sugars make nucleosides more water-soluble than the free bases they bear

β-N_1-glycosidic bonds in pyrimidine ribonucleosides

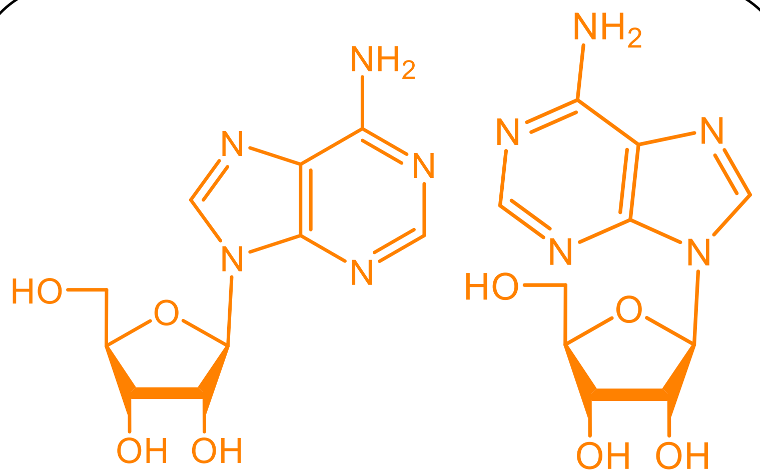
β-N_9-glycosidic bonds in purine ribonucleosides



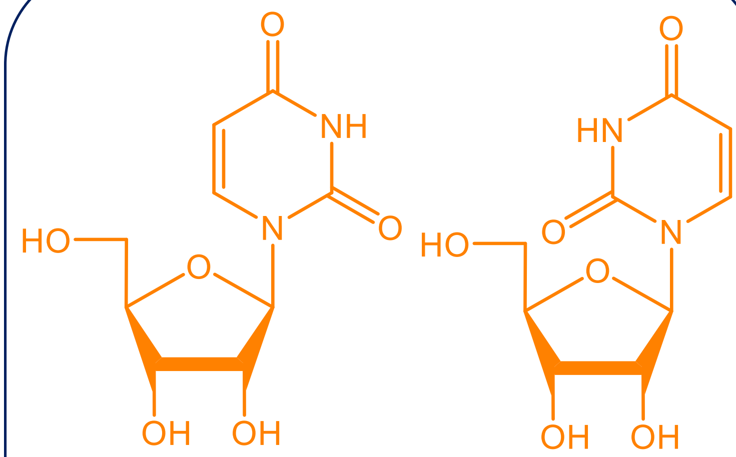


Favored Conformations of Nucleosides.

How to avoid steric clash between the base and pentose rings?



anti-adenosine *syn*-adenosine



anti-uridine *syn*-uridine

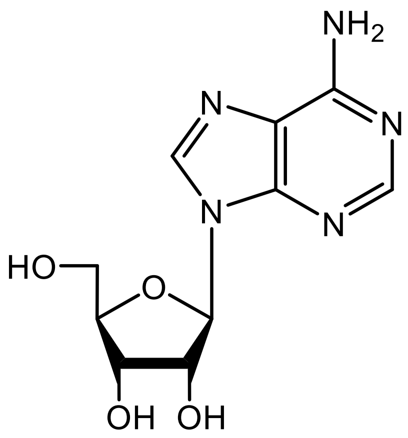
Purine nucleosides
both syn & *anti* are OK

Pyrimidine nucleosides
anti favored

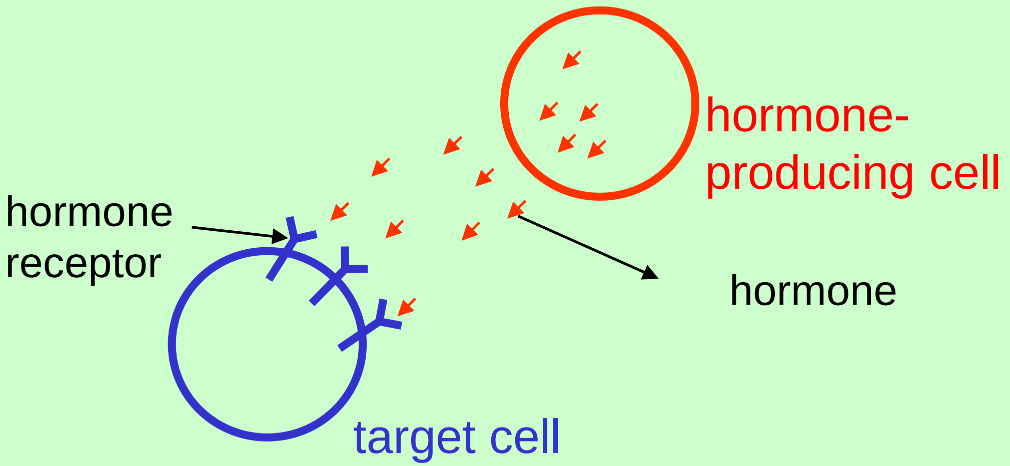
Nucleoside Functions.

- **Precursors of nucleotides**
- Adenosine: can also act as a hormone

    (hormone: blood-borne cellular stimulant)



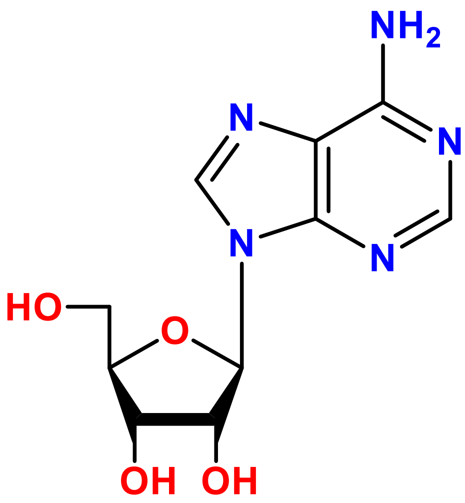
Hormone (e.g. adenosine) is produced by one cell, is secreted into the bloodstream, binds to receptors on another cell surface & initiates changes in that target cell

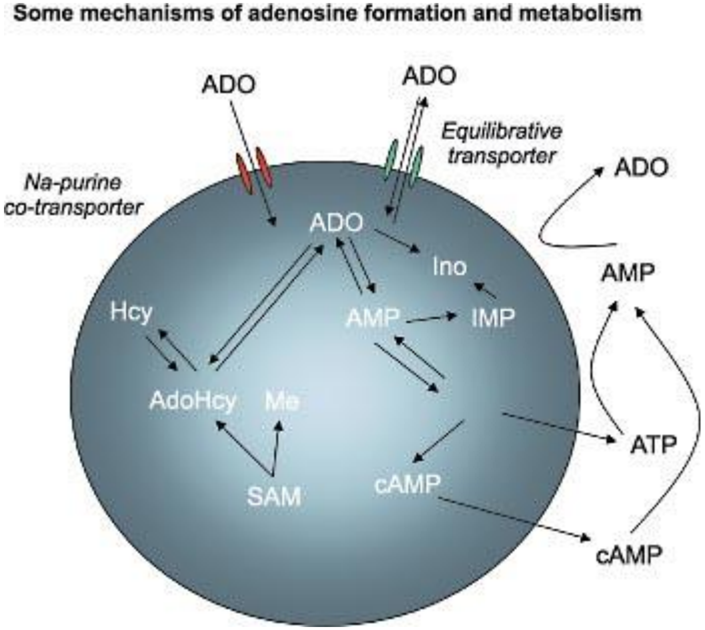


Hormonal Action of Adenosine (ADO).

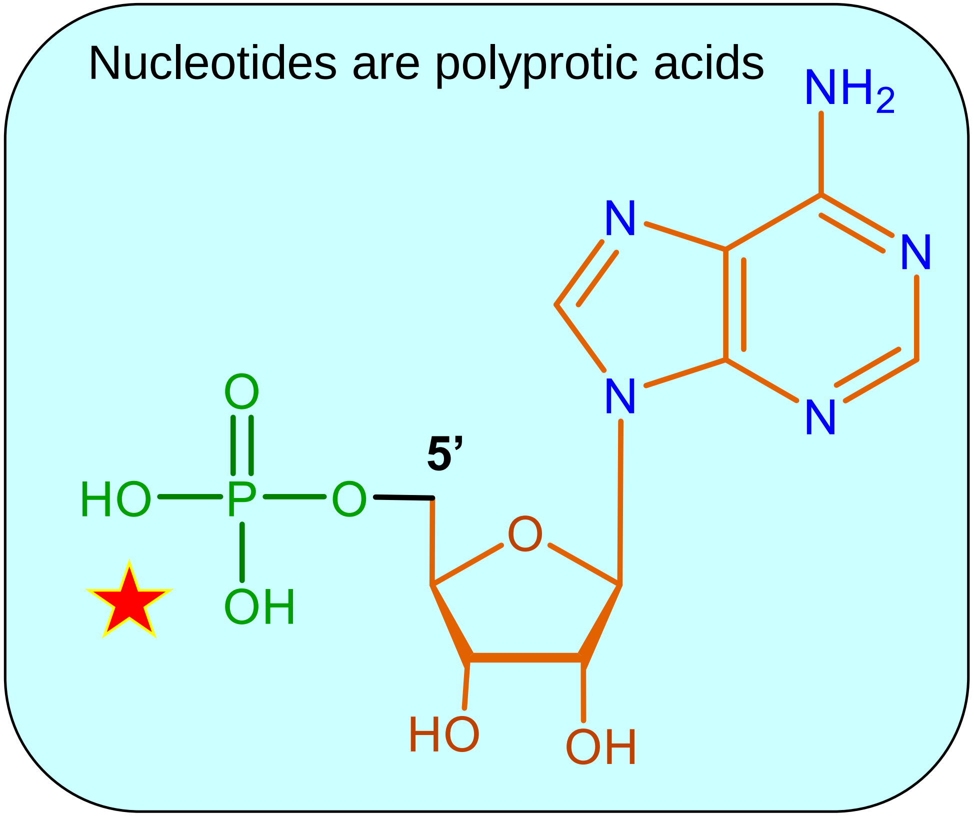
Adenosine is a white crystalline powder. It is soluble in water and practically insoluble in alcohol.

- Induces vasodilatation
- Induces smooth muscle contraction
- Release of neurotransmitters
- Induces sleepiness (countered by caffeine)



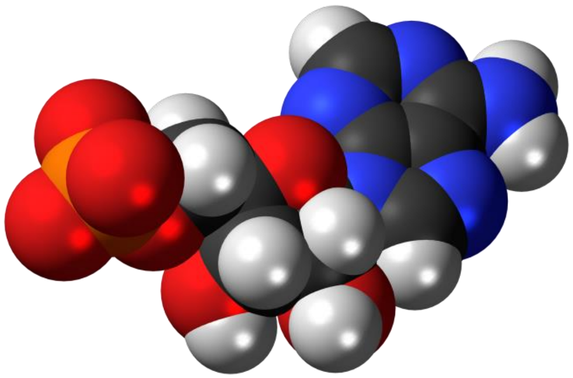


Nucleotides = Nucleoside + Phosphate.



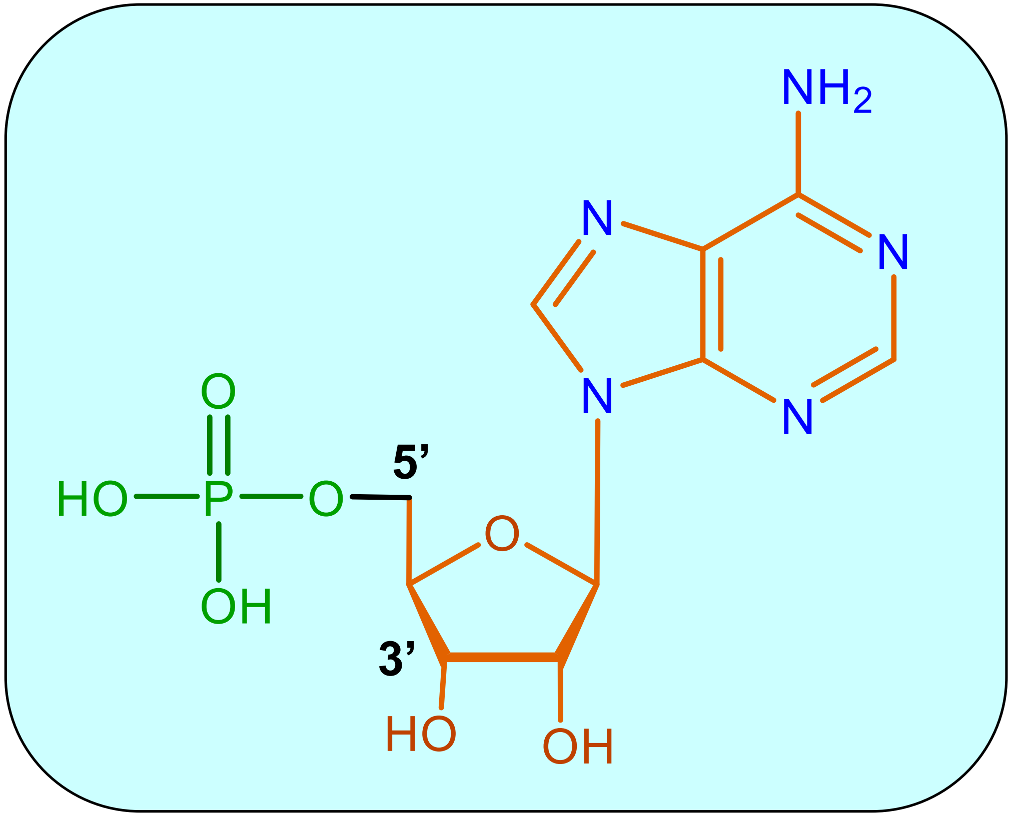
$$R\text{-}O\text{-}PO_3H_2 \overset{K_{a1}}{\rightleftarrows} RO\text{-}PO_3H^- + H^+$$

$$R\text{-}O\text{-}PO_3H^- \overset{K_{a2}}{\rightleftarrows} RO\text{-}PO_3^{2-} + H^+$$



(*e.g.* Adenosine 5'-monophosphate AMP)

Nucleotide Shorthand.



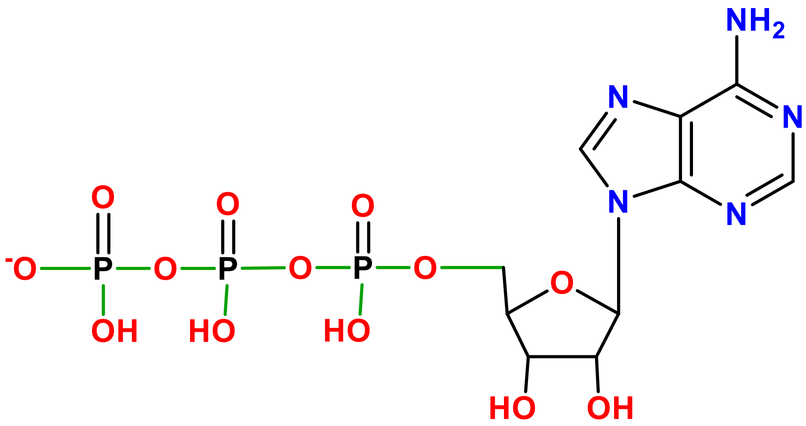
= pA

if diphosphate ppA

if triphosphate pppA

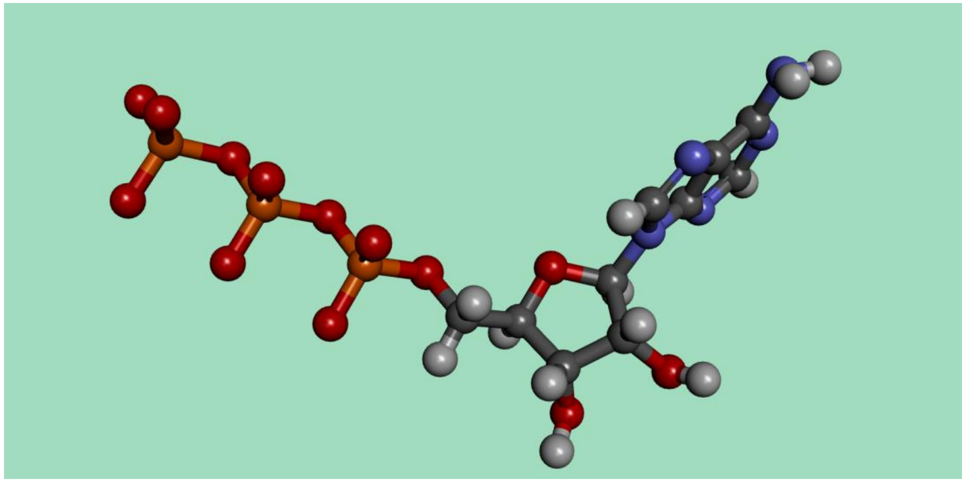
If the phosphate was at the 3' position instead = Ap

Adenosine TriPhosphate (ATP, pppA).



ATP is an high-energy molecule (coenzyme) used as an energy carrier in the cells of all known organisms; the process in which energy is moved.

*It is present in the cytoplasm and nucleoplasm of every cell, and essentially all the physiological mechanisms that require energy for operation obtain it directly from the stored ATP. In animal systems, the ATP is synthesized in the tiny energy factories called **mitochondria** by a process called **glycolysis**.*



Functions of Nucleotides.

- Precursors to the polynucleotides DNA & RNA
- Carriers of energy *via* phosphoryl group transfer

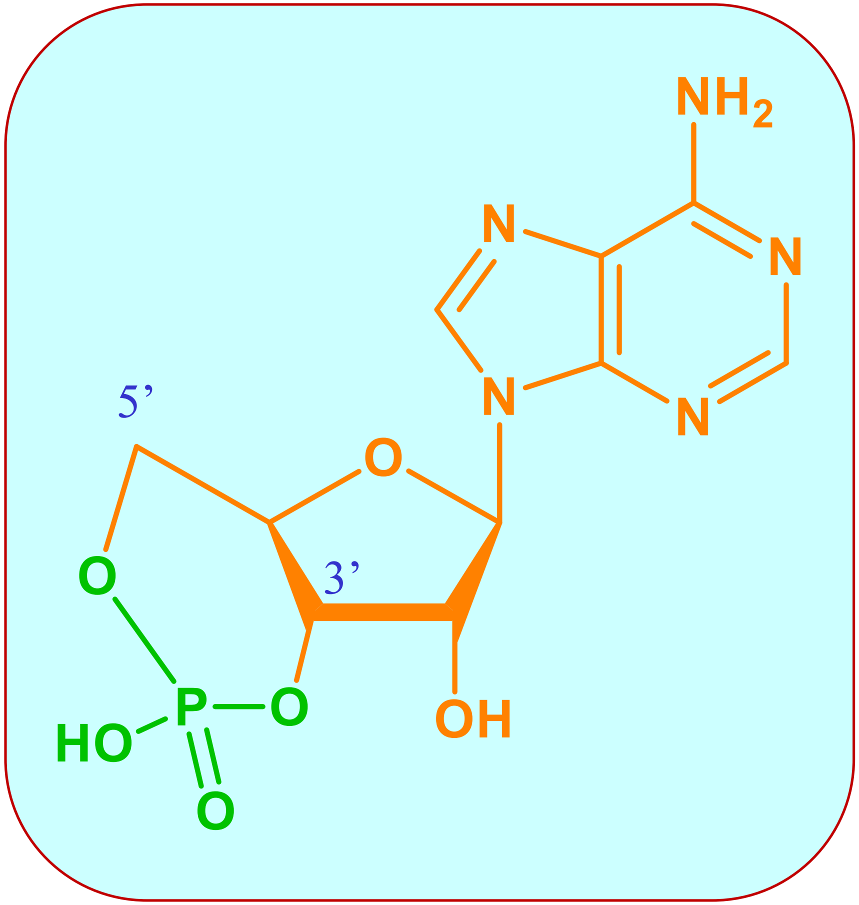
  e.g. ATP + $H_2O \rightarrow$ ADP + Pi + energy

- bases serve as recognition units
  - ATP is central to energy metabolism
  - GTP drives protein synthesis
  - CTP drives lipid synthesis
  - UTP drives carbohydrate metabolism
- Cyclic nucleotides are signal molecules and regulators of cellular metabolism and reproduction

(cyclic AMP or cAMP)

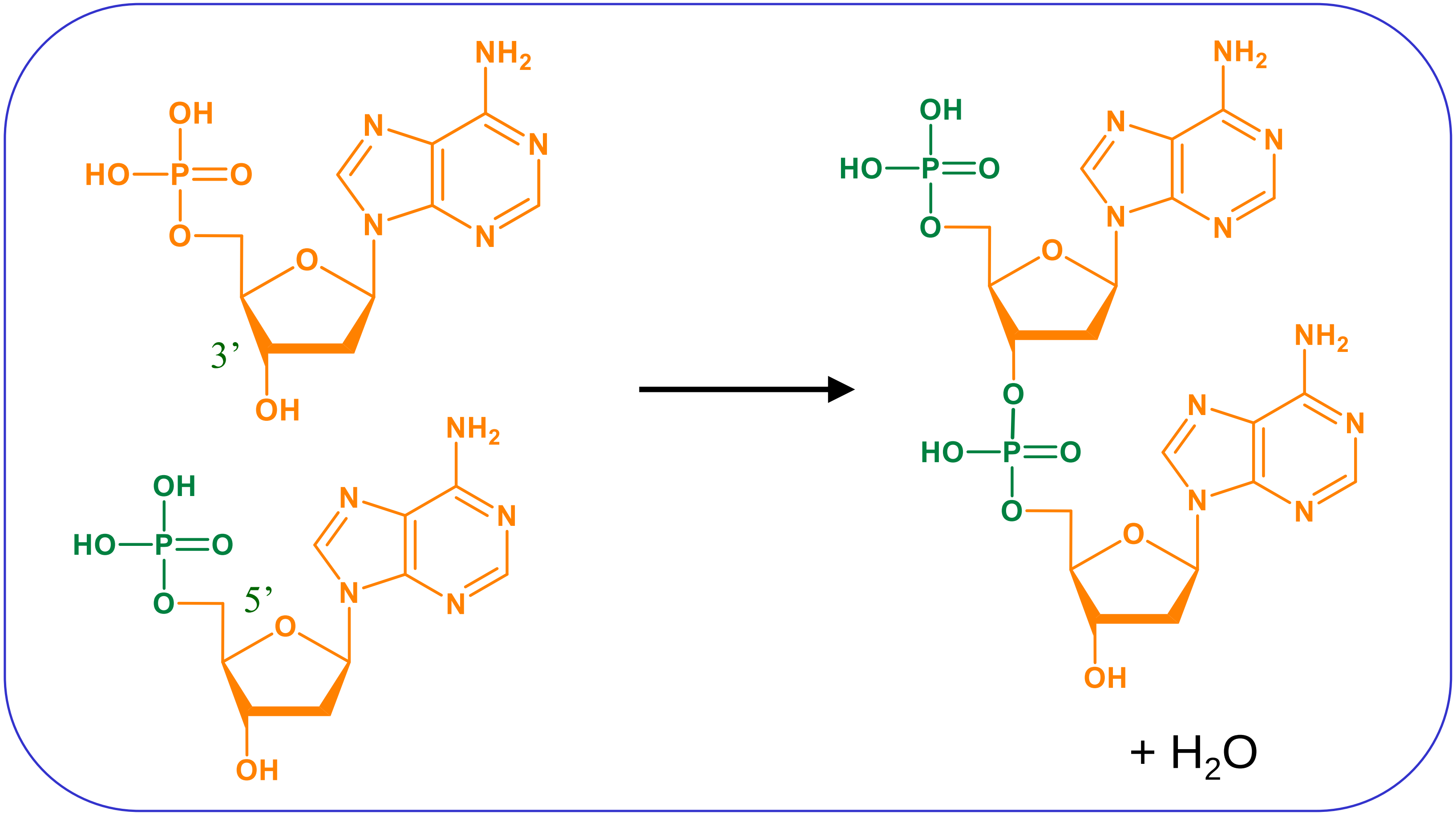
ATP → cAMP + PPi

Function: an important "second messenger" in intracellular signaling



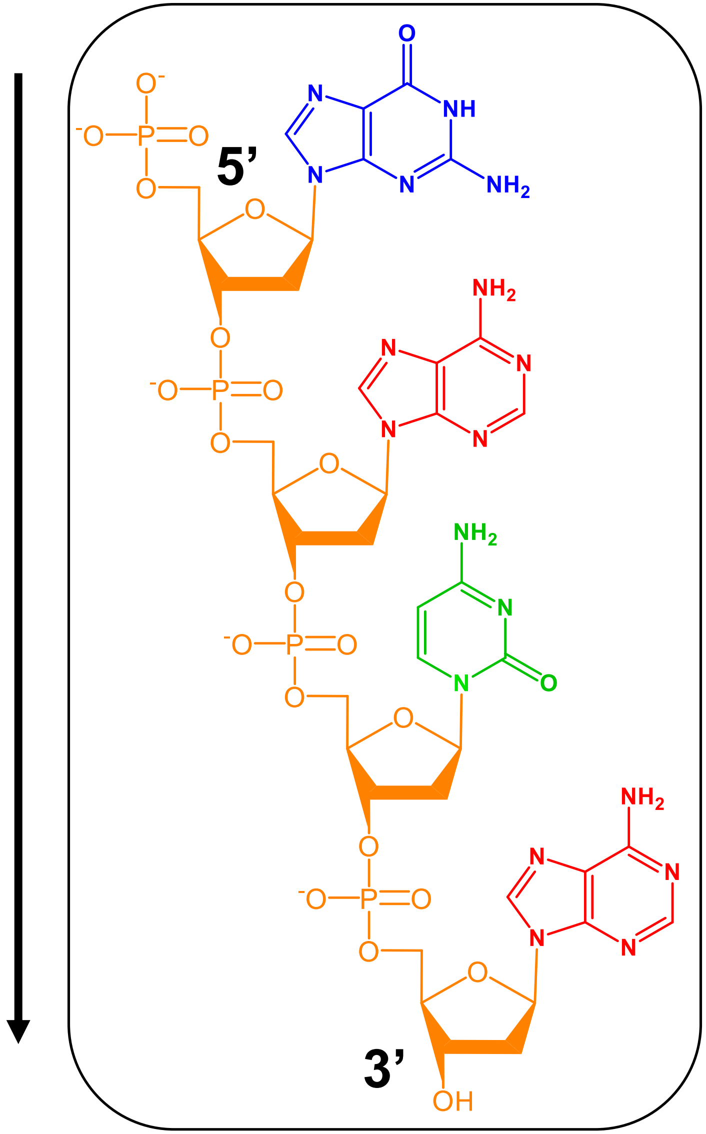
Phosphodiester
6-membered ring

Linking Nucleotides by 5'-3' Phosphodiester Bonds to Dinuclotides.



NpNpNpN + pppN → NpNpNpNpN + 2Pi

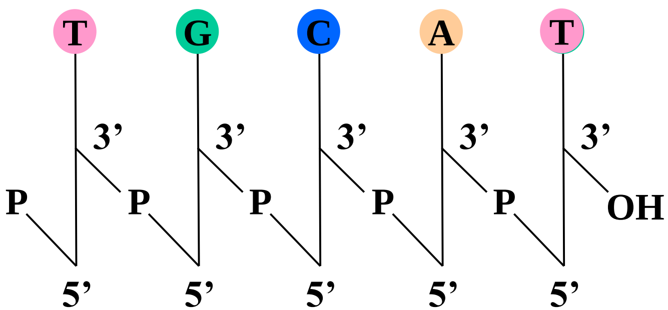
Nucleic Acids: Linear Polymers of Nucleotides.



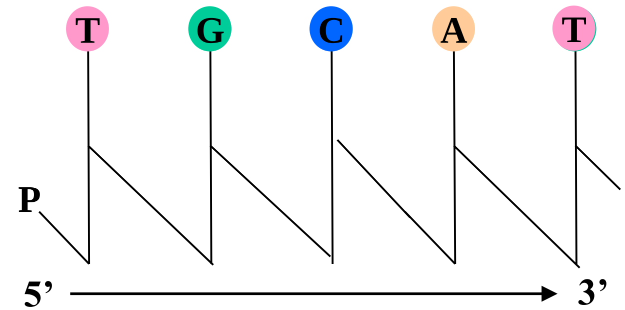
- Polymers linked 5’ to 3’ by phosphodiester bridges
- Sequence is always read 5' to 3'
- In terms of genetic information, this corresponds to "N to C" in proteins
- phosphodiester is weakly acidic: dissociated at neutral pH ∴anionic

The sample shown here is a DNA molecule with the sequence 5’-GACA-3’. The arrow gives the direction of the chain.

Sequence Shorthand.



or simply



or

5’pTGCAT-3’

or

pTGCAT

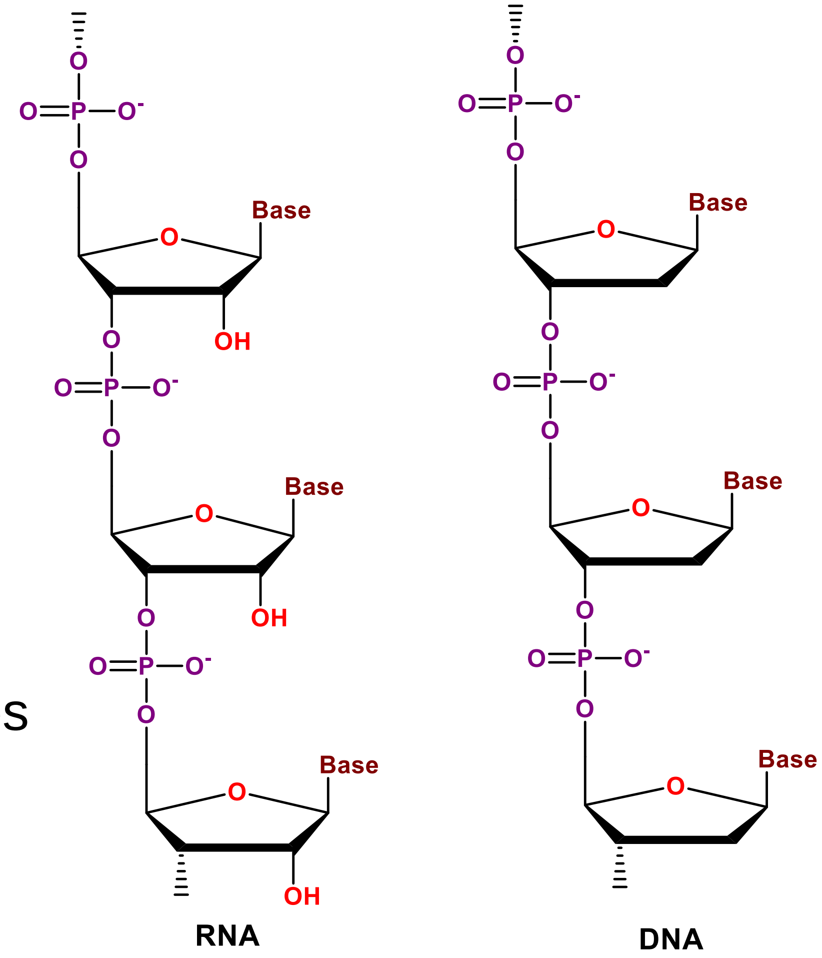
Classes of Nucleic Acids.

DNA - one type, one purpose: genetic material

- possesses primary & secondary structure (helices), but no tertiary structure

RNA - 3 types, 3 purposes

- primary, secondary & tertiary structures all occur
- ribosomal r-RNA - the basis of structure and function of ribosomes
- messenger m-RNA - carries the message
- transfer t-RNA - carries the amino acids



The DNA Double Helix: a Brief History.

Two interwound chains, stabilized by hydrogen bonds between chains & stacking of the bases

"Base pairs" arise from H bonds:

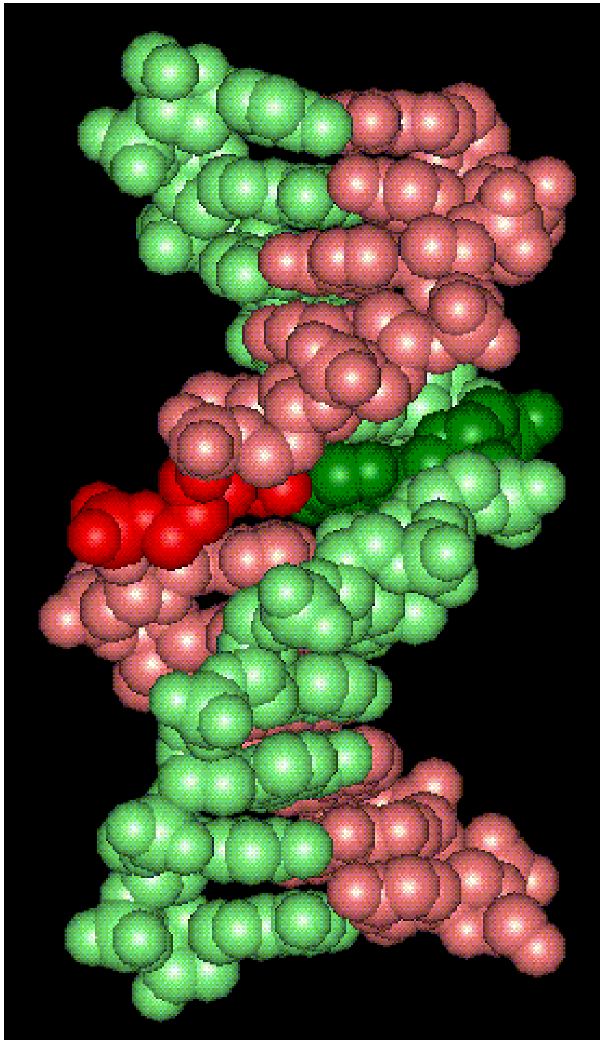
- **A** on one strand pairs with **T** on the other
- **G** pairs with **C**

Erwin Chargaff had the base content data, but didn't understand its implications

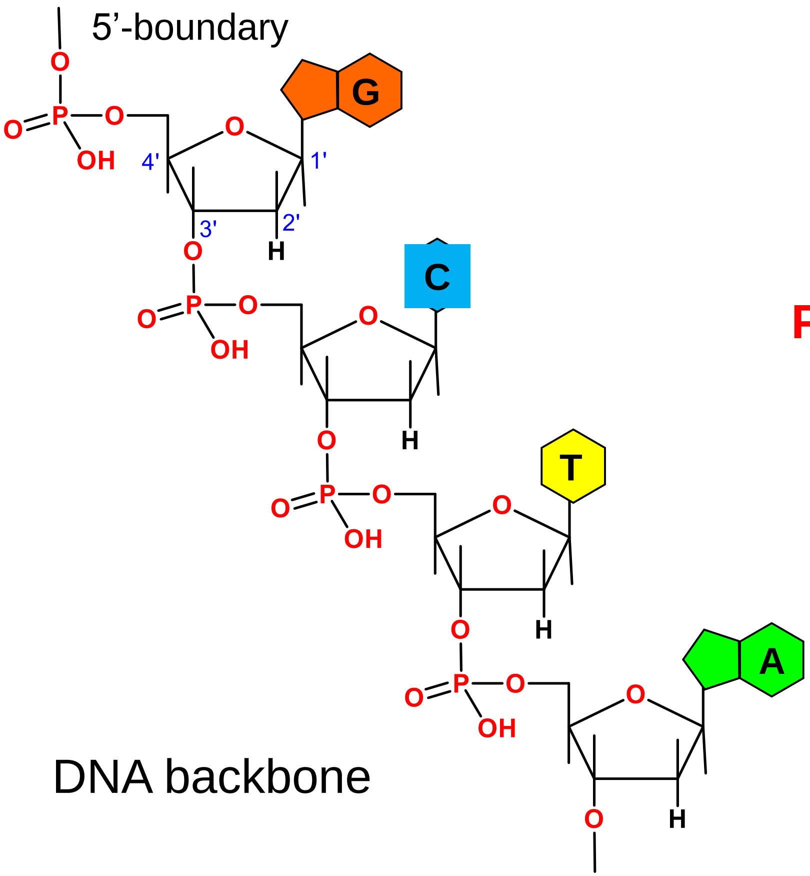
Rosalind Franklin's X-ray fiber diffraction data provided crucial structural constraints

Francis Crick
James Watson } correctly interpreted the clues

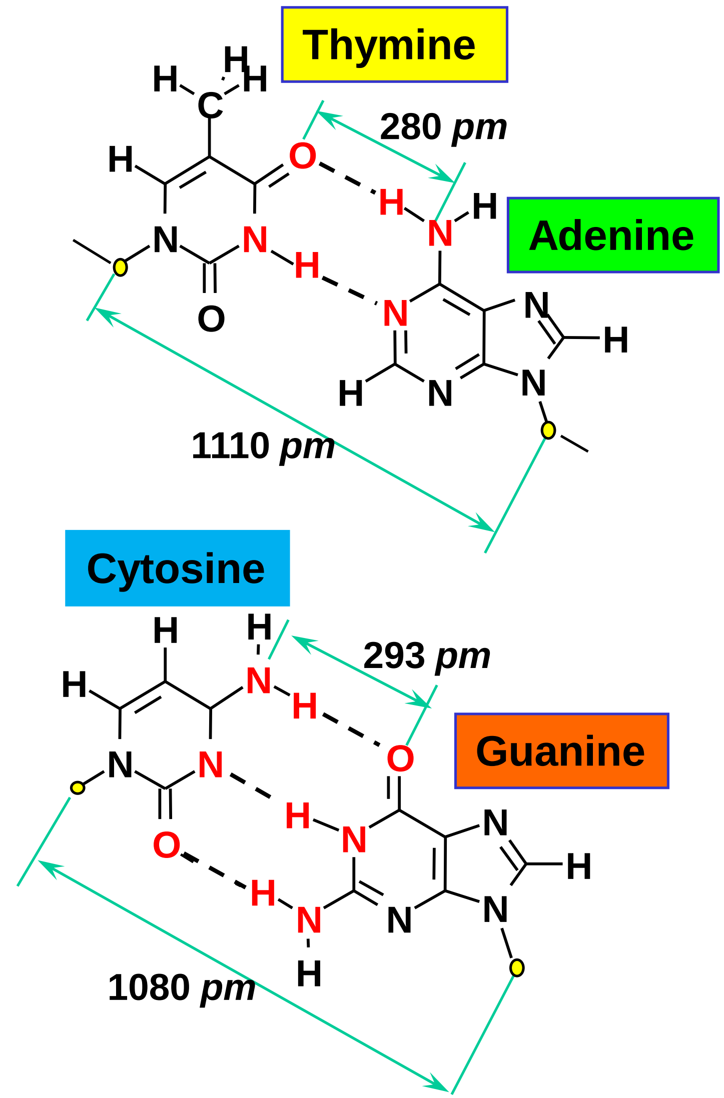
Linus Pauling blew it (for once)



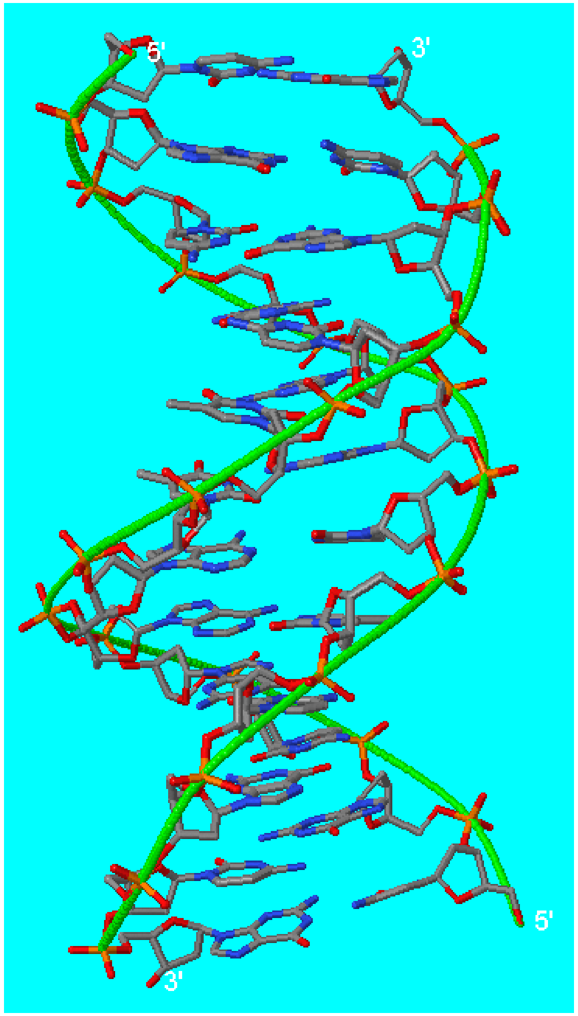
Molecular Recognition Between Nucleic Bases.

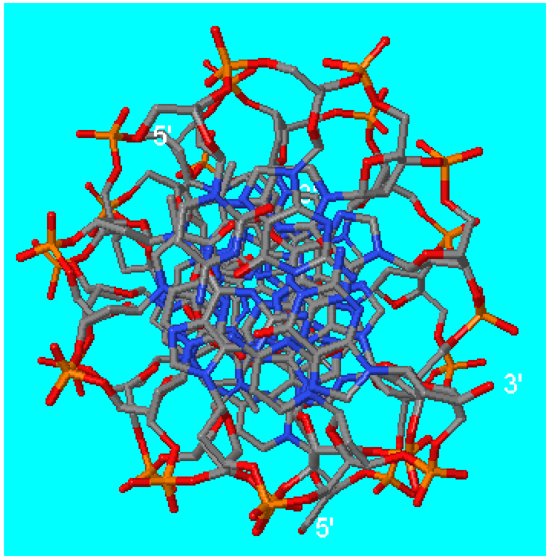


PARING



DNA.



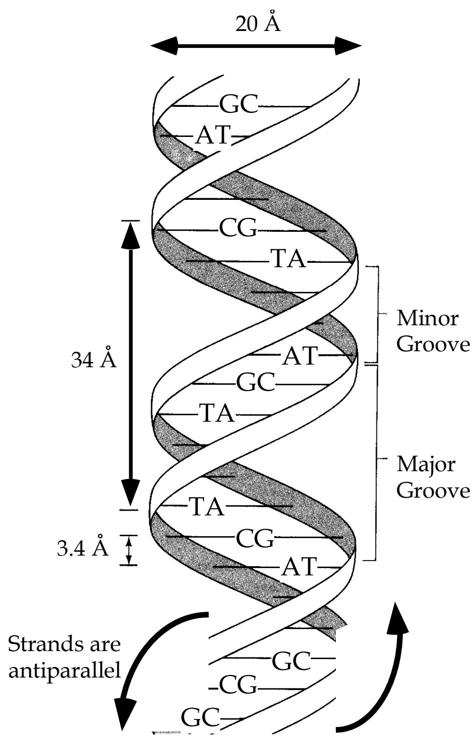


Vision through the axis

Lateral vision where is Evidenced the double chain (Double stranded DNA)

DNA - Semi-conservative Replication (Meselson-Stahl, 1958).

CGCGTTGACAACTGCAGAATC

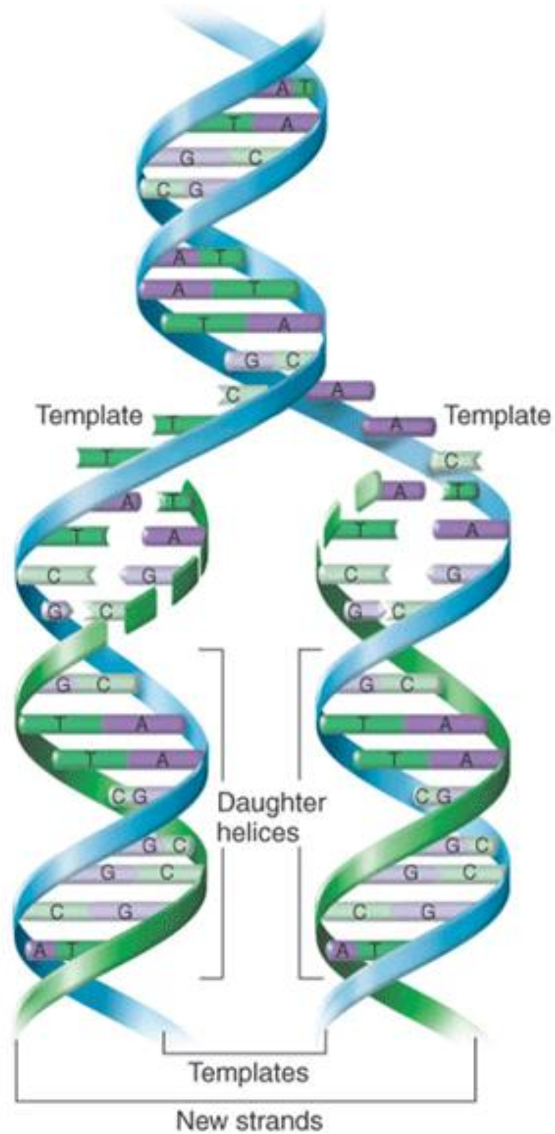


1. Original double helix

2. Strands separate

3. Complementary bases align opposite templates

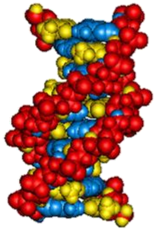
4. Enzymes link sugar-phosphate elements of aligned nucleotides into a continuous new strand



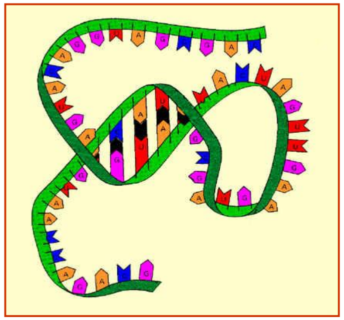
Two Important Mechanisms.

Transcription :

DNA

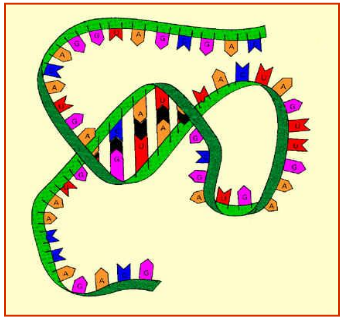


Genetic Information



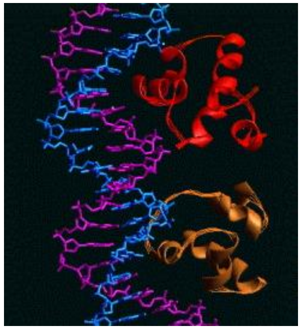
messenger RNA

Traduction :



messenger RNA

Proteins



chemical reactions,
metabolism

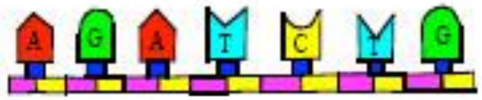
A gene = a fragment of DNA which codes for a protein

The Standard Genetic Code.

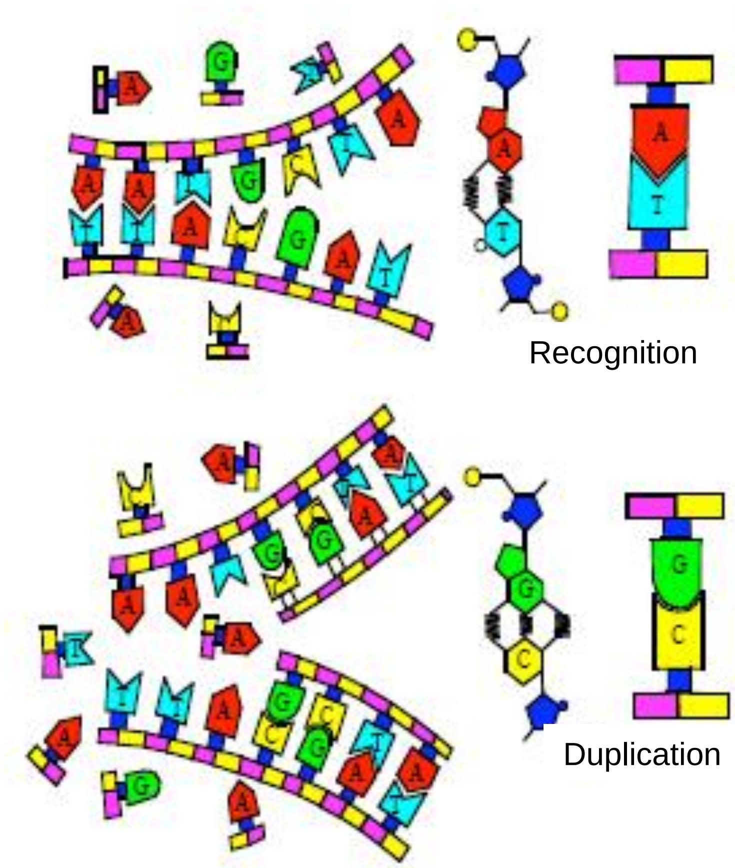
	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr Y	UAU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr Y	UAC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UAA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr H	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr H	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr Q	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr Q	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

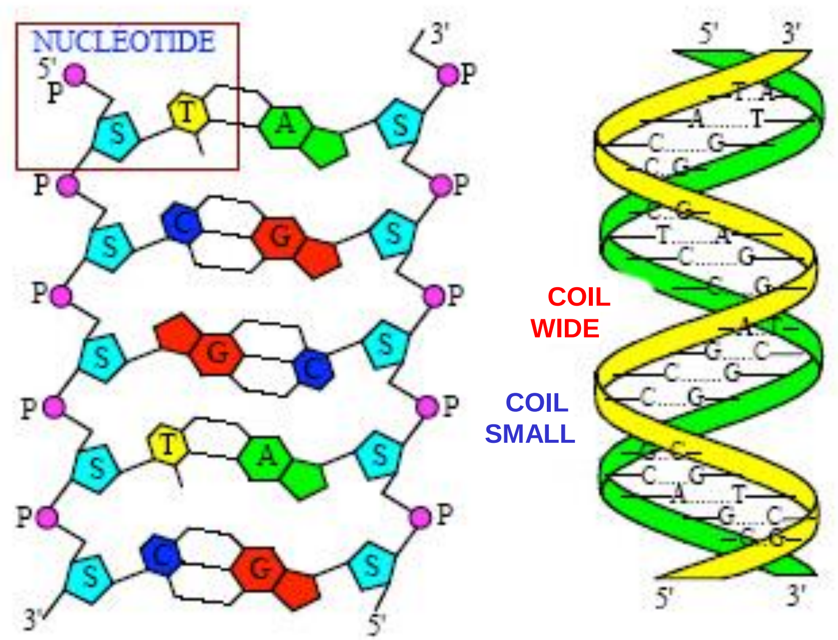
- translation start codon
- translation stop codon
- hydrophobic amino acids
- hydrophilic non charged amino acids
- negatively charged amino acids
- positively charged amino acids
- cysteine

Auto Assembling and Molecular Recognition by DNA Molecule.



Assembling

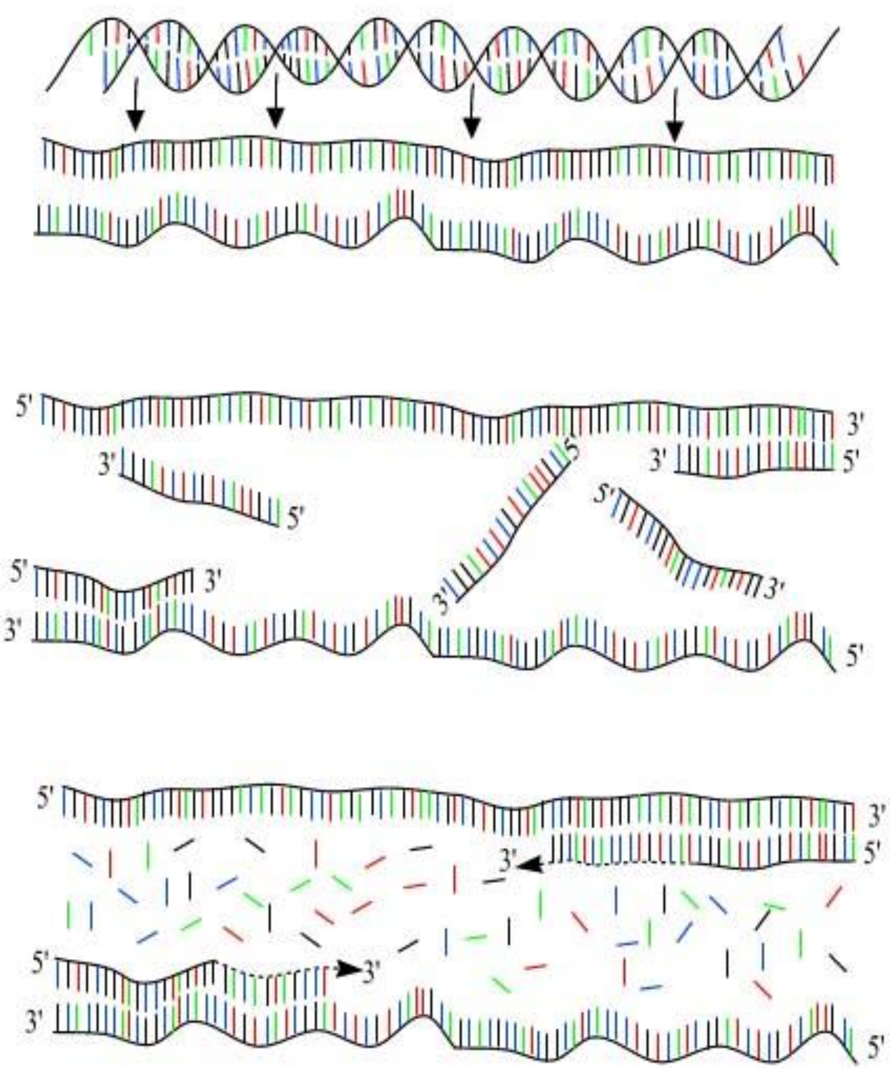




Double alpha helix

Support for genetic information

PCR: Polymerase Chain Reaction.



30-40 cycles of 3 steps:

Step 1: denaturation

1 minute 94°C

Step 2: annealing

45 seconds 54°C

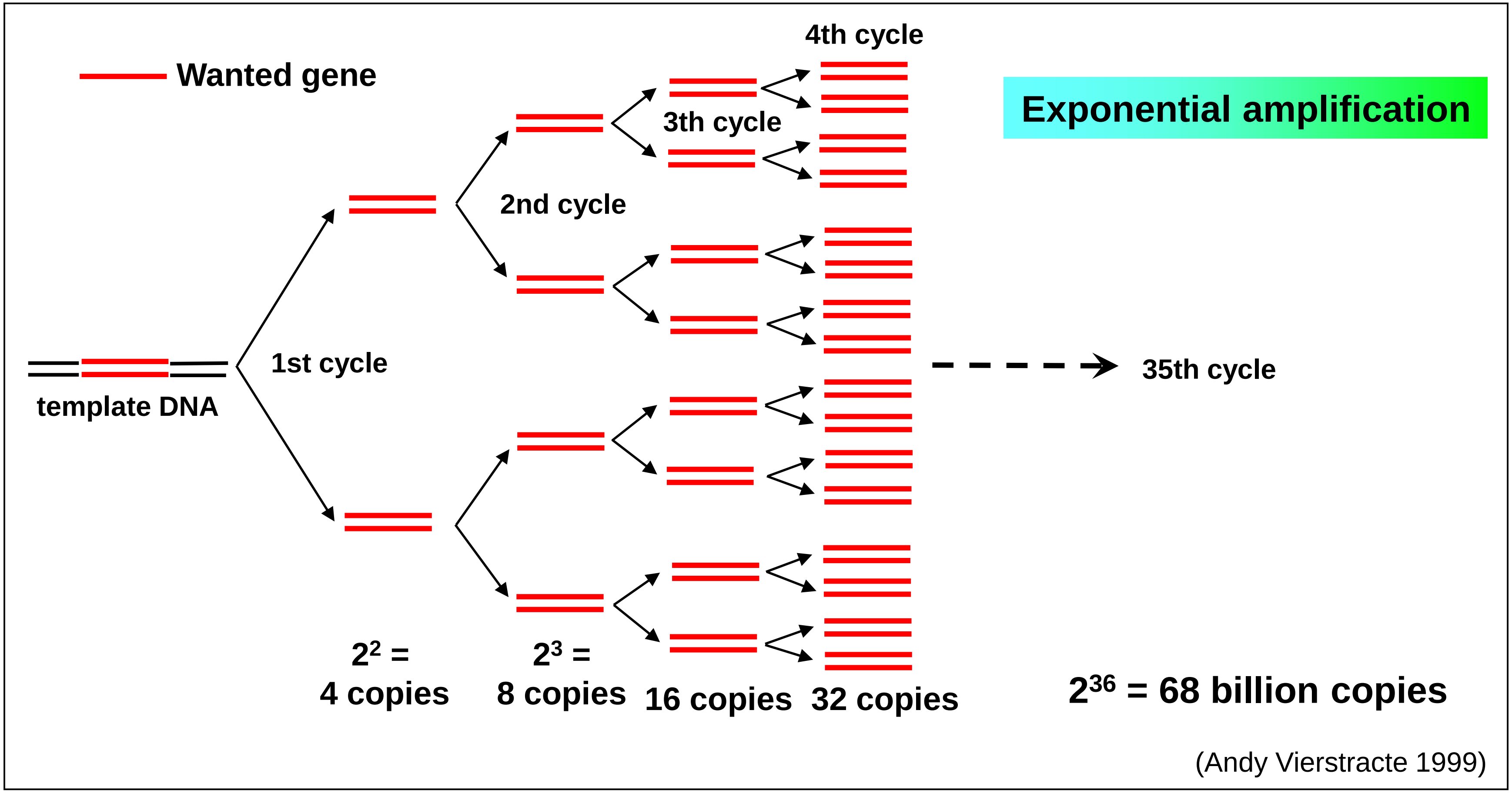
Forward and reverse primers!!

Step 3: extension

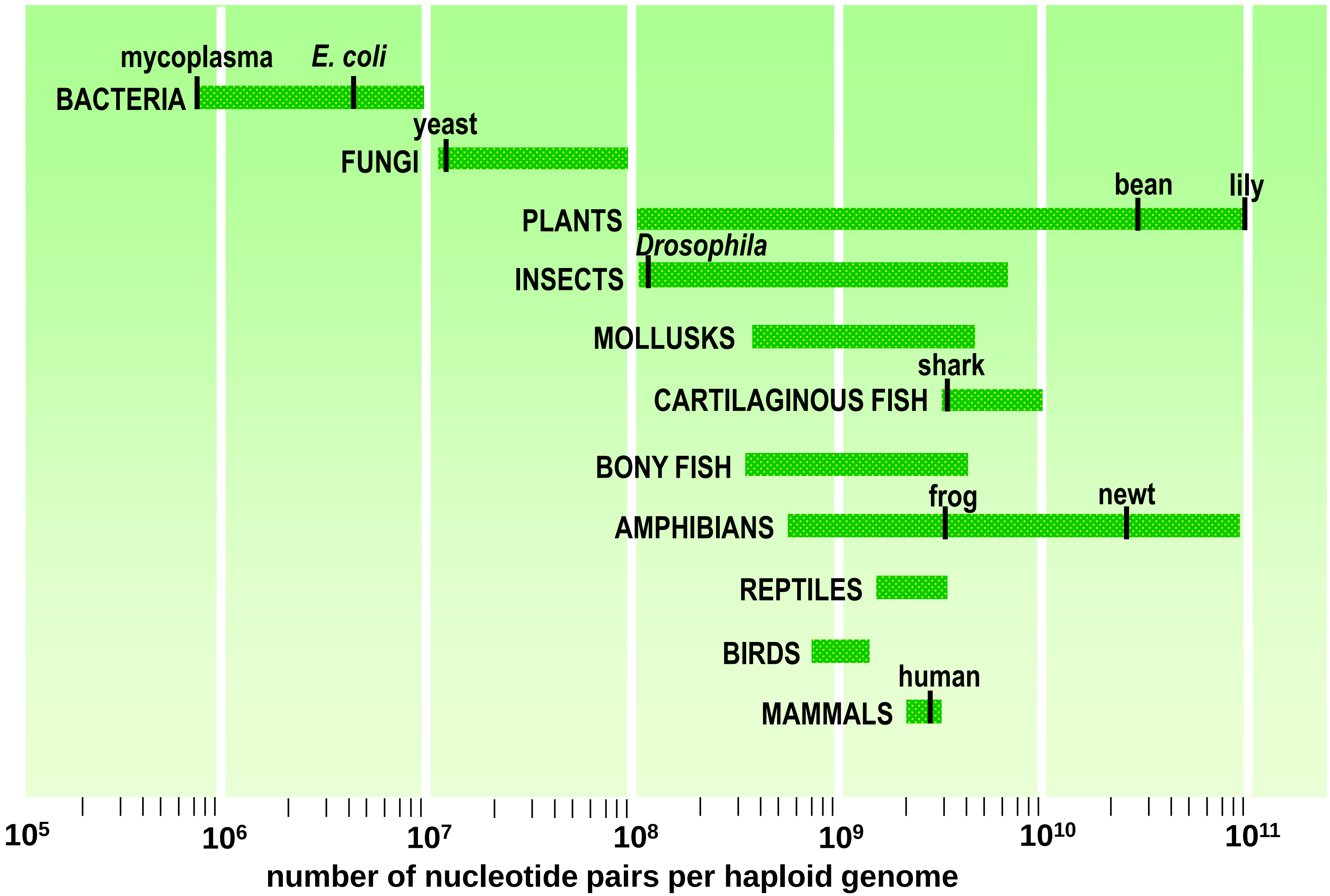
2 minutes 72°C

only dNTP's

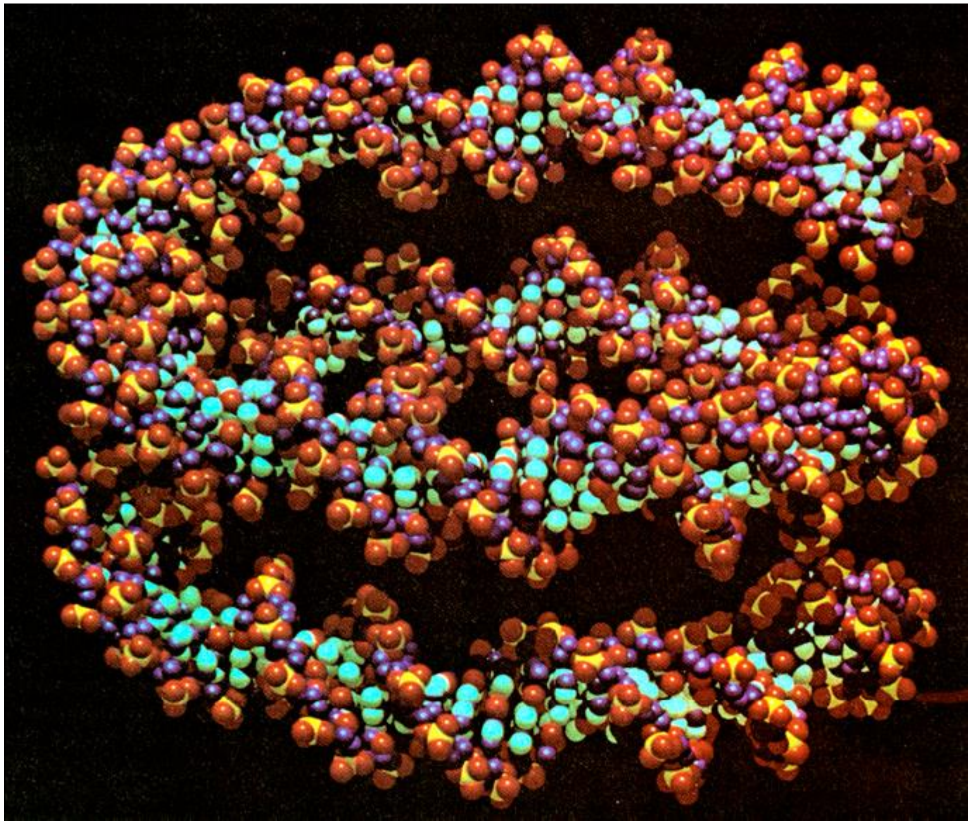
PCR Amplification.



Genome Dimension in Number of Base Pairs.



Super-coils of DNA.



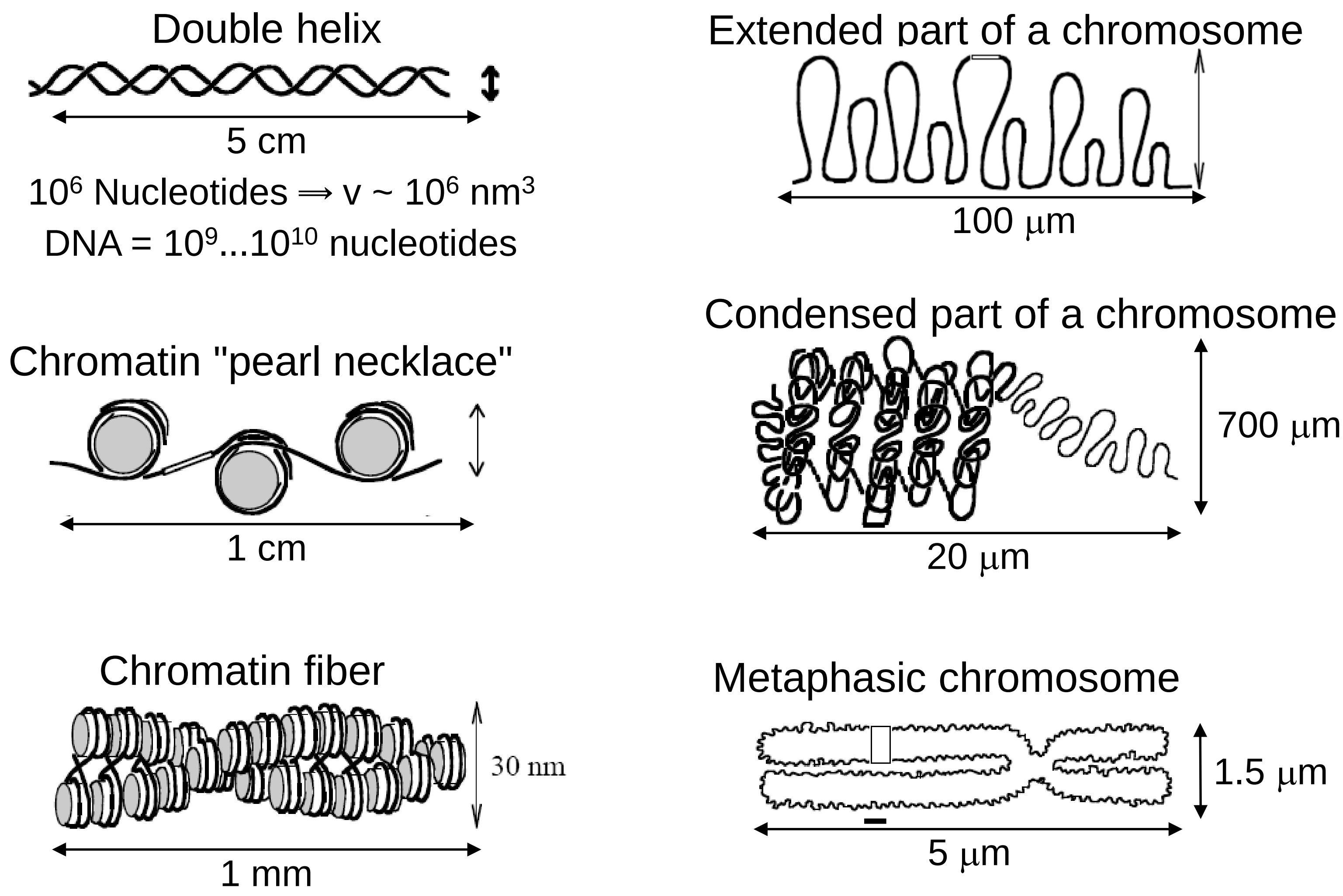
Human

$3.3\ 10^{9}$ pairs of bases

1 base each 3.4 Angstrom

Length = 1 meter !!!!
All inside the cell (~1 μm)

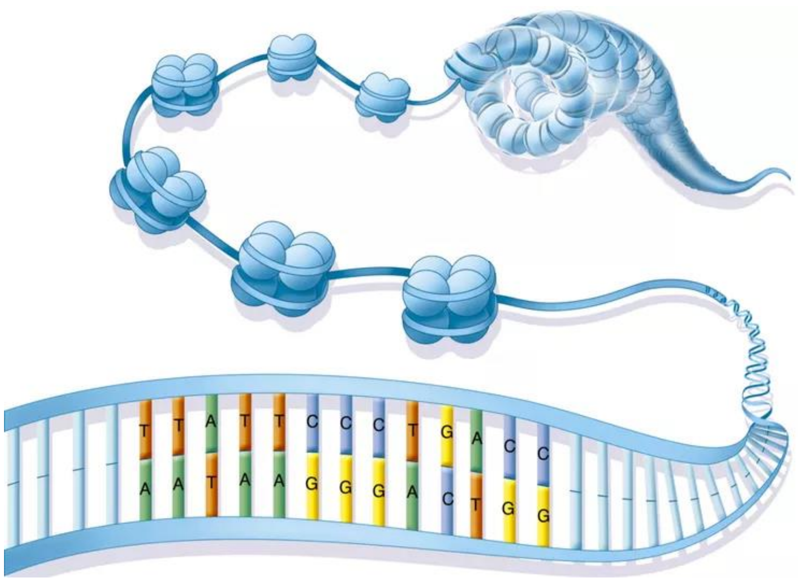
Alternative Packing of DNA Molecule.



Histones and Chromatin.

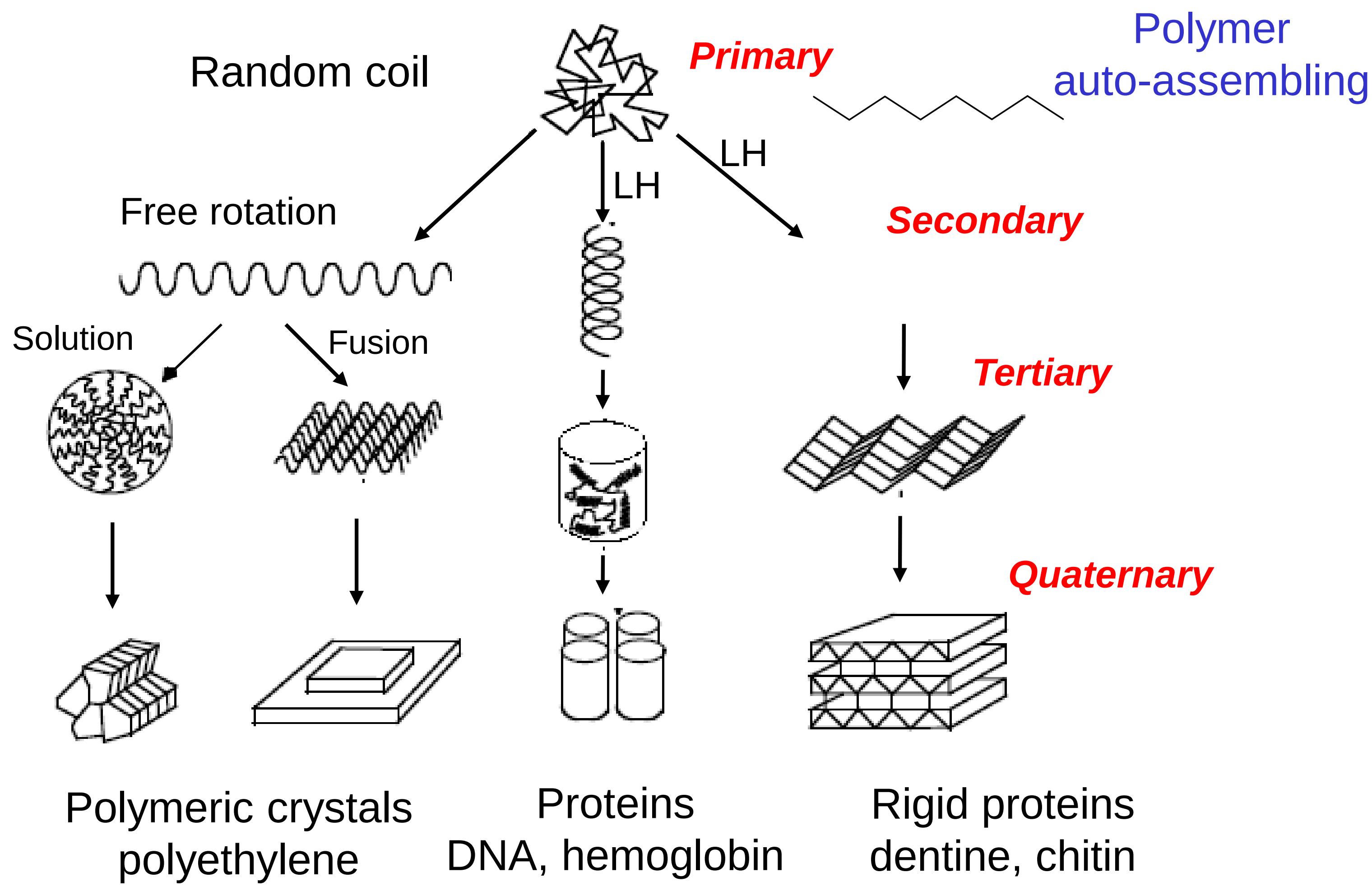
Chromatin is a mass of genetic material composed of DNA and proteins that condense to form chromosomes during eukaryotic cell division. Chromatin is located in the nucleus of our cells.

The primary function of chromatin is to compress the DNA into a compact unit that will be less voluminous and can fit within the nucleus. Chromatin consists of complexes of small proteins known as histones and DNA. Histones help to organize DNA into structures called nucleosomes by providing a base on which the DNA can be wrapped around. A nucleosome consists of a DNA sequence of about 150 base pairs that is wrapped around a set of eight histones called an octamer. The nucleosome is further folded to produce a chromatin fiber. Chromatin fibers are coiled and condensed to form chromosomes.



Cooper, Geoffrey. "The Cell: A Molecular Approach." 8th Edition, Sinauer Associates (an imprint of Oxford University Press), October 9, 2018.

Autoassembling in Polymers: Flexible Macromolecules.



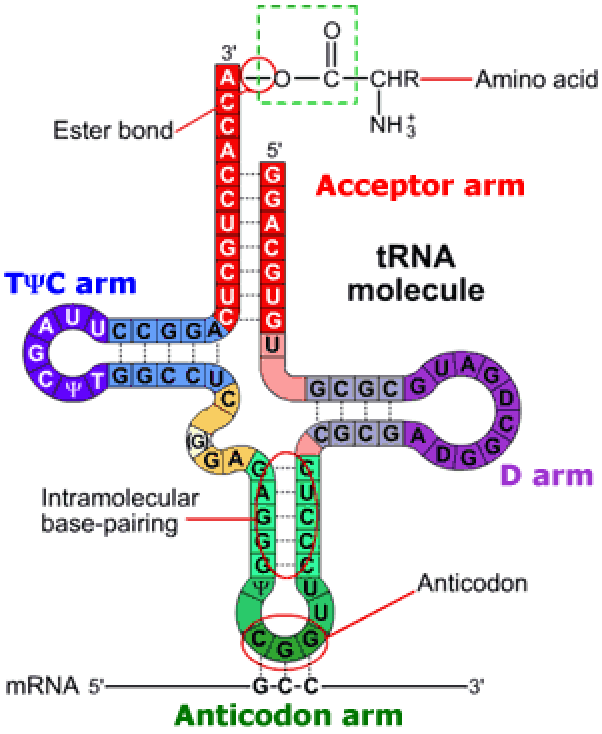
RNA - Types of RNA.

In all prokaryotic and eukaryotic organisms, three main classes of RNA molecules exist:

1) Messenger RNA(m RNA)
2) Transfer RNA (t RNA)
3) Ribosomal RNA (r RNA)

The other are:

- small nuclear RNA (SnRNA),
- micro RNA (mi RNA)
- small interfering RNA(Si RNA)
- heterogeneous nuclear RNA (hnRNA).



Transfer RNA (t RNA)

Storage/transfer of genetic information

- **Genomes**
  - many viruses have RNA genomes

    single-stranded (ss-RNA)

    [e.g., retroviruses (HIV)]

    double-stranded (ds-RNA)

- **Transfer of genetic information**
  - m-RNA = "coding RNA" - encodes proteins

Structural

- e.g., r-RNA, which is a major structural component of ribosomes
- BUT - its role is *not* just structural, also:

RNA Functions (2).

Catalytic

RNA in the ribosome has *peptidyltransferase* activity

- Enzymatic activity responsible for peptide bond formation between amino acids in growing peptide chain
- Also, many small RNAs are enzymes ***ribozymes"***

Regulatory

Recently discovered important new roles for RNAs

In normal cells:

- in "defense" - esp. in plants
- in normal development

As tools:

- for gene therapy or to modify gene expression

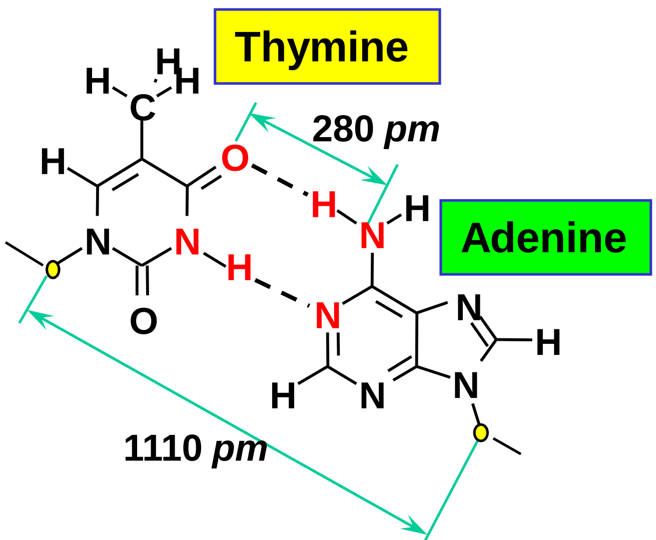
RNA Types & Functions.

Types of RNAs	Primary Function(s)
m-RNA - messenger	translation (protein synthesis) regulatory
r-RNA - ribosomal	translation (protein synthesis) <catalytic>
t-RNA - transfer	translation (protein synthesis)
hn-RNA - heterogeneous nuclear	precursors & intermediates of mature mRNAs & other RNAs
sc-RNA - small cytoplasmic	signal recognition particle (SRP) tRNA processing <catalytic>
sn-RNA - small nuclear snoRNA - small nucleolar	mRNA processing, poly A addition <catalytic> rRNA processing/maturation/methylation
regulatory RNAs (si-RNA, mi-RNA, etc.)	regulation of transcription and translation, other?

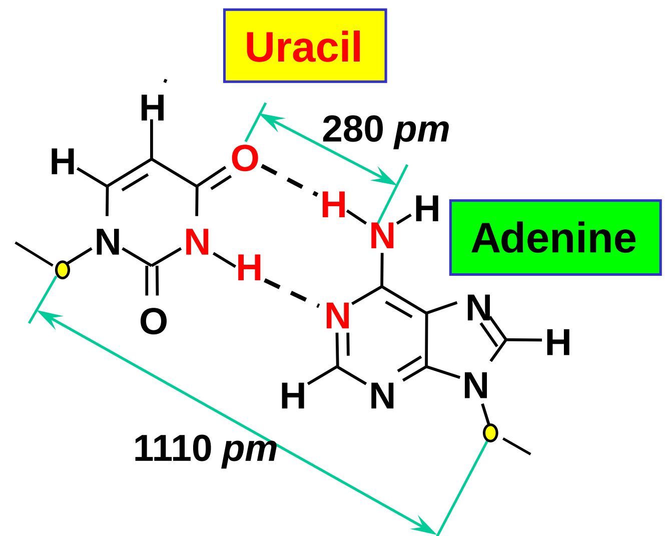
RNA Structure.

The major bases found in DNA and RNA:

DNA	RNA
Adenine	Adenine
Cytosine	Cytosine
Guanine	Guanine
Thymine	Uracil (U)

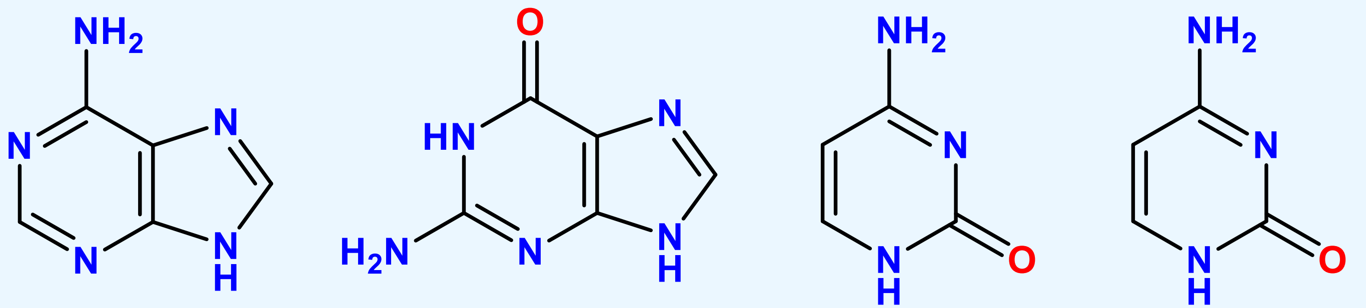


thymine-adenine base pair



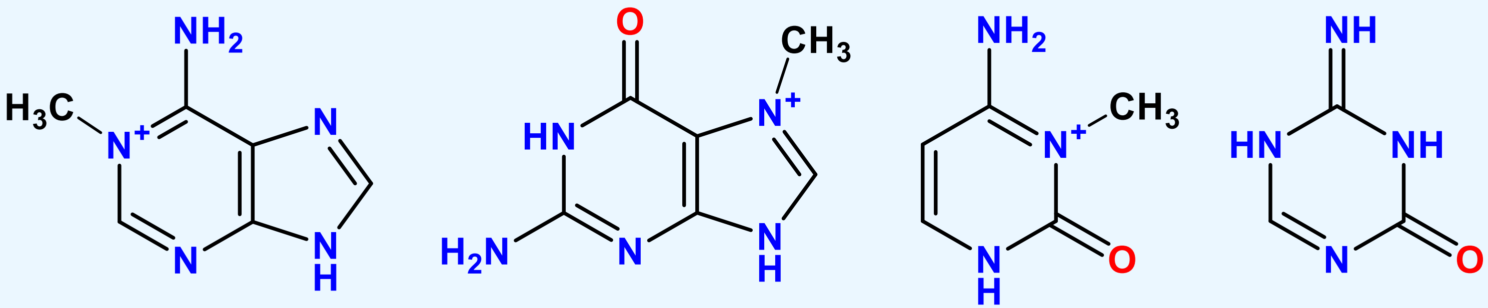
uracil-adenine base pair

Nucleoside bases found in RNA



adenine (A) guanine (G) cytosine (C) uracil (U)

Examples of modified bases found in tRNA



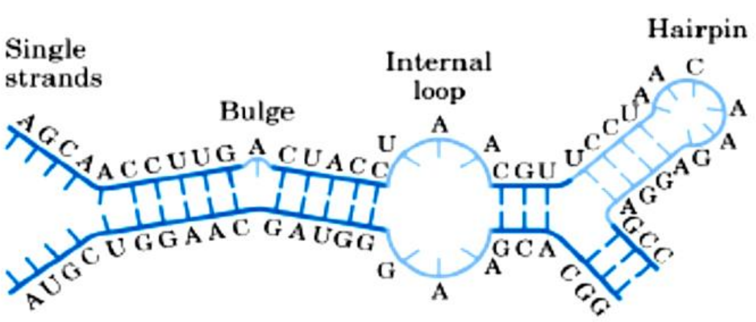
1-methyladenine (m^1A) 7-methylguanine (m^7G) 3-methylcytosine (m^3C) pseudouracil (Ψ)

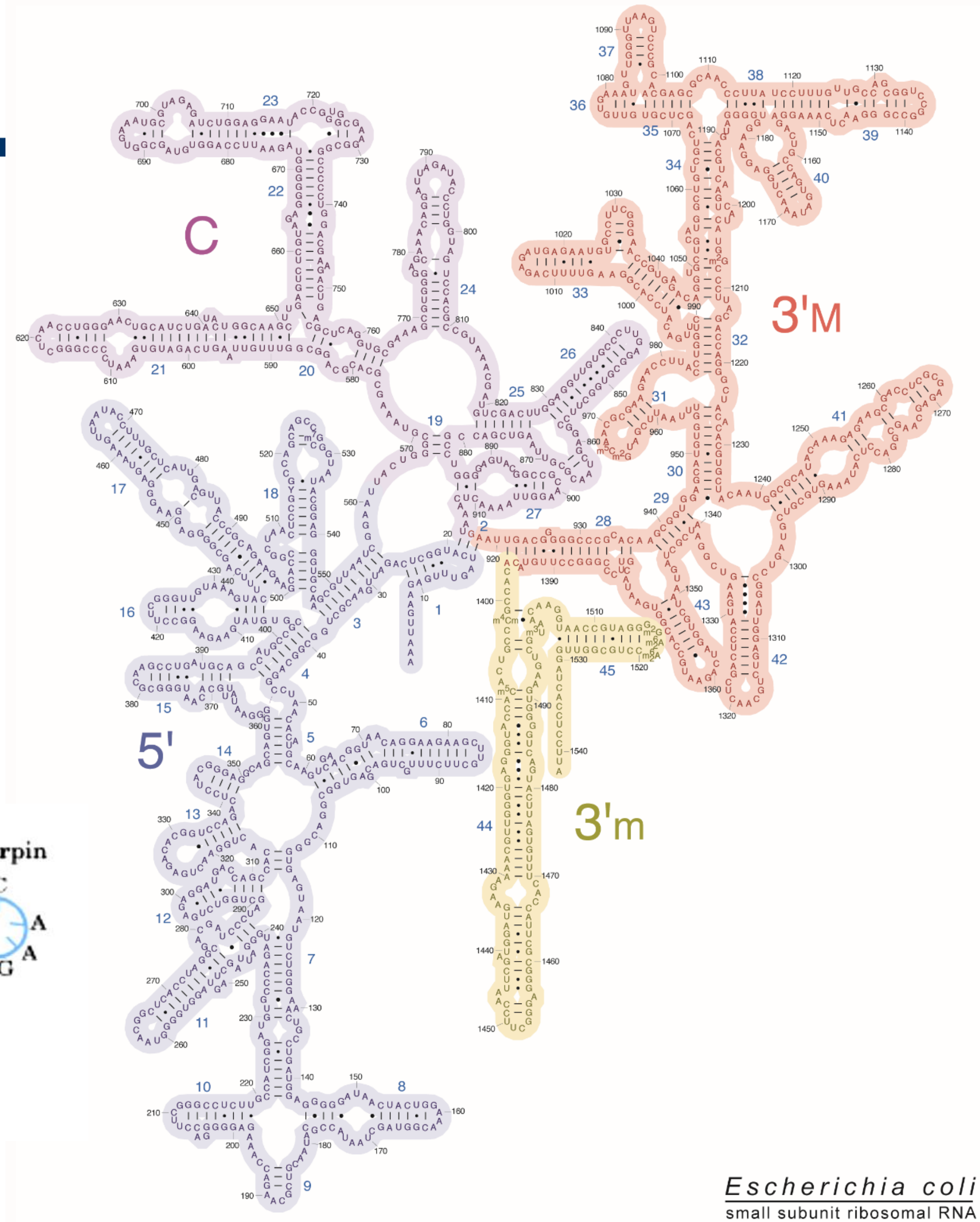
Dihydrouridine 1-methylguanosine 2-thiocytidine 5-methylcytidine Ribothymine

E-Coli 16S - RNA Secondary Structure.

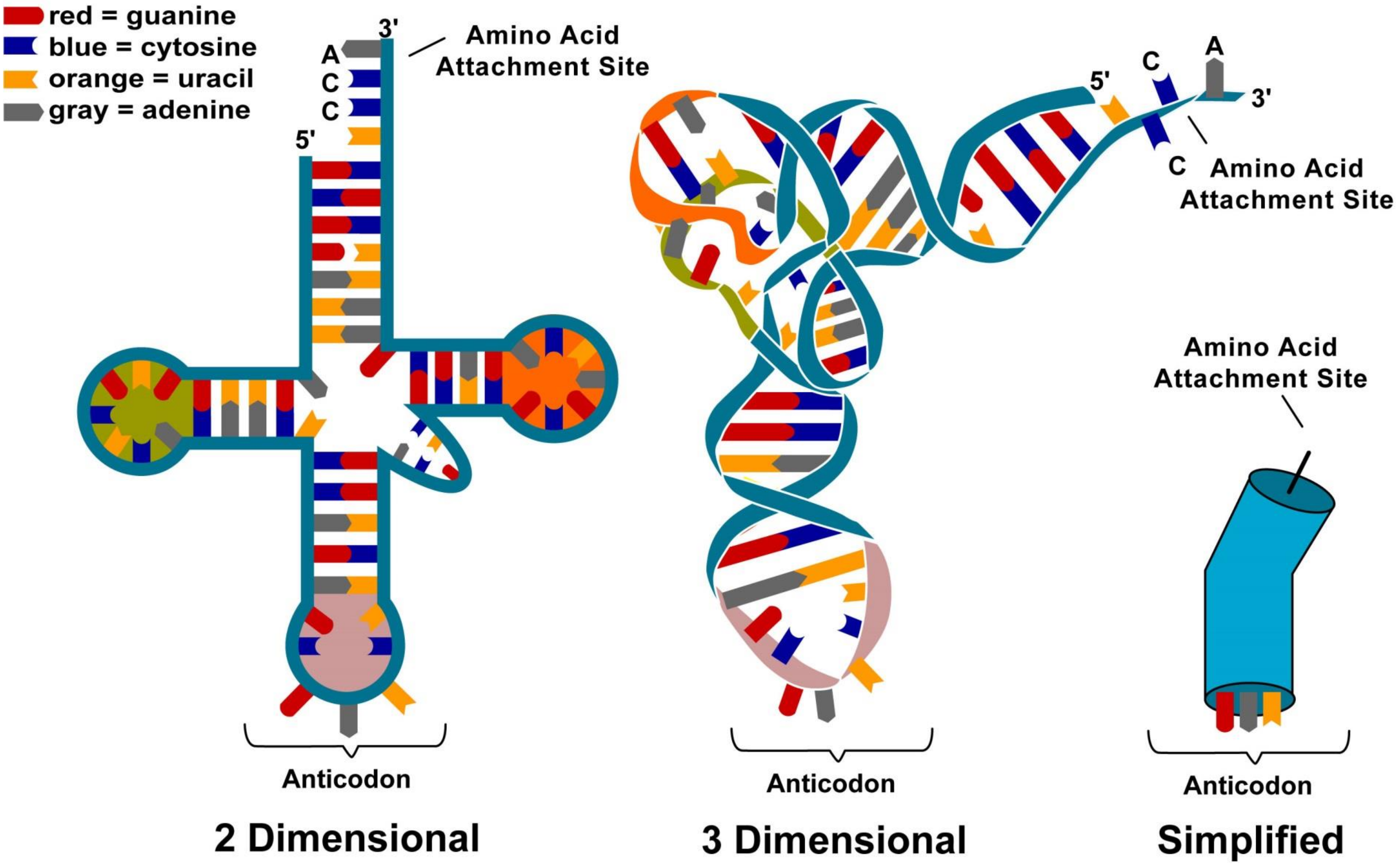
Single stranded bases within a stem are called a bulge of bulge loop if the single stranded bases are on only one side of the stem.

If single stranded bases interrupt both sides of a stem, they are called an internal (interior) loop.



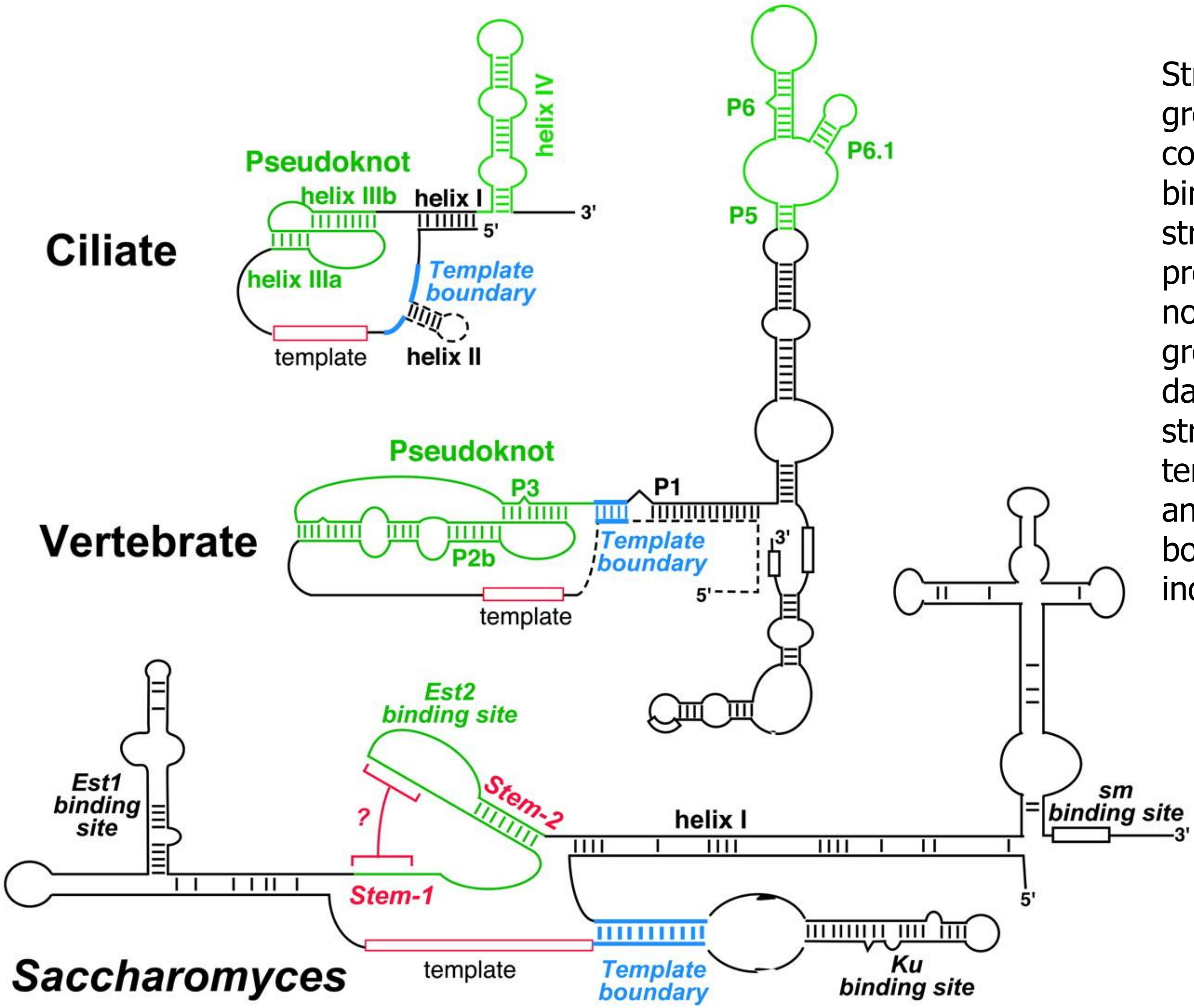


RNA Tertiary Structure.

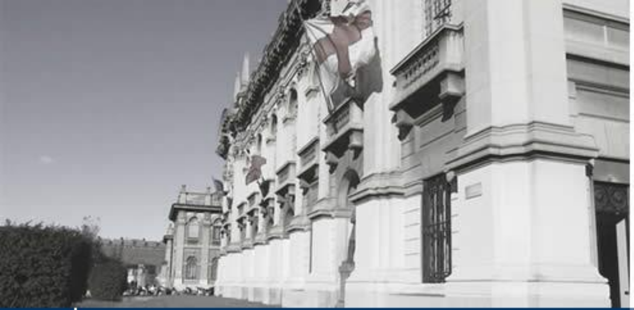


Dept. Biol. Penn State ©2002

Secondary structures of ciliate, vertebrate, and yeast (Saccharomyces) telomerase RNAs.



Structural elements in green represent conserved regions that bind to TERT. The structures that are present in some but not all species within a group are shown by dashed lines. The structures that define template region (red) and the template boundary (blue) are indicated.



POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry





Polysaccharides.

Prof. Attilio Citterio

Dipartimento CMIC "Giulio Natta"

See the related file: E4_15 polysaccharides.pdf

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry

POLITECNICO DI MILANO

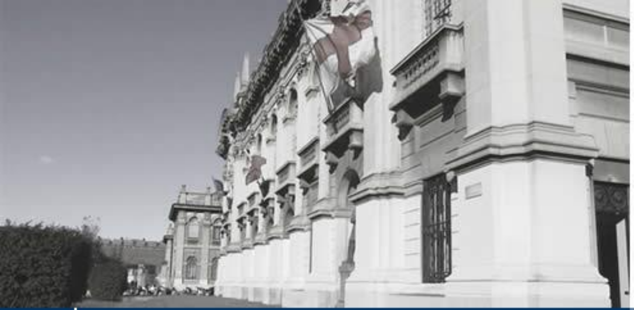
    

Protein Based Biopolymers.

Prof. Attilio Citterio

Dipartimento CMIC "Giulio Natta"

See the related file: E5_15 proteins.pdf



School of Industrial and Information Engineering
Course 096125 (095857)
Introduction to Green and Sustainable Chemistry

POLITECNICO DI MILANO

Biodegradable Plastics - Polylactic Acid and Poly(beta-alkanaoates).

Prof. Attilio Citterio
Dipartimento CMIC "Giulio Natta"
https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/

Biodegradable Plastics.

Three main categories:

- Chemically synthesised polymers: PGA, PLA, polyvinyl alcohol, poly(ethylene oxide), poly(ε-caprolactone) ⇒ do not match all the properties of plastics
- **Starch**-based biodegradable plastics: blends of starch and plastic ⇒ only partially degradable
- **Polyhydroxyalkanoates** ⇒ similar properties and completely biodegradable

Main disadvantage: **PRICES** ⇒

- Synthetic plastics < 1 €/Kg
- Polylactic acid 3.00 - 4.00 €/Kg
- Starch compounds 2.00 - 4.00 €/Kg
- Polyhydroxyalkanoates > 5.00 €/Kg

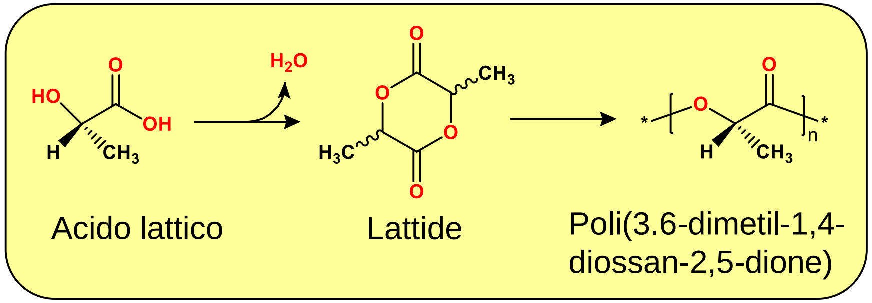
Polylactic Acid.

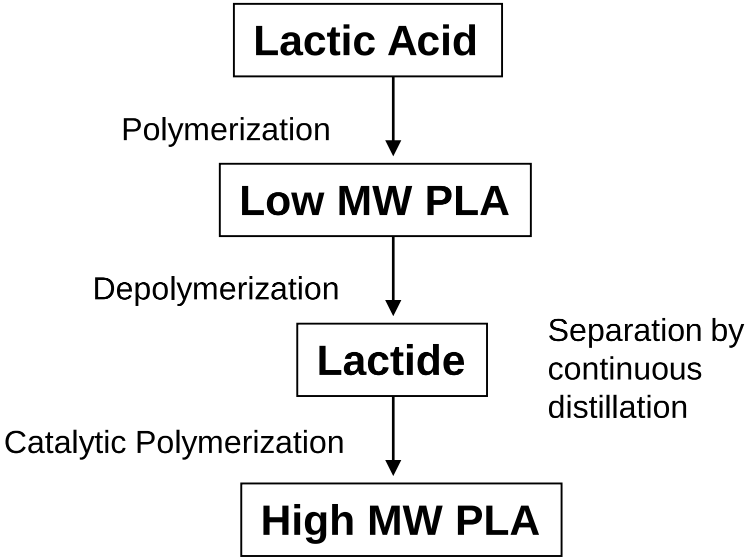
Polylactic acid (PLA) is not a new polymer, it is known from 1932.

Producing low molecular weight PLA is an easy process, however make high MW PLA is a more complex task.

Cargill-Dow has developed a new process which involves a selective depolymerization of low molecular weight PLA to a cyclic intermediate (lactide), which is purified by distillation.

The catalytic opening of lactide ring allow to prepare controlled MW PLA in continuous.

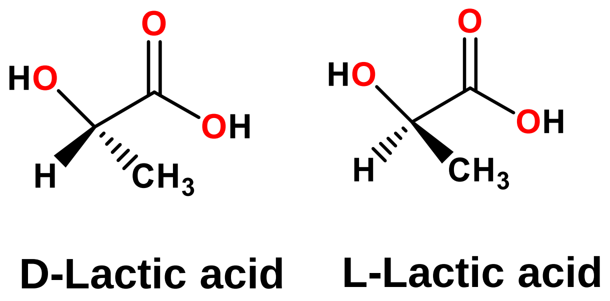


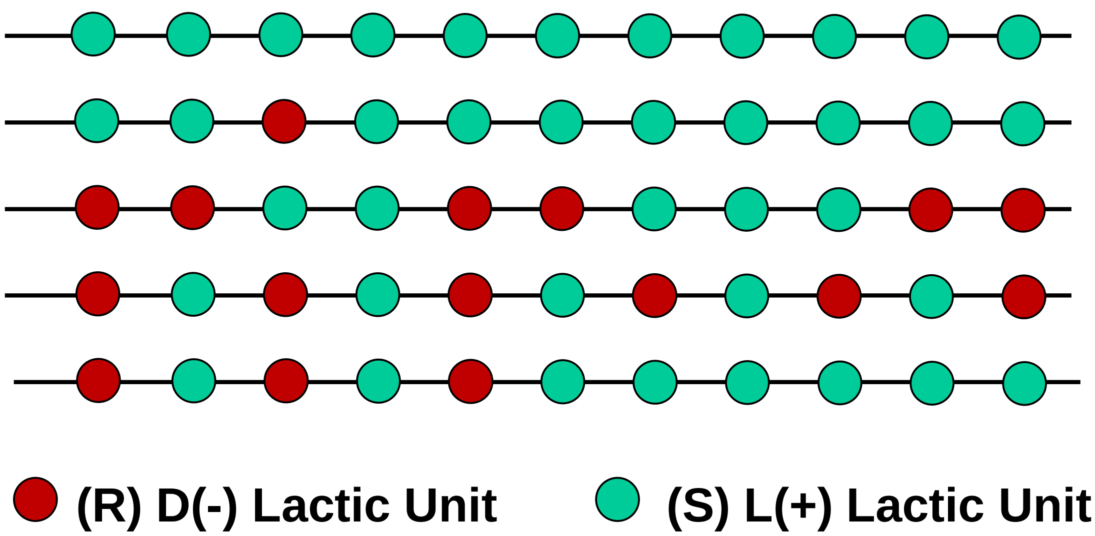


J. Lunt, Polymer Degradation and Stability, 59, (1998), 145-152
http://www.cargilldow.com/home.asp

Polylactic Acid – A Family of Polymers.

Lactic acid is an optically active molecule (the central carbon is asymmetric) and exists in two enantiomeric forms (L and D lactic). Polymers with high L levels (natural) can be used to produce crystalline products, whereas the higher D materials (> 15%) are amorphous.



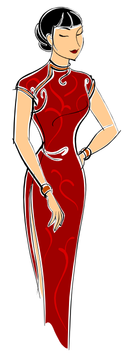


Polylactic acid (PLA) for Plastics Production.

Corn → *Starch* → *Unrefined Dextrose* → ***Fermentation*** → *Lactic acid* → ***Monomer Production*** → *Lactide* → ***Polymer Production*** → *PLA* → ***Polymer Modification***

Film

Apparel



Polymer Grades

Fiber
Film
Thermoforming
Bottle
Woven
Non-woven
Etc.

PLA materials have mechanical properties between polystyrene and PET.

Coating with Polylactic Acid.

From a cooperation between Mitsui Chemicals Inc. and Cargill-Dow, LLC, SANYO in 2003 has realized the first optical disk in bio-plastic (polylactic acid).

Corn was used as raw material to obtain polylactic acid with appropriate optical and structural properties.

A mean of 85 maize seeds needs to make a disk and a maize-cob to make 10 disks. Word production of maize is about 600 millions tons, less than 0.1% is needed to make 10 billions of disks (the present word demand).

SANYO 環境対応型ディスク

Biodegradability of PHB and PLA Products.

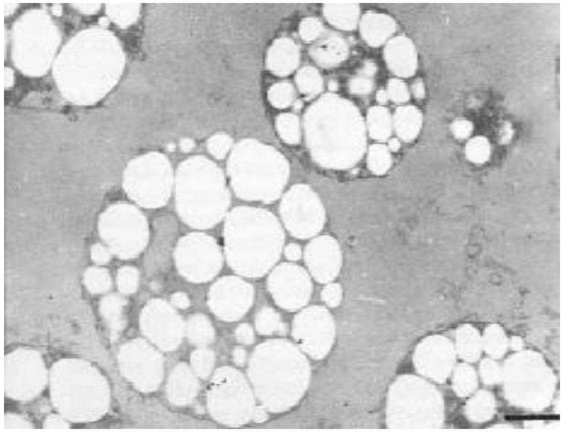
- Produced naturally by soil bacteria, the PHB are degraded upon subsequent exposure to soil, compost, or marine sediment.
- Despite their biodegradability the PHB still have good resistance to water and moisture vapor, and are stable under normal storage conditions and during use.
- PHB is used as nutrient only when phosphates, nitrogen, salts, water and heat allow the microorganisms to grow.
- These conditions are present in the compost and, in part, in soils, but not in the conditions of typical uses of articles formed by injection or extrusion. Therefore these materials are stable to use for years.
- PLA is hydrolyzed auto catalytically in humid environments at temperatures higher then the glassy transition (55°C). The lactic acid subunit is produced, which is used by microorganisms as food.

Biodegradability of PHB and PLA Products (2).

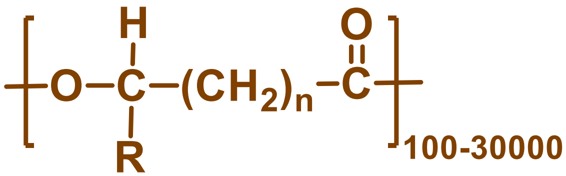
- The biodegradation rate of materials depends on the thickness and on environment temperature: in soil (mean temperature 8-15°C) objects biodegrade quite slowly (years), in not cured composts (with wide temperature excursions) faster and in professional composts (50-65°C) in weeks.
- Generally artifacts in PHB biodegrade at a rate similar to that of wood.
- PLA products degrade well only in professional composting structures (with temperature control).

Polyhydroxyalkanoates (PHA) – Characteristics.

- Linear polyesters;
- Thermoplastics;
- 100% resistance to water;
- Molecular weight : 2×10^5 - 5×10^6;
- Biodegradable;
- Biocompatible;



- Two types of PHA:
  - scl-PHA ⇒ if R= H, CH_3, C_2H_5, C_3H_7
    R = CH_3 ⇒ PHB poly-3-hydroxybutyrate
    R= C_2H_5 ⇒ PHV poly-3-hydroxyvalerate
  - mcl-PHA ⇒ if R= $(CH_2)_3CH_3$ to $(CH_2)_8CH_3$



- scl-PHA bear similar characteristics to polypropylene and mcl-PHA are similar to low density polyethylene

Microbial Polyesters.

3-PHB $-[-O-\overset{*}{C}H(CH_3)-CH_2-C(O)-]_n-$

R-configuration

High crystallinity, 50-80 %
Orthorhombic unit cell
Thermoplastic, T_m = 175°C
Processable from melt
Moldable
Biodegradable

PHA (functiona-lized) $-[-O-\overset{*}{C}H(CH_2R)-CH_2-C(O)-]_n-$

$M_w = 10^5 - 10^6$ Dalton

4-PHB $-[-O-CH_2-CH_2-CH_2-C(O)-]_n-$

Okamura, Marchessault in: Conformation of Biopolymers 1967

Physical Properties: Comparison PHA – PP.

Property	PHB	PP
T_m [°C]	175	176
Crystallinity [%]	80	70
M_W [Dalton]	5×10^5	2×10^5
T_g [°C]	4	- 10
Density [g/cm^3]	1.250	0.905
Tensile strength [MPa]	40	38
Extension at break [%]	6	400
UV resistance	good	poor
Solvent resistance	poor	good
Source	Sugar, Molasses	Petroleum
Cost [$/lb]	3.50	0.40

Howells, E.R. Chem. Ind. (London) 1982, 15, 508

Synthesis of PHB.

(microbial)

Sugar

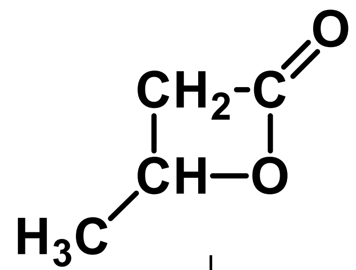
↓

Microbial fermentation
Aqueous medium, 30 °C

↓ Optically pure
100% R-configuration

Recovery

(chemical)



↓

Stannous octanoate,
Organic solvent, 120 °C, p

Racemic mixture ↓

Purification

$$-[-O-\overset{*}{C}H(CH_3)-CH_2-C(O)-]_m-$$

Hydrolytically degradable

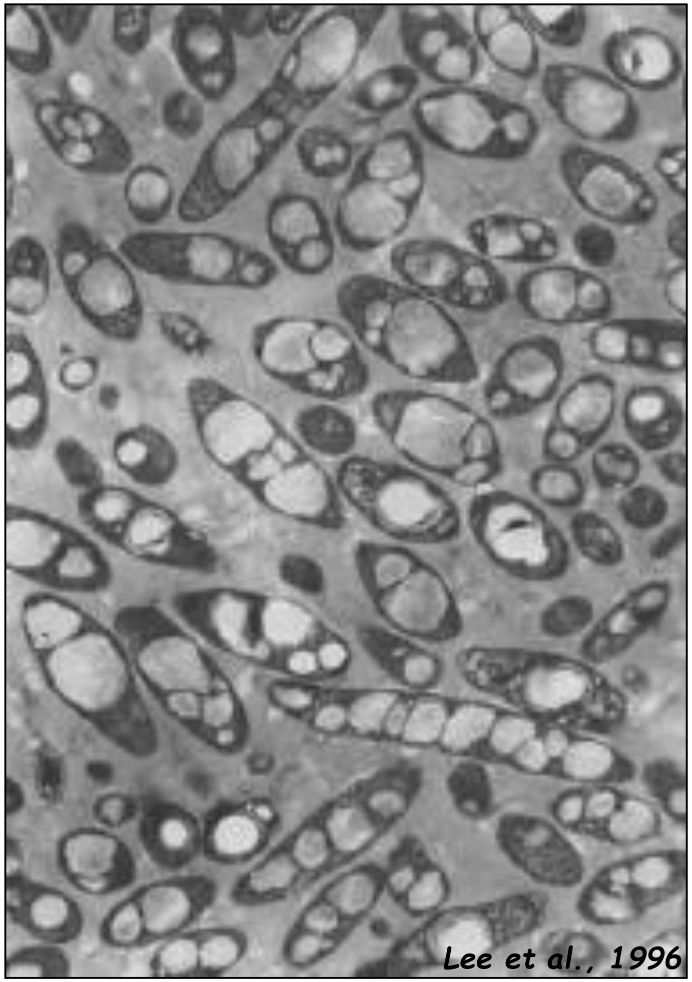
PHB Accumulating Microorganisms.

- Alcaligenes
- Azotobacter
- Bacillus
- Rhodospirillum

(also archaebacterium)

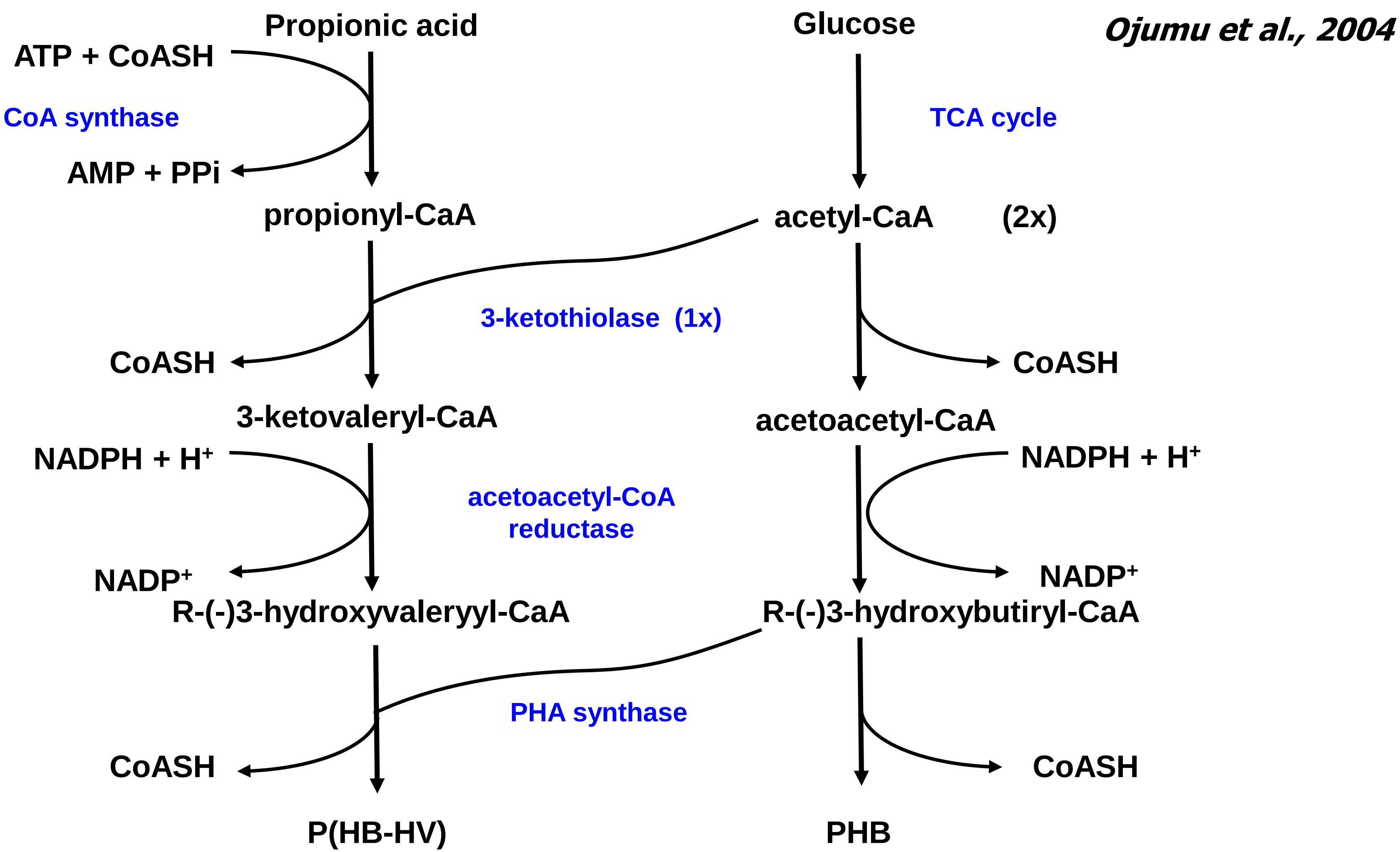
Granules can make up to 90% of dry weight of biomass

Example of short-length PHB produced in activated sludge



Alcaligenes eutrophus now *Ralstonia eutropha*

PHB Biosynthesis.



The biosynthetic pathway of PHB and P(HB-HV) in *Alcaligenes eutrophus*

PHA BioSynthesis.

Non-limiting conditions:

Carbon source (glucose) —glycolysis→ **Pyruvate**

—Citric acid cycle→ **Biomass + Energy** { Cell Proliferation, No polymer accumulation

Limiting conditions:

Carbon source (glucose) —glycolysis→ **Pyruvate**

→ **Acetyl-CoA** —β-ketothiolase→

Acetoacetyl-CoA —Acetoacetyl-CoA reductase→

β-hydroxybutyryl-CoA —PHB synthase→ **PHB**

Side Chains in PHA.

In general:

Bacteria can be divided in two different groups:

- Bacteria that produce short-chain PHAs, x = 0,1
- Bacteria that produce long-chain PHAs, x > 3

$$-[-O-\overset{*}{C}H(CH_2R)-CH_2-C(O)-]_n-$$

Special cases:

R. rubrum	**R = C_3H_7 (x = 2)**
	R = C_4H_9 (x = 3)
B. thuringiensis	**R = CH_3 (x = 0)**
	R = C_6H_{13} (x = 5)

Scholz, C. et al. *Polym.Bull*. **1995**, 34 577-584

PHA Synthesis.

Alcaligenes eutrophus grown on gluconate

Depleted Nutrient	P(3HB) accumulation (g/g of protein / hour)
Oxygen	0.10
Ammonium	0.40
Sulfate	0.49
Magnesium	0.21
Phosphate	0.27
Potassium	0.23
Iron	0.22

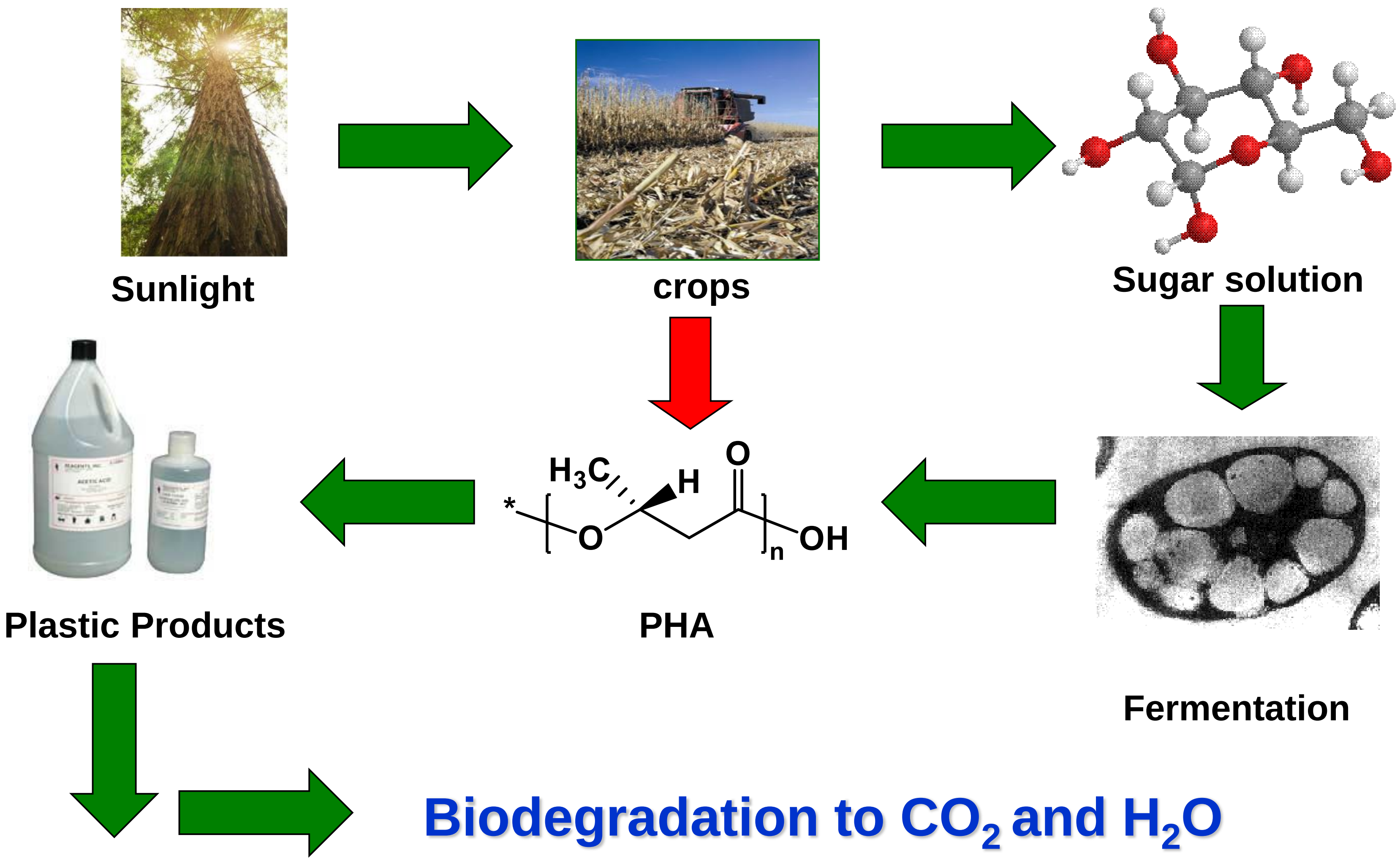
Steinbuechel, A. and Schlegel, H.G. Appl. Microbiol. Biotechnol. 1989, 31, 168-175

Isolation of PHAs.

Solvent Extraction	Alkaline Hypochlorite Treatment	Enzyme Treatment
Quick, efficient, High yields Granules disintegrated	Efficient Potential decrease in molec. weight Granules intact	Rather elaborate procedure Granules intact
Organic solvents	Caustic compounds	Lysozyme

Purification of the polymer is key for applications.

Polyhydroxyalkanoates (PHA’s).



Polyhydroxyalkanoates (PHA) – LCA.

- PHAs grown in corn stover studied
  - Collect grain
  - Harvest polymer
- Process involves:
  - Fertilisers and pesticides (?)
  - Harvesting and drying corn stover
  - Extracting PHA
  - Recycle solvent
  - Purifying the plastic
  - Blending the plastic to make resin
- Use of wheat straw as source of renewable energy

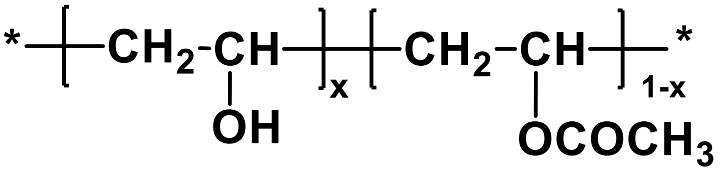
The findings:

- 1 kg of PHA requires 300% more energy than to make 1 kg of PE
  - 2.65 kg of fossil fuel for PHA
  - 2.29 kg for PHA from microbial fermentation
  - 2.2 kg fossil fuel for PE (50% of which ends up in product)
- By burning wheat straw to power process results in Greenhouse gas saving
- However, it may be better environmentally to simply use renewable energy in a fossil fuel based process...

T. U. Gerngross, Nature Biotechnology, 17, (1999), 541 - 544

Oxo-Biodegradable: Poly(vinylalcohol) (PVA).

Poly(vinylalcohol) (PVA) is a synthetic organic polymer made from repetitive units of vinyl alcohol monomer. Cannot be prepared from this monomer but can be indirectly obtained by alkaline hydrolysis of polyvinyl acetate (PVAc).



<u>Properties</u>

Physical properties of commercial PVA depend on 5 factors;

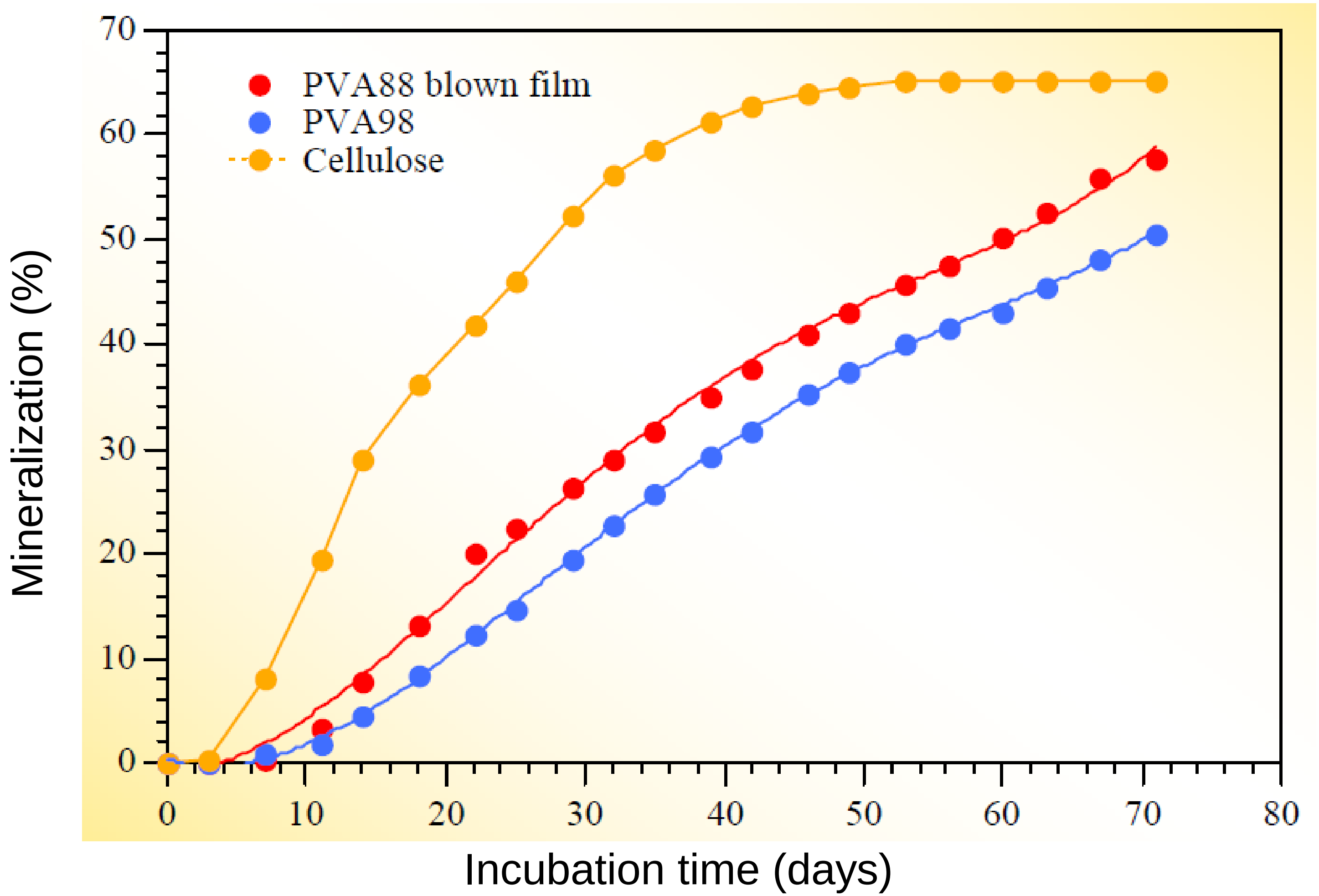
- molecular weight
- hydrolysis degree (the number of non converted acetate units 1-x)
- degree and type of chain breaking
- cross linking degree (between the polymer chains)
- type, form and concentration of various additives

Changing these factors, properties as strength, brittleness, barrier characteristics to gases and water solubility can be controlled.

Biodegradability of Poly(vinylalcohol).

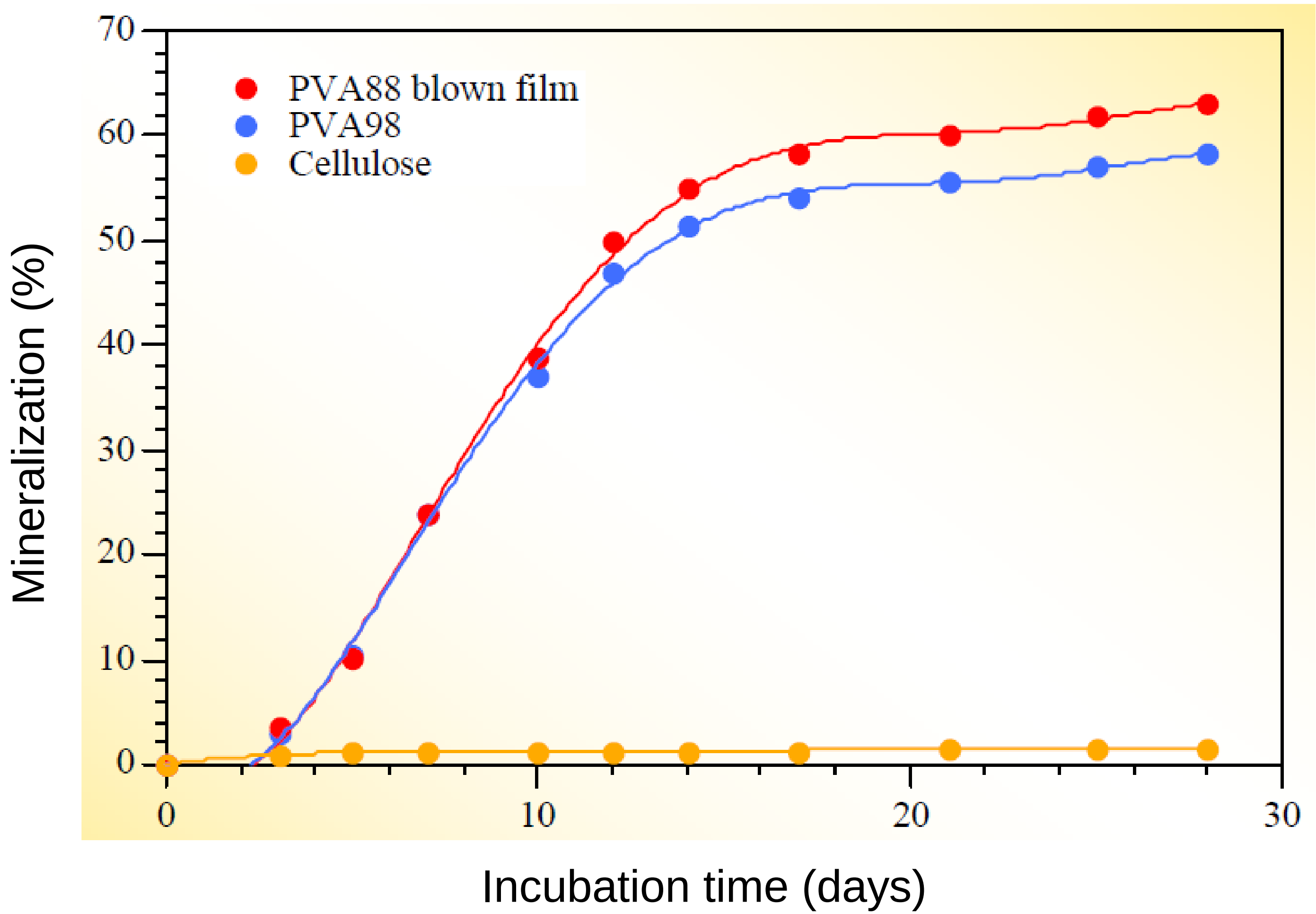
- PVA can be degraded using thermal, mechanical, photochemical, ultraviolet, biological and chemical processes. As concern biodegradation, a number of micro-organisms, at least 20 different genera of bacteria and a number of moulds and yeasts have known to degrade PVA. These organisms can occur in both artificial environments, such as anaerobic digesters, activated sewage sludge and composts, and natural environments such as aquatic systems and soil.
- The micro-organisms use PVA as a food source by producing a variety of enzymes that are able to react with it. The ultimate end products from this process are the naturally occurring substances carbon dioxide, water and biomass. Unusually, the degradation of polyvinyl alcohol takes place at random points along the entire length of the polymer chain at once. This is believed to be the reason for its relatively fast degradation. Other synthetic polymers, when biodegraded, are usually attacked gradually from both chain ends.

PVA Biodegradation in Liquid Culture.

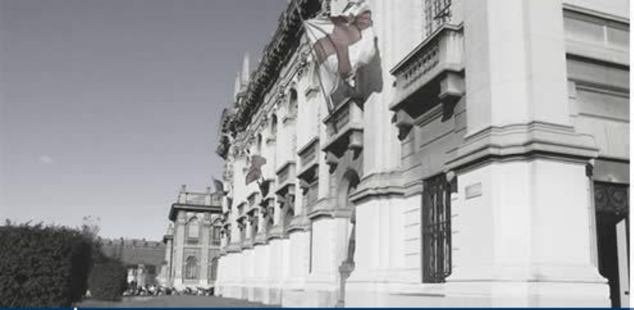


Inoculum: Paper Mill Sewage Sludge

PVA Biodegradation in Liquid Culture.



Inoculum: Acclimated Microorganisms



School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry

POLITECNICO DI MILANO

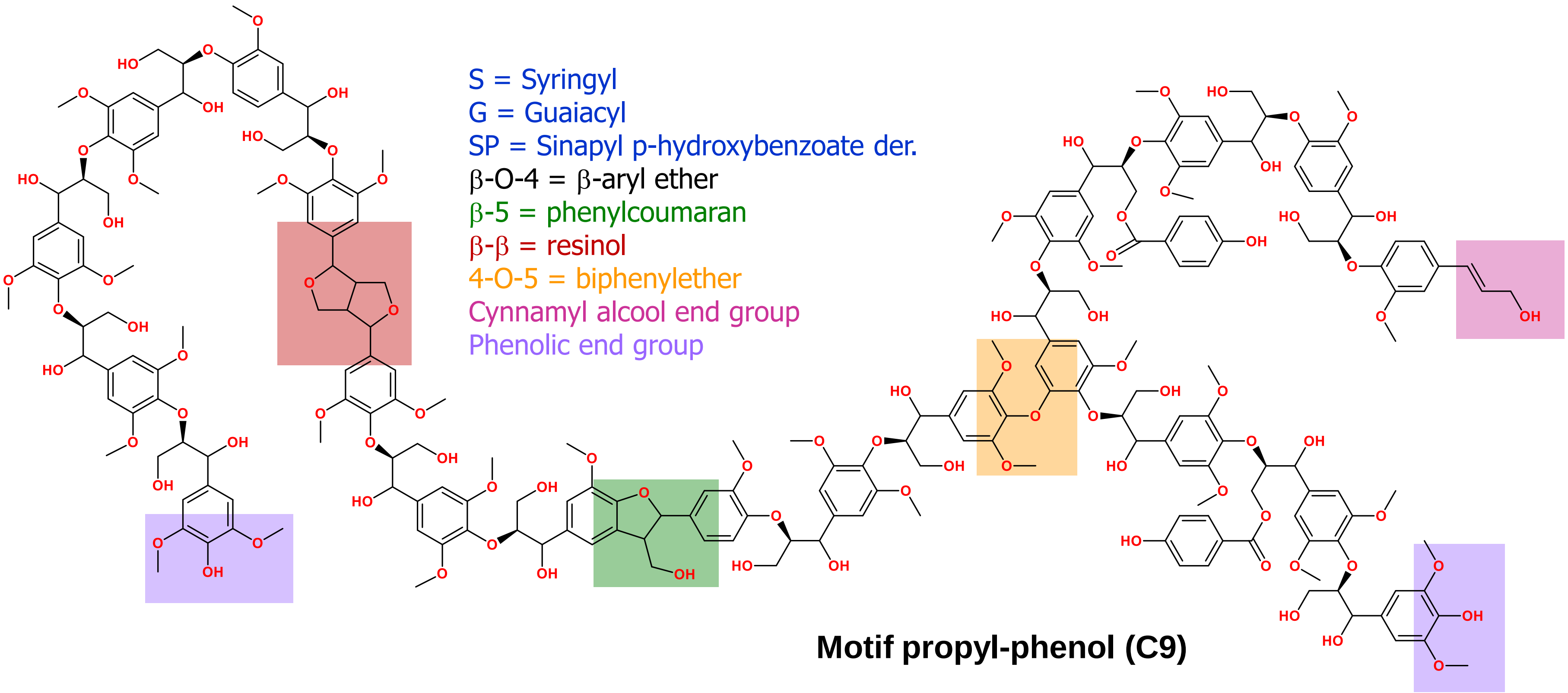




Lignin, Natural Rubber, and Natural Fibers.

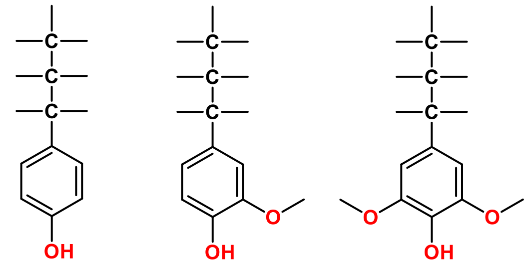
Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

Lignin: amorphous cross-linked polymer with high molecular weight.



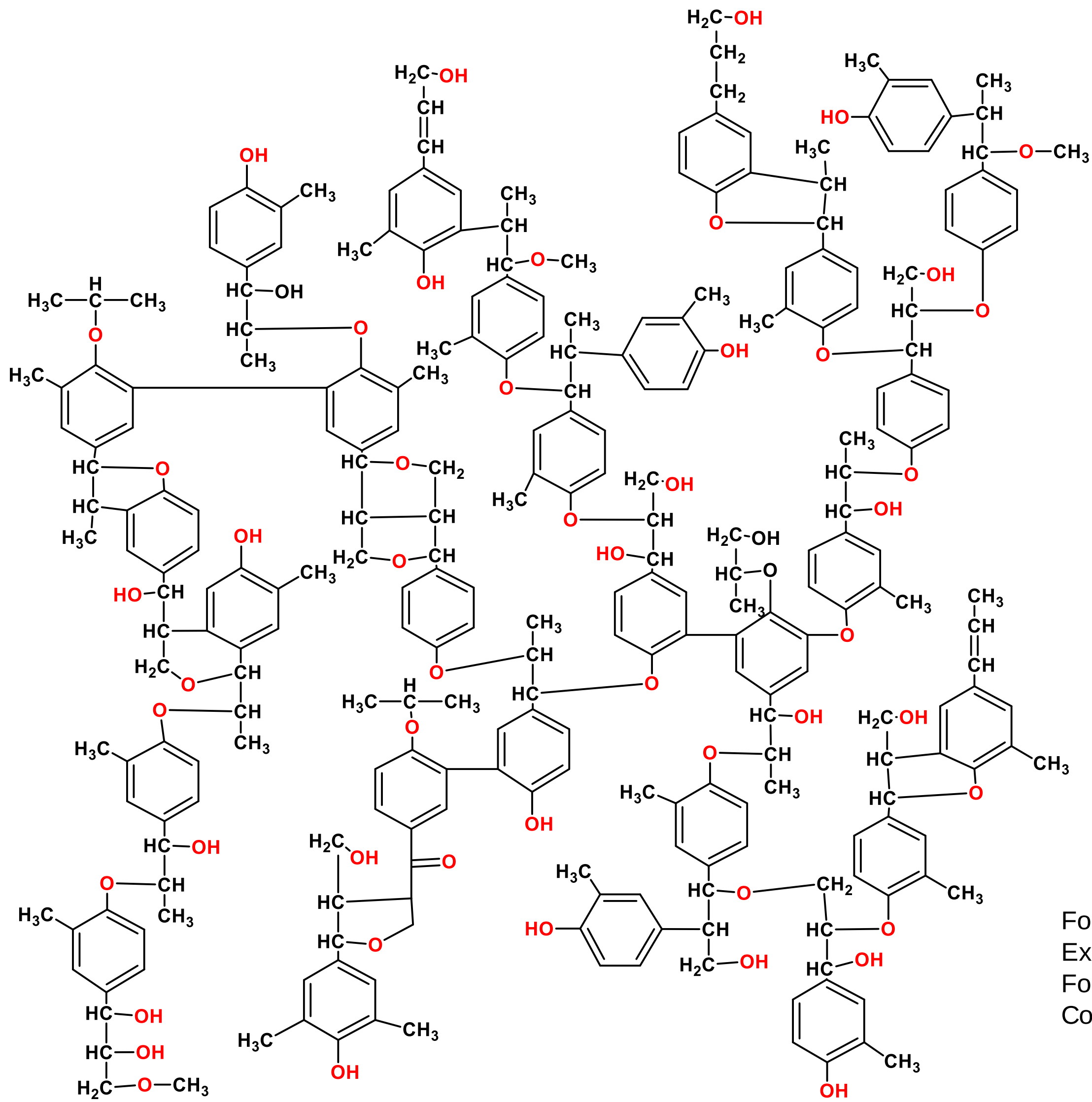
Motif propyl-phenol (C9)

Monomers of lignin



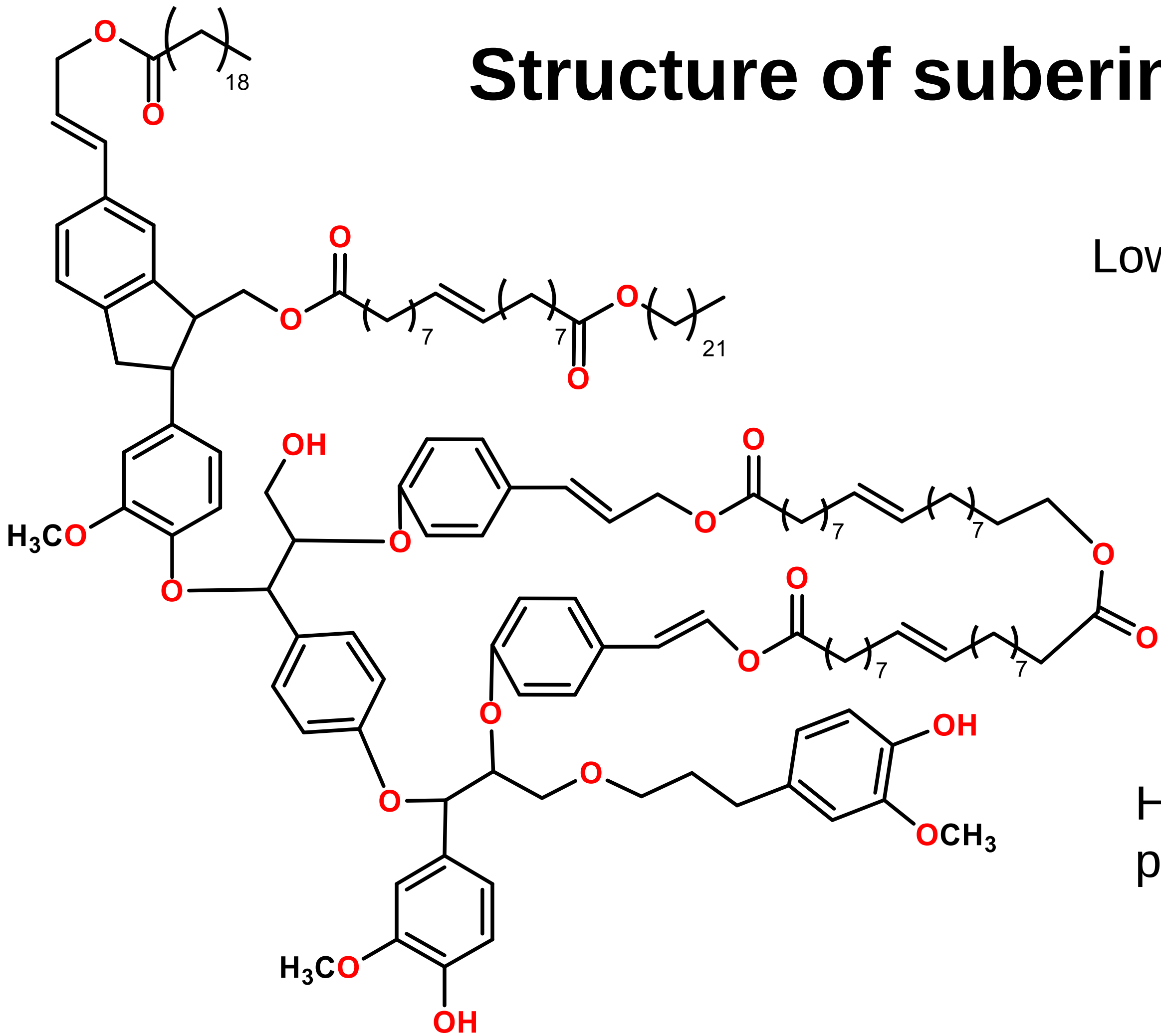
Annual plant | Resinous | Foliage

Lignin of Soft Wood.



Formula Weight : 4477
Exact Mass : 4474,1354995(1)
Formula : $C_{275}H_{308}O_{54}$
Composition : C 73,77% H 6,93% O 19,30%

Structure of suberine

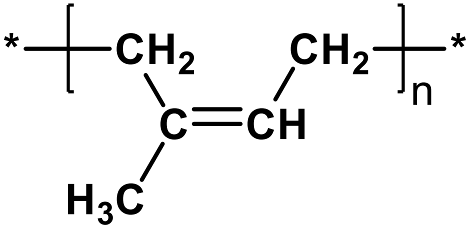


Low density

Hydrophobicity and plasticity

Natural Rubber.

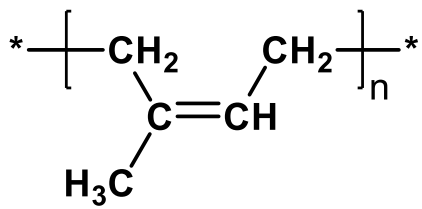
- Natural rubber consists of 1,4-cis-polyisoprene, extracted from the rubber tree (Hevea brasiliensis).
- It is produced in the tree by the biocatalyst hydroxynitrilelyase (2-hydroxyisobutyronitrile acetone-lyase).
- Synthetic rubber accounts for 75 % of rubber usage. However, natural rubber has advantages of elasticity, resilience and thermal properties.
- Natural rubber is easily broken down in the environment, however vulcanization (treatment with sulfur) renders it resistant to biodegradation.



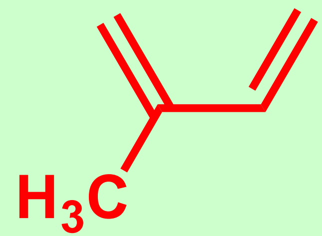
ECONOMY & ECOLOGY IN Bio-Isoprene SYNTHESIS and NR RECOVERY.

Tradit.

Hevea brasiliensis



Polyisoprene

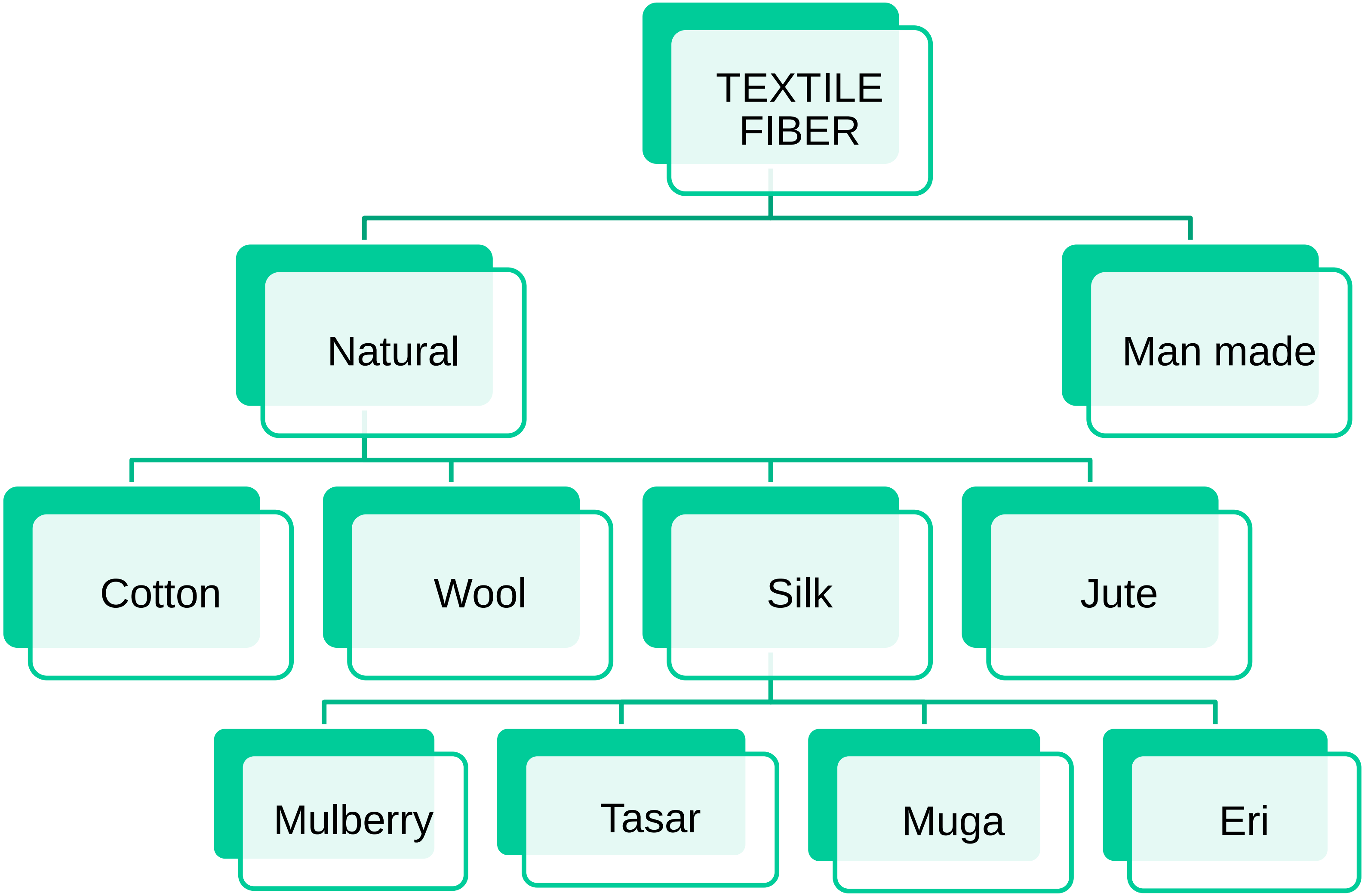


Polymer.

Bio-isoprene

Alternative Sources

Natural Fibres.



Natural Fibers for Biocomposites.

Natural fibres are classified according to their origin:

1. Vegetable or cellulose
2. Animals or protein
3. Minerals

Natural fibers (hemp, flax, china-reed, etc.) as reinforcing materials

Economics

- Glass Fibers (~ \$ 2/kg)
- Natural Fibers (~ \$ .44 - \$ .55/kg)

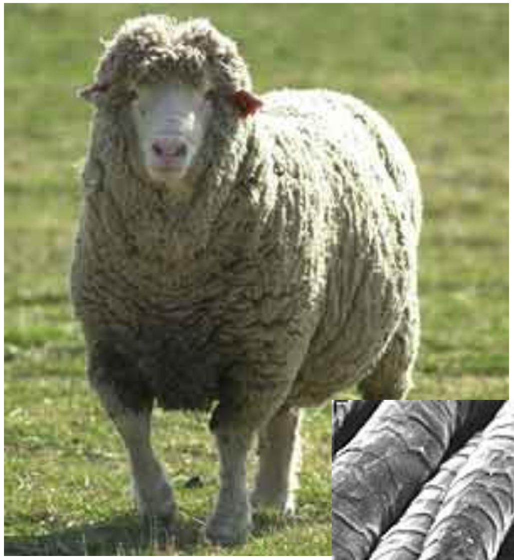
Weight reduction

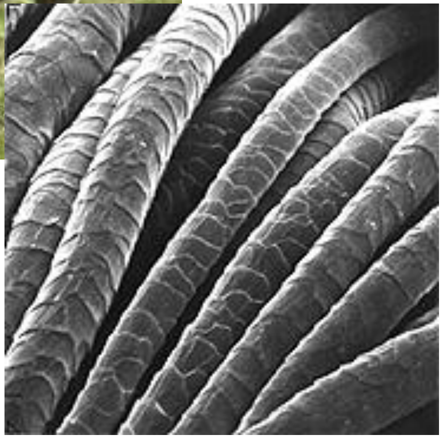
- Glass Fibers 2.5-2.8 g/cm^3
- Natural Fibers 1.2-1.5 g/cm^3

Protein Based Natural Fibres: Silk and Wood.

Silk: fibre produced by insects (Bonbyx Mori) to protect larva

Wool: hair, produced by several animals for cold protection, composed of the protein keratin.





Cellulose Based Natural Fibres: Cotton and Flax.

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Composite Materials.

Composite definition

- A composite is a material comprised of two or more physically distinct materials with at least one material providing reinforcing properties on strength and modulus (typically $CaCO_3$, SiO_2, Carbon black, Clay).

Natural Composites

- Bone
- Wood
- Bamboo: Natures fiber glass due to pronounced fibrillar structure which is very apparent when fractured.
- Muscle and other tissue

Engineering Composites

- Reinforced concrete beams
- Thermoset composites: Thermoset resins (polyurethanes, polyesters, epoxies)
  - Glass fibers, Carbon fibers, Synthetic fibers, metalfibers, or ceramic fibers
- Thermoplastic composites (polypropylene, nylon, polyester, TPU, polyimide)
  - Glass fibers, Carbon fibers, Synthetic fibers, metalfibers, ceramic fibers but also organic: kevlar, natural fibers (cellulose, hemp, flax, etc.)