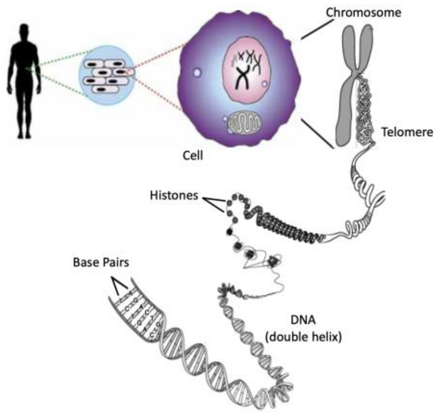
Nucleic Acids and Molecular Biology



Biochemistry Boot Camp 2021: Session #7

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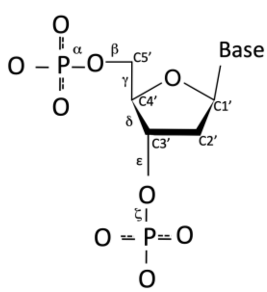
Deoxy-Ribose Nucleic Acids (DNA and RNA)



- DNA and RNA polymers of (deoxy) ribose nucleotides
- DNA - chromosomes, mitochondria and chloroplasts
- DNA - Carries the genetic information

- DNA ______________ -> RNA ______________-> Protein

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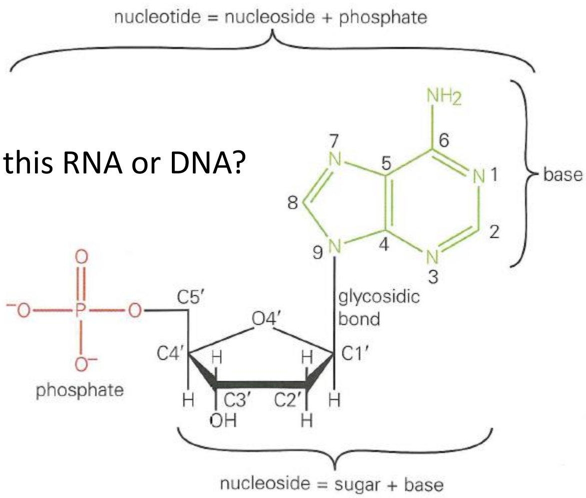
Nucleotide Structure

Question: Is this RNA or DNA?



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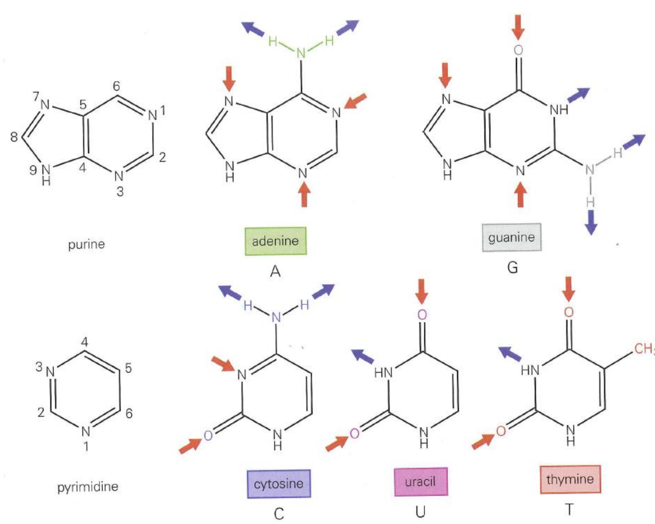
Nucleic Bases



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Nomenclature (Scientific Names)

	Base	Nucleoside	Nucleotide	Nucleic Acid
Purine	Adenine	Adenosine	Adenylate	RNA
		Deoxyadenosine	Deoxyadenylate	DNA
	Guanine	Guanosine	Guanylate	RNA
		Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines	Cytosine	Cytidine	Cytidylate	RNA
		Deoxycytidine	Deoxycytidylate	DNA
	Thymine	Thymidine	Thymidylate	
		Deoxythymidine	Deoxythymidylate	DNA
	Uracil	Uridine	Uridylate	RNA

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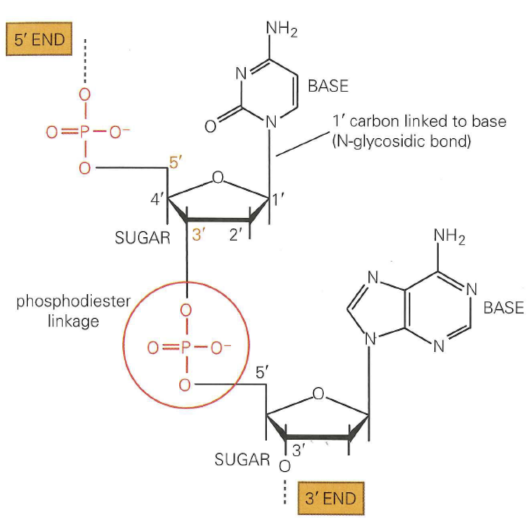
Nucleic Acids are also Polymers



DNA & RNA Polymerase: Build up DNA and RNA from nucleoside triphosphates (5' → 3' synthesis)

Convention: RNA/DNA typically is read from 5' to 3' direction (e.g. 5'-ATTGCAAC-3')

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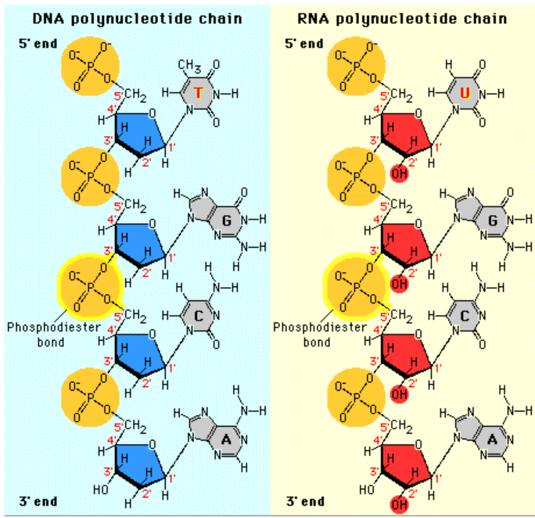
DNA vs RNA



- DNA less reactive
- RNA is easily attacked by enzymes

Science, www.phschool.com (Accessed on June 02, 2014)

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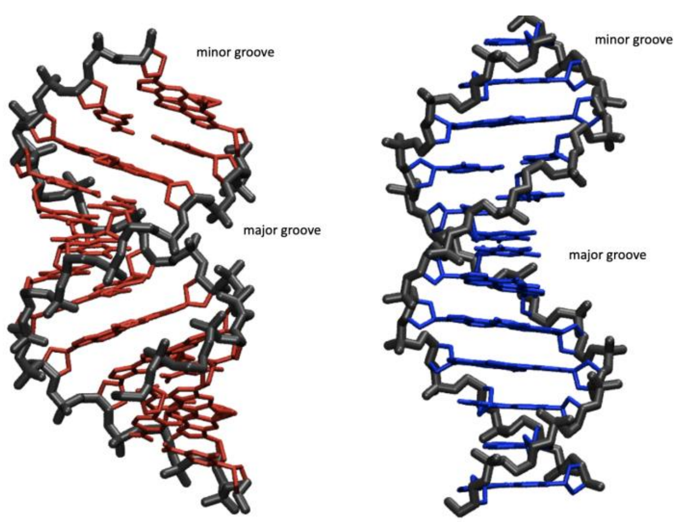
DNA and RNA are Similar but Different



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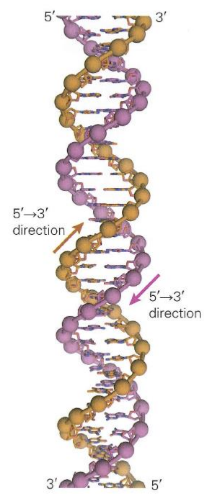
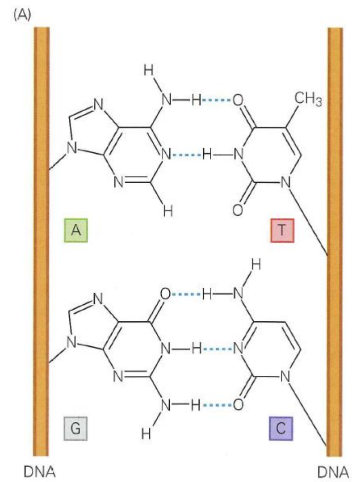
Watson –Crick Base Pairing (Antiparallel) Double Helix





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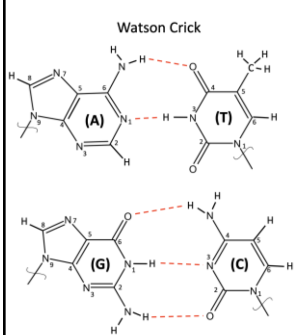
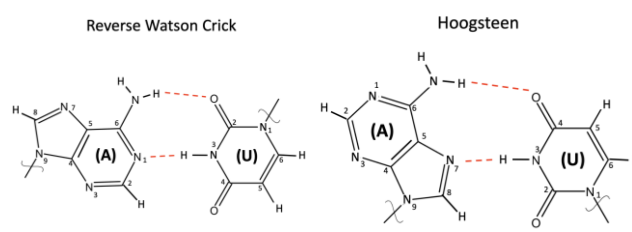
Base Pairing





- Watson-Crick base pairing
- RNA can “hybridize” with DNA, forming mixed strands
- **Example:** What’s the reverse complement to AUCCGCCTT?

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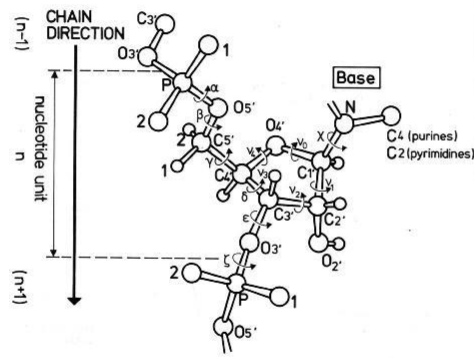
Nucleic Acid Structure

- Bases are planar
- Nucleic acids
  - 5 backbone torsion angles
- Proteins
  - 2 backbone torsion angles
- Nucleic acid structure can be much more complex compared to protein



Saenger, W. *Principles of Nucleic Acid Structure.*

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Nucleic Acid Sugar Pucker

- ν angles are related, so sugar ring can be simplified
- Think "chair" and "boat" forms of cyclohexane

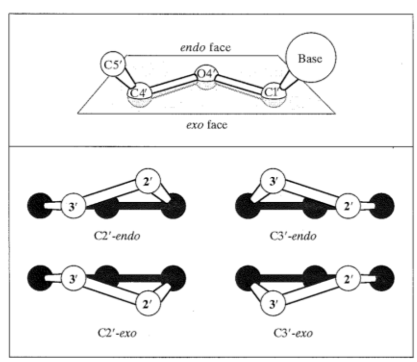

Figure 1.38 Sugar conformations of nucleic acids. The pucker of the sugar ring in RNA and DNA is defined relative to the plane formed by the C1′-carbon, C4′-carbon, and O4′-oxygen of the five-member ring. The *endo* face lies above the plane, toward the nucleobase, while the *exo* face lies below the plane.

van Holde, *et al*. *Principles of Physical Biochemistry.*

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Nucleic Acid Primary Structure

- **Just like proteins:** the sequence of bases

5’-dAdGdTdTdCdAdCdCdC-3’ (DNA)

AGTTCACCC

5’-AGUUCACCC-3’ (RNA)

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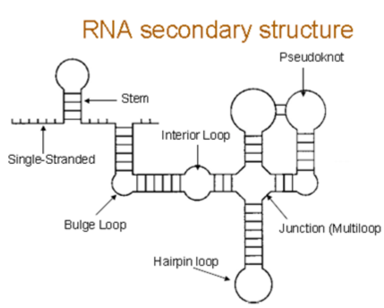
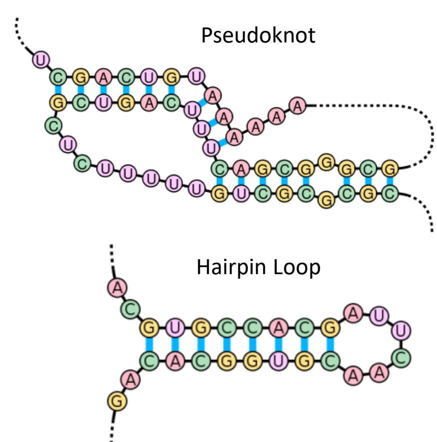
Secondary Structure





- Base pairing motifs

Source: Wikipedia, “RNA Secondary Structure,” “Nucleic Acid Secondary Structure”

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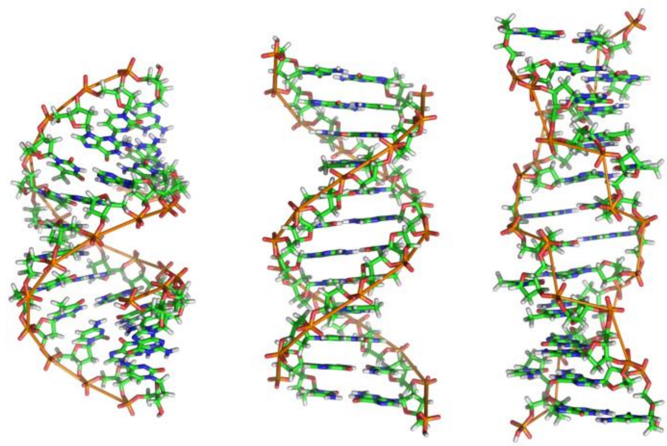
Tertiary Structure



A Form DNA B Form DNA Z Form DNA

Source: Steven Carr, www.mun.ca

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Tertiary Structure

	Average Torsion Angles for Nucleic Acid Helices (in °)						
Structure Type	**Alpha**	**Beta**	**Gamma**	**Delta**	**Epsilon**	**Zeta**	**Chi**
A-DNA (fibres)	-50	172	41	79	-146	-78	-154
GGCCGGCC	-75	185	56	91	-166	-75	-149
B-DNA (fibres)	-41	136	38	139	-133	-157	-102
CGCGAATTCGCG	-63	171	54	123	-169	-108	-117
Z-DNA (C residues)	-137	-139	56	138	-95	80	-159
Z-DNA (G residues)	47	179	-169	99	-104	-69	68
DNA-RNA decamer	-69	175	55	82	-151	-75	-162
A-RNA	-68	178	54	82	-153	-71	-158

Blackburn and Galt. *Nucleic acids in chemistry and biology.*

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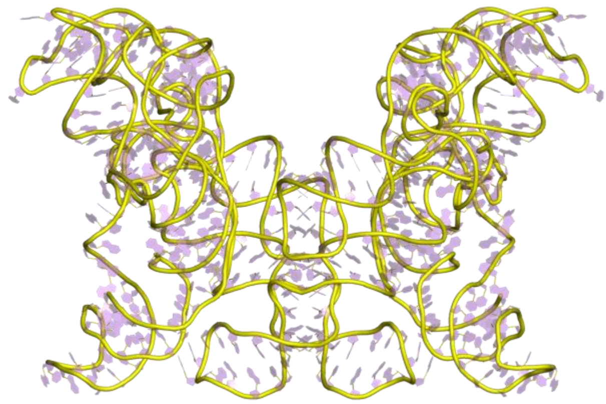
Tertiary and Quaternary Structure



Ribozyme: An RNA capable of catalyzing a chemical reaction

The ribosome contains a significant amount of RNA as well as proteins

Macromolecules can perform incredibly diverse structures!
(And we haven't even mentioned lipids and sugars.)

Wikipedia, "Group I Catalytic Intron." Accessed 8/23/2012.

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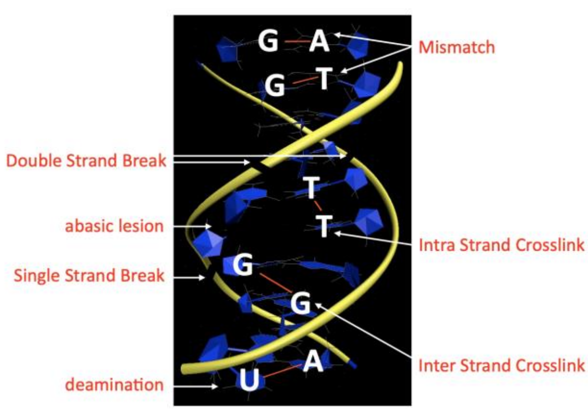
DNA Damage = Major Driving Force in Cancer



- **UV light can generate ~ 100,000 lesions per cell per hour.**
- **Healthy human cells generate ~ 10,000 lesions per cell / day.**
- **Repair pathways for fixing some but NOT all of this damage.**

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Think and Discuss

Why is DNA damage bad?

Could DNA damage ever be good?

DNA and RNA Science Can Help!

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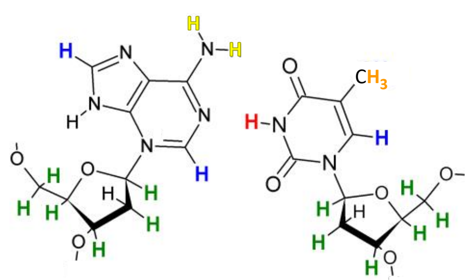
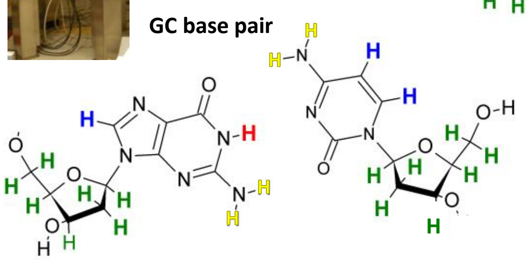
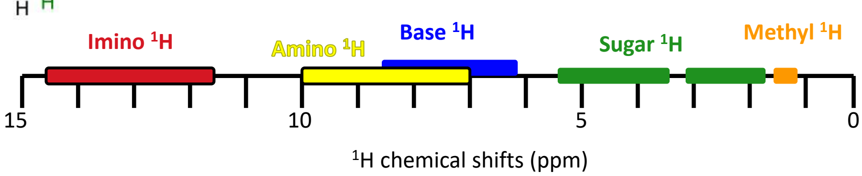
Protons provide information about structure and dynamics



AT base pair

GC base pair





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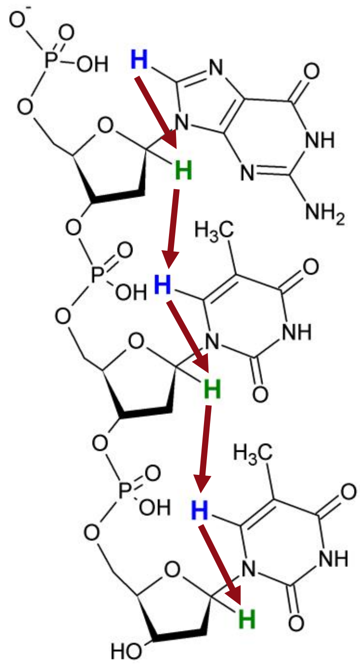
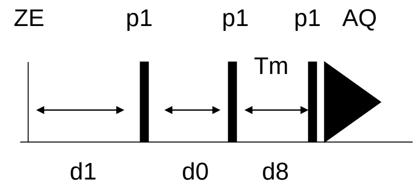
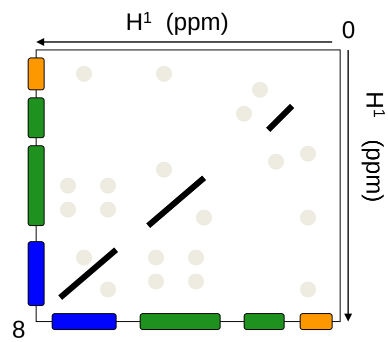
NMR: Base to sugar connectivity







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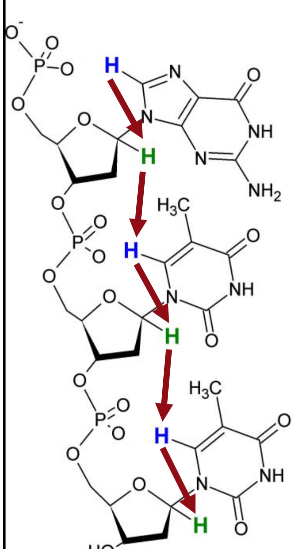
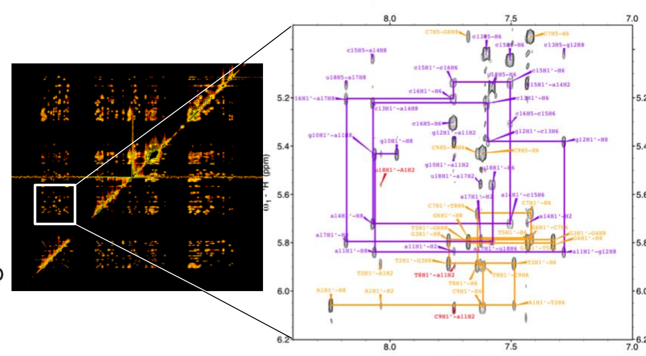
NMR: Base to sugar connectivity

A crazy game of connect the dots !!





Question: How could we use this to understand DNA damage ?

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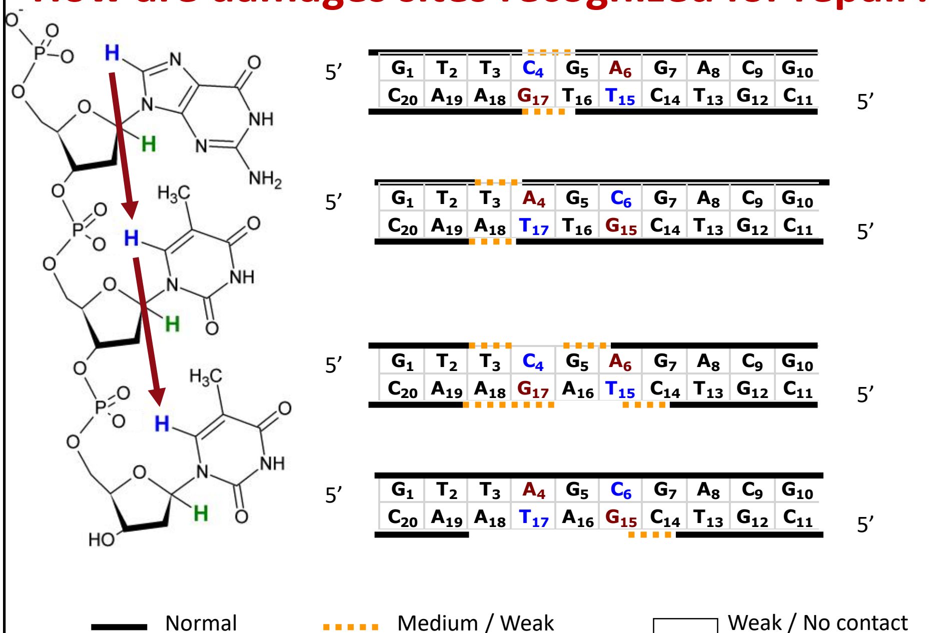
How are damages sites recognized for repair?



Mazurek et al. PNAS 2009

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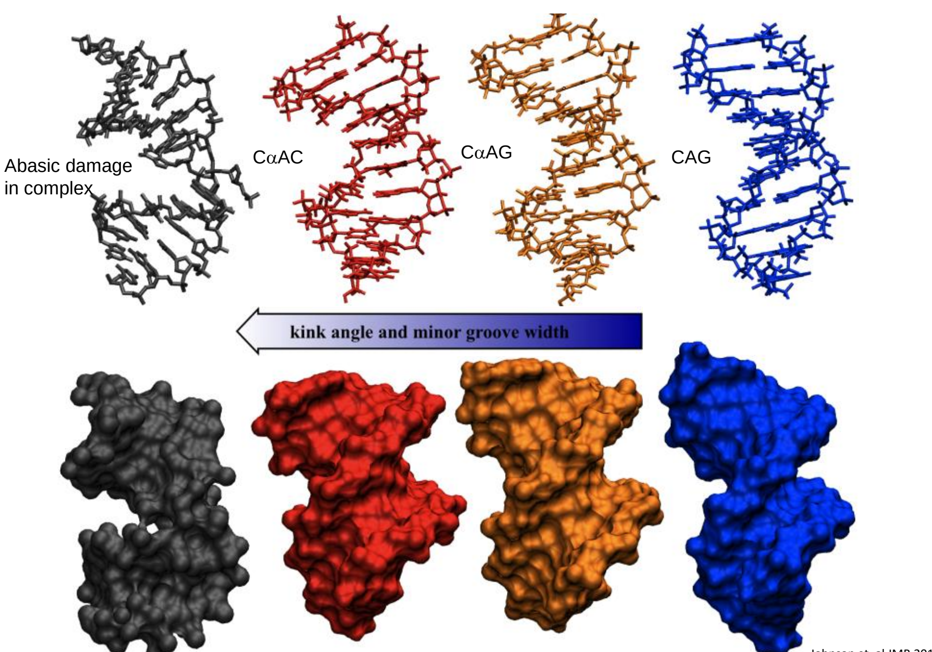
DNA as a Molecular Wire?



Johnson et. al JMB 2012

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Think and Discuss

What technologies have in part been developed based on DNA/RNA structural biology advancements?

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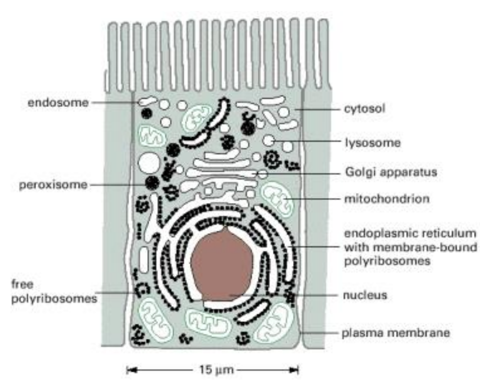
Review of Intro Biology

- Parts of a eukaryotic animal cell
- Has a nucleus where DNA is stored
- Membrane-bound organelles



Alberts, *et al*. *Molecular Biology of the Cell,* 4th Edition.

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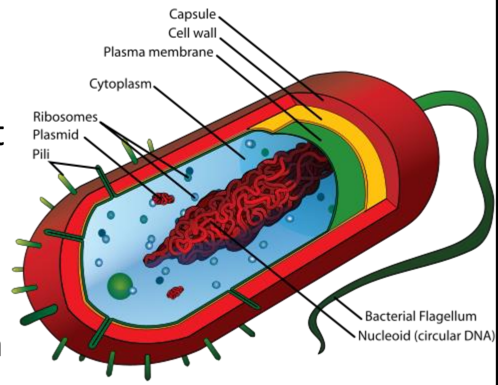
Review of Intro Biology

- Parts of a prokaryotic bacterial cell
- No nucleus: DNA is not linear but circular (no ends)
- No organelles, but ribosomes, etc. exist in the cytoplasm



Source: Wikipedia, "Bacterial Cell Structure."

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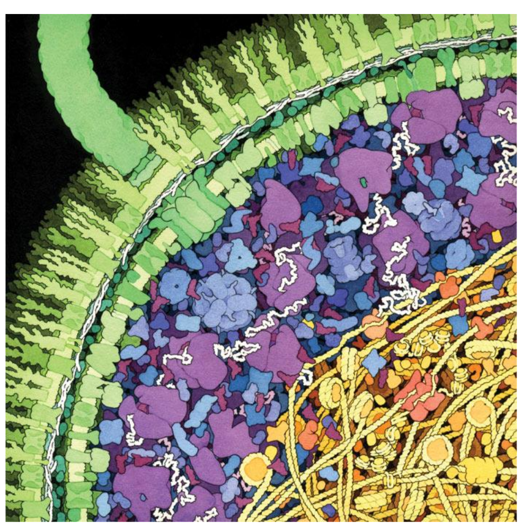
It's Crowded in There!



Source: Goodsell, D. http://mgl.sripps.edu/people/goodsell/illustration/public/

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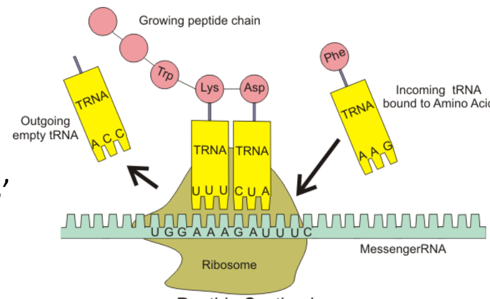
Central Dogma

- DNA → mRNA "Transcription"
  - Synthesized RNA Polymerase
  - RNA formed from 5' to 3'
- mRNA → Protein "Translation"
  - Synthesized by ribosome
  - New proteins formed from NT to CT



Peptide Synthesis

Trick: Reading the DNA in the "standard way", one can easily identify the codons for peptide synthesis.

Source: Wikipedia, "Ribosome"

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Genetic Code

nonpolar | polar | basic | acidic | (stop codon)

Standard genetic code

1st base	2nd base: U		2nd base: C		2nd base: A		2nd base: G		3rd base
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

Source: Wikipedia, "Genetic Code"

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Different Reading Frames

```
reading frame:                123
                              |||
                              acttacccggacta
first reading frame             T  Y  P  G  L
second reading frame             L  T  R  D
third reading frame               L  P  G  T
```

Source: http://www.ncbi.nlm.nih.gov/Class/MLACourse/Original8Hour/Genetics/readingframe.html

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Think and Discuss

Our biochemistry experiments are normally done in aqueous buffer. Is this a good model for the inside of a cell?

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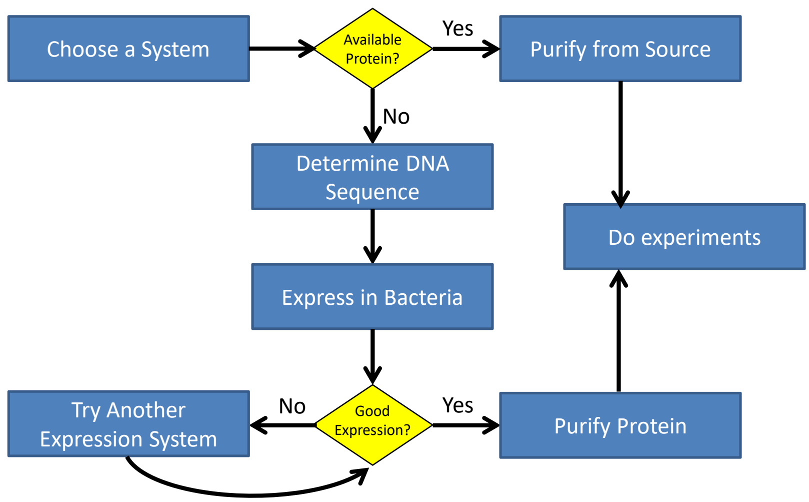
Biochemistry Research Flow Chart



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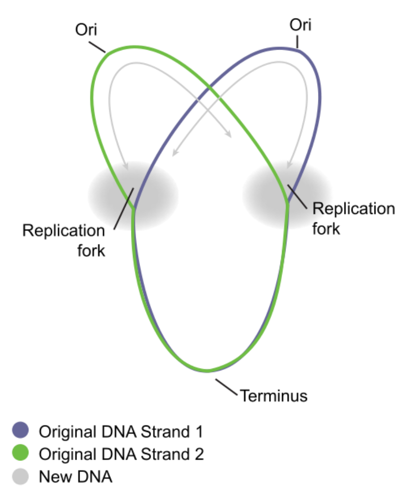
Bacterial DNA: Features

- Chromosome is *circular*
- Replication starts at the *origin of replication* (Ori, TTATCCACA)
- **Plasmid:** *Any* circular DNA in the bacterial cell can be replicated if it has an Ori



Source: Wikipedia, "Circular Bacterial Chromosome"

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The Lactose (lac) Operon

- **Idea:** Bacteria only want to produce proteins if they are needed
- Why metabolize lactose (hard) when glucose (easy) is available?
- **Operon:** A set of genes (proteins) under the control of other genes in the cell

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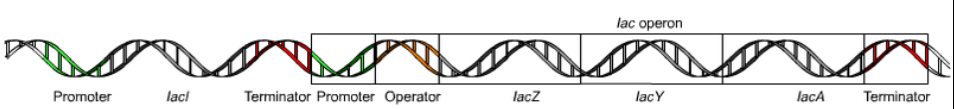
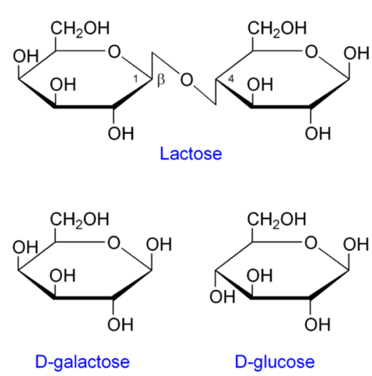
The Lactose (lac) Operon



Proteins:

- **lacI** (lac repressor): binds at operator when no lac present; prevents binding of RNA polymerase at promoter
- **lacZ** (β-galactosidase): converts Lac in to Gal and Glc by hydrolyzing glycosidic linkage
- **lacY** (β-galactoside permease): Pumps Lac into the cell



Source: Wikipedia, "Lac Operon"

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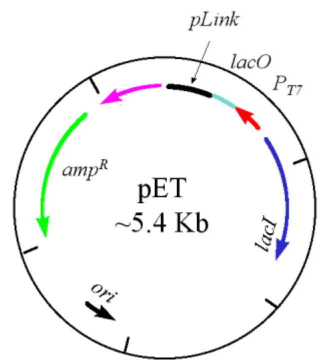
Bacterial Expression Vectors

- pET Plasmid Genes
  - Origin of replication
  - Lac repressor (lacI)
  - RNA Pol promoter (P_{T7})
  - Lac Operator (lacO)
  - Polylinker – where your DNA sequence goes (pLink)
  - Ampicillin resistance (amp^R)



- Is this plasmid persistent?

Source: Mike Blaber, BCH5425 Course Notes

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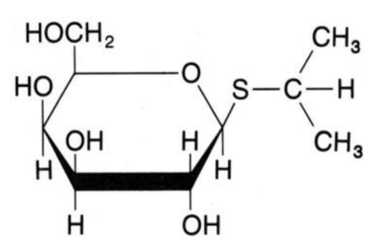
Inducible Expression

- **IPTG:** Turns on protein expression without being hydrolyzed



Isopropyl-β-D-thiogalactoside (IPTG)

- Protein expression can be switched on when desired

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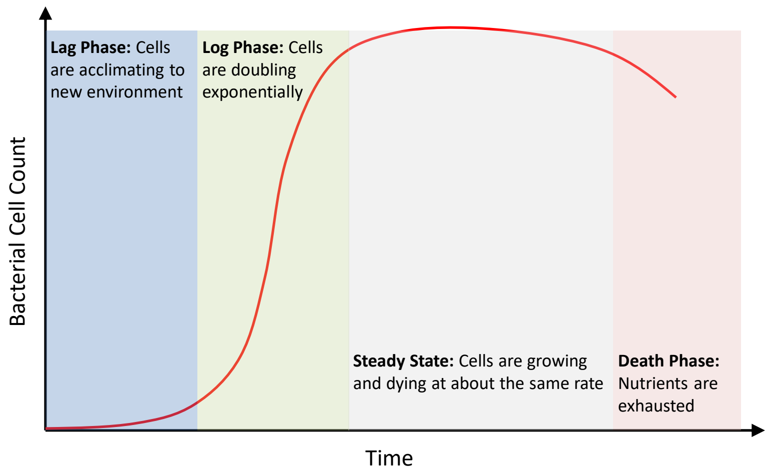
When Should I Induce?



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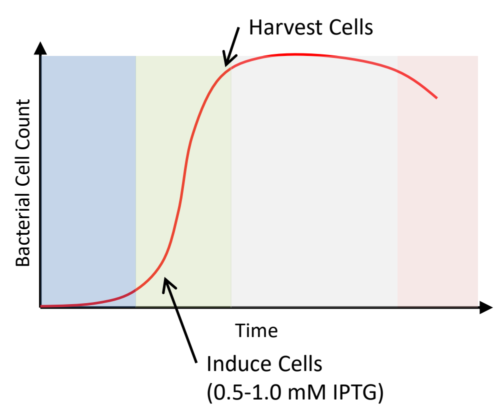
When Should I Induce?

- Protein expression is greatest during log phase
- Inducing at lag phase may unnecessarily cripple your cells
- Typically, induce at an OD_{600} of 0.5-0.6
- Always follow your lab's protocols!



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Think and Discuss

Why is Ampicillin resistance necessary for the function of the pET vector system?

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Summary

- DNA structure is as varied as protein structure, and nucleic acids can catalyze chemical reactions (“ribozymes”)
- Bacterial and animal cells store and process DNA slightly differently, although both use similar ribosomes and the same genetic code
- Modern molecular biology allows us to express virtually any gene using bacterial expression systems

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