How did the midterm go?

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2012 Lecture Outline for Biology 1202B

Week of	Lecture	Lecture Topic	Instructor
Feb 13	11	DNA (Chapter 13)	Dr. Brenda Murphy
	12		
Feb 20	Conference Week - All lectures, labs, tutorials cancelled		
Feb 27	13	Transcription (Chapter 14, 15)	Dr. Brenda Murphy
	14	Translation (Chapter 14, 15) miRNA (Chapter 15)	
Mar 5	15		
	16	DNA Technology (Chapter 16)	
Mar 12	17		
	18	Application of DNA Technology	
Mar 19	19	Stem Cells, Hematopoiesis (Page 196, 779, 1090)	
	20	Immunology (Chapter 44)	
Mar 26	21		
	22	Application of Immunology	
Apr 2	23	Q&A	

Confirmation of Final Exam
Bio 1202B

Tuesday April 17

7:00pm -10pm

Comprehensive Final Exam

Format For My Section is Multiple Choice

Do not book travel during exam period

Dr. Murphy
prepares & delivers webct lectures
and
prepares self assessments & exams

Anything else regarding the course, direct to Dan Lajoie in NCB Rm 301

dlajoie@uwo.ca

Bio 1202B
Lecture 11
DNA Structure and Organization
Chapter 13

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Human Genome

1952 **Hershey & Chase**

DNA inherited material

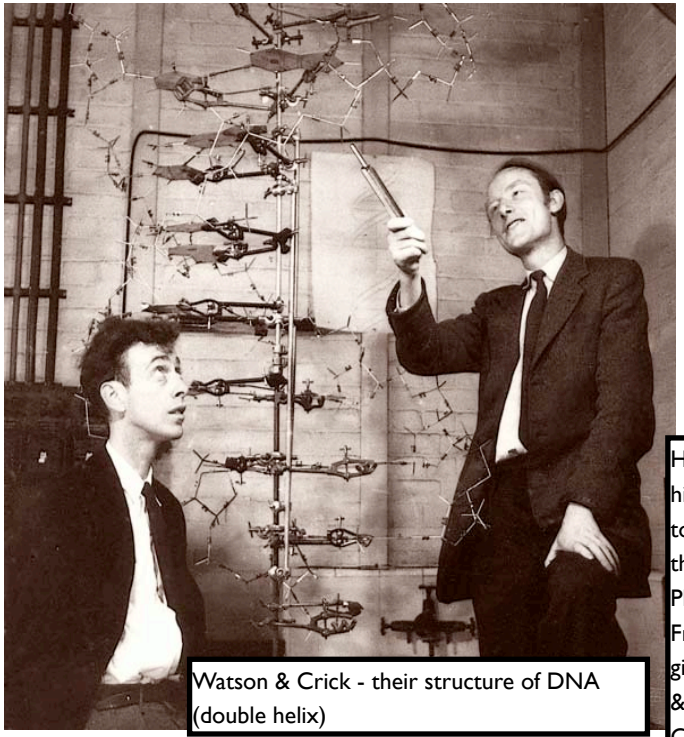
1953 **Watson, Crick & Franklin**

DNA structure double helix

1956 - Tjio & Levan - 46 Chromosomes in nucleus
1963 - Margit & Sylvan Nass - mitochondrial DNA (mtDNA)

What makes up human genome?
- nucleotides
- where is it ? cell
- nuclear chromosomes are linear (46)
- mitochondria has DNA (mitochondrial DNA)
- genetics is new field in medicine, not many have training in basic genetics
- 1952 - up until then they didn't know if it was DNA or protein.. thought it was protein b/c it seemed to have unlimited repretoire
- 1953 - Franklin : her work was really instrumental
- Watson & Crick took everyone elses work & put into a comparative analysis
- mature red blood cells doesnt have 46 chromosomes… this is b/c mature red blood cells don't have nucleus

DNA Structure



Watson & Crick - their structure of DNA (double helix)

a. Rosalind Franklin

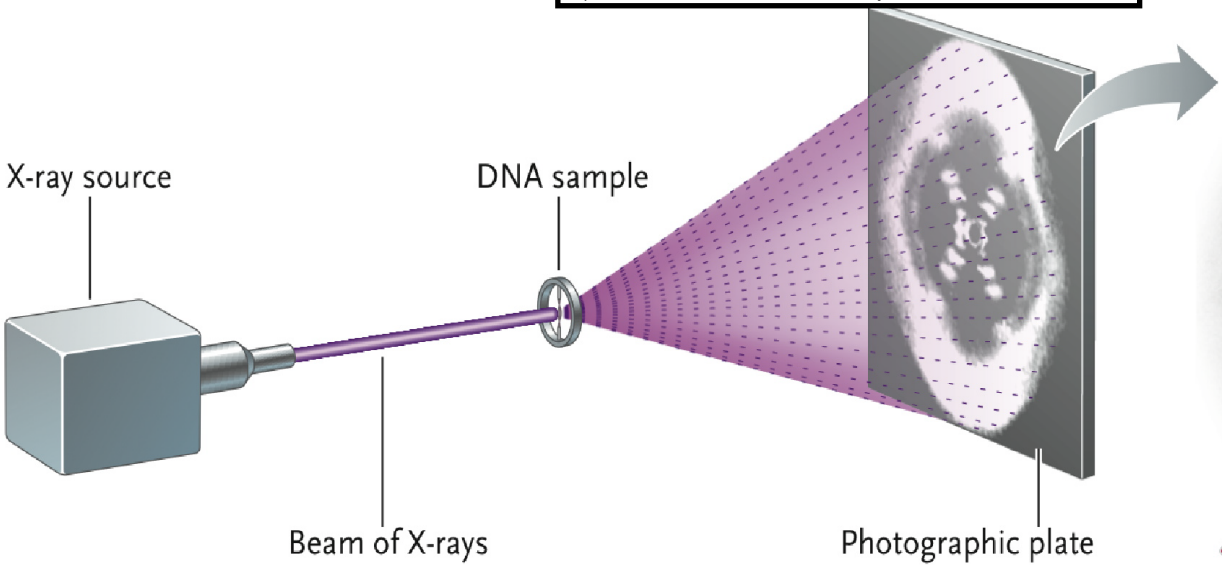
Had xray source & DNA in crystal.. had beans of light hitting things.. beams of light diffract & reflect & hit things to create an image… putting photographic plate allowed them to capture the image

Photo 51 reveals pattern DNA makes.. position of atom

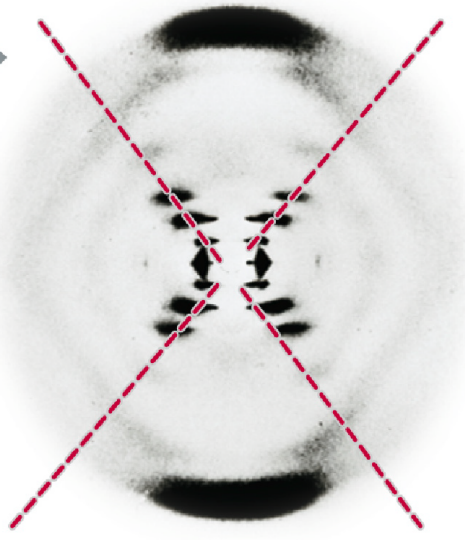
Franklin didn't give her info to watson & crick.. she didn't give it to them… she gave it to the head of her university & one of her colleagues took it and gave it to Watson & Crick and they won the nobel prize

- Franklin didn't win the nobel prize b/c she was dead (@ 30).. died from cancer.. b/c of xrays

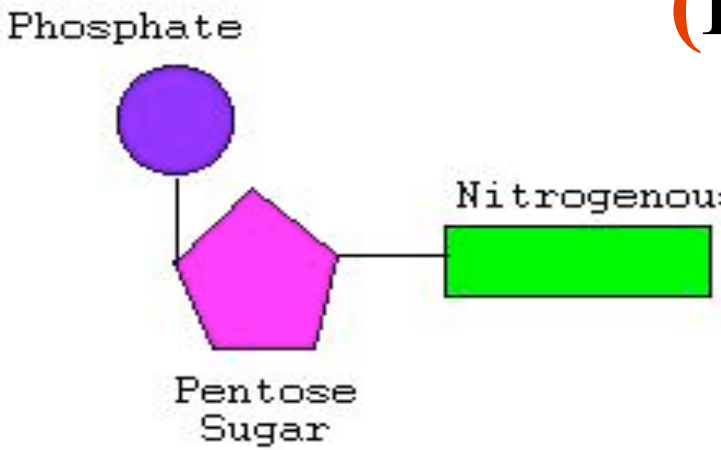
b. X-ray diffraction analysis of DNA



Franklin's DNA diffraction pattern



Nucleotides = building blocks of nucleic acid (DNA & RNA)



Nucleotides: three structures
Pentose sugar : 5 carbons

Triphosphate

3 phosphates

Deoxyribose Sugar

missing oxygen on DNA's sugar

Ribose Sugar

have oxygen (RNA)

Nitrogenous Bases

- **Guanine (G)**
- **Adenine (A)**
- **Thymine (T)**
- **Uracil (U)**
- **Cytosine (C)**

GATC : sequencing of DNA
in RNA we have uracil.. other nitrogenous base

side : sugar & base without the phosphate

DNA & RNA are made of dNTP = deoxynucleoside triphosphate

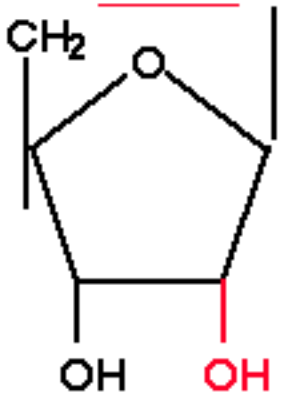
i.e. dATP, dCTP, dGTP, dTTP , dUTP

Differences between RNA and DNA

1) ribose sugar

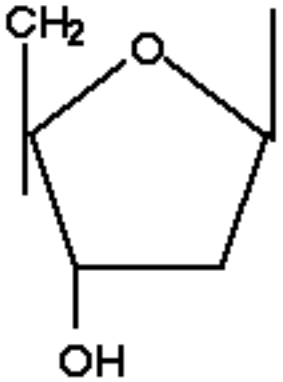
- can see that we have an OH group on C2 in RNA (makes it unstable)
- DNA is more stable b/c it doesn't have the OH group there
- RNA is unstable
- clinically RNA has to be removed fast (24 hours) to be tested because that's how long it's stable for

RNA



(ribonucleic acid)

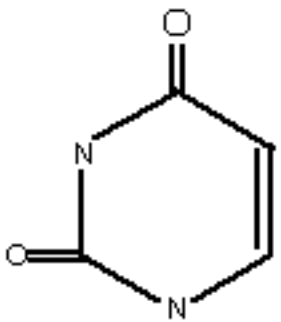
DNA



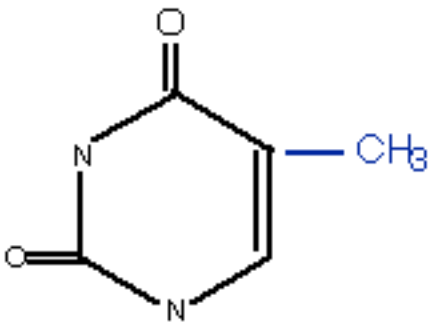
(deoxy ribonucleic acid)

2)T and U

- thymidine has methyl group



uracyl



thymidine

3) strand

single

double

What are dATP, dGTP, dCTP, dTTP and dUTP?

deoxynucleoside triphosphates (dNTP)

Nucleotide = Nucleoside + phosphate

sugar + base

deoxyadenosine

deoxy : represents nucleosides (means it's a nucleoside)

deoxyguanosine

deoxythymidine

deoxyuridine

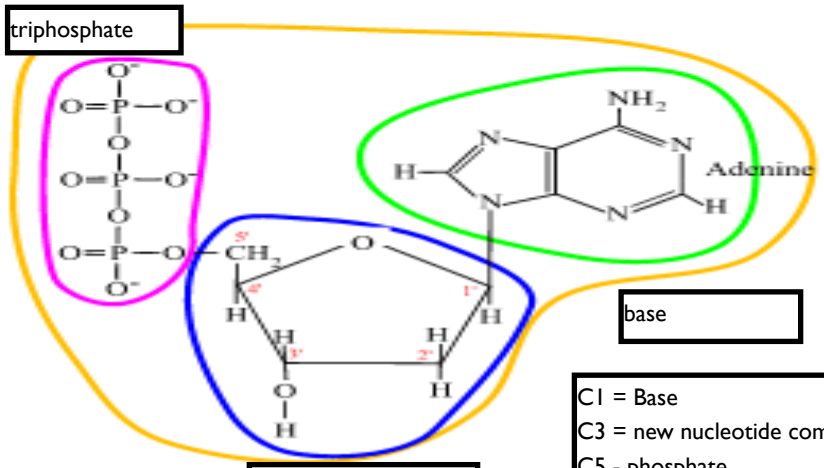
deoxycytidine

The components of nucleotides

Nucleotide = base + sugar + phosphate

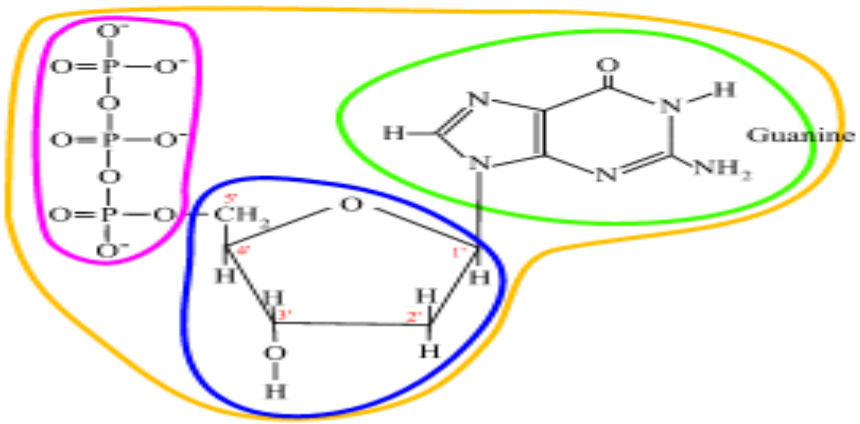
4 different dNTP's (deoxynucleoside triphosphate) :

deoxyadenosine triphosphate = dATP

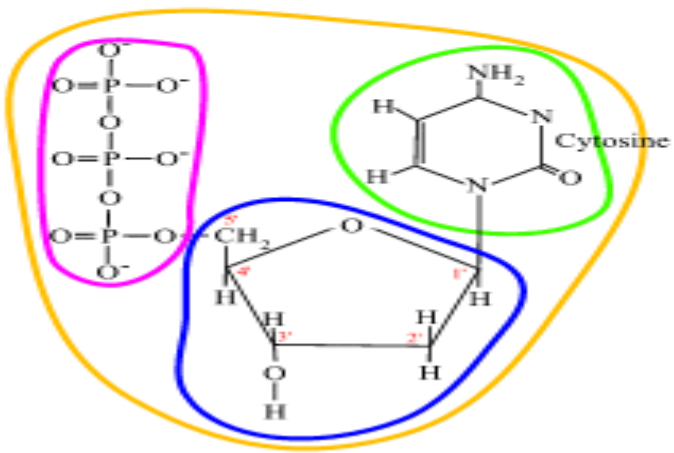


C1 = Base
C3 = new nucleotide comes in
C5 - phosphate

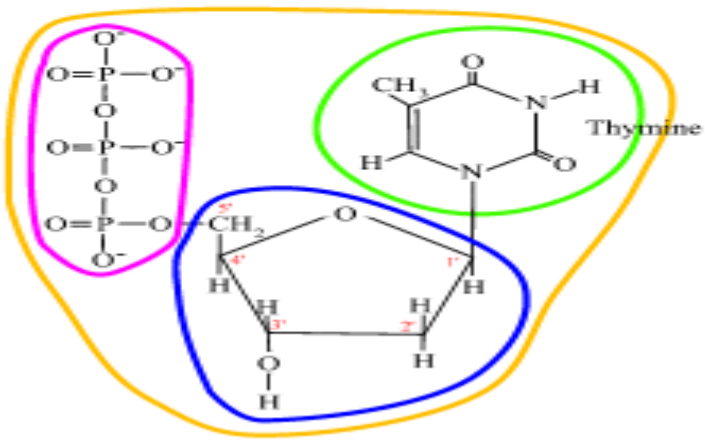
deoxyguanosine triphosphate = dGTP



deoxycytidine triphosphate = dCTP



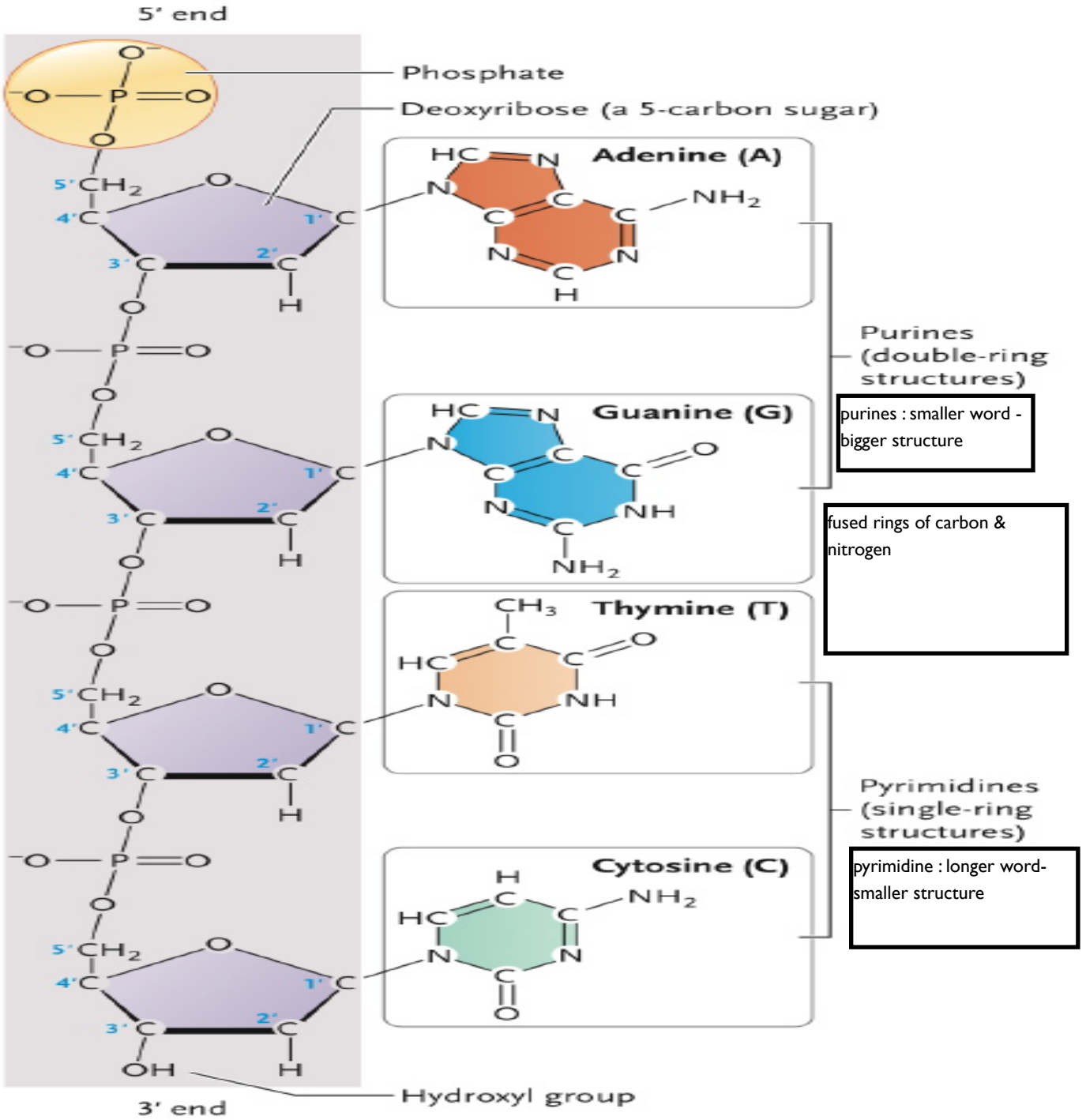
deoxythymidine triphosphate = dTTP



(Andy Vierstraete 1999)

Figure 13.4
The 4 nucleotide subunits of DNA, linked into a polynucleotide chain

- 5' end of a strand & 3' end.. this is called the polarity of the DNA
- 5' end has a phosphate group added… C5 position holds the phosphate
- 3' has an OH… C3 position holds the OH
- diagram shows four nucleotide subunits of DNA in a polynucleotide chain… chain is 5'-3'
- triphosphates goes down to mono when new nucleotidecomes in
- shows us that we have a sugar phosphate backbone (left side of picture)
- phosphate bridges 3'C to 5'C from two different sugars… ex phosphate in between bases on nucleotides… phosphate acts as a bridge between two sugars.. we have a phosphate between 3' & 5' of neighbouring sugars
- bond is referred to as a 3'-5' phosphodiester bond



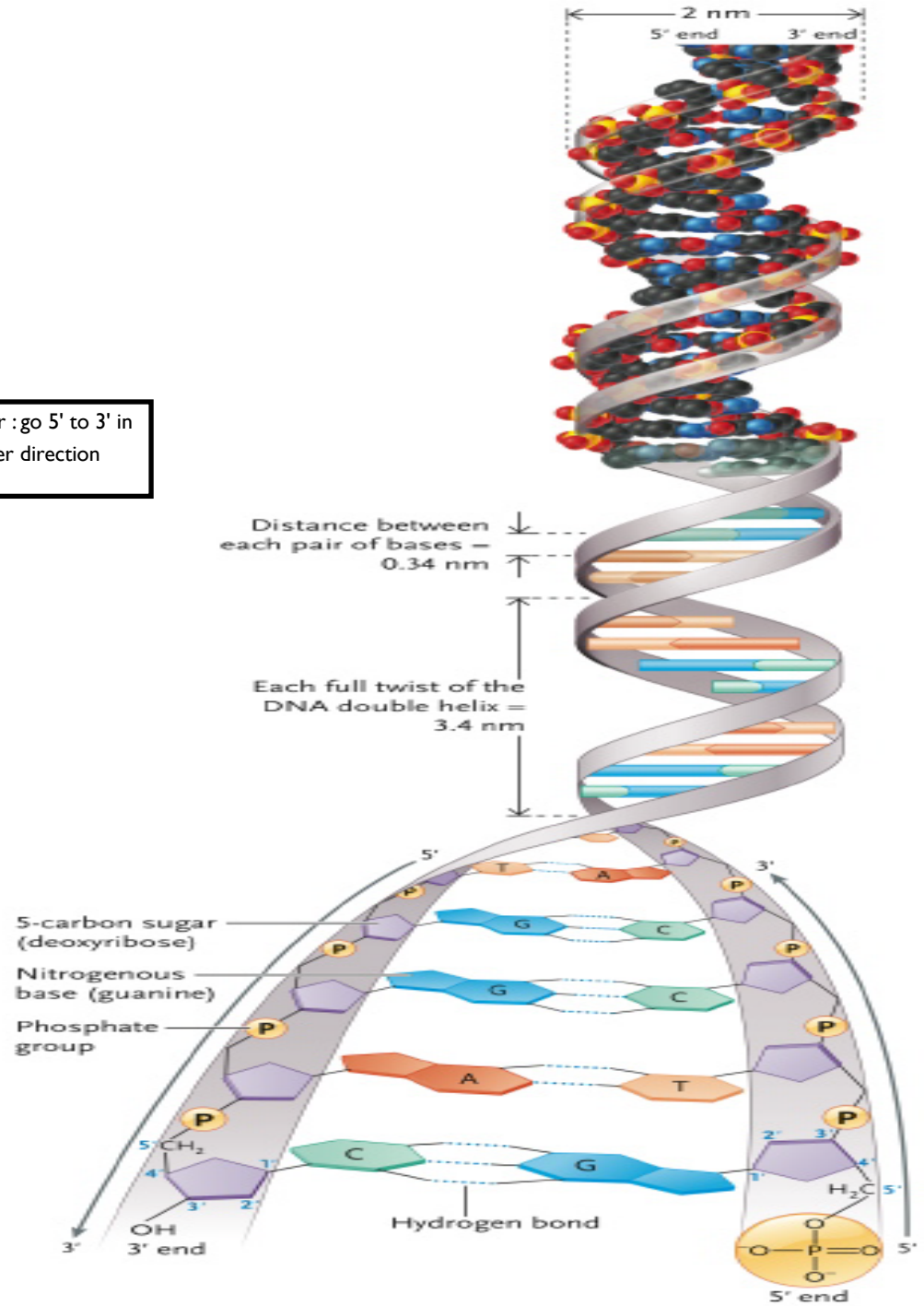
purines : smaller word - bigger structure

fused rings of carbon & nitrogen

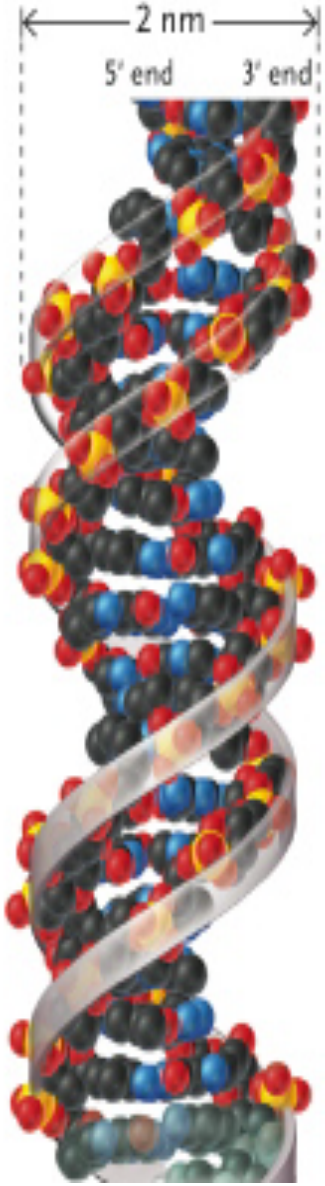
pyrimidine : longer word- smaller structure

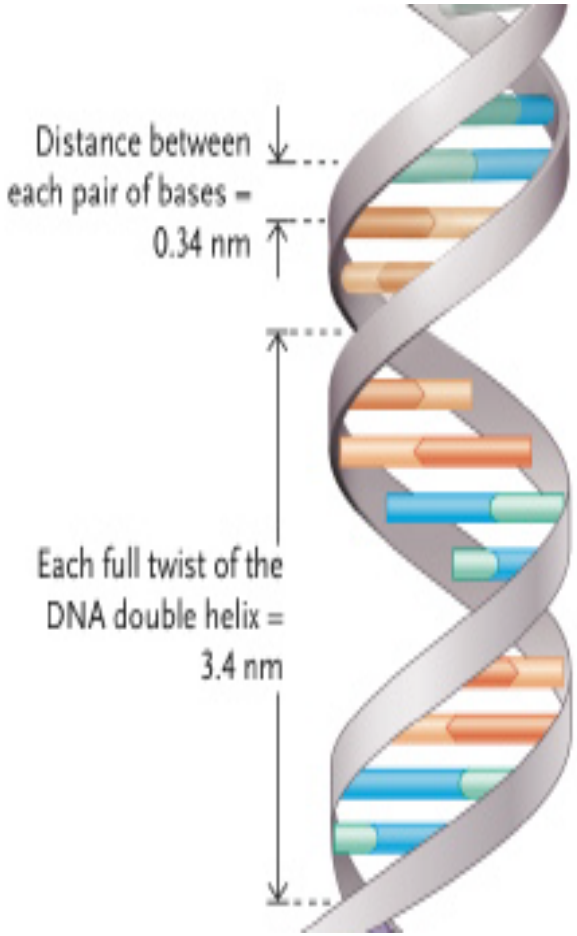
Figure 13.6
DNA double helix

anti parallel when come together : go 5' to 3' in one direction and 3' to 5' in other direction



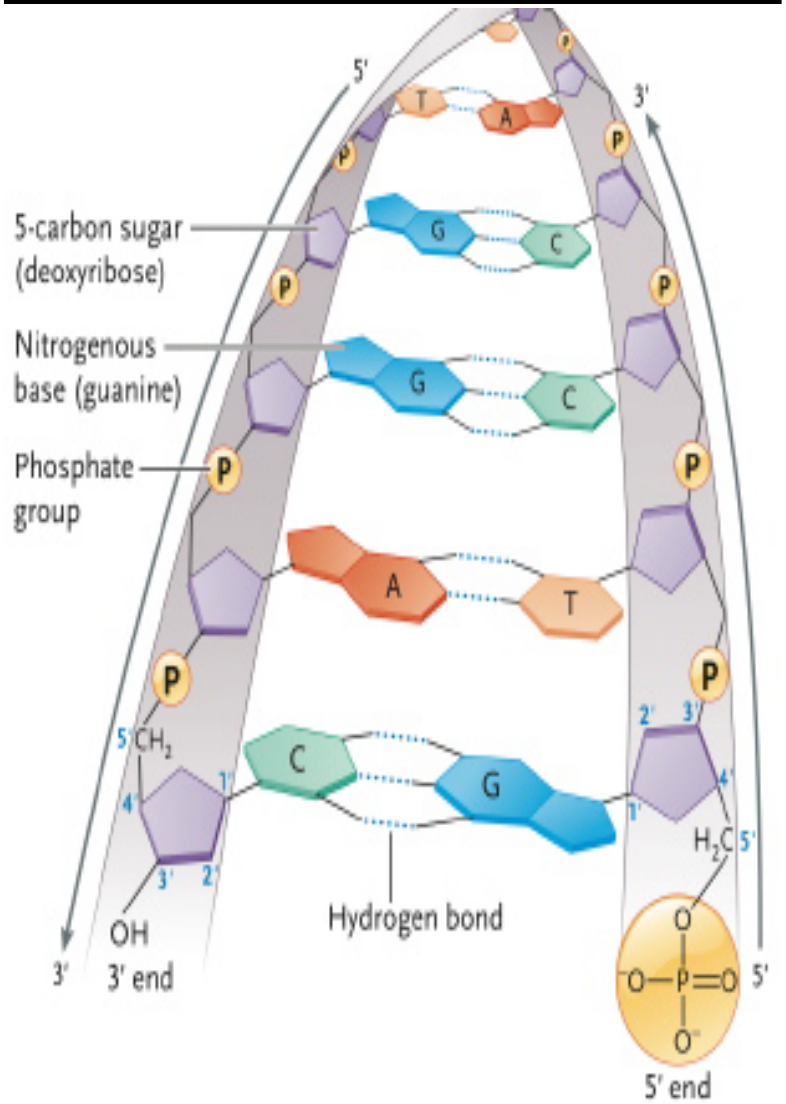
ribon structure again





Chargaff's rule : found the amount of purines = amount of pyrimadines… this was found before they knew it was a double helix

- guanine binds with cytosine (3 hydrogen bonds)
- adenine binds to thyamine (2 hydrogen bonds)
- why do they bond this way? b/c its too big.. purines w/ purines we have too much crowding.. too tight… two pyramidines are too loose, too small binding
- if they do bind, its called non watson & crick binding.. weird binding with purines



Two polynucleotide chains twist around each other in a right handed way

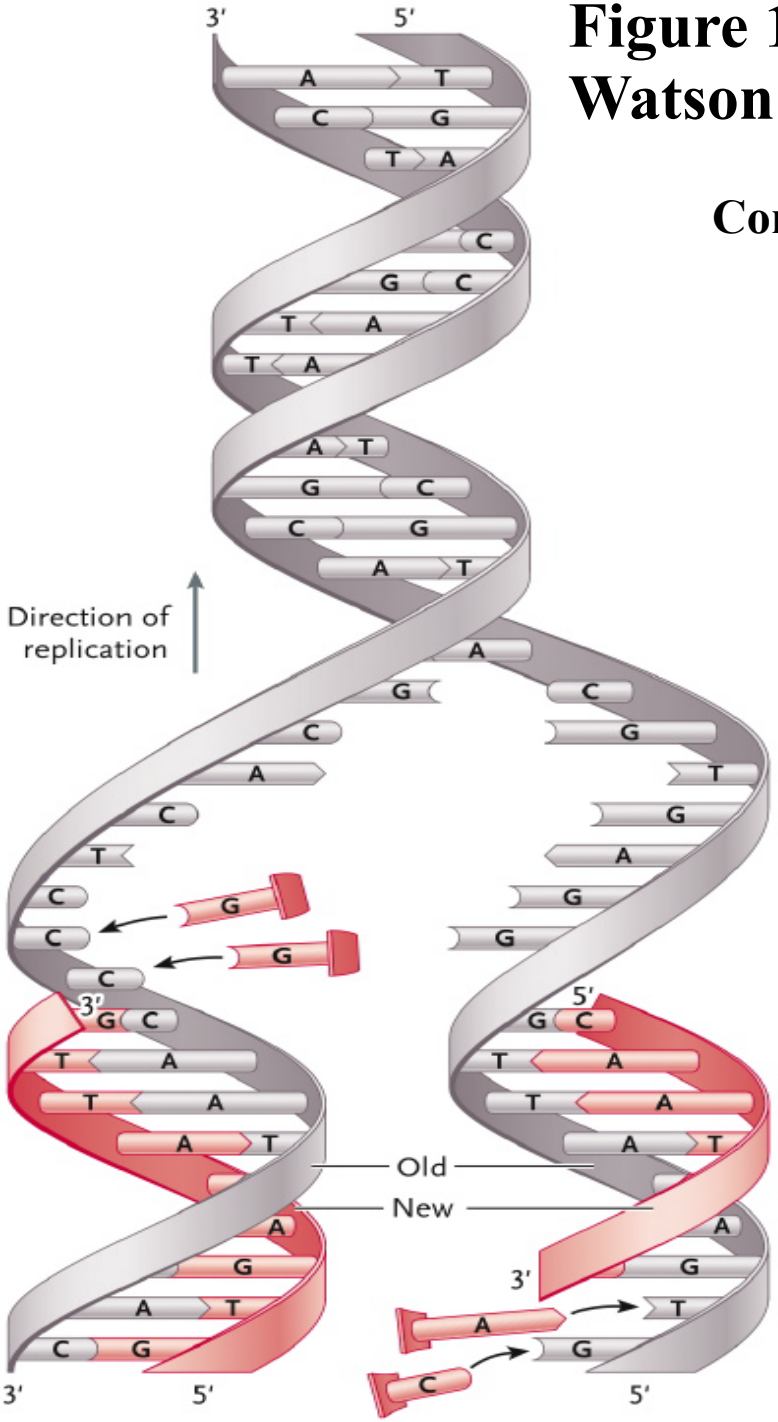
Never be tested on this… DNA normal structure is right handed DNA

Difference between right & **left-handed** helices:

If you move along a helix in the direction of your right hand's thumb, and the helix turns in the direction of your right hand's fingers, then it's a right-handed helix,

Picture the helix vertical; if the front strands move from the lower left to the upper right, then it is a right-handed helix.

Figure 13.7
Watson & Crick's model for DNA replication



Complementary base pairing in the DNA double helix. A pairs with T. G pairs with C

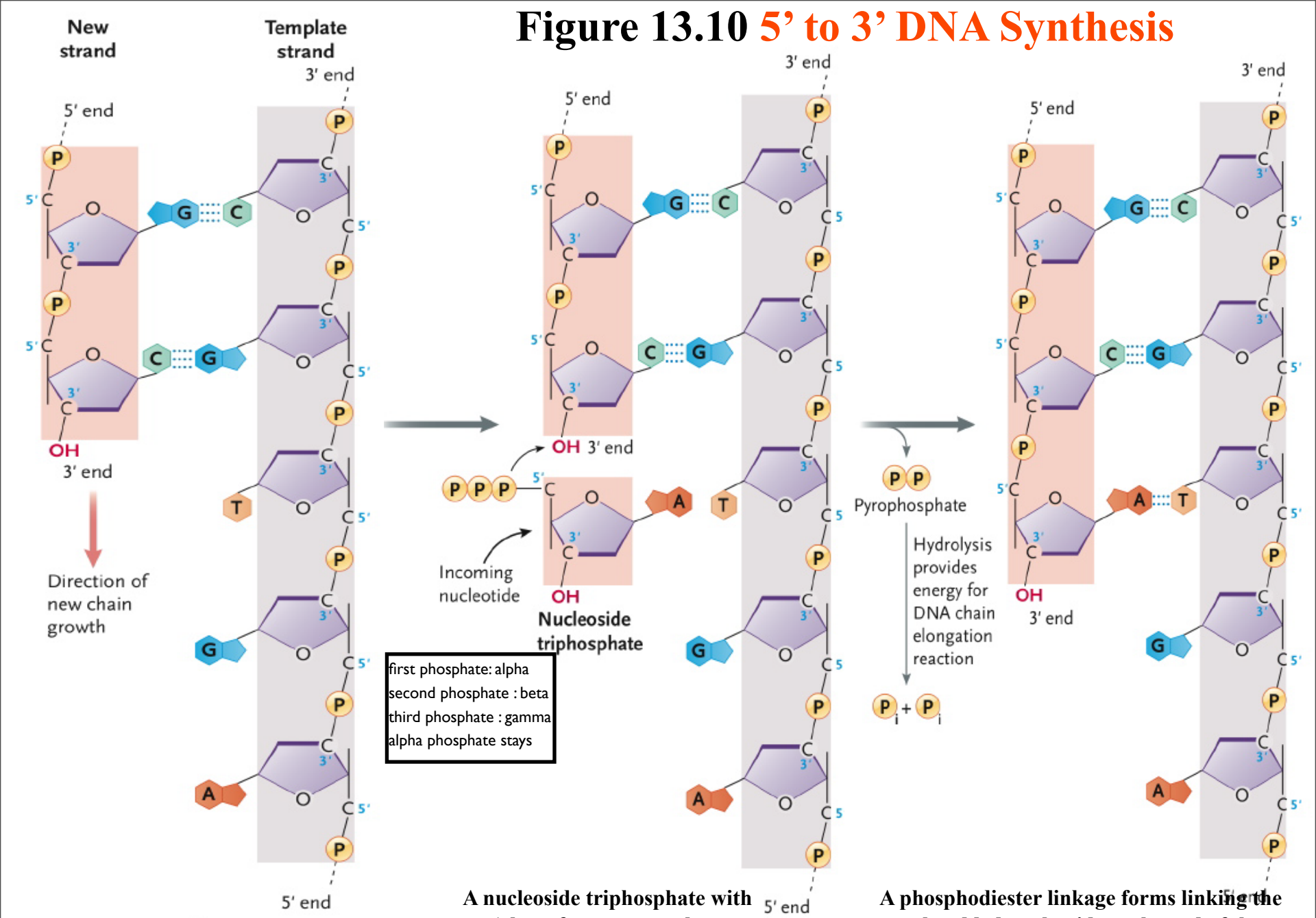
The two chains unwind & separate

Each "old" strand is a template for the addition of complementary bases..

The result is two DNA helices that are exact copies of the parental DNA molecule with one "old" strand & one "new" strand.

Semi-conservative replication

Figure 13.10 5' to 3' DNA Synthesis



The template strand with two nucleotides of the new strand assembled.

A nucleoside triphosphate with an A base forms a complementary base pair with the next nucleotide of the template strand.

A phosphodiester linkage forms linking the newly added nucleotide to the end of the primer, lengthening the strand by one.

Eukaryote DNA Organization

Chromatin is the building block of chromosomes

75% **Chromosomal Proteins**

37.5% each ½ Histones

½ Nonhistones

15% **DNA**

10% **RNA**

aka DNA is not pure to it's own.. has a lot of proteins..
- to extract DNA we have to get protein out & RNA
- histones : important for compaction of DNA
- nonhistones : important for turning genes on & off… transcription factors

Histones compact DNA structure

i.e. 2 metres of DNA into a cell 10μm in diameter

small positive charged, basic proteins

attracted to DNA negative charged phosphates

5 histone proteins exist in most eukaryote cells

H1, H2A, H2B, H3 & H4 — form a histone core

Nucleosome Core = ~2 turns of DNA wound around 2 molecules of H2A, H2B, H3 & H4 to form a "bead like" structure under electron microscope

* note it doesn't include H1
- 8 molecules that make up a histone core

147 base pairs of DNA wrapped around an octamer of histone molecules — 8 histome molecules

Nucleosome = Nucleosome Core plus linker (DNA between nucleosome cores) & H1 form a "bead on a string" structure under electron microscope

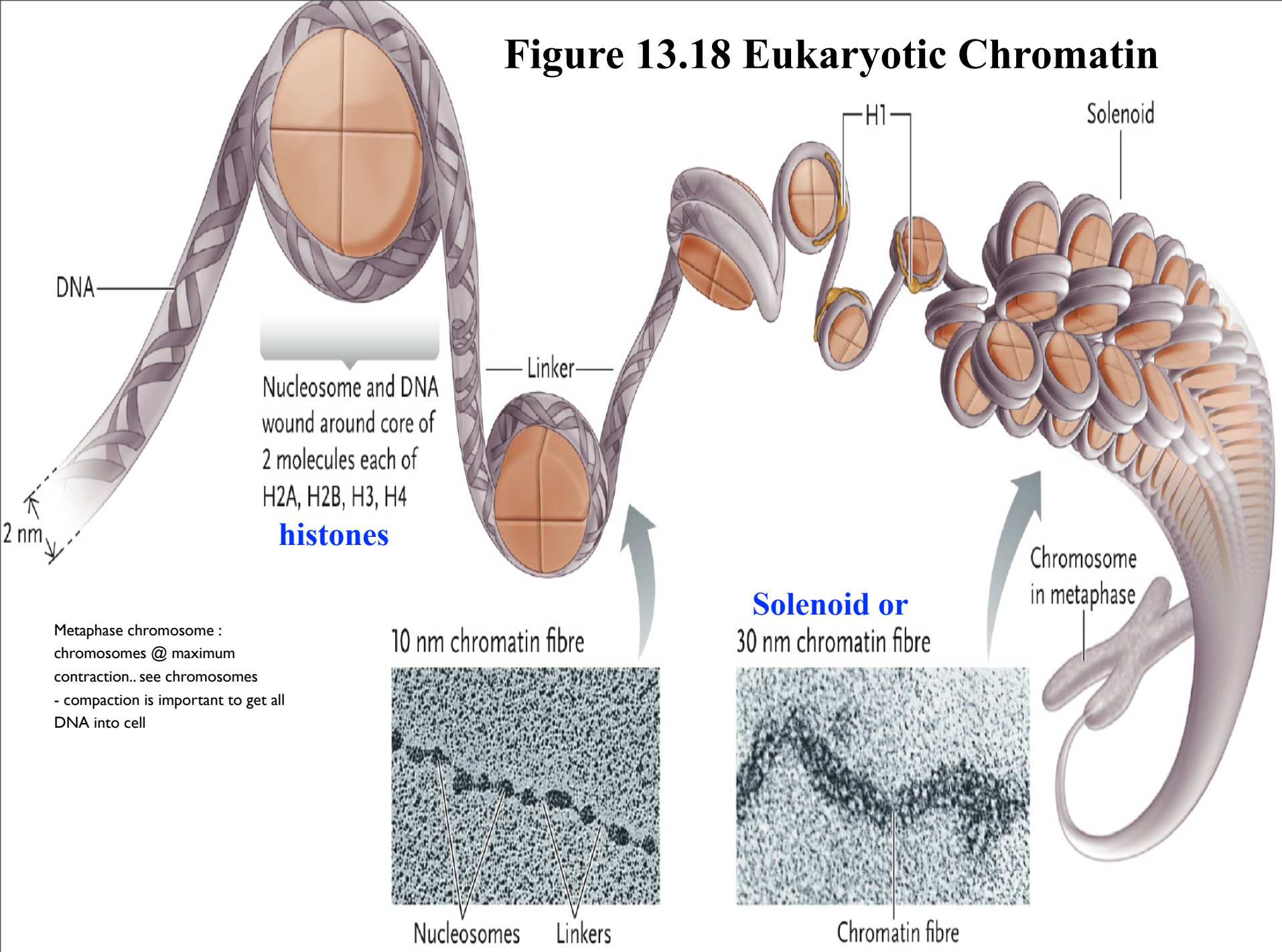
200bp of DNA

bp = base pair

H1 is important for anchoring compaction

Nucleosome protein amino acid sequence is conserved in all eukaryotic organisms so assume these protein are performing the same function

Figure 13.18 Eukaryotic Chromatin



Metaphase chromosome : chromosomes @ maximum contraction.. see chromosomes - compaction is important to get all DNA into cell

More Levels of Compaction

DNA

Histones to form Chromatin Fibre

In interphase nuclei, chromatin fibres differentiate into

Euchromatin (loosely packed, genes expressed)

DNA is still packed but a little loser… can still be expressed (transcribed)

Heterochromatin (densely packed, genes not expressed)

In mitotic or meiotic cells, chromatin fibre fold & pack into thick, rod-like **chromosomes**

Nonhistones regulate gene activity

Proteins, other than histones, associated with DNA

Some alter accessibility to the gene by loosening or tightening the binding of histones to DNA

i.e. Histone acetylases (loosen histone binding)

Histone deacetylases (tighten histone binding)

Others alter gene regulation

i.e. transcription factors (MeCP2, SP1, HOX, SRY) bind to promoter regions to enhance or repress the transcription of a gene

Prokaryote DNA Organization

Most prokaryotic cells have a **circular chromosome**

don't have histome proteins that are compacting it..

Bacterial DNA is **not organized into nucleosomes**, but there are "+" charged proteins that combine with bacterial DNA to compact it into an irregularly shaped mass called the **nucleoid**. DNA is organized into loops providing some DNA compaction.

DNA of the nucleoid is suspended directly in the **cytoplasm**, so no surrounding membrane