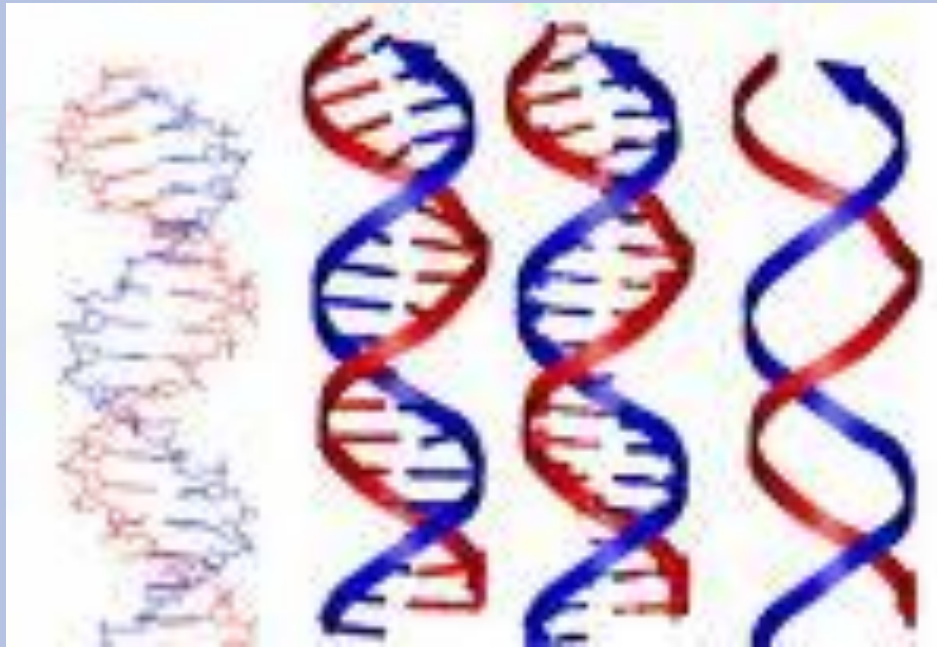


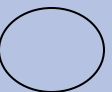
Vet-Medlink 2009 Pathology Project



Introduction and Background

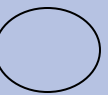
Why a Pathology Project?

- Extend your 'A' level studies
- Allow you to try some research
- Opportunity to submit a research paper and have it published
- Opportunity to make your personal statement stand out



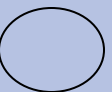
RNAi

RNA interference



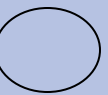
DNA

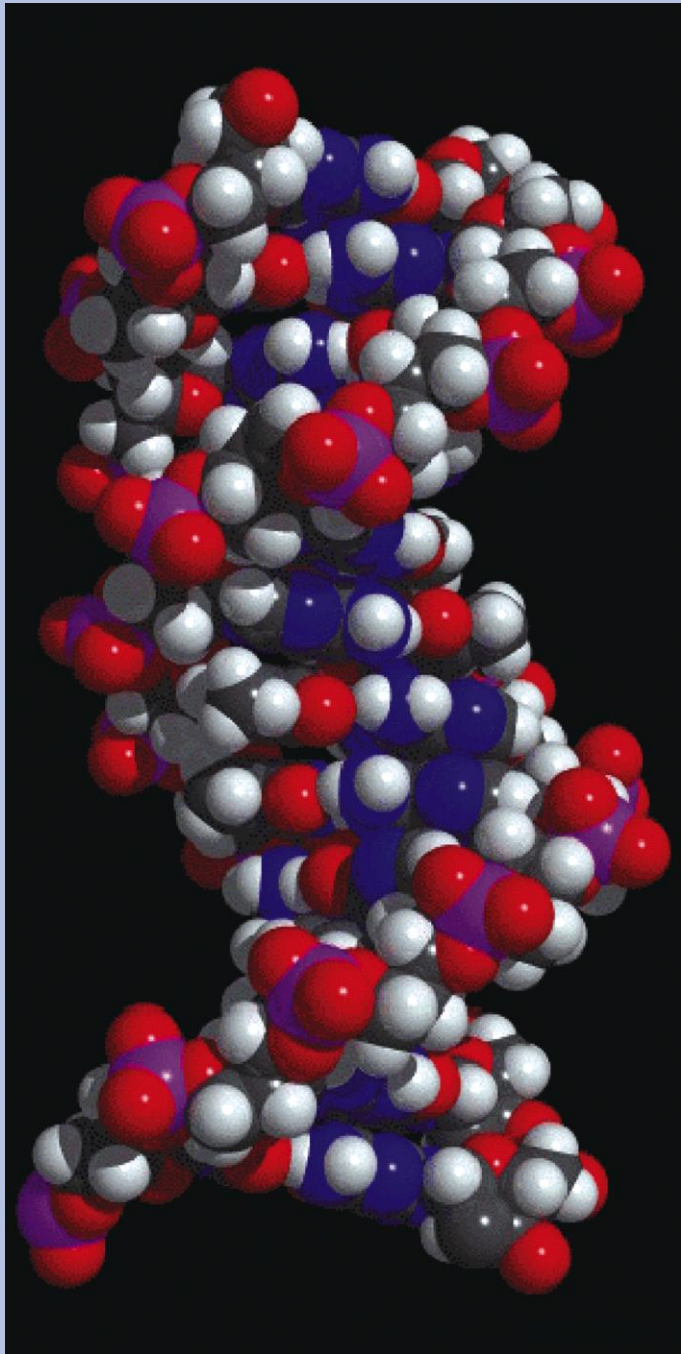
Deoxyribose Nucleic Acid



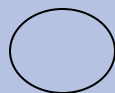
DNA

What does DNA do?

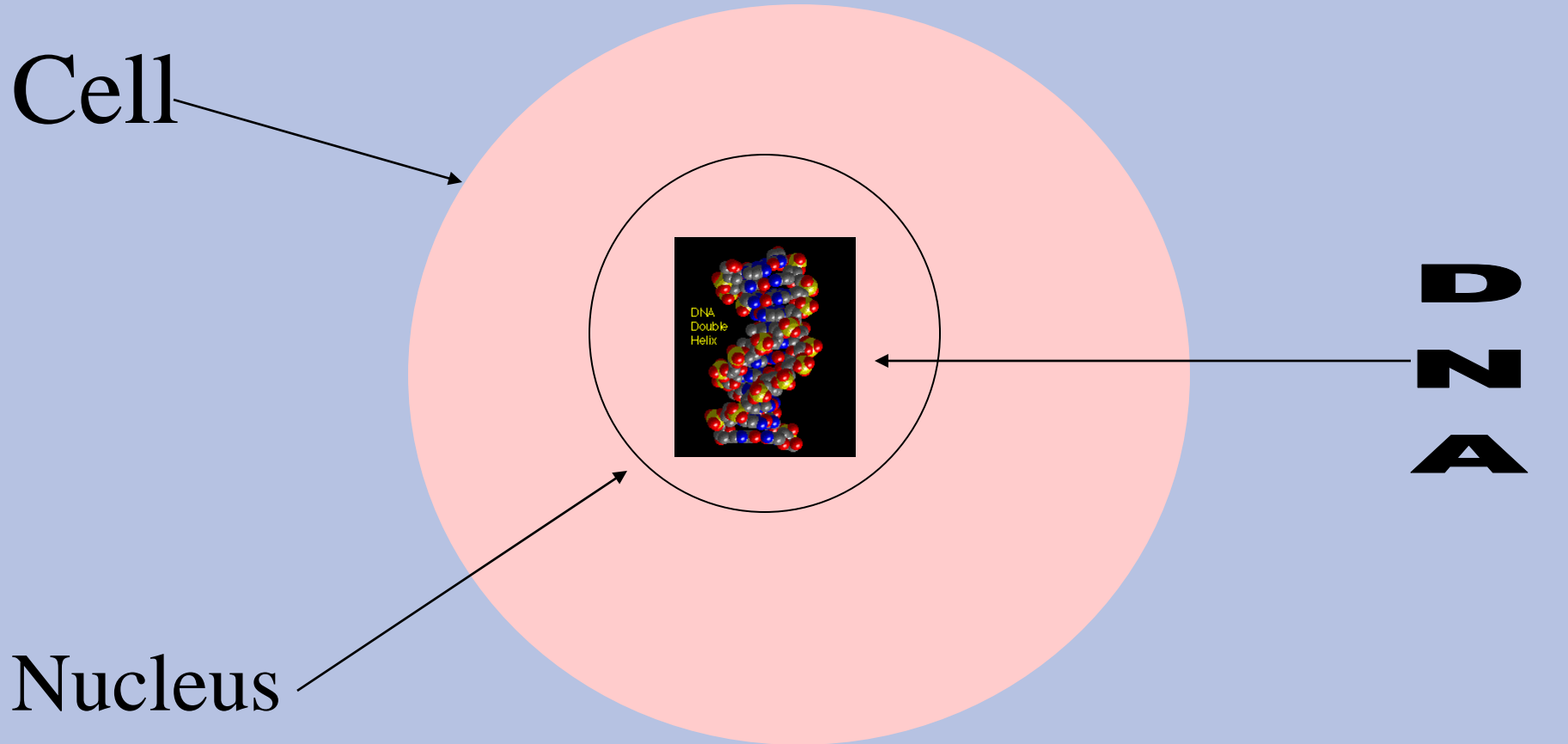




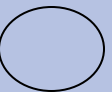
**A
N
D**

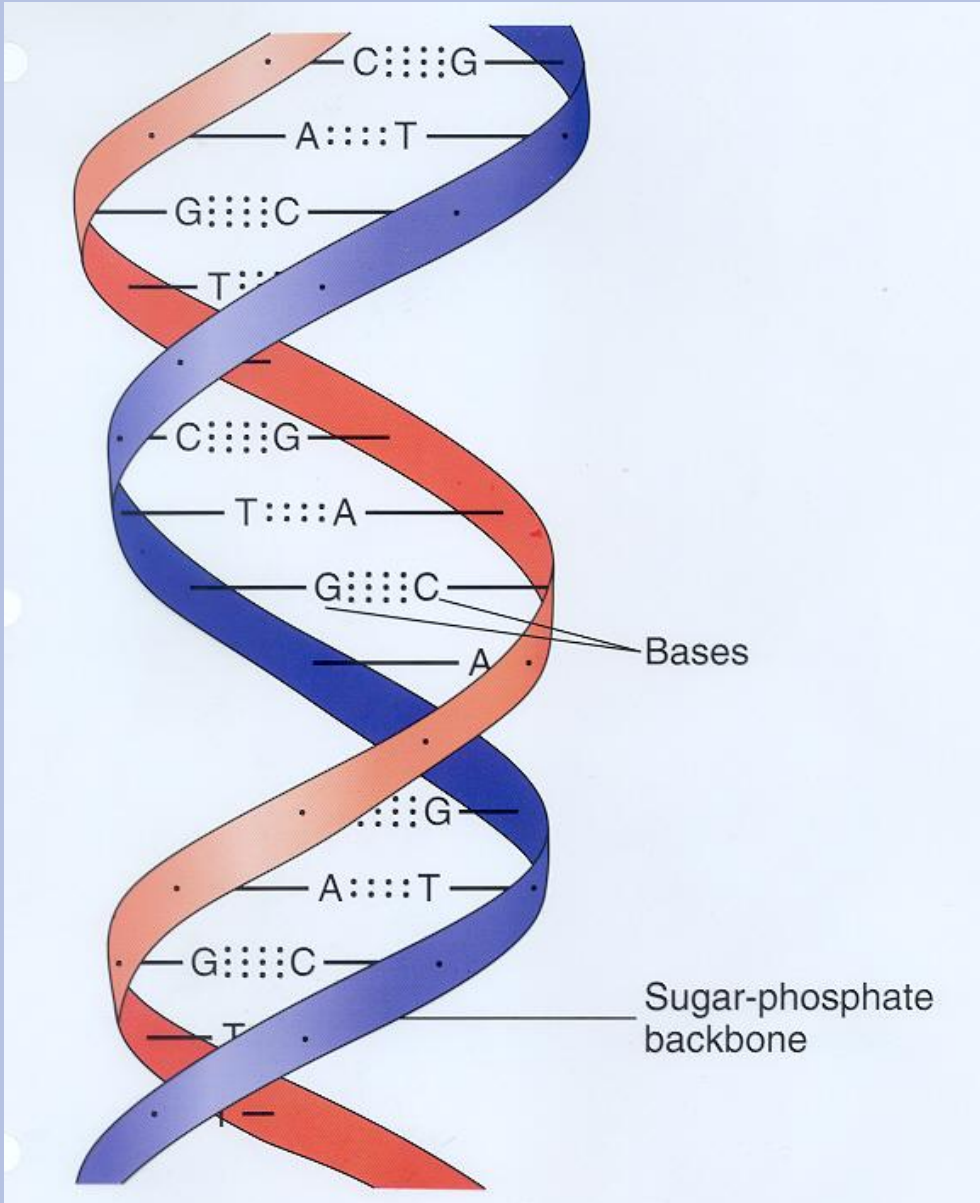


The Basics of DNA

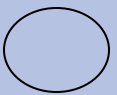


D
N
A

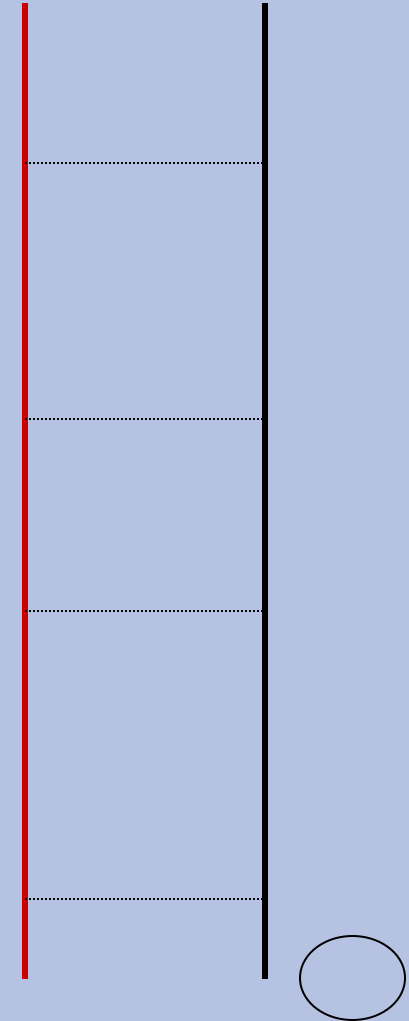
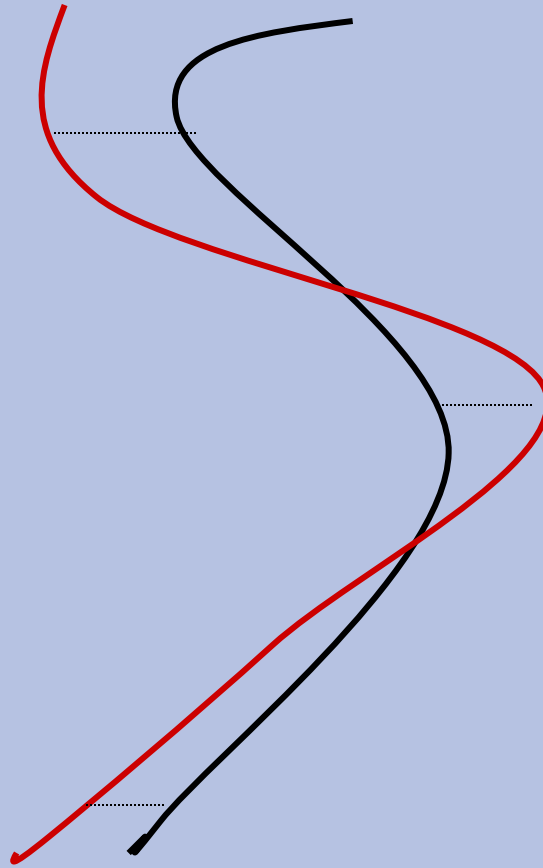
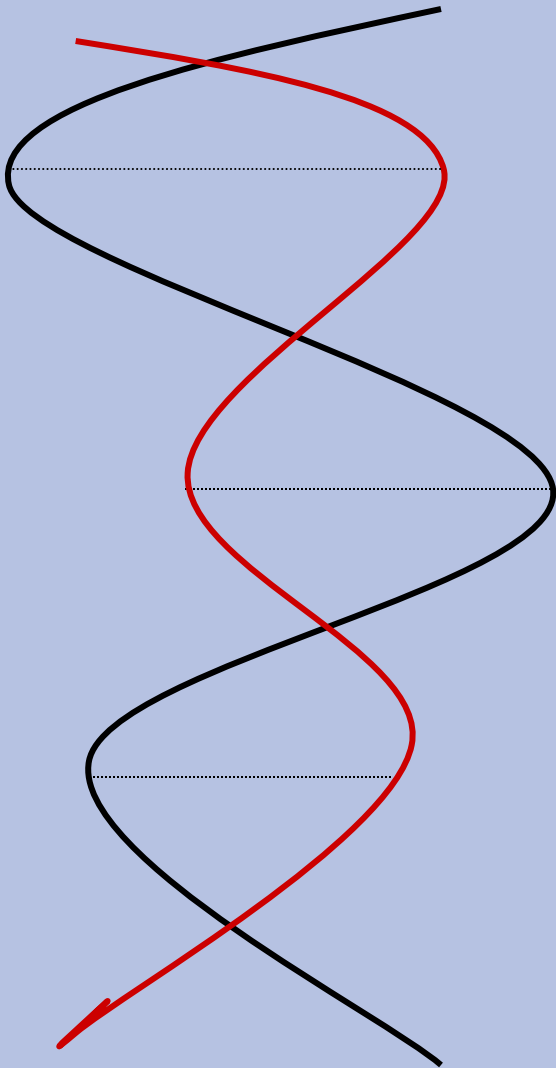




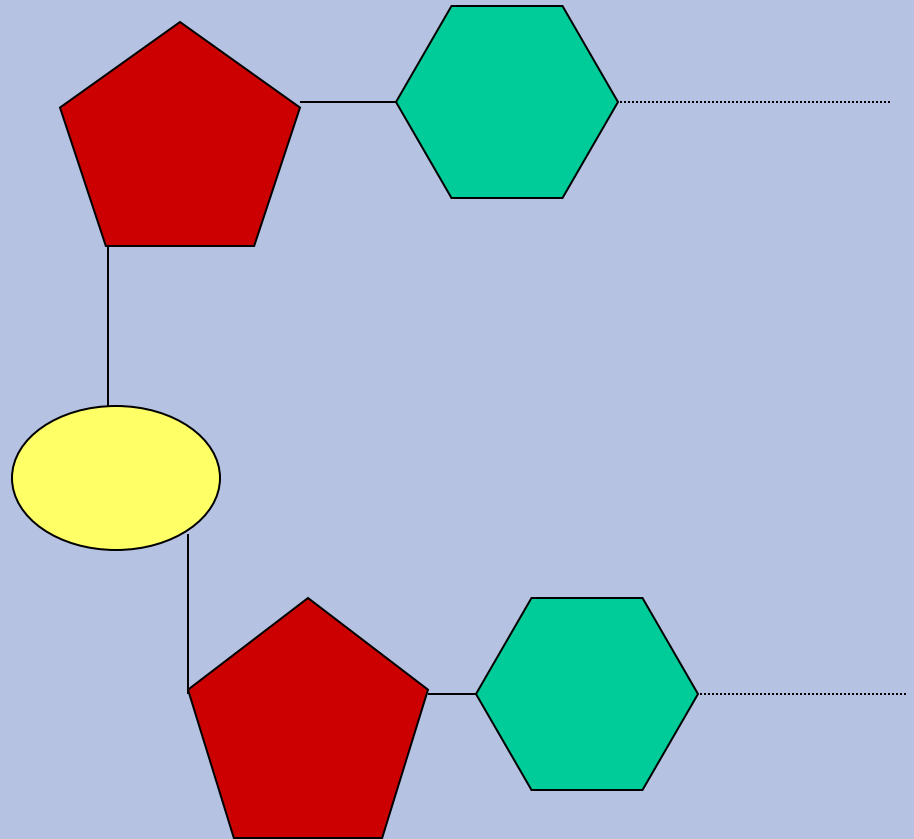
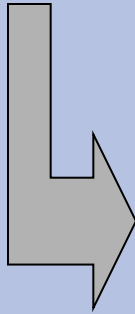
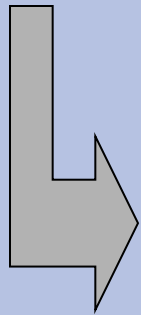
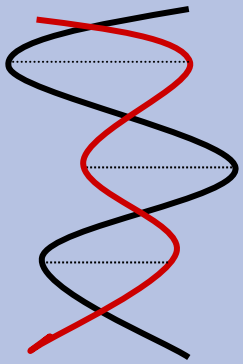
D
N
A



Unwind the Double Helix

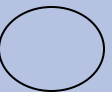


Single Strand of DNA



DNA Strand

Molecular View

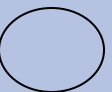


Nucleotides

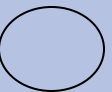
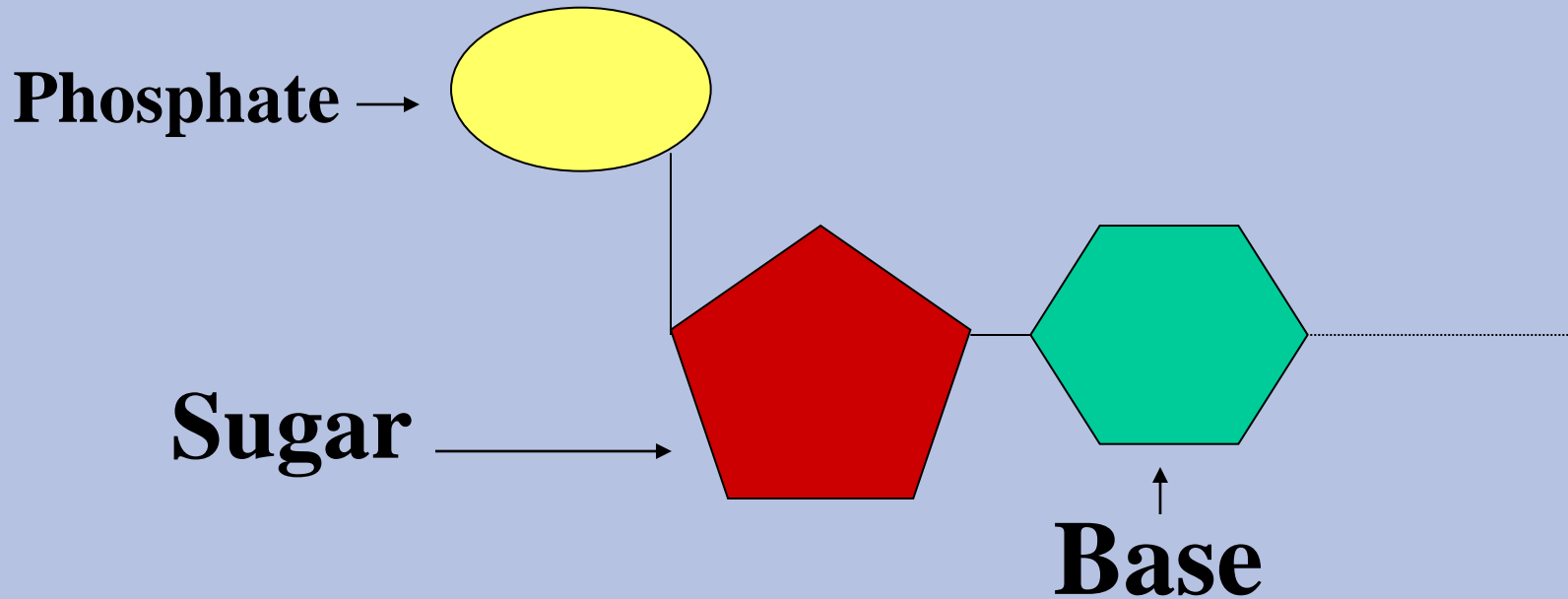
DNA & RNA

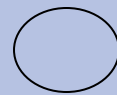
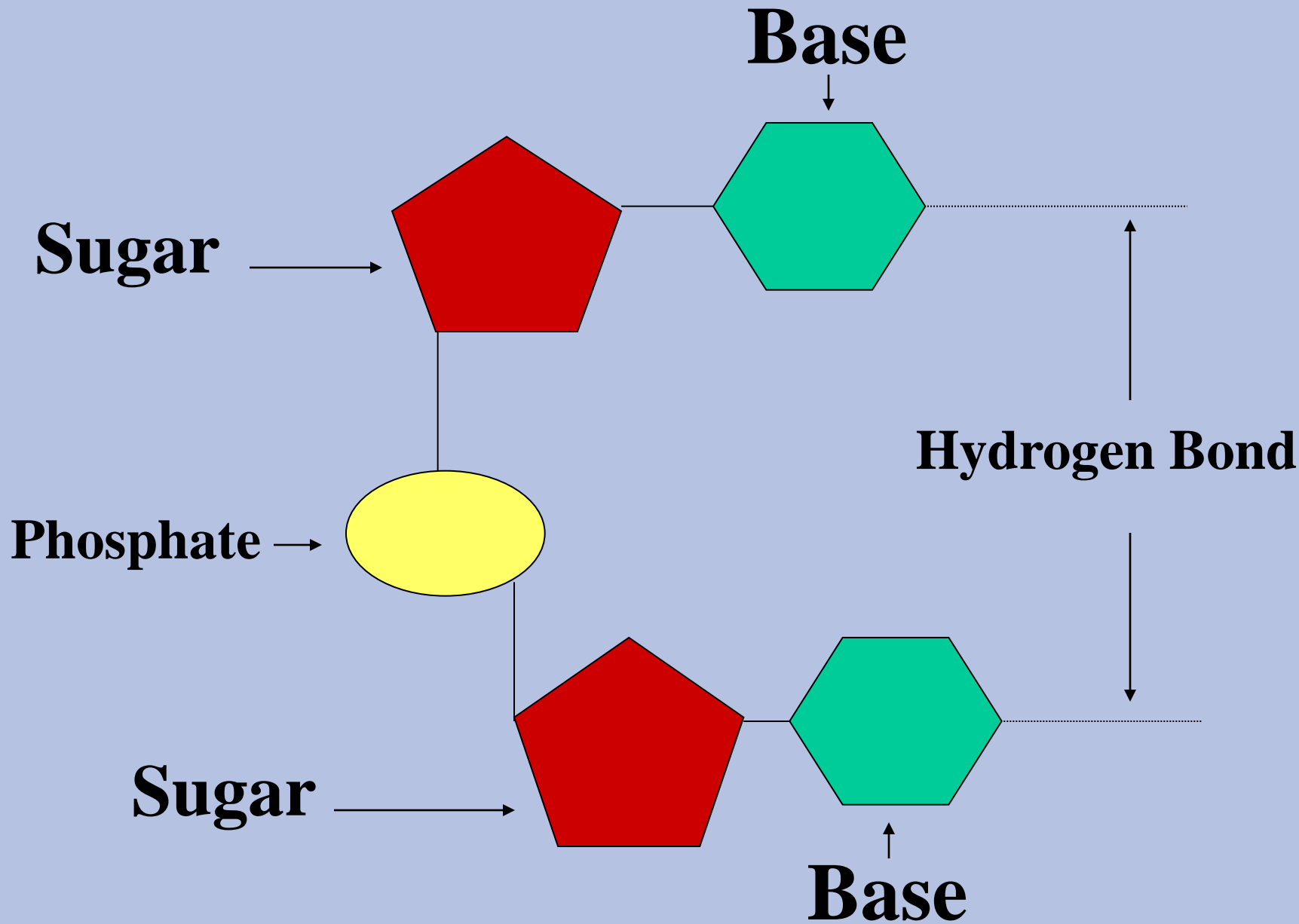
Are polymers (chains) of nucleotides

- *Nucleotides are made of the following:*
 - A Base
 - A Sugar
 - A Phosphate



A Nucleotide





BASES

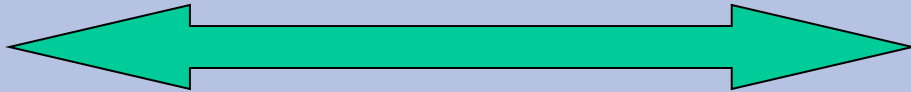
A



T

Adenine

G



C

Guanine

T



A

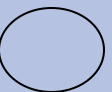
Thymine (Uracil in RNA)

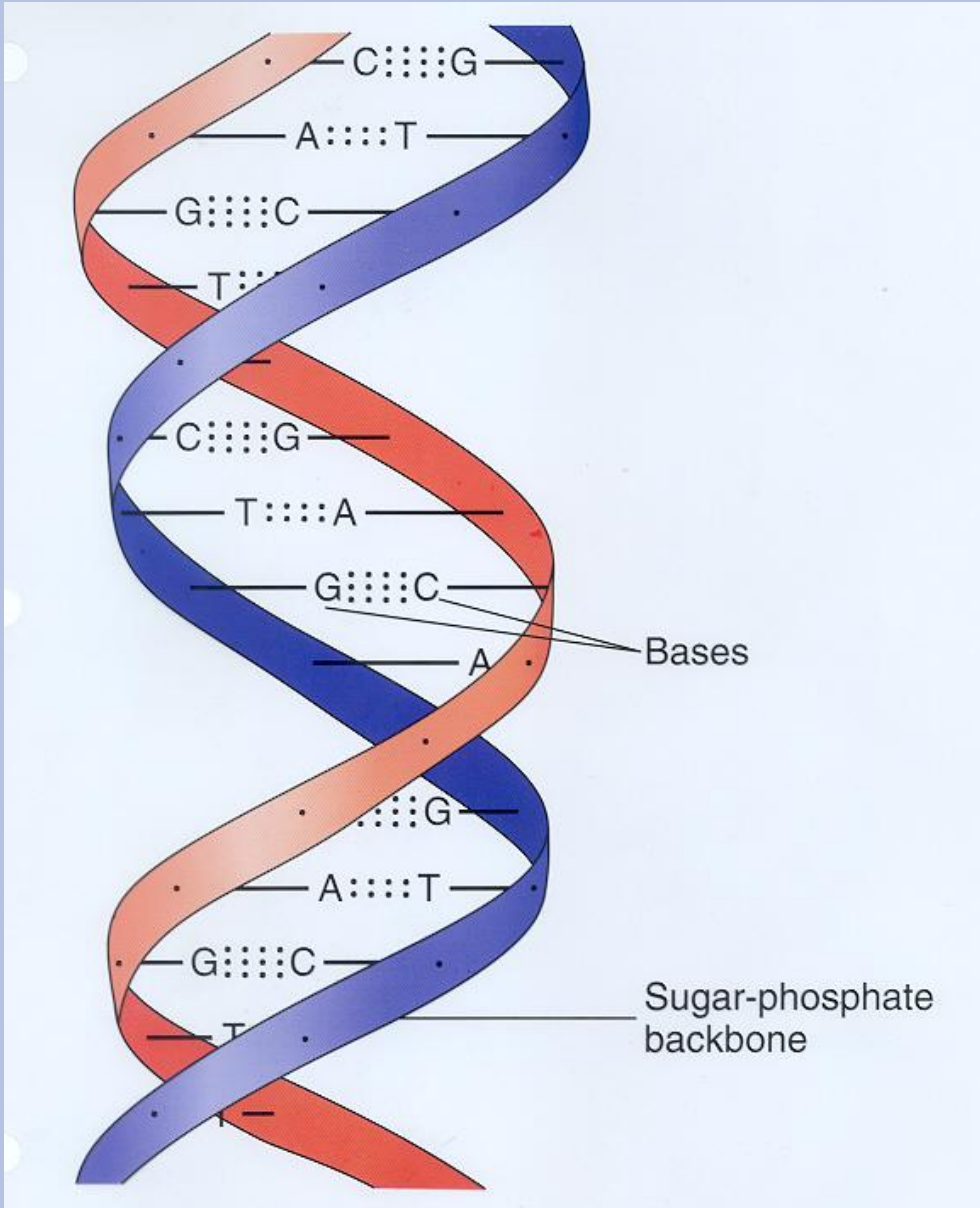
C



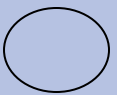
G

Cytosine

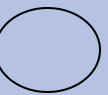


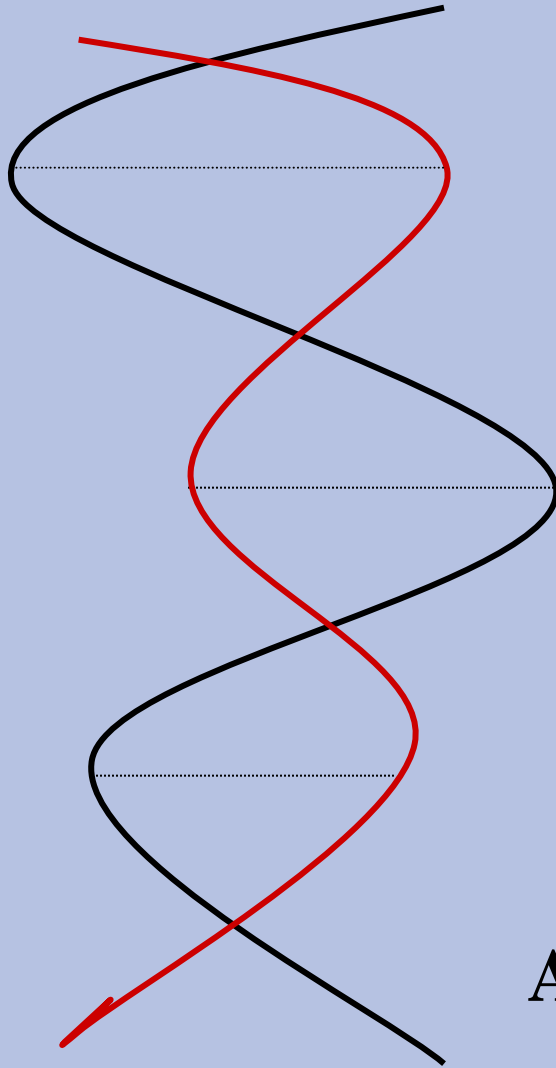


A
Z
D



**The Role Of DNA
In
The Cell**





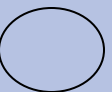
Genes are sections of DNA



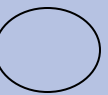
That encode for proteins e.g. enzymes



Allowing & controlling 1000s of complex chemical reactions in the cell.



Genes
To
Proteins
The
Process



DNA



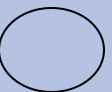
Transcription



Translation



Protein



TRANSCRIPTION

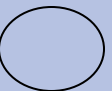


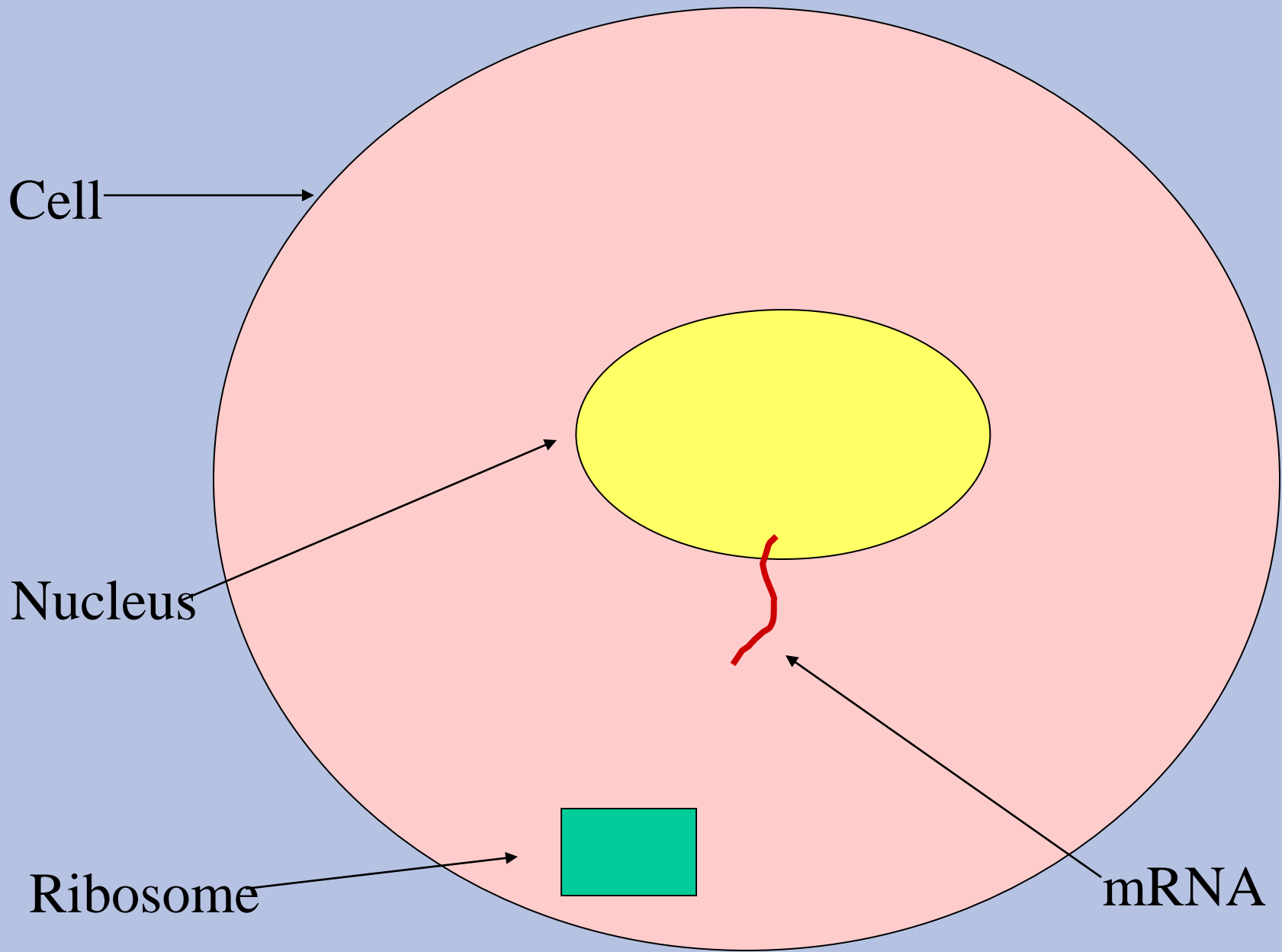
DNA spirals separate (RNA polymerase)

Copies base instructions to:



mRNA (messenger RNA)



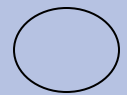


Cell

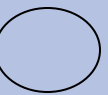
Nucleus

Ribosome

mRNA



**TRANSLATION
TAKES PLACE
AT THE RIBOSOME
WHEN THE MESSENGER
RNA ARRIVES
PRODUCING NEW
PROTEINS**



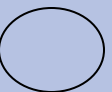
TRANSLATION

COULD BE STOPPED

AT THE RIBOSOME

IF THE mRNA

NEVER ARRIVES



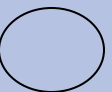
**This means any gene can be
switched off at any time**

A Cure for Cancer

A Cure for foot and mouth

A Cure for any Virus

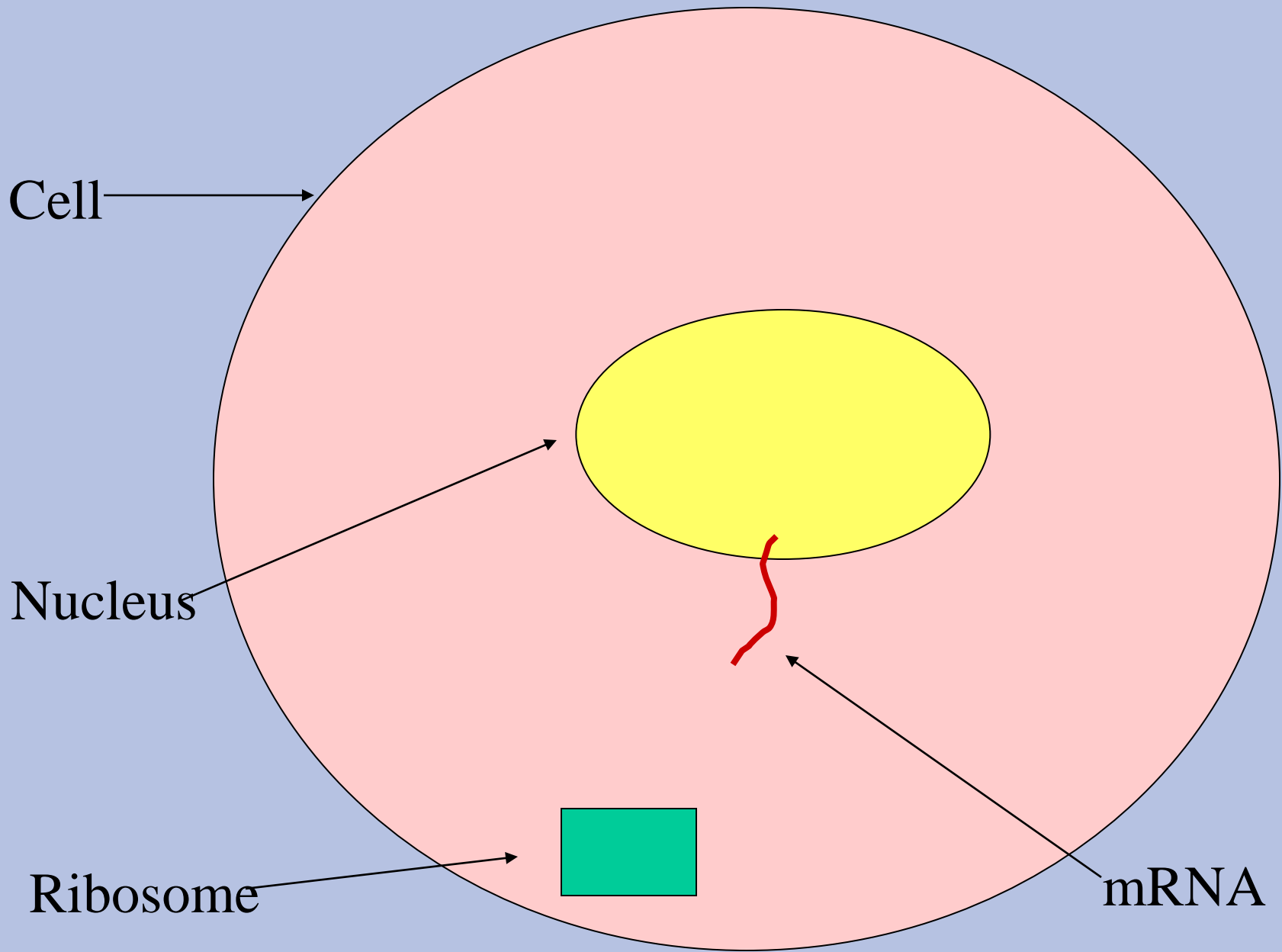
A Cure for ?



RNAi

Responsible for
Turning Genes Off





REVIEW SO FAR

Transcription Process

Halted



How Does RNAi Work?

The Discovery



How to make Petunias a deeper purple?



**Add in extra genes responsible for
the colour**

The result?



This effect is called co-suppression

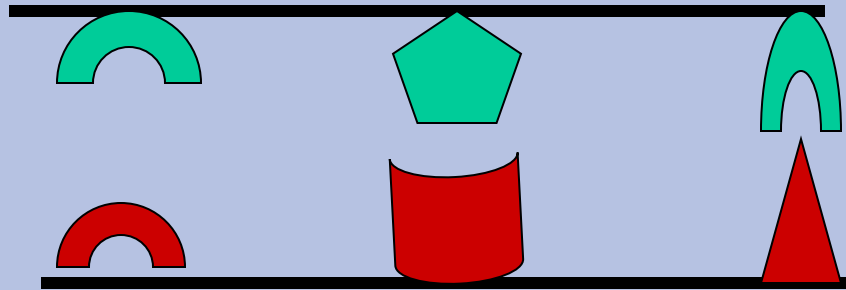


Viral Defence
In
Plants and
Invertebrates



1. Viral forms contain RNA
2. A virus has a characteristic genetic
...fingerprint
3. Some viral forms are double-stranded

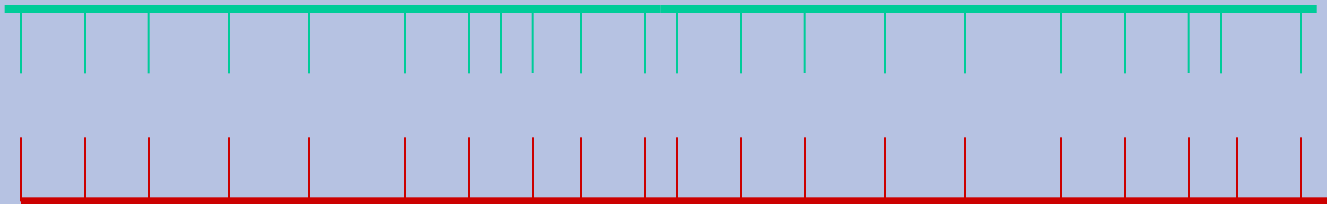




dsRNA

Double-Stranded
RNA

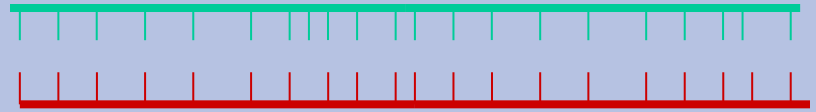
Invades the cell



The Defence



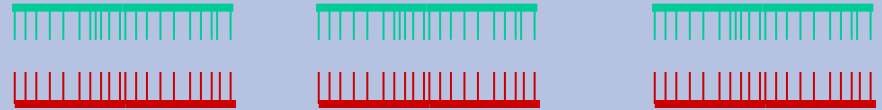
dsRNA invades the cell



Is identified → 2 → Enzyme DICER chops dsRNA

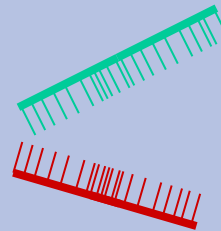


Activates
RISC

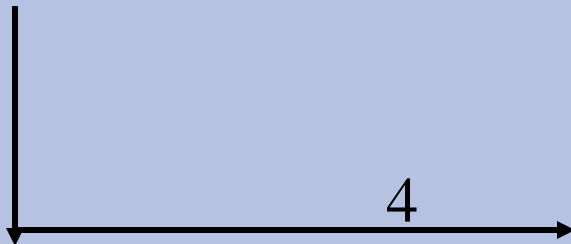


RNA-induced Silencing
Complex

3



{ RISC opens the strands and copies fingerprint



Matching RNA destroyed



BUT
Humans
DO NOT
EMPLOY
This Defence



Viral Defence in Humans

Dicer and RISC are *both* present in higher animals

BUT: The presence of long chains of dsRNA provokes a complex **interferon** based inflammatory response that **shuts** down **all protein production**



THE PROBLEM



The RNAi pathway does exist in humans

Specifically cells, via RNAi pathway, can ‘learn’ and destroy specific genetic fingerprints carried by foreign RNA *AND VERY IMPORTANT their own mRNA*

BUT the *RNAi pathway does not come into play* because the presence of *long strands of dsRNA triggers the interferon response*, shutting everything down



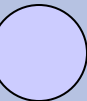
The Solution



Introduce **short double strands** of RNA into the cell (**siRNA**) smaller than 23 nucleotide pairs in length.

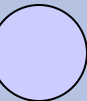
siRNA does **NOT** trigger the *inflammatory* response

But **DOES** trigger the *interference* response.



In 1998 Richard Fire and Craig Mello of The Stanford School of medicine publish an article in Nature describing how RNAi works from their studies in nematodes - worms.

In 2006 Richard Fire and Craig Mello are awarded the Nobel Prize for Medicine for their pioneering work

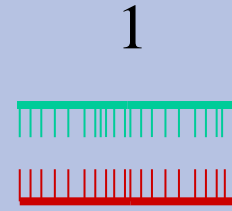


WHY
is this a
Revolution in
Medicine?



siRNA introduced into cell

With *specific* fingerprint



E.g. foot and mouth virus

Is identified

2

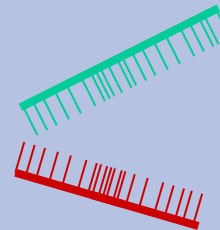
Activates

RISC

RNA-induced Silencing

Complex

3



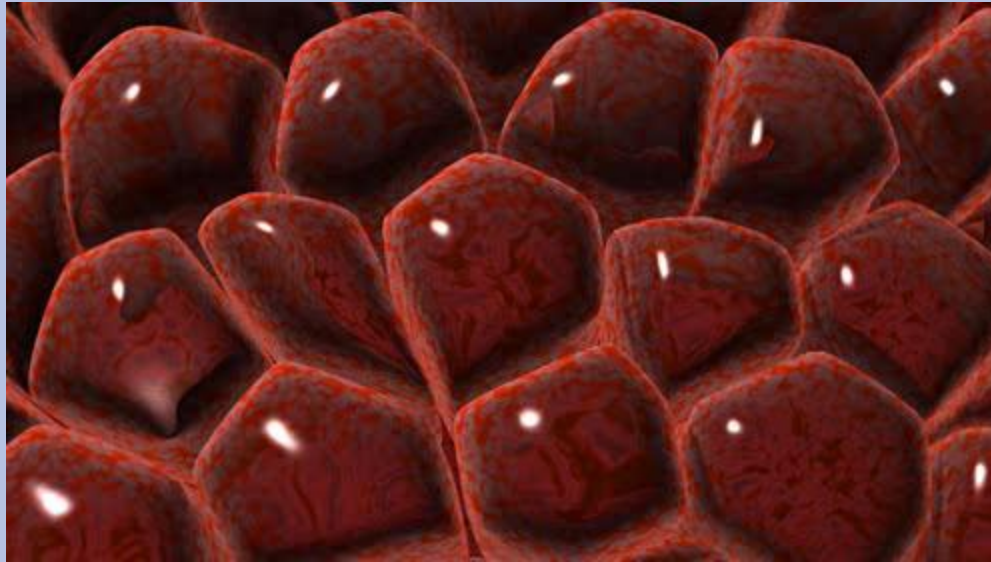
RISC opens the strands and copies fingerprint

Matching RNA destroyed

Including *mRNA* ***i.e. can turn off ANY gene***



RNA interference – the movie



THE POSSIBILITIES

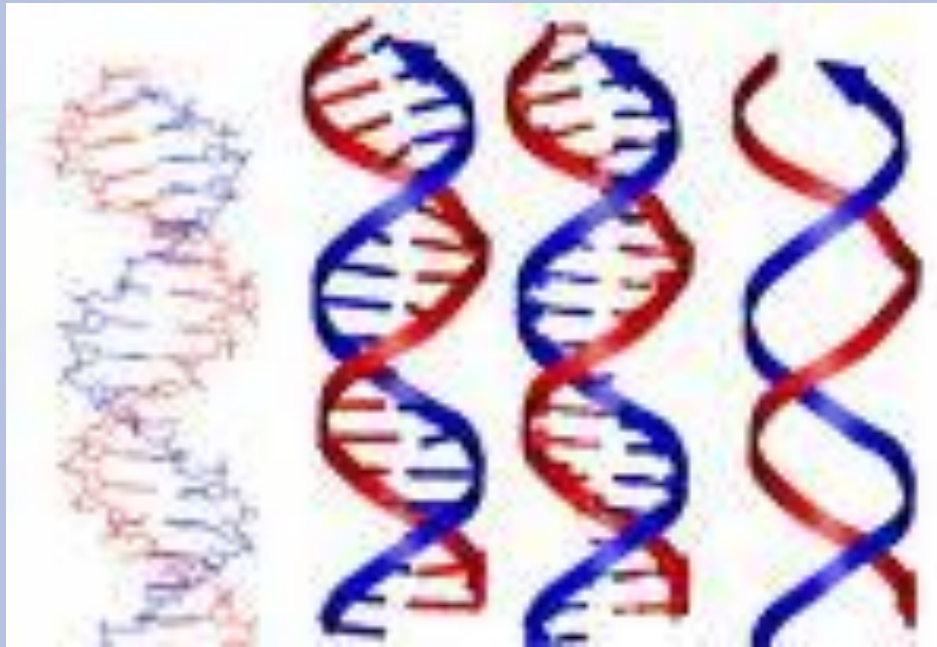


SUGGESTIONS:

- **Stop** almost any virus
- **Turn off** cancer cells
- **Find** the function of a gene
- **And** ???
- **More in Part 2**



Vet-Medlink 2009 Pathology Project



Current Research

PROBLEMS TO SOLVE



Problems

- How to deliver the siRNA complexes to all cells requiring them?
- siRNA are very short and very specific, how to cope with changes in say viral RNA?
- This does NOT always cure and it may be necessary to keep a level of siRNA present ----- how?



YOUR RESEARCH



Project Suggestions:

- **Solve** one of the above **problems** using a **specific** example, e.g. Foot and Mouth, HIV
- Show how *gene silencing* could solve a problem in chronic disease conditions, e.g. Hepatitis B (HBV) is a result of the activation of the protein Fas, which mediates cellular death.
- Propose and outline a **novel use** for the above method of gene silencing.
- Consider some of the ethical issues involved in this research or possible future applications



The Research Project

Now it is YOUR turn!

Question:

How can I carry out a sophisticated research project with no lab, no budget and limited time?

Answer:

Do a *Gedankenexperiment*

Gedankenexperiment

Gedanken: German for *thought*
hence *Thought Experiment*

Mostly used in physics a
Thought Experiment aims to solve
theoretical problems through
reasoning based on known facts

The Research

- Follow up from this lecture with your own research - e.g. Google
- Starting with any of my suggestions, or those arising from your research propose your own uses of RNAi research
- No 'Star Trek' science permitted!
- Feel free to explore ethical issues

The Research



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The RNA Interference Resource

RNA Interference (RNAi): The mechanism by which double-stranded RNA (dsRNA) specifically suppresses the expression of a gene bearing its complementary sequence.

New to RNAi? Start Here!

[The Mechanism of RNA Interference \(RNAi\)](#)

[RNAi: A "How To" for New Users](#)

[Getting Started with RNAi](#)

[siRNA Decision Tree](#)

[RNA Interference and Gene Silencing: History and Overview](#)

Ambion Articles

[Top Ten Ways to Optimize siRNA Delivery in Cultured Cells](#) (5/06)

[Define the Best Conditions for siRNA Delivery in Cultured Cells](#) (5/06)

[siRNAs in the Format You Need](#) (5/06)

[Silencer® siRNA Libraries and RNA Interference Screening Projects](#) (5/06)

[Getting Started with RNAi](#) (1/06)

[\[more \(categorized\)...\]](#)

Ambion Publications

[High-throughput RNAi screening in vitro: From cell lines to primary](#)

Ambion Resources

[siRNA Database](#)

[siRNA Design Tools](#)

[RNAi Reference List](#)
Updated every 2 weeks

[NEW! In Vivo siRNA Resource](#)

[siRNA Delivery Resource](#)

[RNAi Research Guide \(PDF\)](#)
[\[Request a hardcopy\]](#)

[Request a Free RNA Silencing Poster](#)

Ambion Products

[siRNA Products & Services](#)

[All RNAi Products](#)

NEW!

[Silencer® Human Phosphatase siRNA Library V3--0.25 nmol size](#)
A set of >800 siRNAs targeting 267 human phosphatases. For your convenience, the siRNAs (0.25 nmol of each) are delivered dried in the wells of 96 well plates.

[\[more NEW products...\]](#)

Silencer E-newsletter

An email newsletter containing our most up-to-date information about RNAi and our RNAi products.

[Silencer Archive](#)
[Subscribe](#)

Ambion Webcasts

[RNAi Basics and siRNA Experimental Design](#)
Broadcast January 12, 2006
(WebEx Format)

Ambion Press Releases

[Ambion to Provide MitoCheck Consortium a Genome-Wide siRNA Library](#) - 7/12/05

[Ambion Kinase siRNA Library Used in Key Cellular Trafficking Study](#) - 7/7/05



RNAi in the News

[Developing axons and growth cones display RNA interference](#) 7/13/06

[Pathway Toward Gene Silencing Described In Plants](#) 7/13/06

[Interfering RNA Silences Genes In 'Slippery' Immune Cells](#) 5/8/06

[RNA Interference Genetic Screen Suggests New Targets For Cancer Therapies](#) 3/29/06

[New RNAi Tools Enable Systematic](#)

The Research Report

- Use the VetMedlink site for information
- Details lecture slides, notes, references etc
- How to submit: by Word attachment to email
- When to submit: by Sunday March 14 2010
- Teamwork is good, expected, but not penalised
- Format of paper & length of paper - template
- Electronic publication details
- Notice board for self-support and questions

The Research Report ... and finally!

- When to submit by: Sunday March 14 2010
- Maximum length 4000 words
- Details on website from Jan 21 2010
- Teamwork - collaborate between schools
No more than 3 in a team
- Notice board for self-support and questions
- Marked by July 1 2010
Best papers published by September 2010

