# Lecture 4

## DNA Structure

April 7, 2016
Pyle

# Discovery of “DNA” as a unique entity

DNA was first discovered by a Swiss biochemist Fredrich Miescher in 1871. He isolated an acid and alkali-soluble material from white blood cells found in pus in surgical bandages. He found DNA was very large, acidic, and rich in phosphorus.

The term “nucleic acid” was coined in 1889 by one of Miescher’s student Richard Altmann

It took almost another half century for scientists to recognize the function and importance of DNA

# Nature of the Gene and the Discovery of DNA

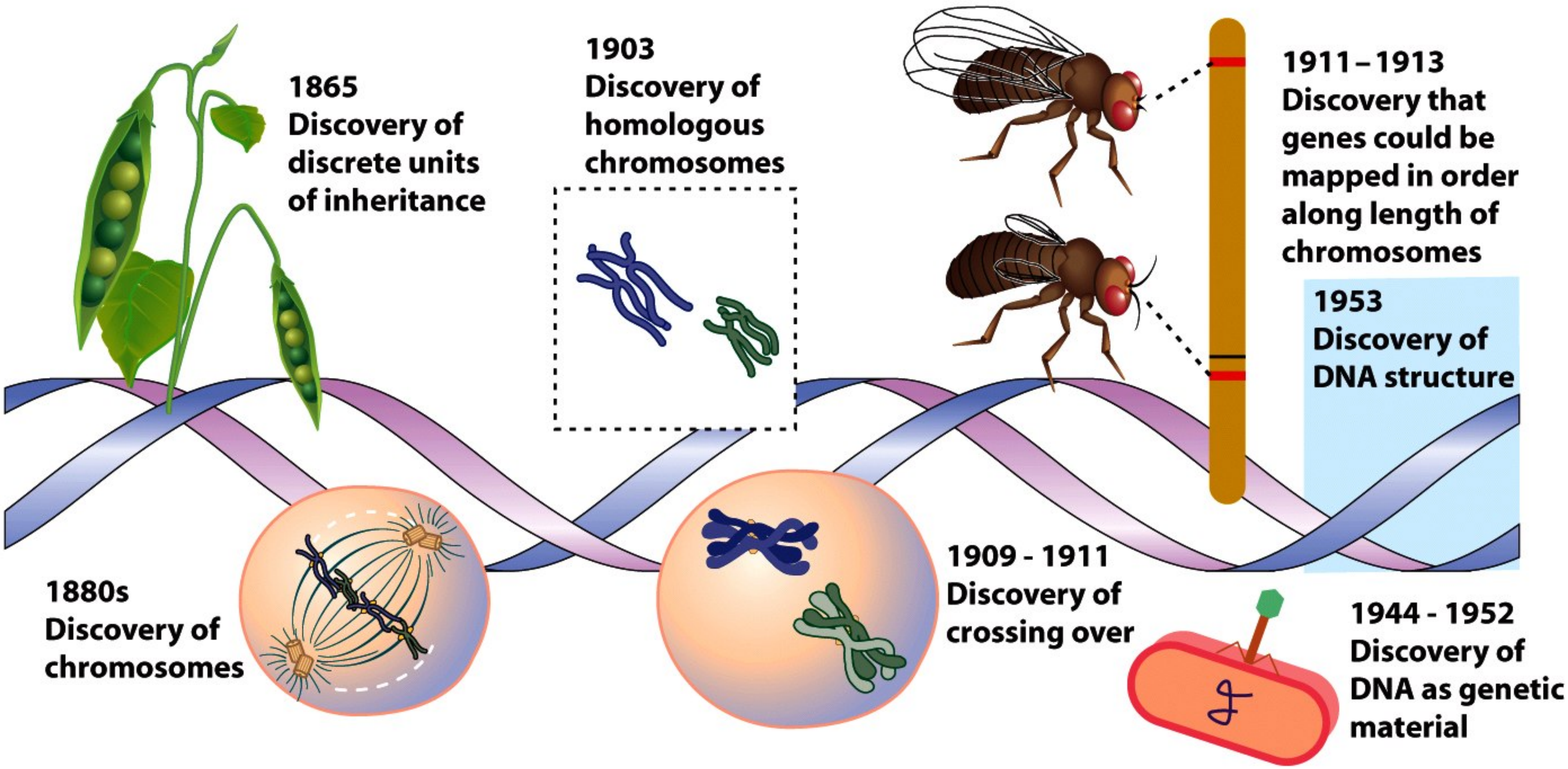
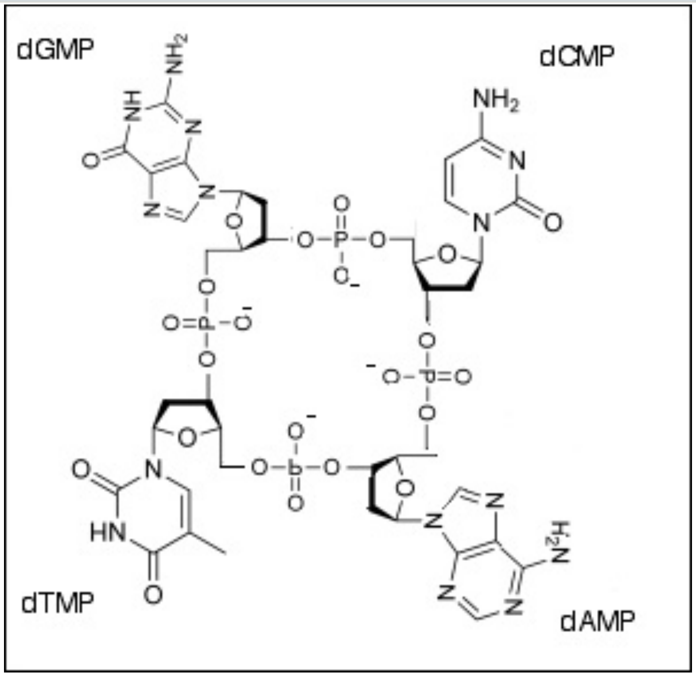



Figure 10-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

The finding that DNA was the genetic molecule carrying all hereditary information led to enormous momentum to discover the structure of DNA!

# The race for identifying DNA structure




Levine- decribed the chemical components of DNA but
Incorrectly thought the structure was composed of circular tetranucleotides

Many thought since proteins contained 20 aa that this must be the genetic material b/c it would allow for more diversity (than 4 nucleotides) and that DNA was just a structural component of chromosomes

# The race continues..

-Linus Pauling, a prominent American scientist, had envisioned that DNA might be a triple helix – three strands of nucleotides wrapping around each other.

- Francis Crick and James Watson had published their own incorrect version of a triple helix model. However, the diffraction pictures at the time were all relatively poor quality and resolution.

-As the technique was further refined, a brilliant chemist named Rosalind Franklin working at King's College in England, was able to take much higher-resolution X-ray diffraction pictures.

-Chargaff also reported that the amount of Adenine (A) always equals the amount of thymine (T) and the amount of cytosine (C) always equals the amount of guanine (G).

# Watson-Crick and the model of DNA structure

1. Rosalind Franklin and Maurice Wilkins obtained the x-ray differaction picture of the DNA
2. James Watson and Francis Crick proposed the 3-D structure of DNA molecule in 1953.
3. Watson, Crick, and Wilkins got the Noble Prize for solving the DNA structure in 1962.

# DNA-Deoxyribonucleic acid

1. Nucleosides, nucleotides, and polynucleotide
2. DNA double helix

   5’-3’ polarity

   Anti-parallel nature of the double strand

   Complementary nature of the double strand

One strand is oriented “5’ to 3’” and the other strand is “3’ to 5’. This is in reference to the 5’-3’ connections in the phosphate-sugar backbone. The machinery of the cell also uses this orientation to select which direction to read the genetic information contained in the nucleotide sequence. Imagine trying to read an English sentence going from right to left!!

3. Other important properties of DNA

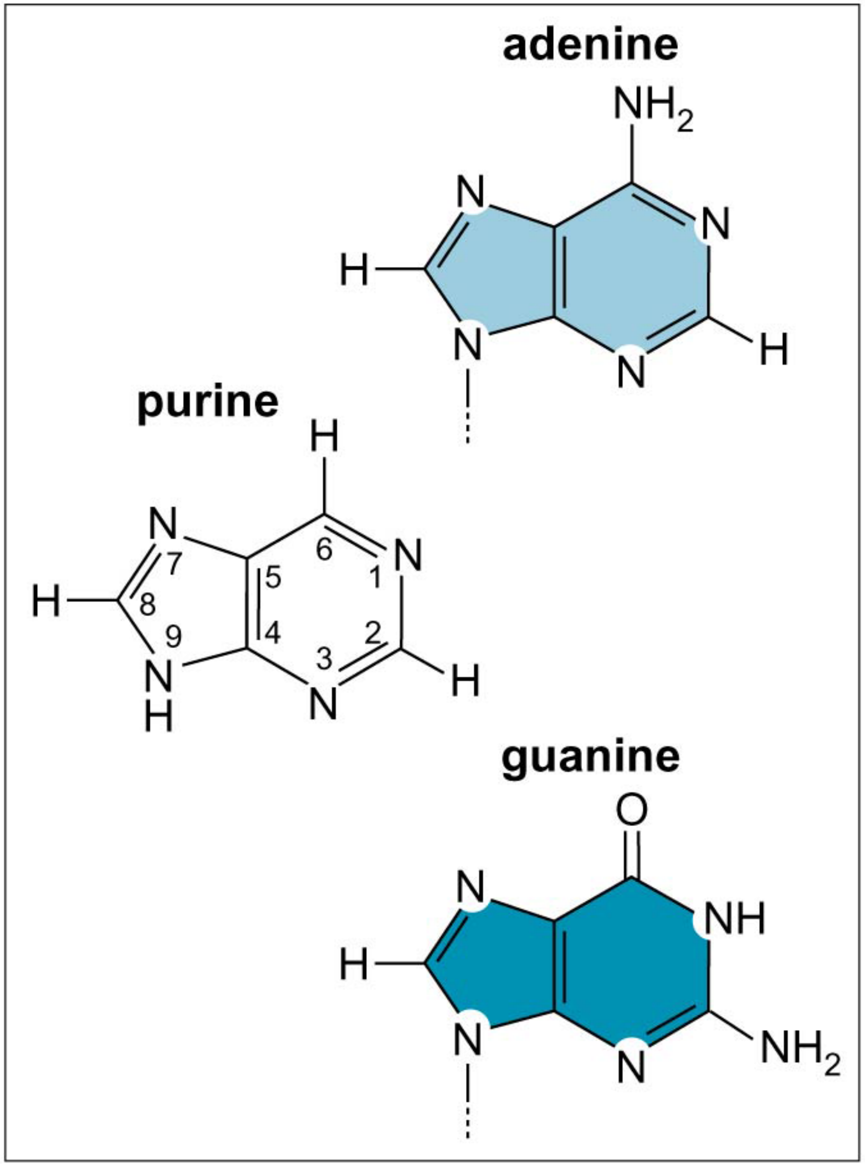
   Denature and renature

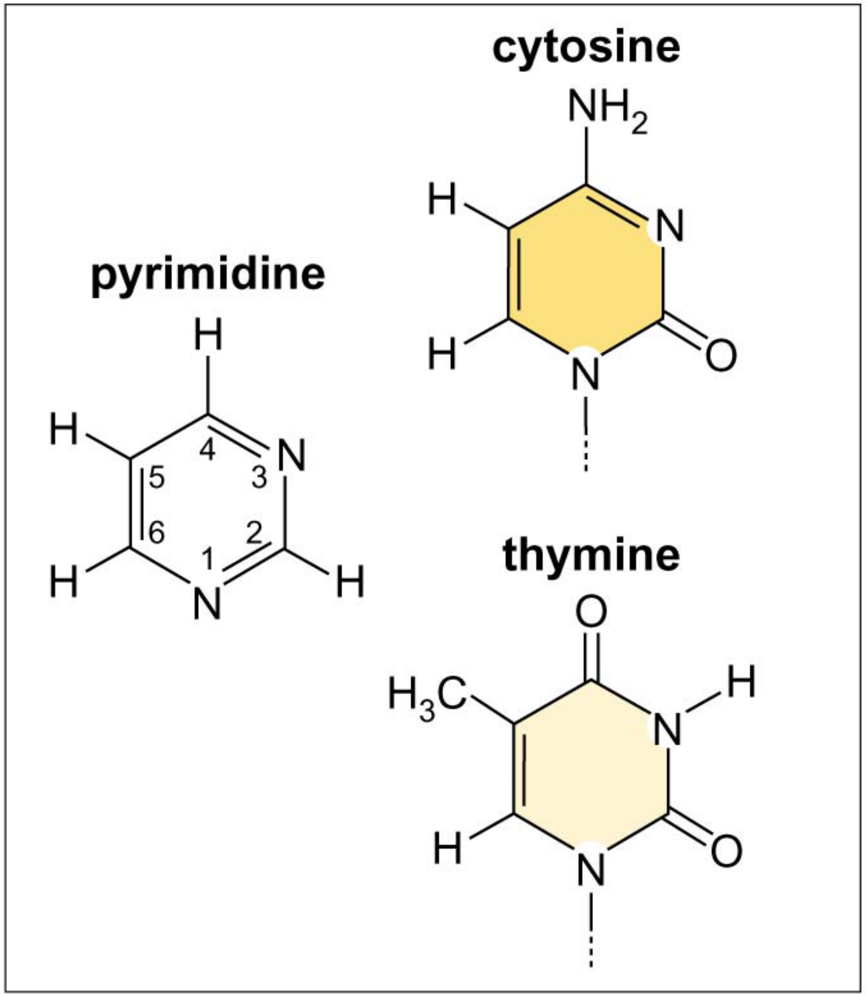
   sequence and sequence homology

   supercoil

https://www.hhmi.org/biointeractive/chemical-structure-dna

# Four Bases make up DNA:purines or pyrimidines

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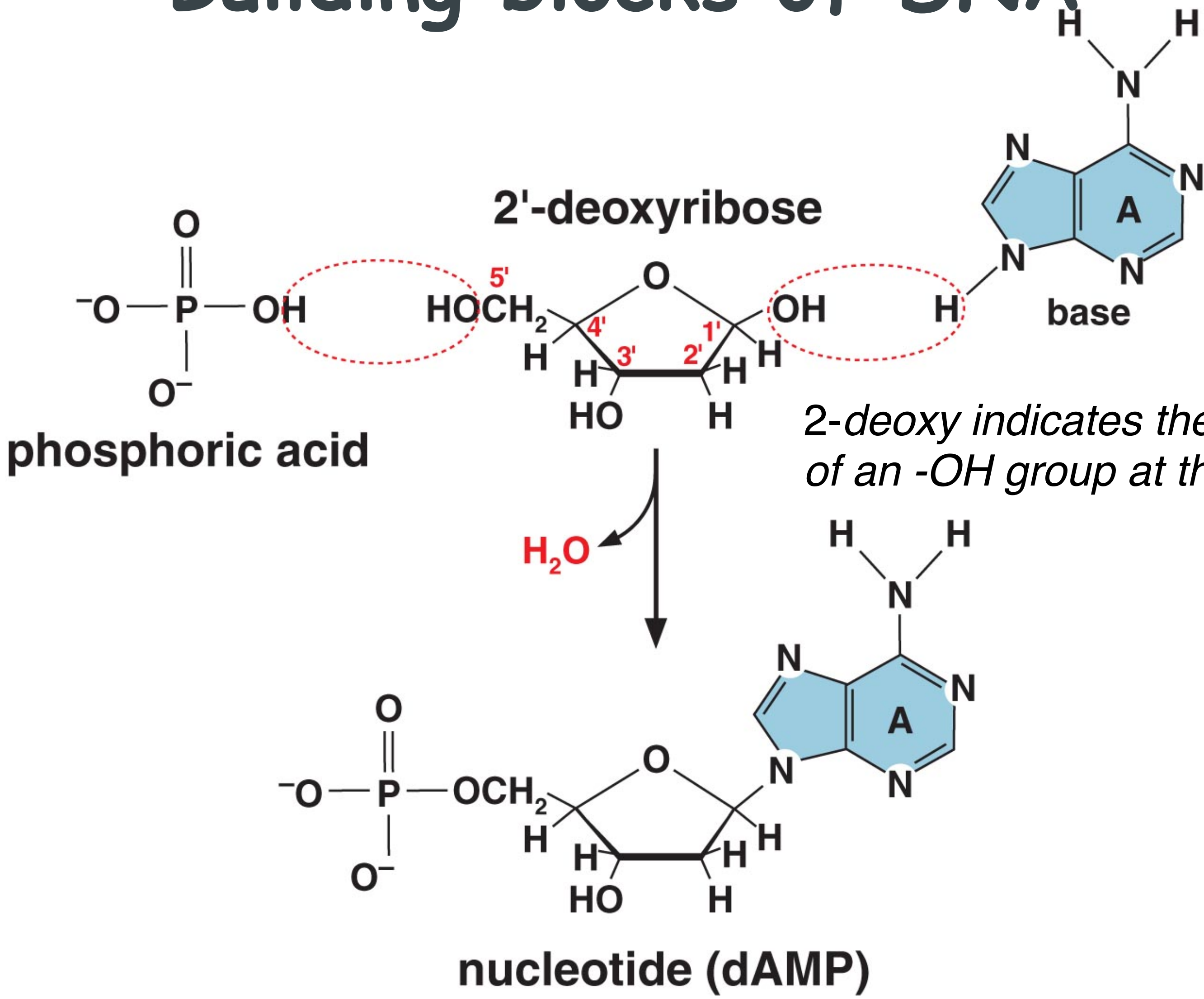
# Building blocks of DNA




*2-deoxy indicates the lack of an -OH group at the 2 position*

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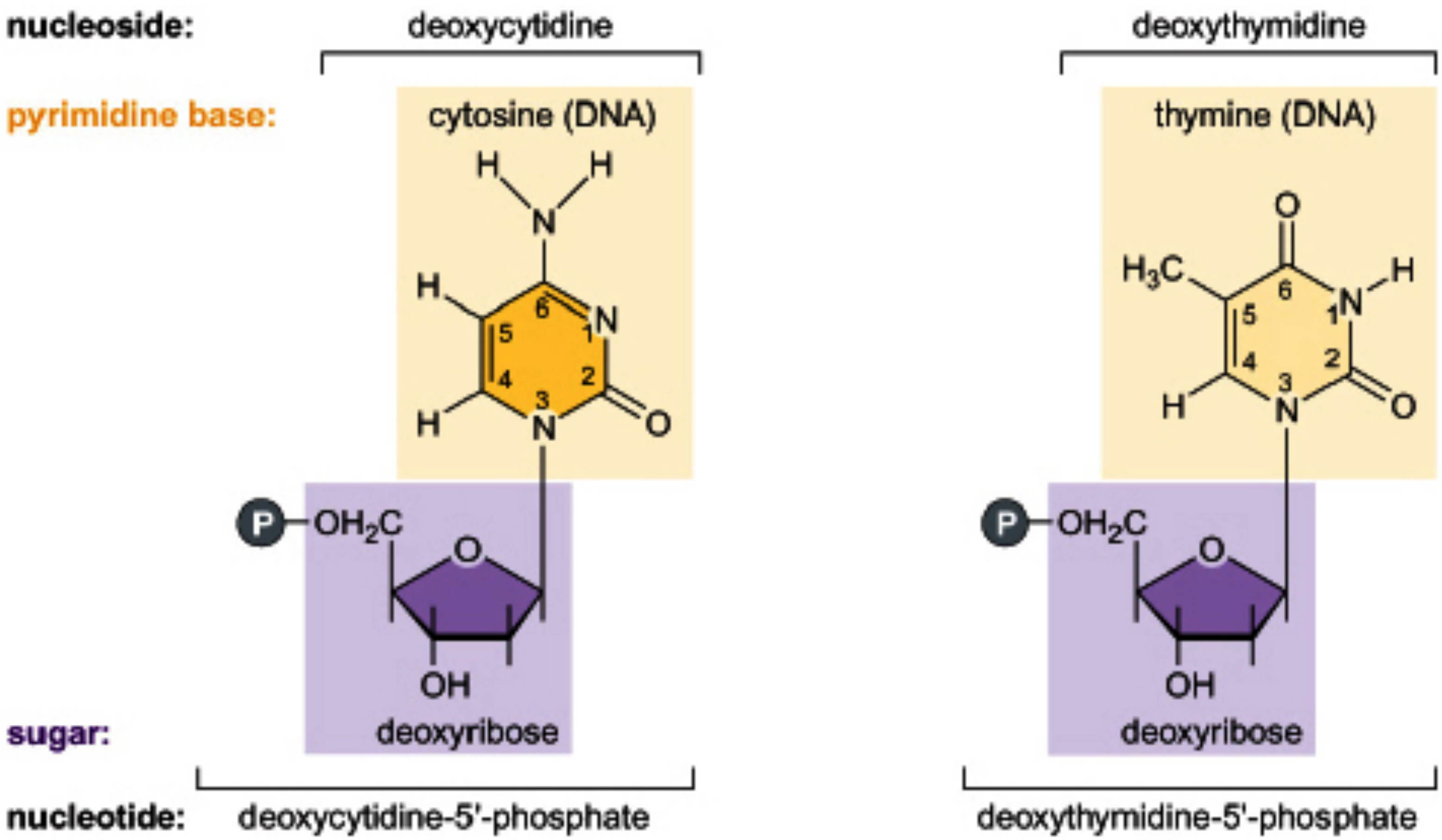
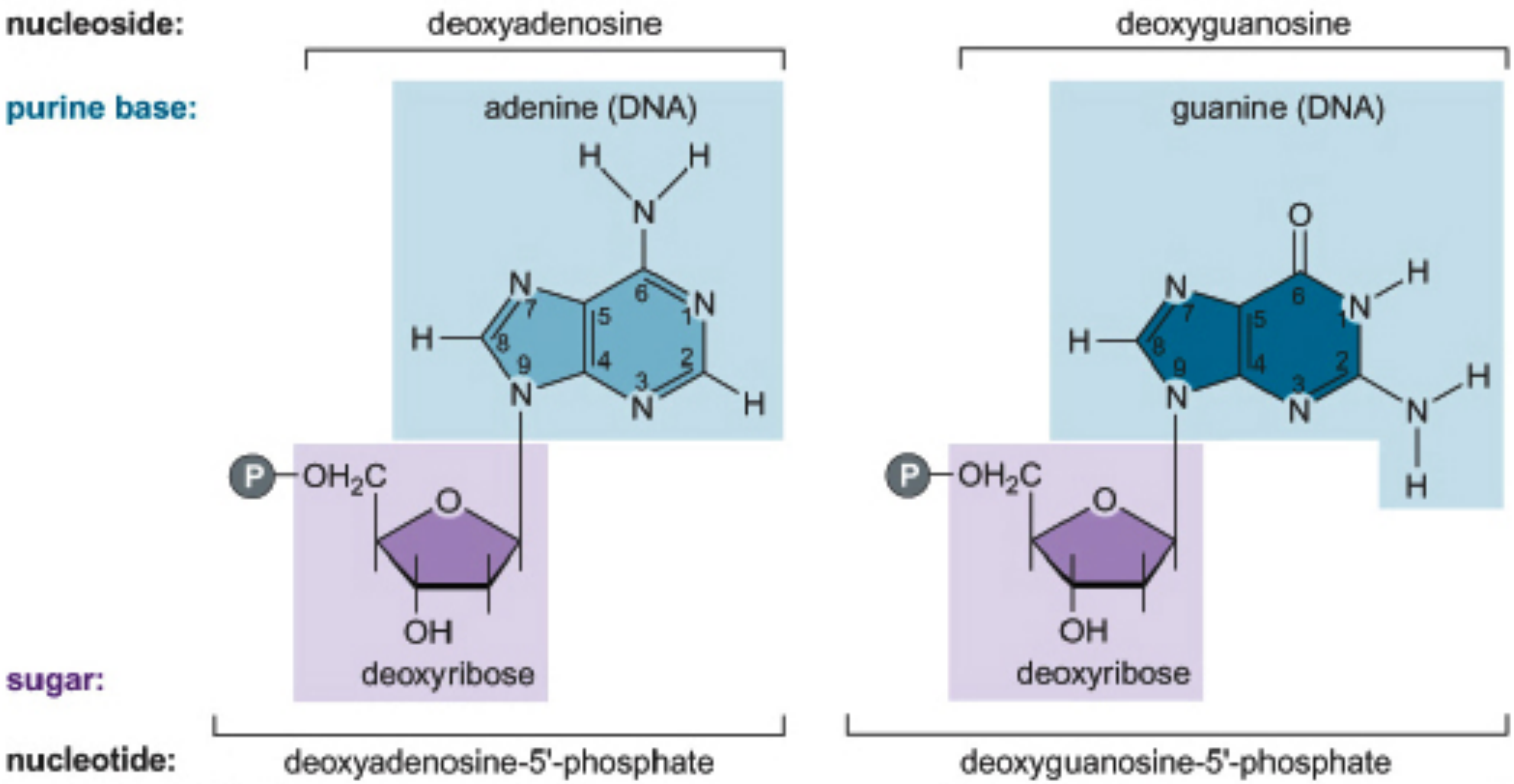



M: mono
D: di
T: tri
-phosphates

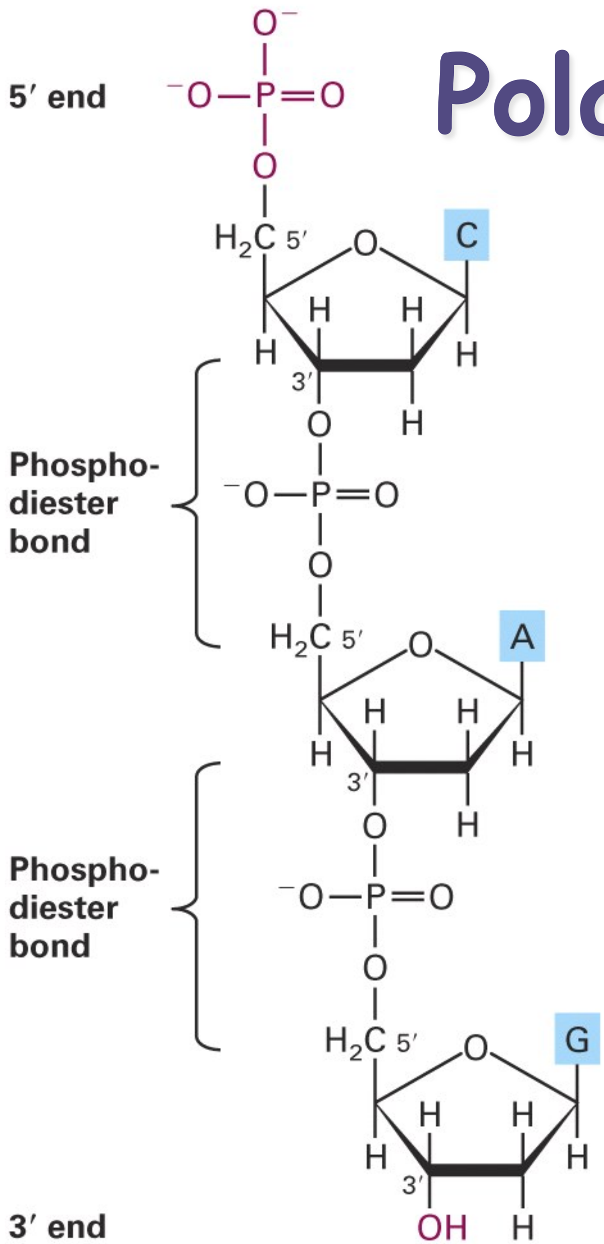
**dCMP** **dTMP**

Nucleoside= sugar + base

Nucleotide= sugar + base + phosphate


nucleoside:
deoxyadenosine
deoxyguanosine
purine base:
adenine (DNA)
guanine (DNA)
sugar:
deoxyribose
deoxyribose
nucleotide:
deoxyadenosine-5'-phosphate
deoxyguanosine-5'-phosphate

# dAMP dGMP




# Polarity of a DNA Strand

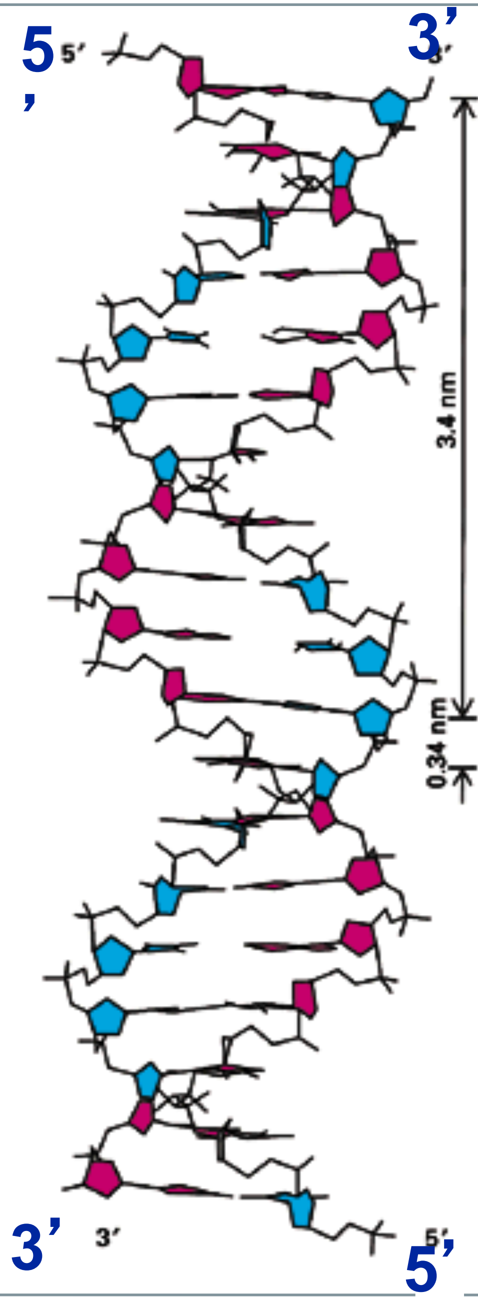
- A DNA strand is a linear polymer of mononucleotides joined by <u>phosphodiester</u> bonds between the 3′ carbon of one nucleotide and the 5′ carbon of the next nucleotide.
- The bases are all joined to the 1′ carbons .
- DNA has polarity: 5′ to 3′ (free 5′ Phosphate and free3′ hydroxyl)

## Biochemical properties of DNA:

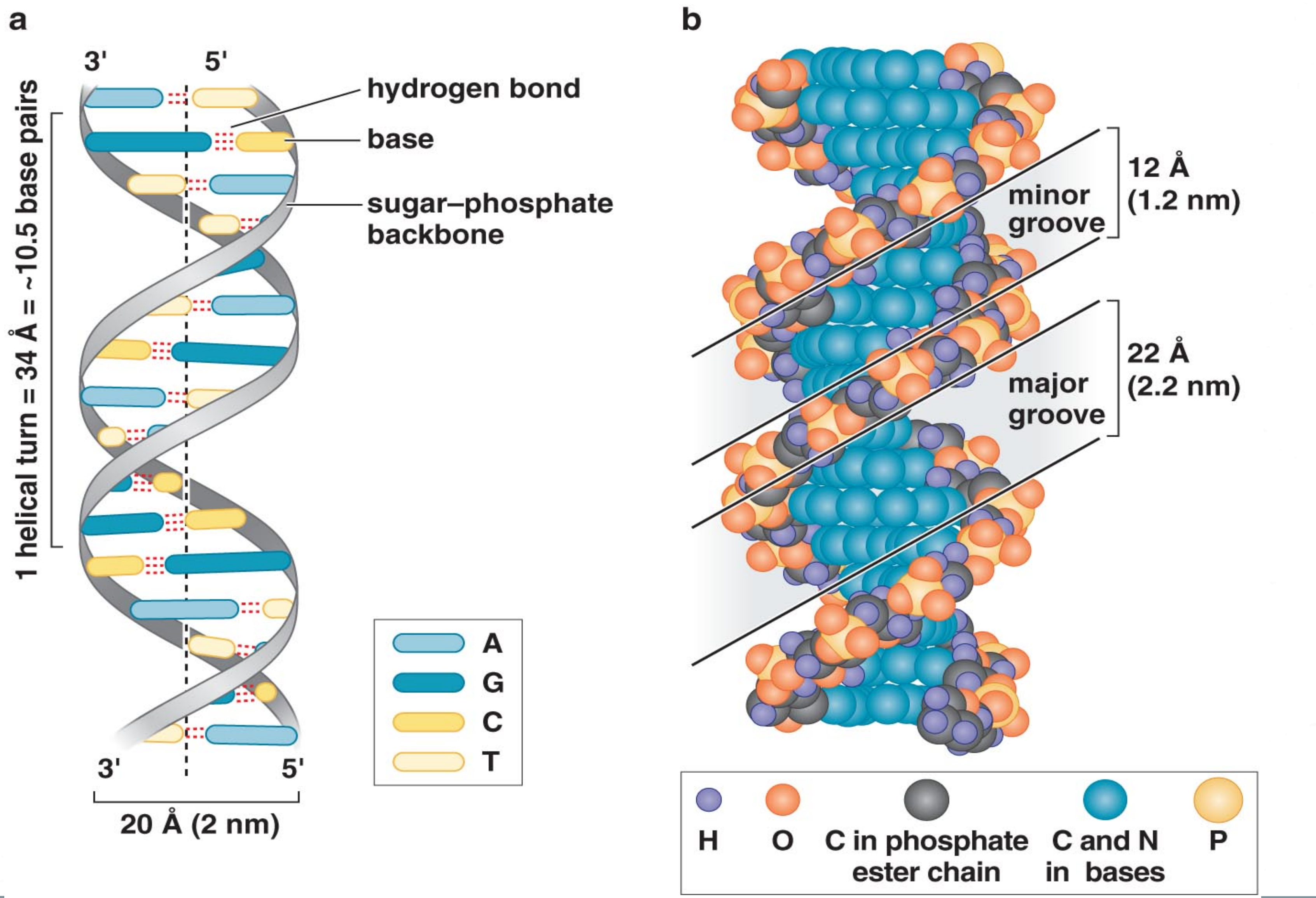
1. **Negatively charged and polar (water loving)**
   Nucleic acids are hydrophilic due to the negatively charged phosphate ($PO3-$) groups along the sugar phosphate backbone.
2. **DNA is precipitated in ethanol/salts, but soluble in water**
   positively charged sodium ions ($Na+$) neutralize the negative charge on the $PO3-$ groups on the nucleic acids, making the molecule far less hydrophilic, and therefore much less soluble in water. ETOH enhances the ability of salt to interact with DNA. Water has a high dielectric constant, which makes it fairly difficult for the $Na+$ and $PO3-$ to come together. Ethanol has a lower dielectric constant, making it much easier for $Na+$ to interact with the $PO3-$, shield it's charge and make the nucleic acid less hydrophilic, causing it to drop out of solution
3. **DNase (deoxyribonuclease) degrades DNA by hydrolysis of phosphodiester bonds**
   A phosphodiester bond is a group of strong covalent bonds between a phosphate group and two 5-carbon ring carbohydrates (pentoses) over two ester bonds.
4. **DNA absorbs UV light (260 nm) due to aromatic ring structure in the bases-**dsDNA (double strand) absorb less at 260 nm than ssDNA (single strand) -b/c the bases become unstacked and can thus absorb more light when ss.

# DNA Double Helix

- Double helix
- Right handed
- Anti-parallel strands
- Strands are held together by hydrogen bonding between base pairs on opposite strands and by hydrophobic stacking interactions between base pairs on the same strand.



# 3-D structure of DNA Double Helix

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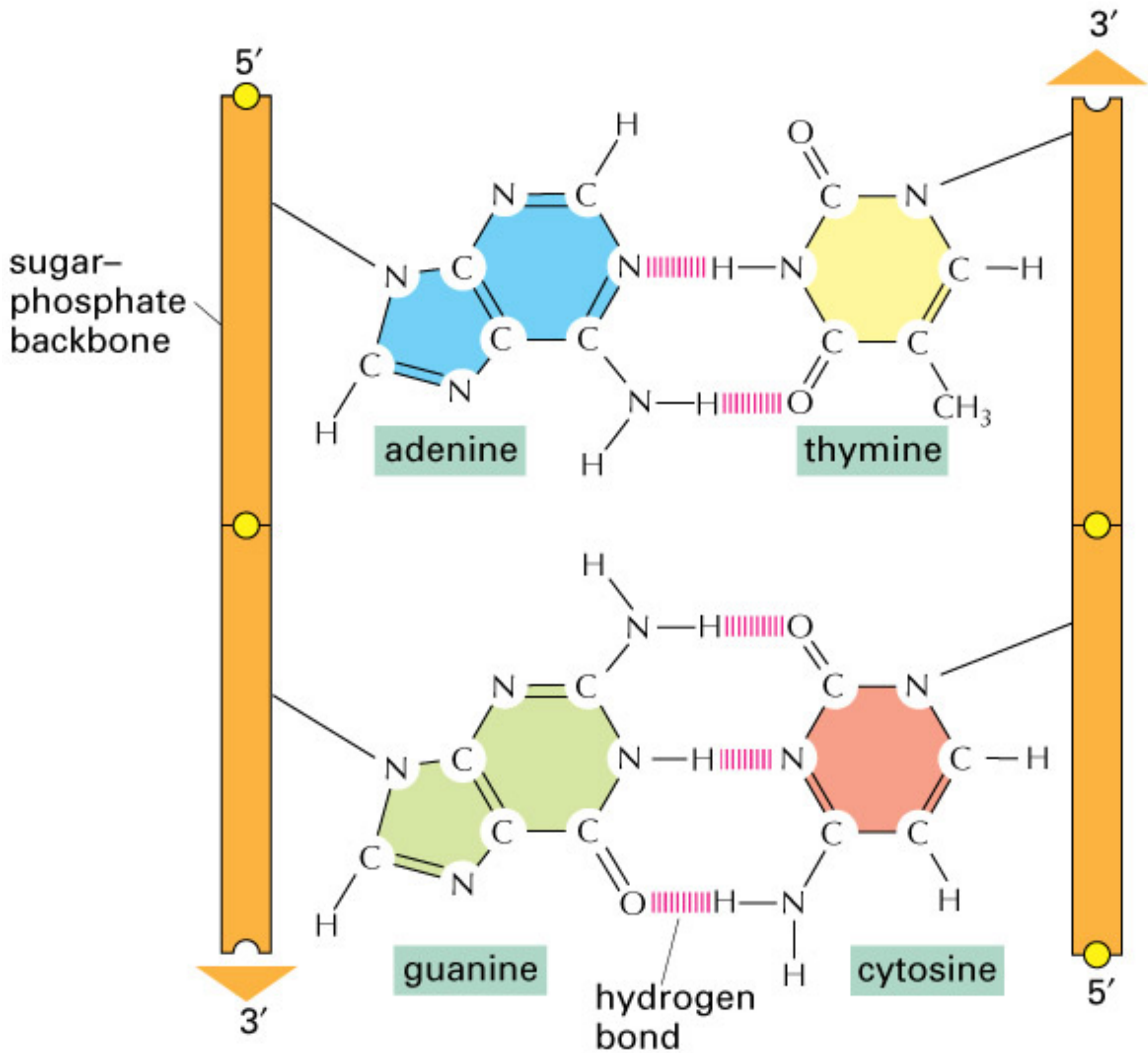
# Hydrogen bonds between the two strands of DNA




- A pairs with T
- A-T base pair has two hydrogen bonds

- G pairs with C
- G-C base pair has three hydrogen bonds

# DNA Base-pairing rules

**The two strands in a DNA are "complementary" to each other: A-T, G-C**

**5' -TGCCAGTTCAGCTAAACTCTG-3'**
**3' -ACGGTCAAGTCGATTTGAGAC-5'**

- **Base-pairing rules (= A-T & C-G), so if you know the sequence of one strand, you can always deduce the sequence of the other strand according to base-pairing rules.**

**•The base composition of DNA is different in different organisms, but the base compositions of different organisms all obey the following rules: = A-T & C-G**

# DNA strand characteristics:

- A DNA is usually presented as
5' (left) to 3' (right) for the top strand
and 3' (left) to 5' (right) for the bottom strand

5' ATGCATGCATGCATGCATGCATGC 3'
3' TACGTACGTACGTACGTACGTACG 5'

- And often you only need to write the top strand (if no 5'/3' is indicated, the left end is always 5')
ATGCATGCATGCATGCATGCATGC
Know how to write the bottom strand!

- The size of DNA:
1 bp (base pair) = 660 Dalton,
1 kb (kilo base-pair, 1,000 bp) = 660 kD
1 mb (1 mega base-pair, 1,000,000 bp) = 6.6 X $10^5$ kD

# DNA can be denatured and renatured

**Denature** (melt):

double strand (ds DNA) → single strand (ssDNA)

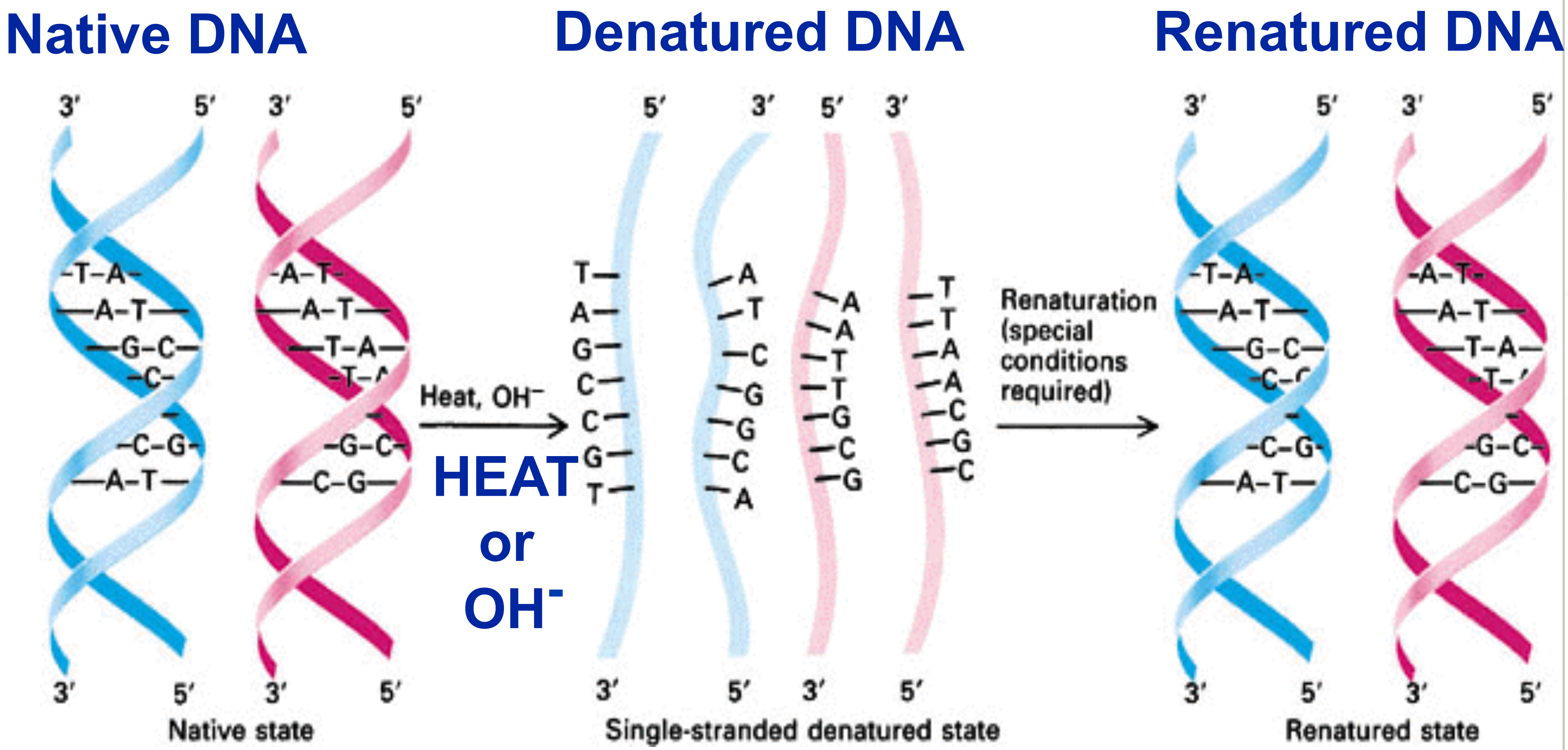
occurs at higher temperature, or lower salt concentration (destabilizes helix by less shielding of PO3- groups), or extreme pH.  These conditions break up H bonds between complementary bases

**Renature** (anneal)

single strand (ss DNA) → double strand (dsDNA)

occurs at lower temperature, higher salt concentration (favors more Na+ and PO3- interactions and helix stabilization), or neutral pH that favors H bond formation

# Denaturation/Renaturation of DNA



# Thermal denaturation of DNA

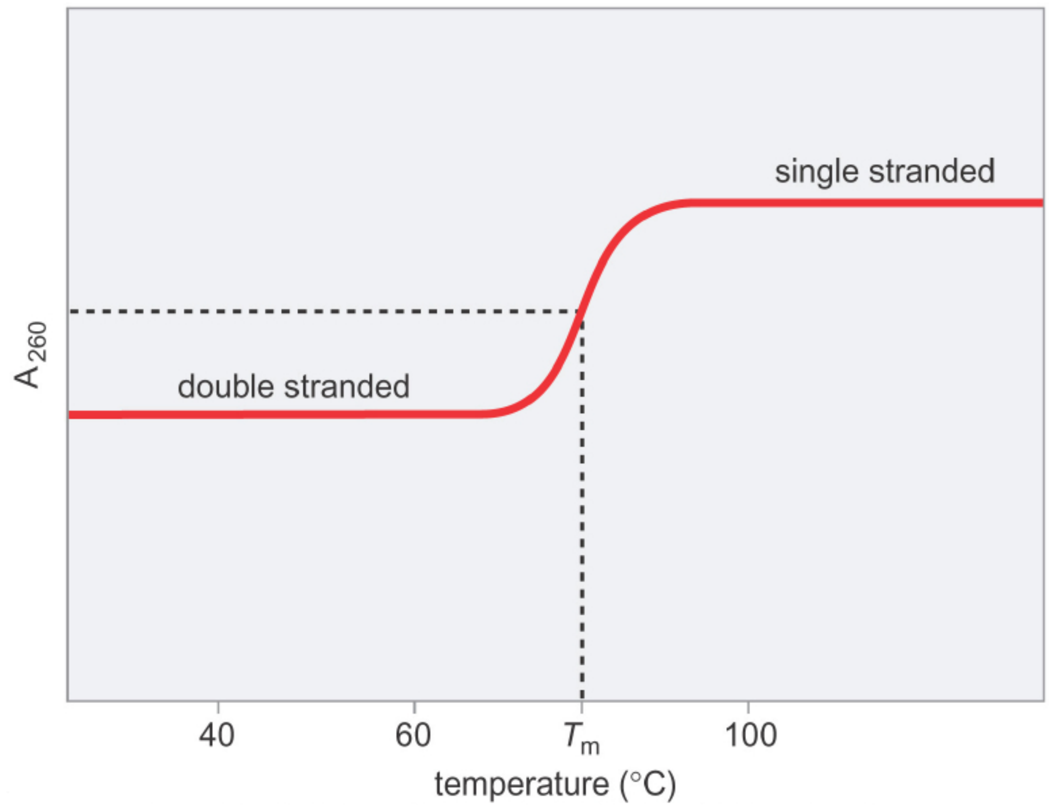
1. Denaturation occurs at a narrow range of temperature

2. The temperature corresponding to half the increase in absorption at 260nm is termed melting temperature--Tm

3. At Tm, 50% DNA is denatured

4. G:C rich DNA in general has higher Tm than A:T rich DNA

5. Perfect matched DNA in general has higher Tm than mismatched DNA.



# DNA Hybridization

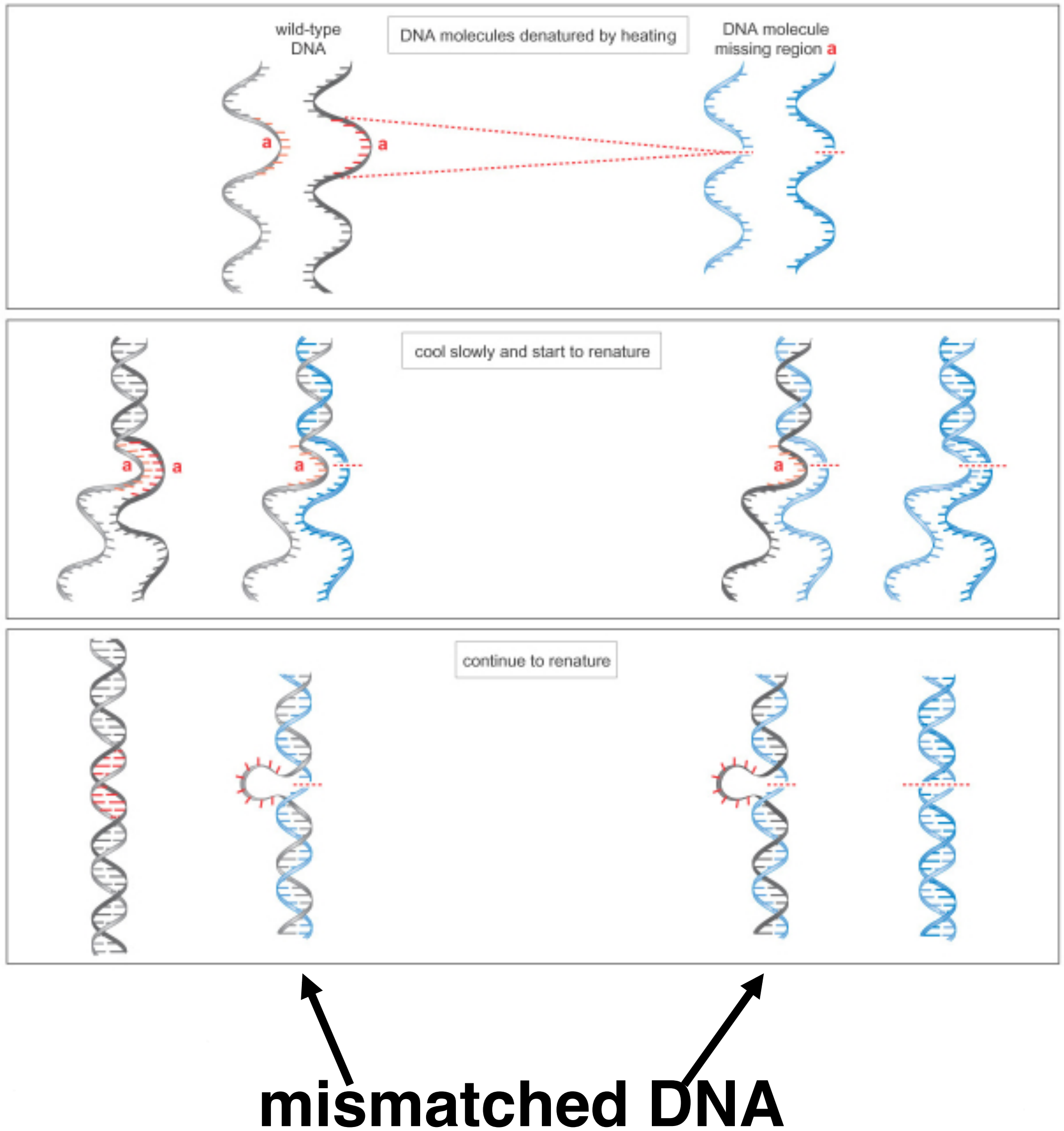
•Hybridization is the annealing of two ssDNA strands

•In a mixture of DNAs, only the complementary strands will reassociate, so hybridization only occurs when the two DNA sequences have homology

**DNA sequence** --the linear order of nucleotides of a DNA strand (always read from 5' to 3')

**DNA sequence homology**—similarity between the sequences of two DNA molecules

Hybridization is the basis of Southern Blots, FISH, DNA microarrays, etc.



# Fluorescence in situ hybridization (FISH) can be used to mark regions of DNA

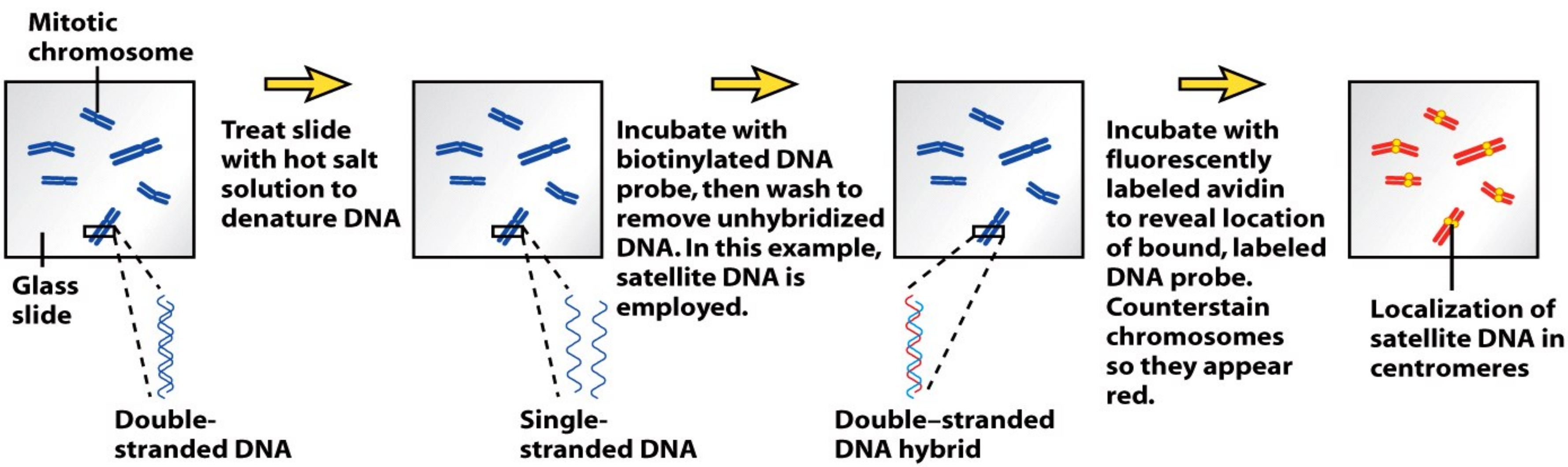



Figure 10-19a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

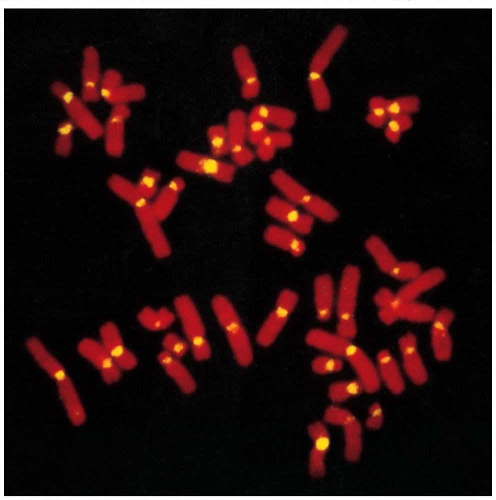


Figure 10-19b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Visualization of satellite DNA

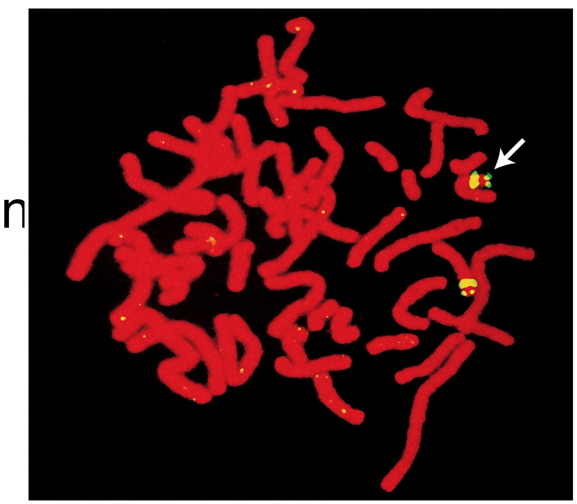


Figure 10-22 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

FISH can be used to detect chromosome abnormalities- i.e. duplication

# DNA Hybrid Formation

DNA 1

5' ATGCATGCATGC
3' TACGTACGTACG

$\xrightarrow{\text{denature}}$ 5' ATGCATGCATGC + 3' TACGTACGTACG

DNA 2

5' ATGCATGC
3' TACGTACG

$\xrightarrow{\text{denature}}$ 5' ATGCATGC + 3' TACGTACG

**Mix & renature**

5' ATGCATGCATGC
3' TACGTACGTACG

5' ATGCATGCATGC
3' TACGTACG

**DNA hybrids**

5' ATGCATGC
3' TACGTACG

5' ATGCATGC
3' TACGTACGTACG

# Watson-Crick model of DNA structure

1. Each DNA molecule has two strands of polydeoxyribonucleotide

2. The two strands spiral around each in antiparallel direction (that means if one strand runs 5' → 3', the other strand must run 3' → 5') to form a right-handed helix.

3. A pyrimidine (one-ring bases: T & C) from one strand always form H bonds with a purine (two-ring bases: A & G) from the other strand. This explains the base composition rules. C:G paring (3 H bonds) is stronger than A:T paring (2 H bonds).

4. Bases are inside the double helix, sugar-phosphates backbones are outside the helix (backbone). Each turn of the helix contains 10 base pairs of deoxyribonucleotides.

5. The double helix is held mainly by H bonds between bases from opposite strands, the H bonds can be broken and reformed.

# DNA Supercoiling

**When a circular DNA molecule is isolated and analyzed using gel electrophoresis, it often migrates as two forms, one migrate faster than the other.**

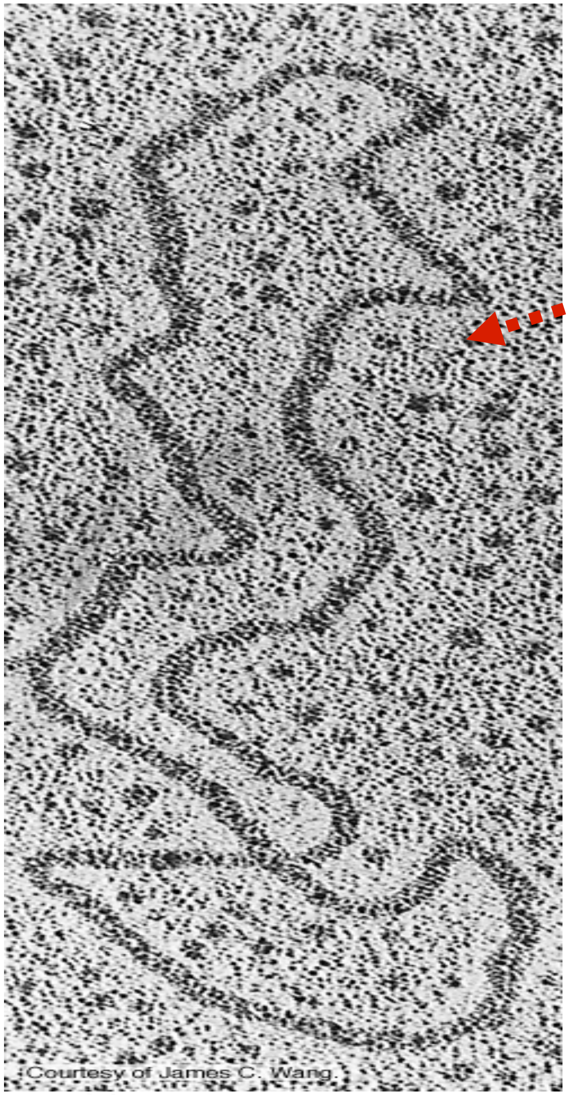
**It was found that the fast-running form of the circular DNA has a more compact structure called a supercoil.**

**Supercoiled DNA is a twisted circular DNA. A very very long linear DNA could also form the supercoiled structure.**

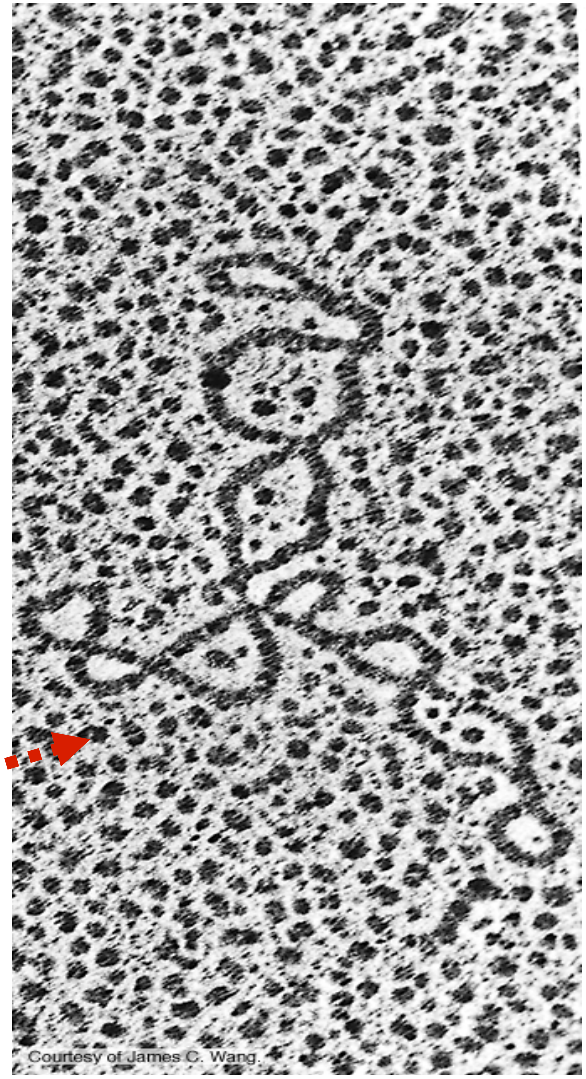
**Supercoiled DNA has more or less than 10 bp per turn**

**Supercoiled DNA provides a basis for the packaging of DNA in the cell, but it represents a problem for DNA replication**

# Relaxed and supercoiled SV40 DNA molecules




relaxed SV40 DNA
(=10 bp per turn)




supercoiled
SV40 DNA
(≠10 bp per turn)

# Analysis of SV40 DNA by agarose gel electrophoresis

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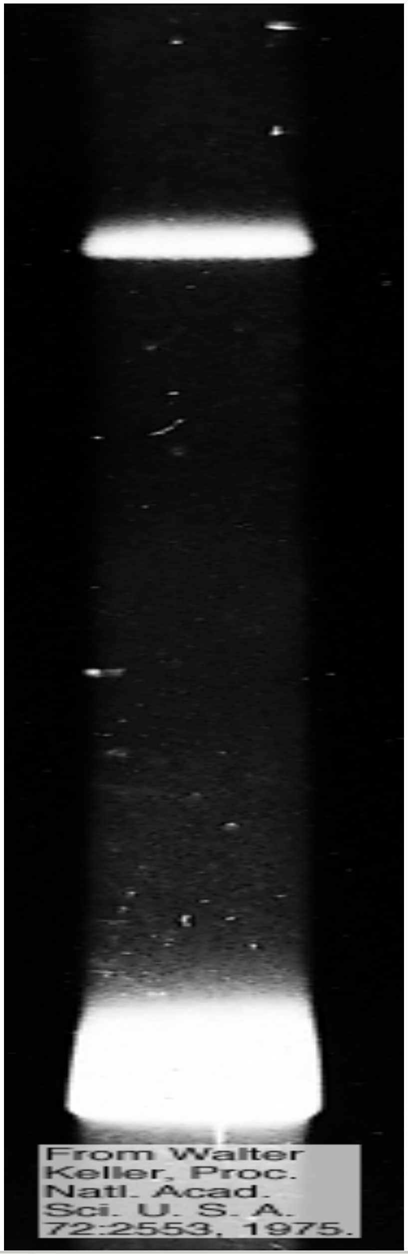


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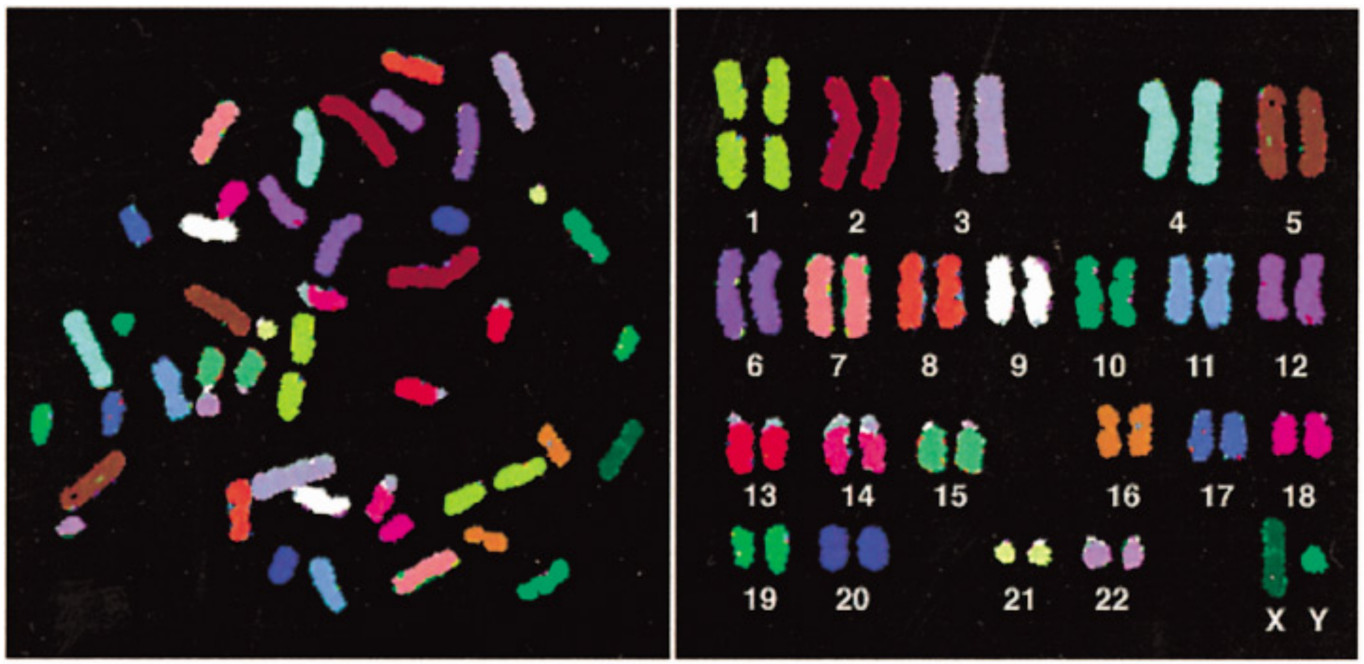
Relaxed SV40 DNA

Supercoiled DNA is a much more compact molecule, so it migrates faster than the relaxed DNA. The two bands shown here belong to the same kind of DNA, the genome of SV40 virus, but migrate differently depending on the degree of supercoiling.

Supercoiled SV40 DNA

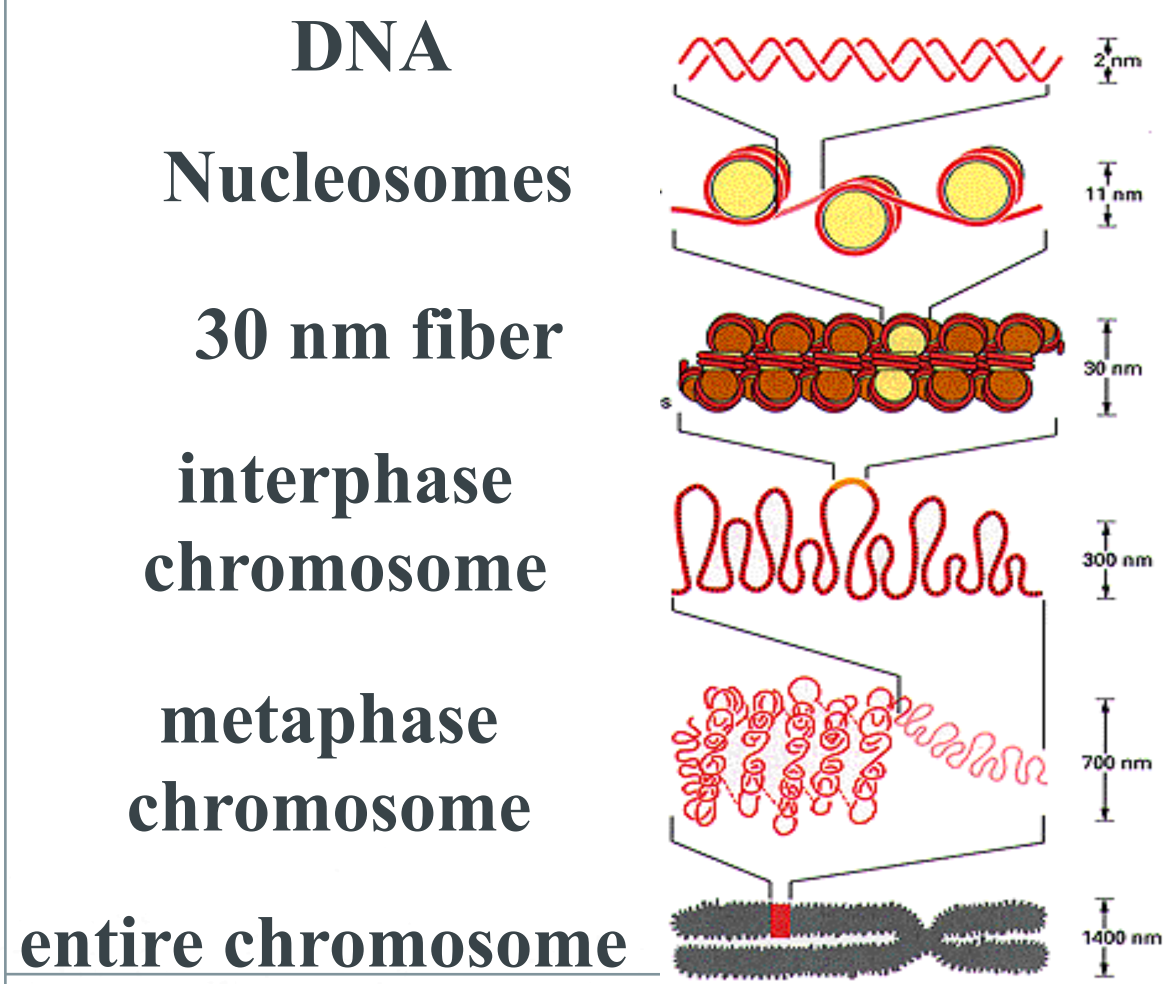
# DNA Content of Cells

- The DNA of a bacterium is about 1000 times as long as the length of the cell and consists of $4 \times 10^6$ base pairs.
- The DNA of a human cell is about 100,000 times the cell diameter and consists of about $3 \times 10^9$ base pairs.

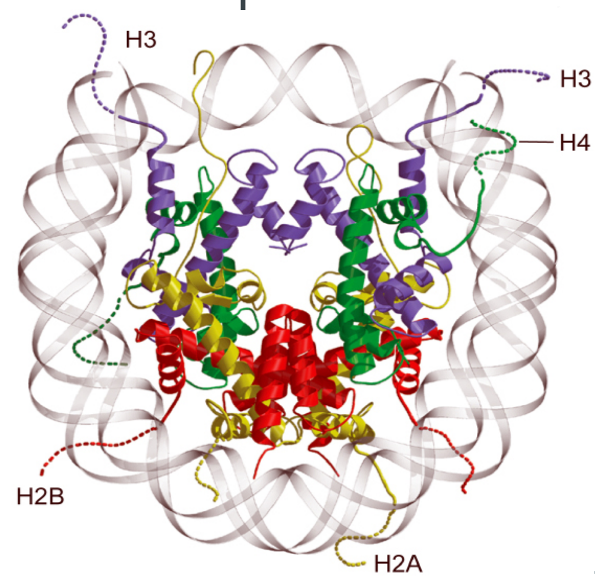


(A) (B)

# Eukaryotic DNA is packed into chromosomes that consist of both DNA and protein




The nucleosome contains the core histone complex (2 each of histone H2A, H2B, H3 and H4) and 150-200 bp DNA



# DNA Packaging

https://www.hhmi.org/biointeractive/dna-packaging

# What exactly is a genome?

A genome is all the genetic information (DNA) for a given species.

- Human genome: all the genetic information on the 22 autosomes plus the X and Y chromosome.
- Most of the genome of higher eukaryotes is made up of sequences that do not code for genes.  In humans, this is about ~95% of the genome!

# The Human Genome and Personalized Medicine

- Fifty five years after Watson co-authored the discovery of DNA, he had his entire genome sequenced!
- Molecular Biology has led to a new era of personalized genetic medicine with full genome sequencing
- Originally 2-4 million taking 30 people and 6 mo to sequence Watson genome in 2008
- Now paired end sequencing for ~$5K!

# Genome sequencing

http://ed.ted.com/lessons/how-to-sequence-the-human-genome-mark-j-kiel

# Implications and Critical Thinking -4

We know that mutations in the DNA sequence can lead to aberrant gene expression and often disease.

But there are instances where the regulation of access to the DNA sequence can regulate how the DNA sequence represents information and function to the cell.

What are these changes?