Lecture 3:

History and Review (II)

DNA the code

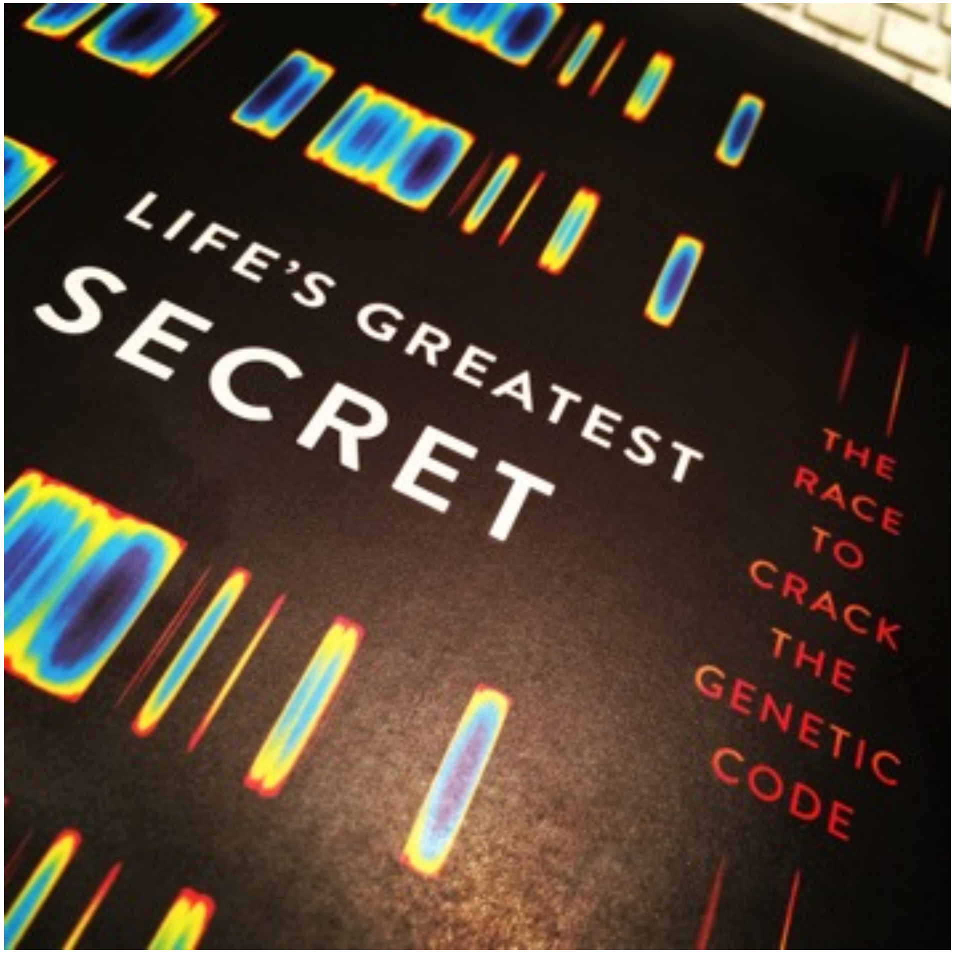
Course 485

Introduction to Genomics

AIMS

- Review concepts related to:
    - Chemical composition of DNA and RNA
    - Double helix DNA structure
    - Genome organizations
    - Histone modifications
    - Crick’s Central Dogma
    - Decoding the code

Good Reads



Life’s Greatest Secret: The Story of the Race to Crack the Genetic Code by Matthew Cobb

What is nucleic acid made of?

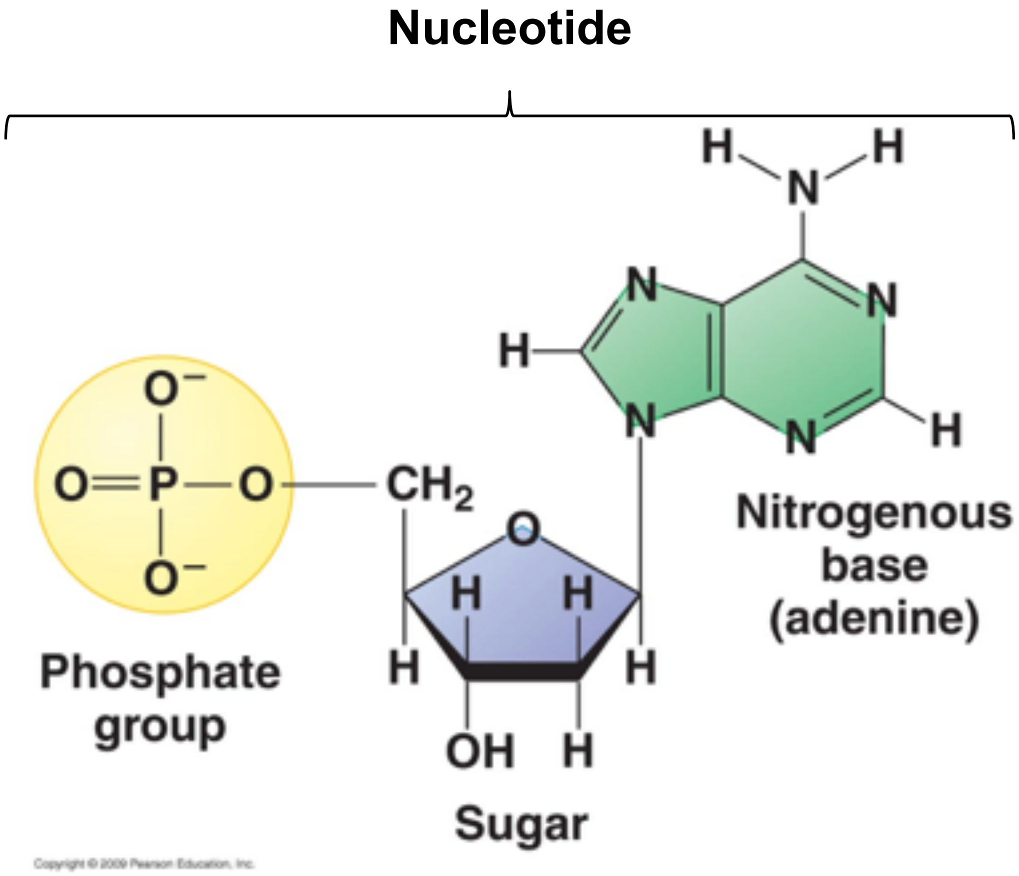
What is the chemical composition of nucleic acids (DNA and RNA)?

DNA and RNA chemical unit

- The chemical unit that makes nucleic acids (DNA and RNA) is called **Nucleotide.**

- A **nucleotide** is composed of:

1. Sugar
2. Phosphate group
3. Nitrogenous base



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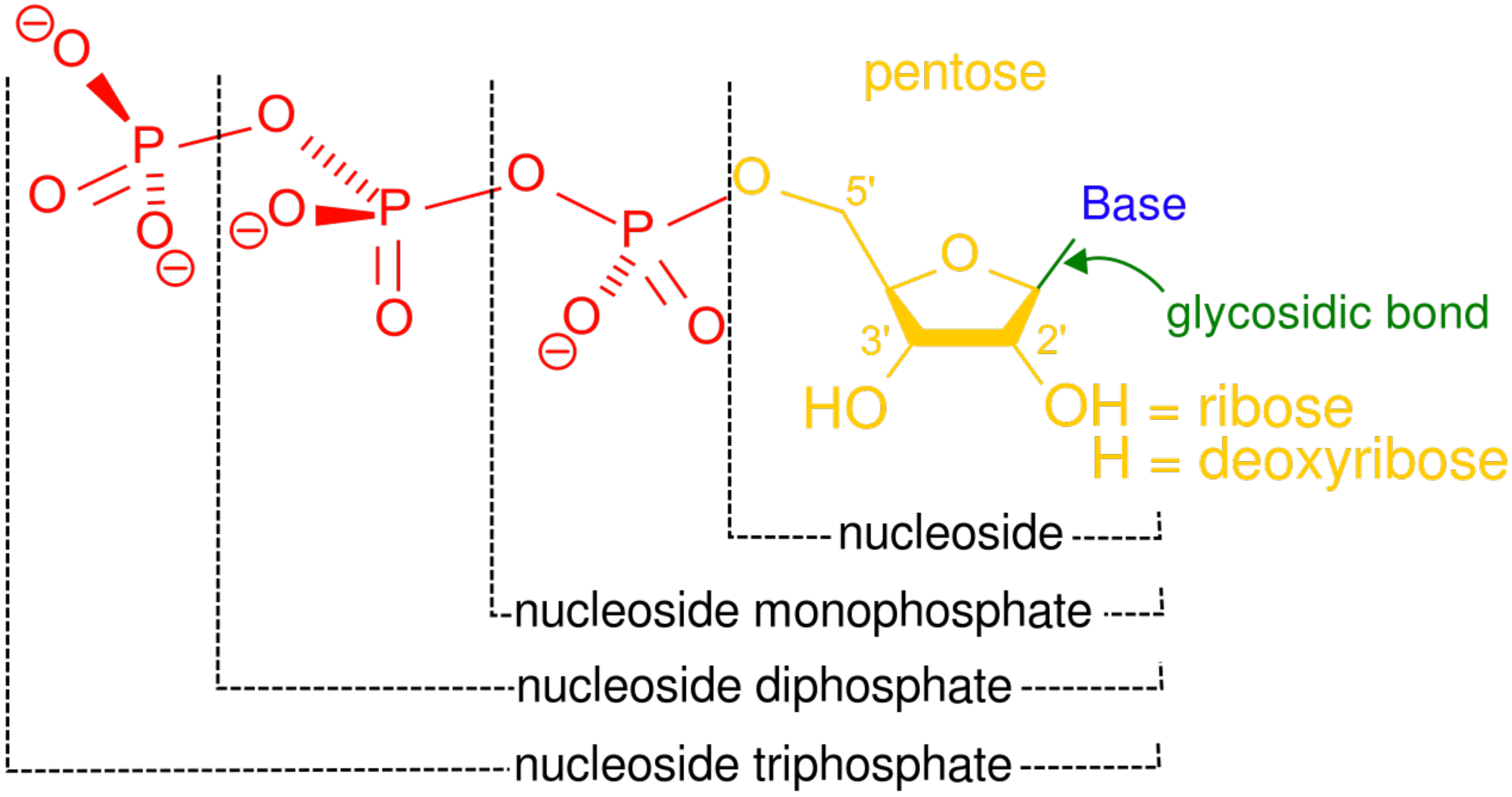
DNA and RNA chemical unit

Do you remember what a nucleoside is?

only for fun :-)

The phosphate group

- A number of phosphate groups can be attached to carbon 5 of the sugar.
- NTPs? dNTPs?



The phosphate group

What is the function os the phosphate groups?

Any relation to replication/sequencing?

The bases

Nitrogenous bases

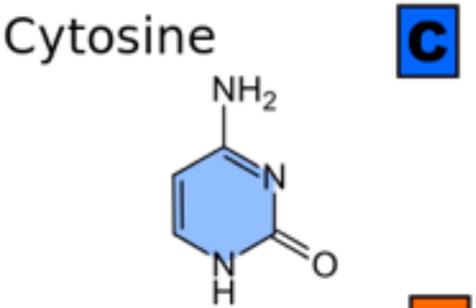
Small bases

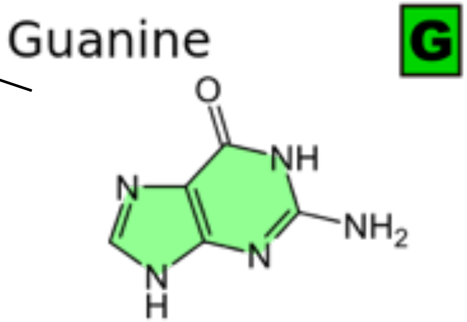
Pyrimidines
One ring

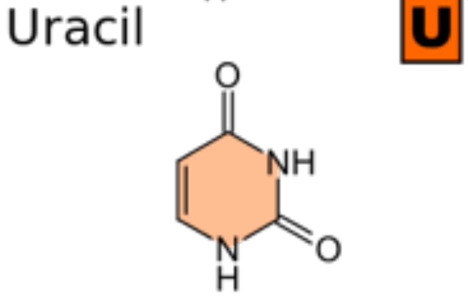
Large bases

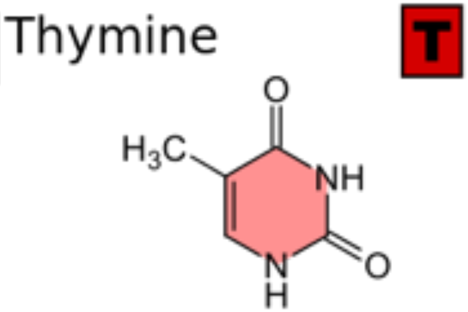
Purines
Two rings

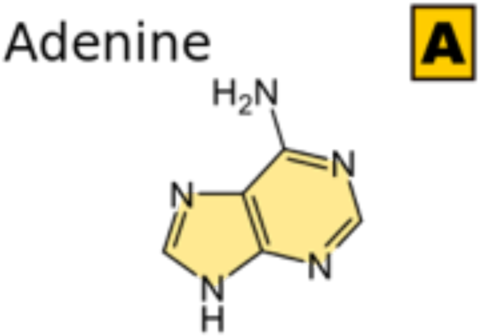
Found in DNA and RNA











Found in RNA

Found in DNA

Naming of DNA bases

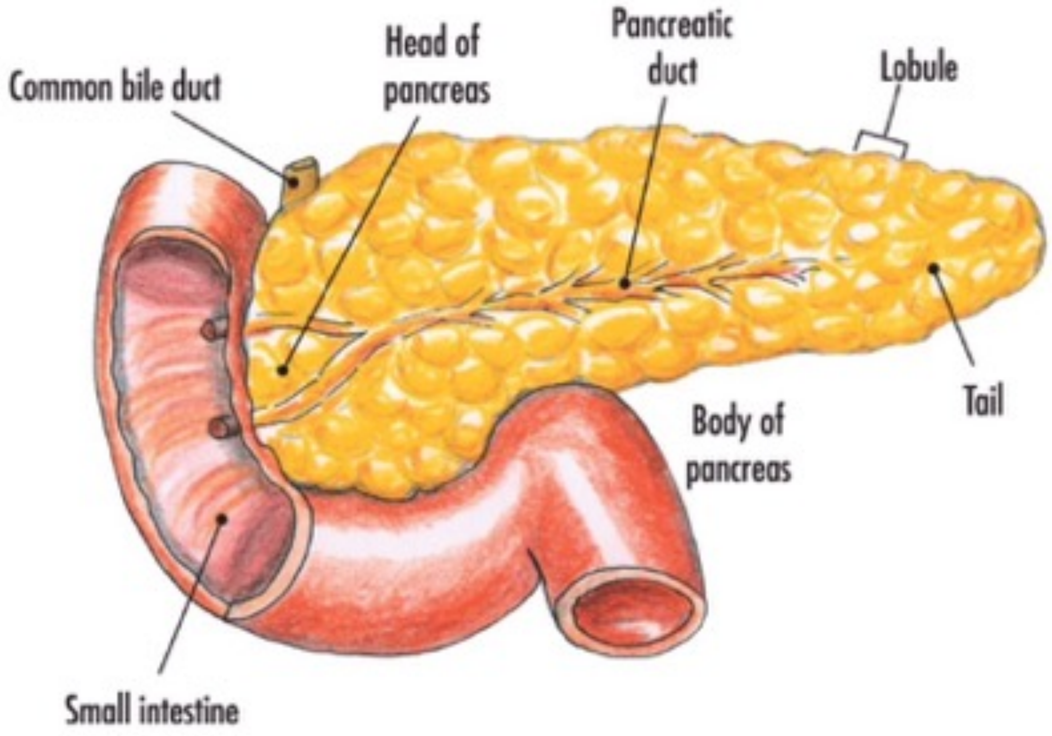
Celebrating an unsung hero of genomics: how Albrech Kossel saved bioinformatics from a world of hurt

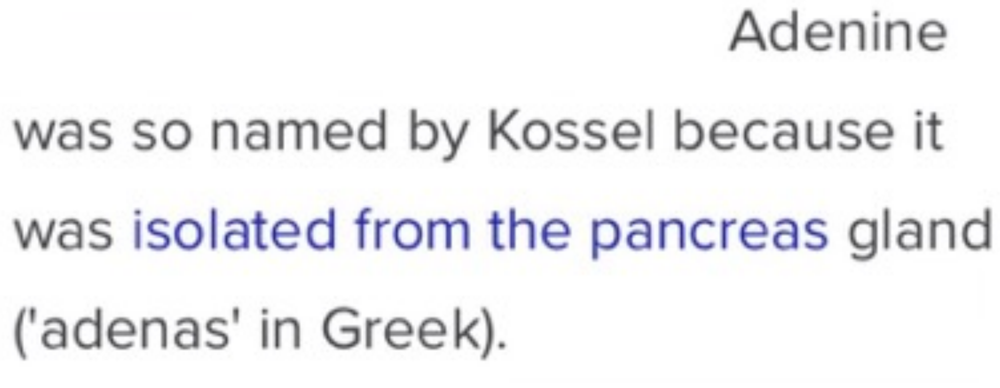
http://www.acgt.me/blog/2014/3/3/celebrating-an-unsung-hero-of-genomics-how-albrech-kossel-saved-bioinformatics-from-a-world-of-hurt

Naming of DNA bases

Guanine had already been named based on where it had first been discovered (the excrement of seabirds known as guano).

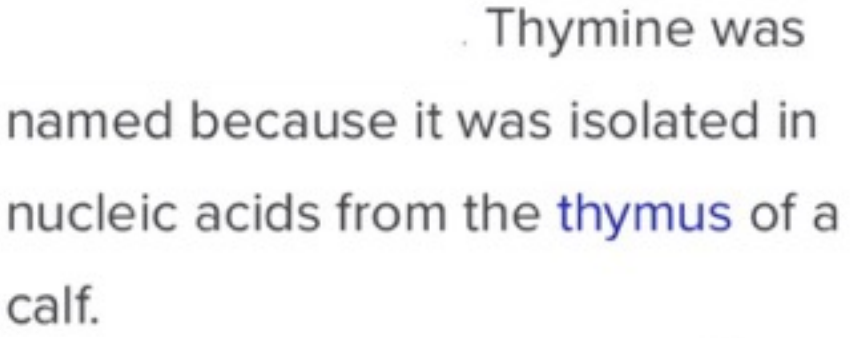
Naming of DNA bases



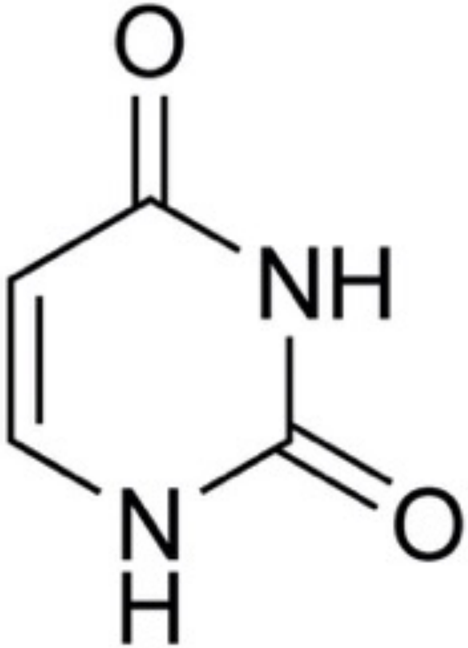

Adenine

was so named by Kossel because it was isolated from the pancreas gland ('adenas' in Greek).

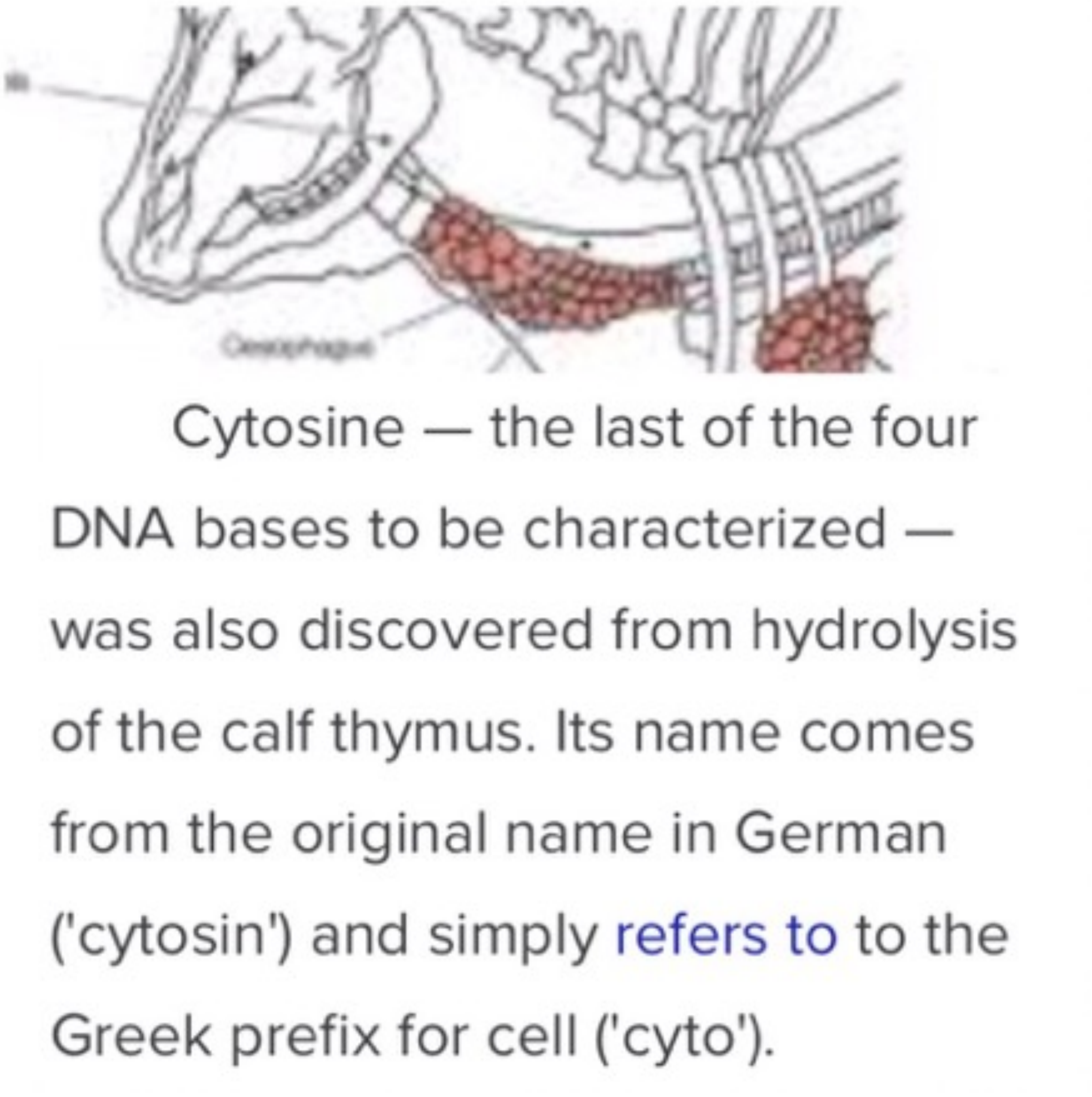
Naming of DNA bases



. Thymine was named because it was isolated in nucleic acids from the thymus of a calf.



Naming of DNA bases



Cytosine — the last of the four DNA bases to be characterized — was also discovered from hydrolysis of the calf thymus. Its name comes from the original name in German ('cytosin') and simply refers to to the Greek prefix for cell ('cyto').

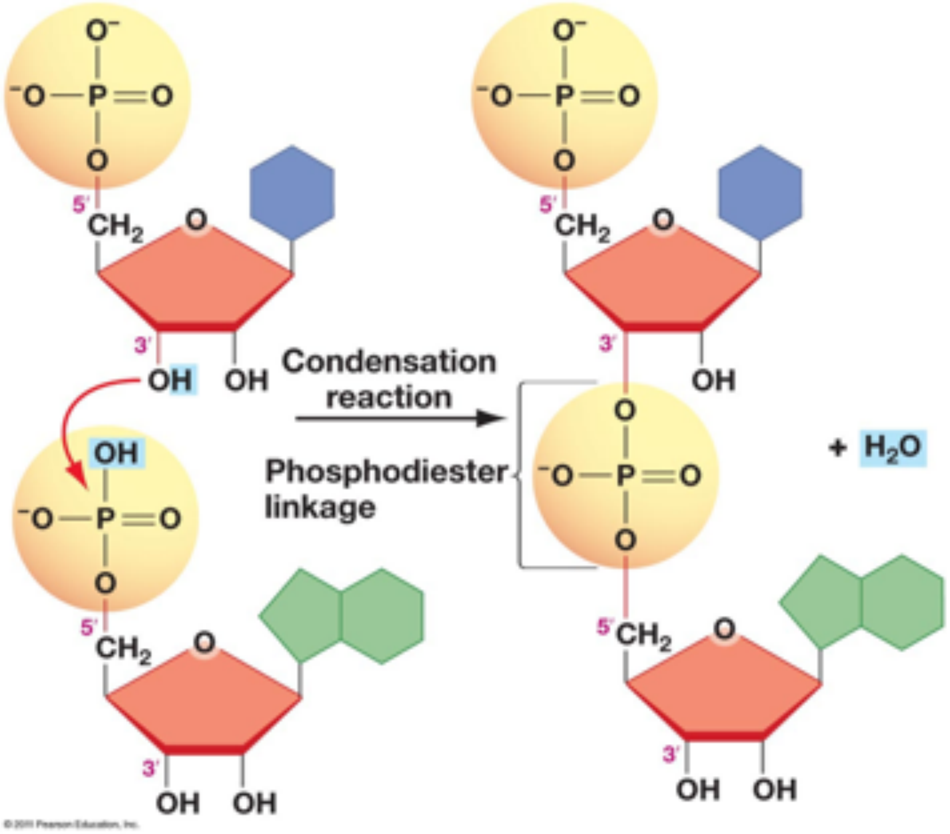
Naming of DNA bases

The naming of the DNA bases made it easier to abbreviate the names of the molecules into unique single letters (A,T,G,C).

The single letter representation of DNA enabled application of information theory via bioinformatics.

Nucleotide linking

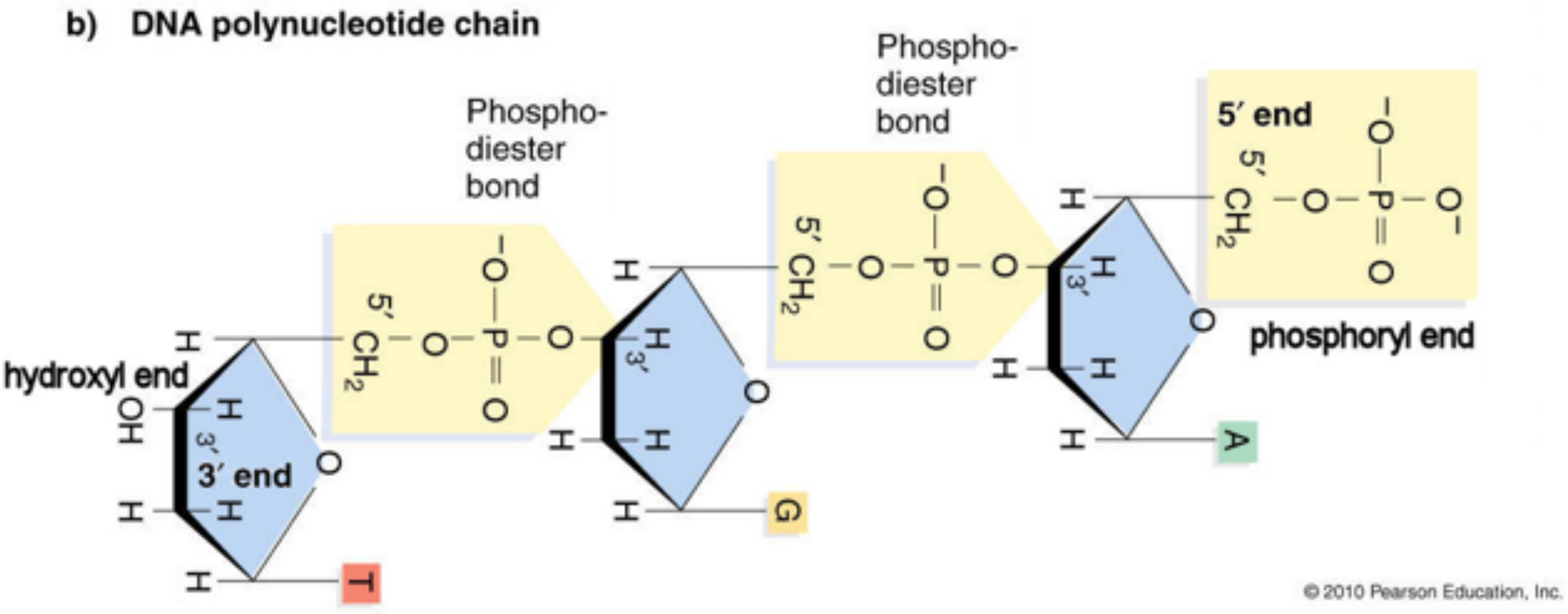
- Nucleotides are linked via **phosphodiester bond.**
- A covalent bond links the phosphate group of one nucleotide to the 3’ carbon of the sugar of another.



Polarity

- **5’ end:** where the 5’ carbon at one end of the molecule has a phosphate group.

- **3’ end:** where the 3’ carbon at the other end of the molecule has a hydroxyl group.



b) DNA polynucleotide chain

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The Race to DNA structure

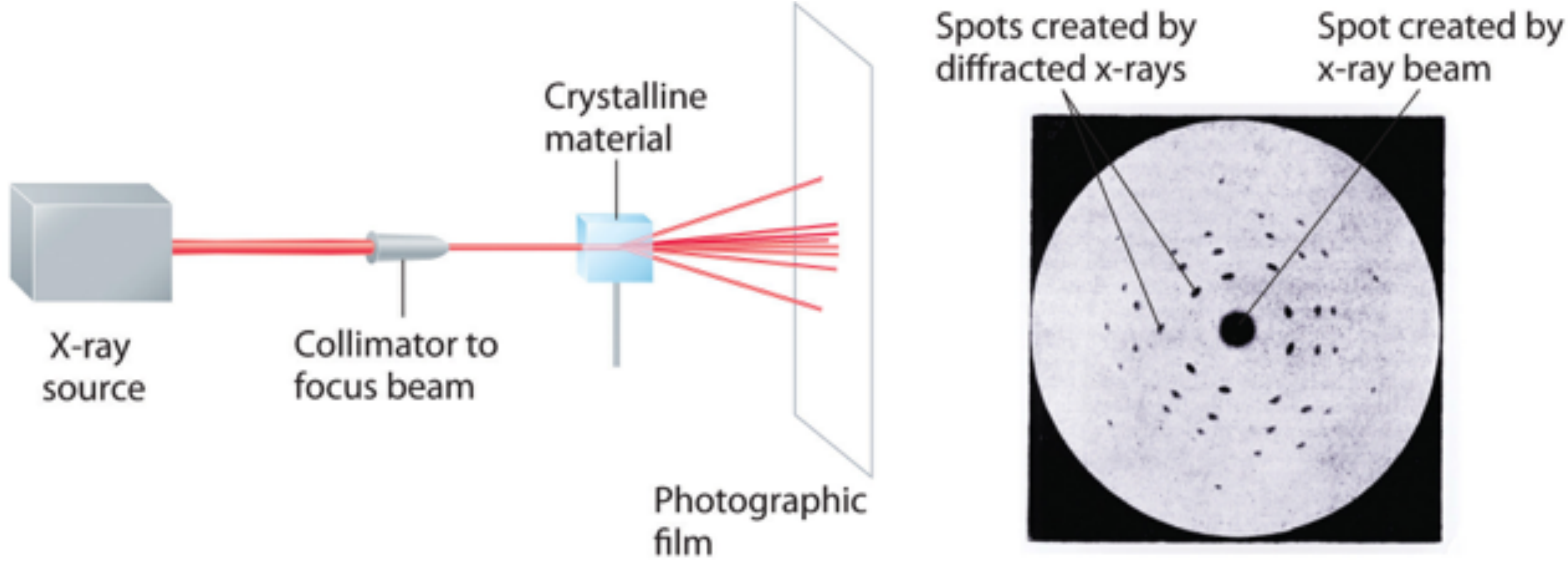
The story of the discovery of the double helix involves these key actors

Molecular structure

How can we learn about the structure of molecules?

X-ray diffraction

X-ray diffraction was the method to study the fine structure of molecules. DNA was no different!

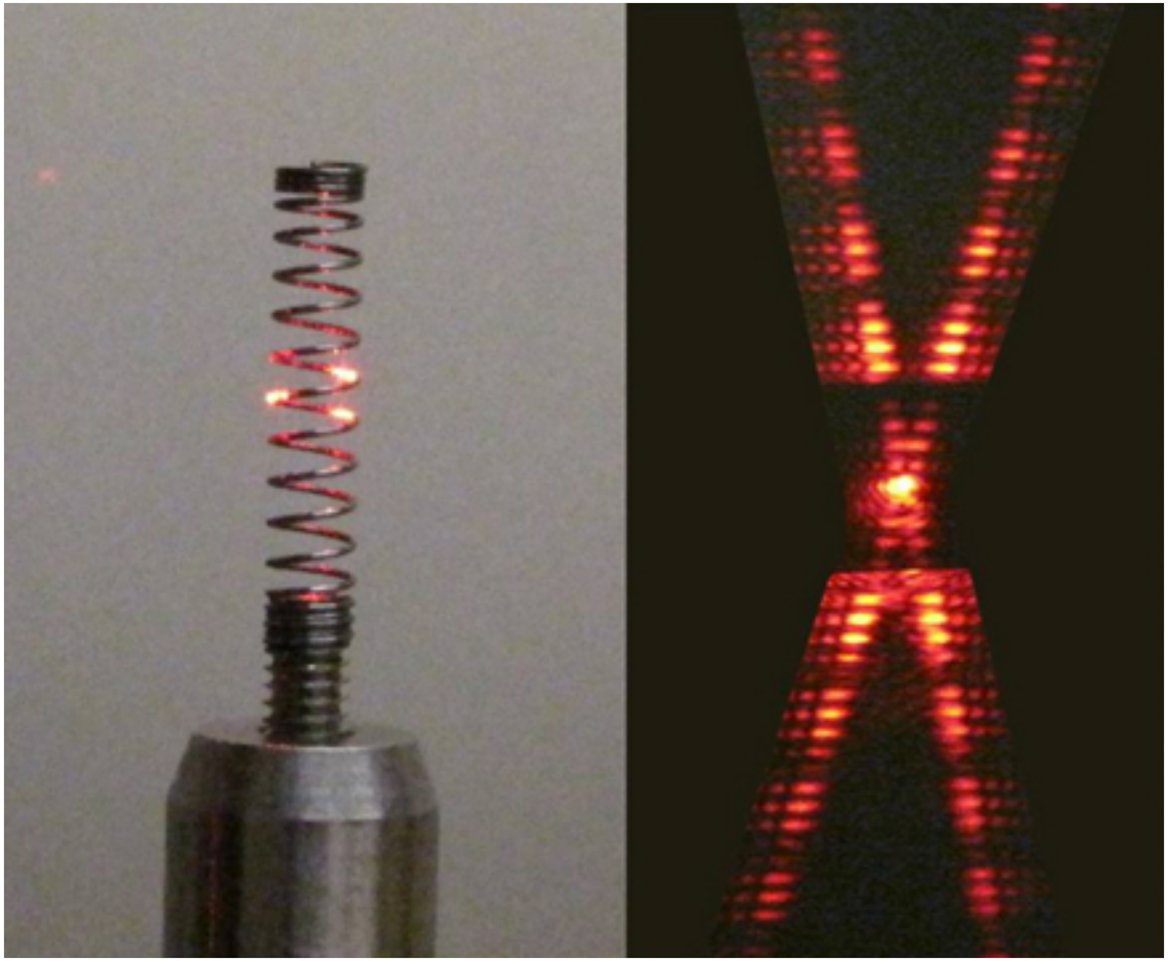


(a) X-ray diffraction

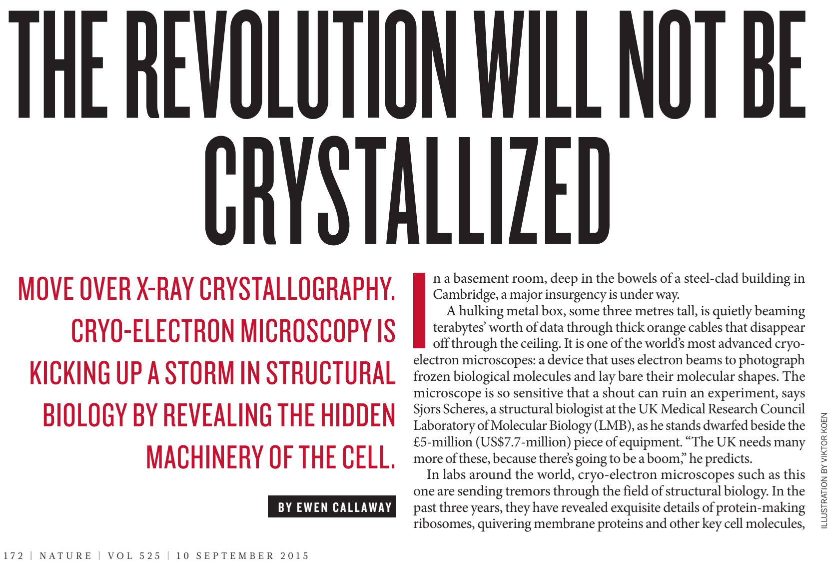
(b) X-ray diffraction pattern captured on photographic film

X-ray diffraction

Look at the pattern resulting!



New Method



THE REVOLUTION WILL NOT BE CRYSTALLIZED

MOVE OVER X-RAY CRYSTALLOGRAPHY. CRYO-ELECTRON MICROSCOPY IS KICKING UP A STORM IN STRUCTURAL BIOLOGY BY REVEALING THE HIDDEN MACHINERY OF THE CELL.

BY EWEN CALLAWAY

In a basement room, deep in the bowels of a steel-clad building in Cambridge, a major insurgency is under way.

A hulking metal box, some three metres tall, is quietly beaming terabytes' worth of data through thick orange cables that disappear off through the ceiling. It is one of the world's most advanced cryo-electron microscopes: a device that uses electron beams to photograph frozen biological molecules and lay bare their molecular shapes. The microscope is so sensitive that a shout can ruin an experiment, says Sjors Scheres, a structural biologist at the UK Medical Research Council Laboratory of Molecular Biology (LMB), as he stands dwarfed beside the £5-million (US$7.7-million) piece of equipment. "The UK needs many more of these, because there's going to be a boom," he predicts.

In labs around the world, cryo-electron microscopes such as this one are sending tremors through the field of structural biology. In the past three years, they have revealed exquisite details of protein-making ribosomes, quivering membrane proteins and other key cell molecules,

ILLUSTRATION BY VIKTOR KOEN

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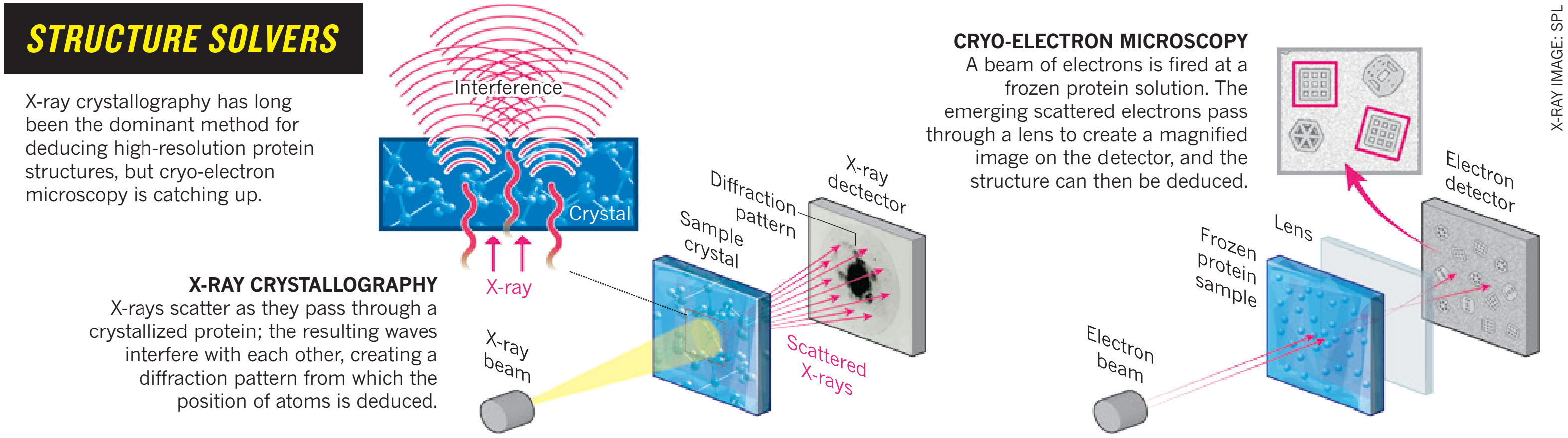


STRUCTURE SOLVERS

X-ray crystallography has long been the dominant method for deducing high-resolution protein structures, but cryo-electron microscopy is catching up.

X-RAY CRYSTALLOGRAPHY
X-rays scatter as they pass through a crystallized protein; the resulting waves interfere with each other, creating a diffraction pattern from which the position of atoms is deduced.

CRYO-ELECTRON MICROSCOPY
A beam of electrons is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, and the structure can then be deduced.

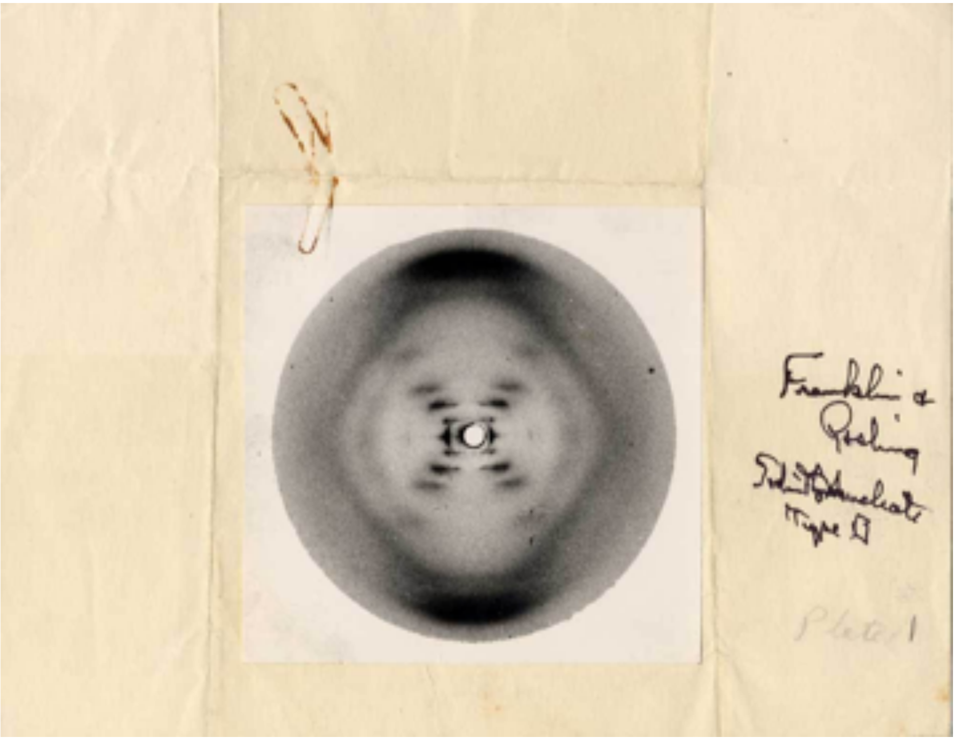
http://www.nature.com/news/the-revolution-will-not-be-crystallized-a-new-method-sweeps-through-structural-biology-1.18335?WT.mc_id=TWT_NatureNews

Franklin/Wilkin

- R. Franklin produced the best diffraction photo (called photo 51).
- Her findings were shared (with or without her approval?) Watson and Crick by Wilkin.

NATURE April 25, 1953

Molecular Structure of Deoxypentose Nucleic Acids



Watson and Crick

- They published a 900 words paper and Franklin and Wilkin also published on the same issue of Nature.

April 25, 1953 NATURE 737

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

DNA structure

1) DNA is a double helix.

2) Two polynucleotides chains.

3) The two chains wind around right handedly - right handed double helix.

4) The two chains are in an anti-parallel orientation. One strand 5’ – 3’ orientation and the other 3’ – 5’).

5) Sugar-phosphate backbone is located on the outside of the helix.

6) The nitrogenous bases located on the inside of the helix.

DNA structure

7) The bases are stacked flat and perpendicular to the axis of the helix. The bases are on top of each other following the twist of the helix.

8) The bases of the two polynucleotides are bonded together via hydrogen bonds on the inside of the helix.

9) Bases of the two polynucleotide chains are base-pairing in a combination that maintains similar diameter of the double helix.

10) A Pyrimidine always basepair with Purine forming **complementary base pairs.**

DNA structure

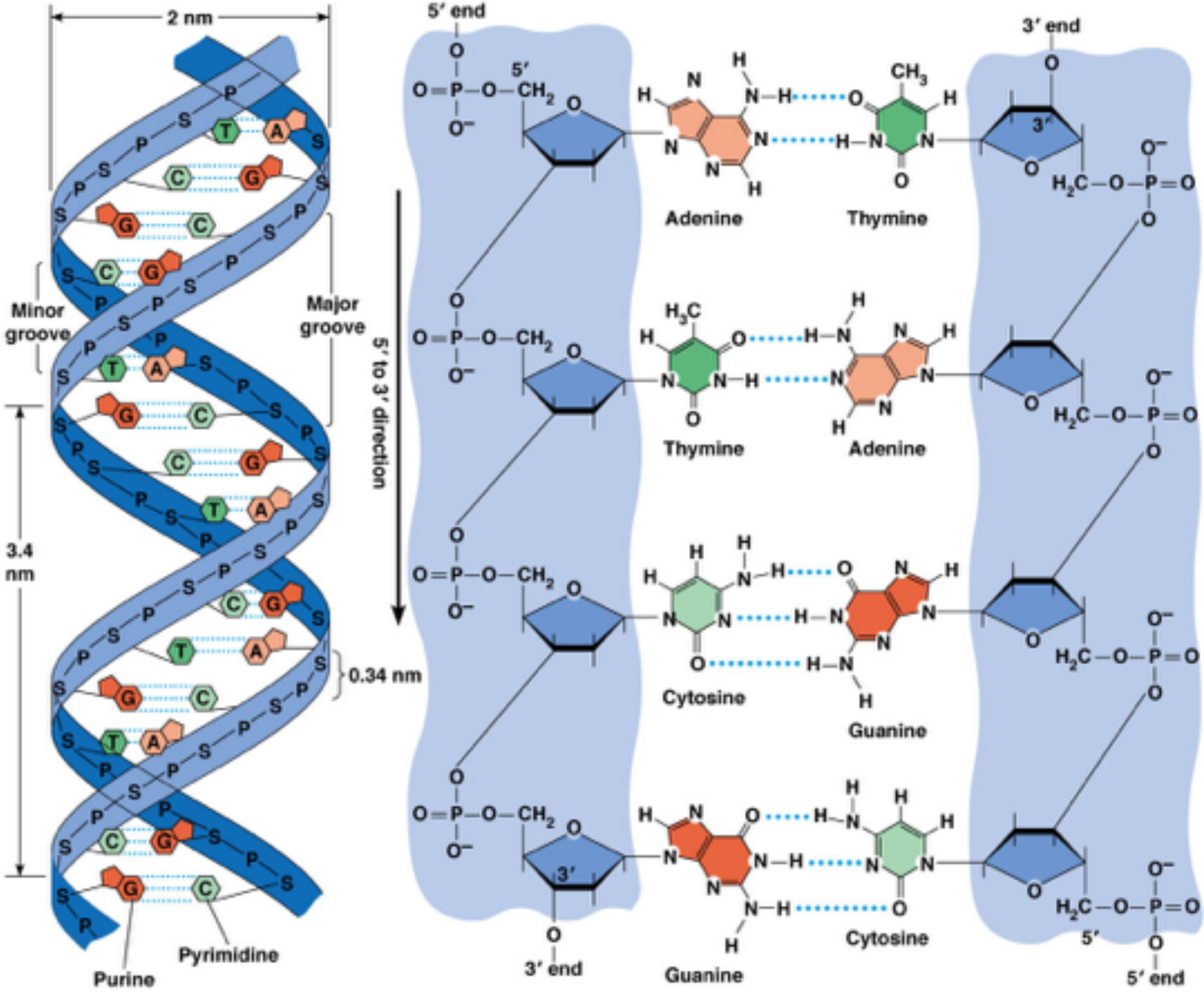
11) Thymine (T) basepair with Adenine (A), and Cytosine basepair with Guanine (G).

12) Two hydrogen bonds involve the base-pairing of (A and T) and three hydrogen bonds between (G and C).

13) The sequence of one chain (strand) is enough to predict the complementary one in the other orientation.

14) A major and minor groove result from the unequal spacing of the phosphate-sugar backbone.

Summary



(a) Double helix

(b) Antiparallel orientation of strands

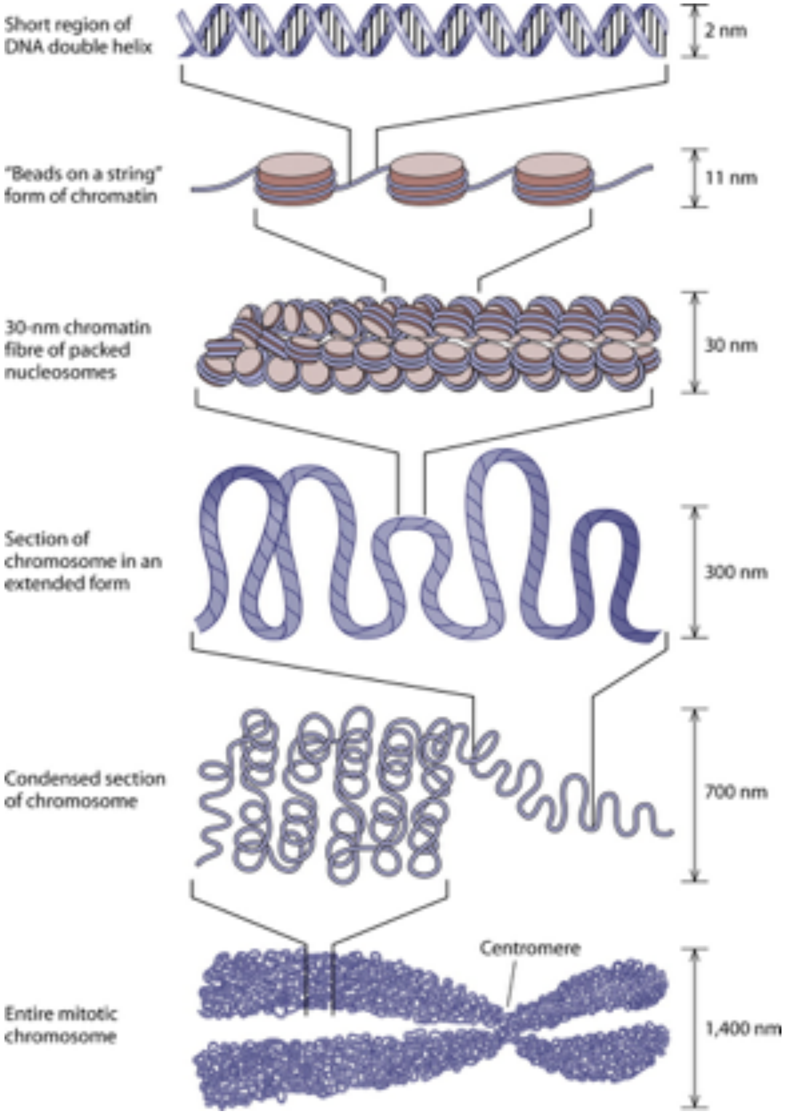
Genome packaging and organization

Do you remember the organization of viral genomes?

prokaryotic genomes?

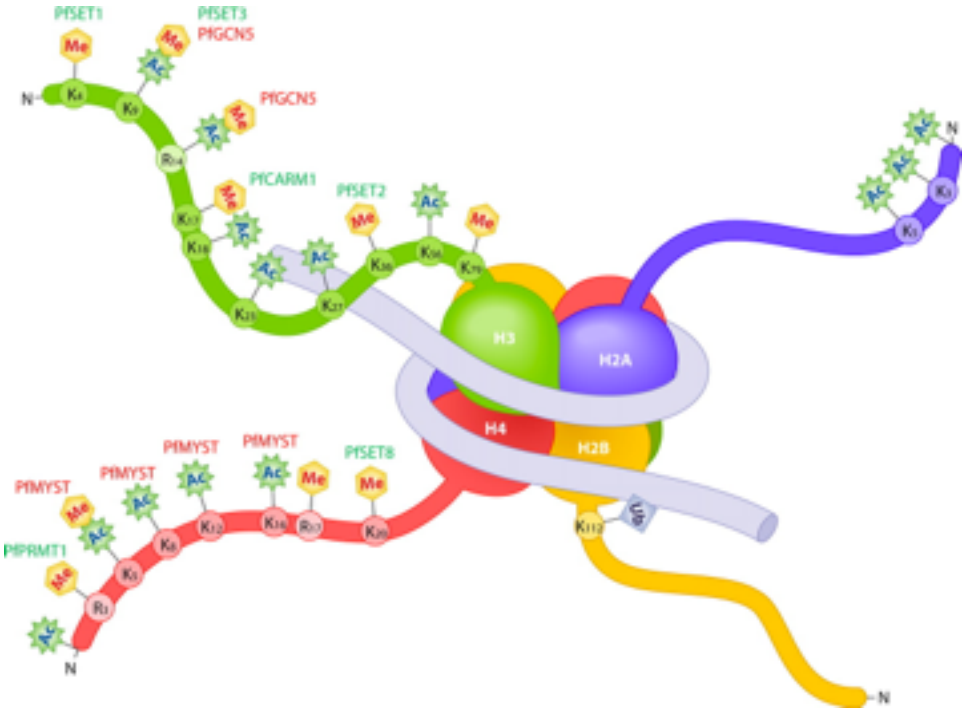
Review of genome condensation

- Double helix.
  - Nucleosomes (DNA histones).
    - Beads on a string.
      - 30 nm chromatin fiber/ solenoid.
        - Eu/Heterochromatin.
          - Metaphase chromosome.

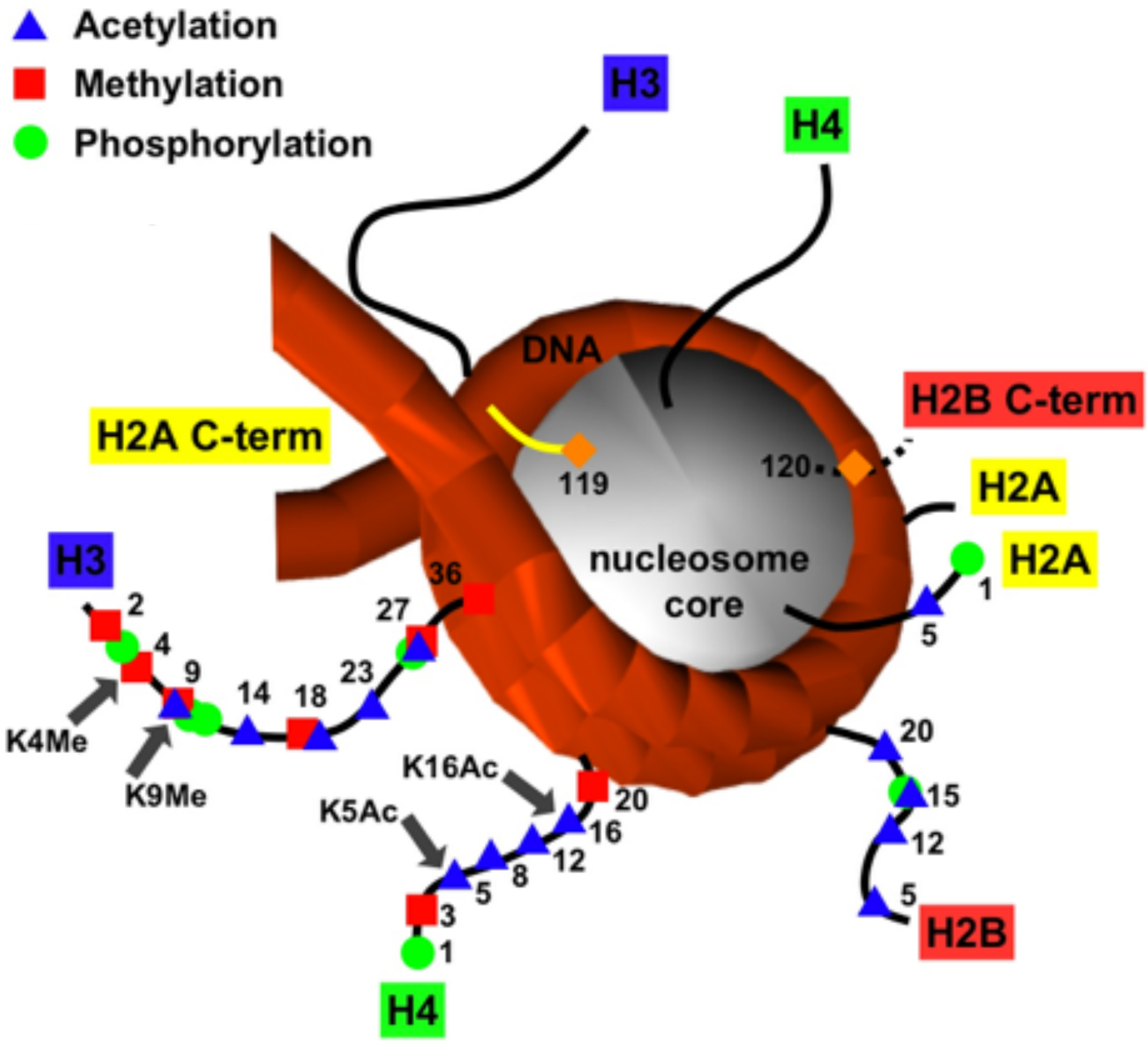


DNA packaging

- A single nucleosome is composed of 8 histone proteins (octamer).
- Histone proteins contain tails of amino acids that can be modified.



Histone tail modifications

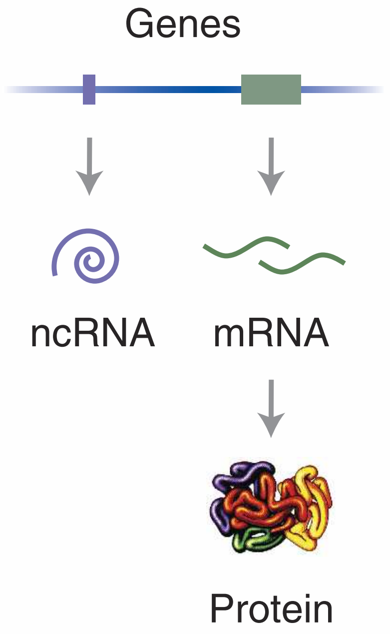


Histone tail modifications

Do you remember the functions/effects of histone modifications?

Are the histone modifications uniform across all chromosomes?

The Central Dogma



Genes

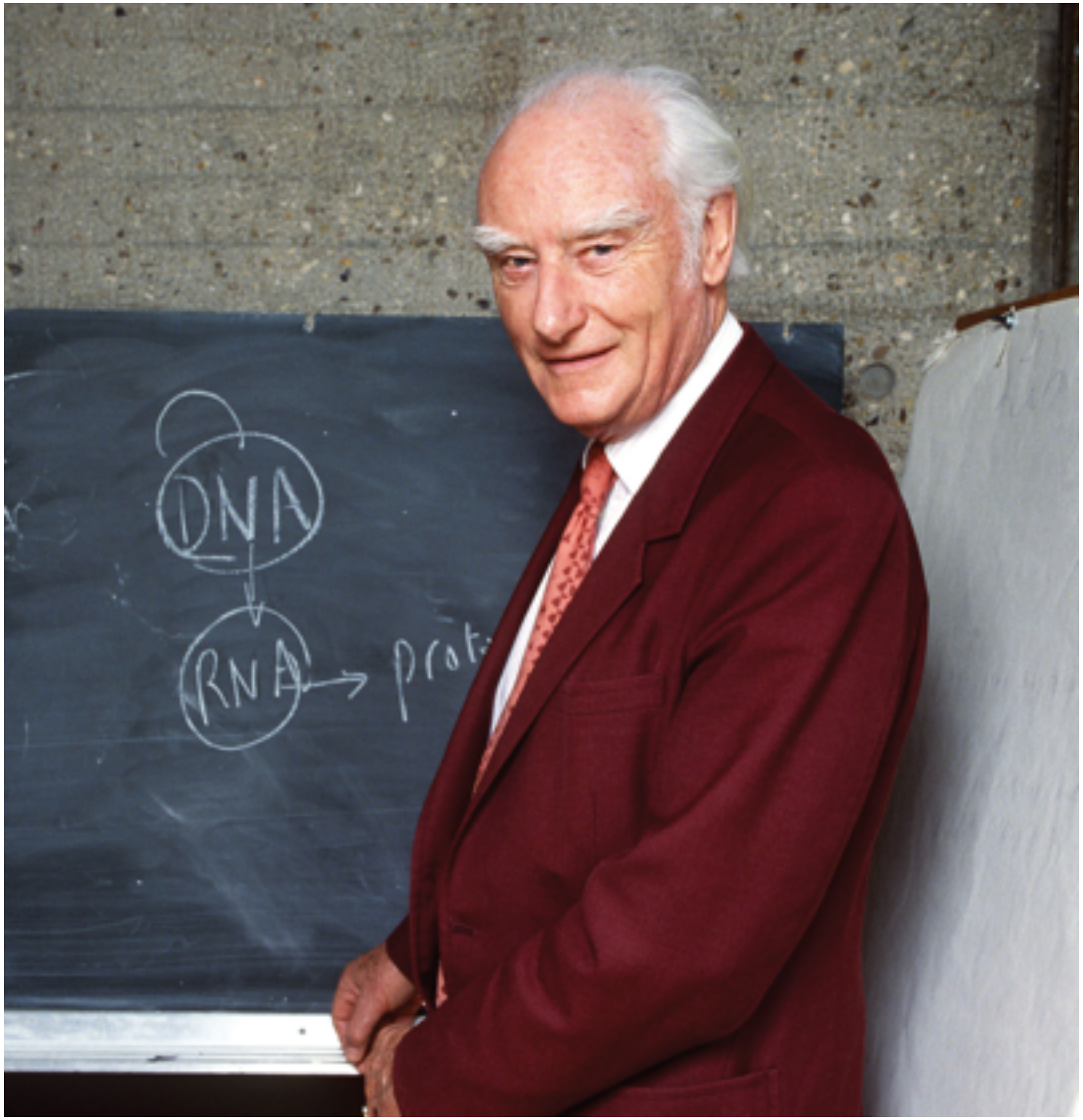
ncRNA

mRNA

Protein

The ‘**Central Dogma**’ of molecular biology was proposed by Francis Crick.

1958

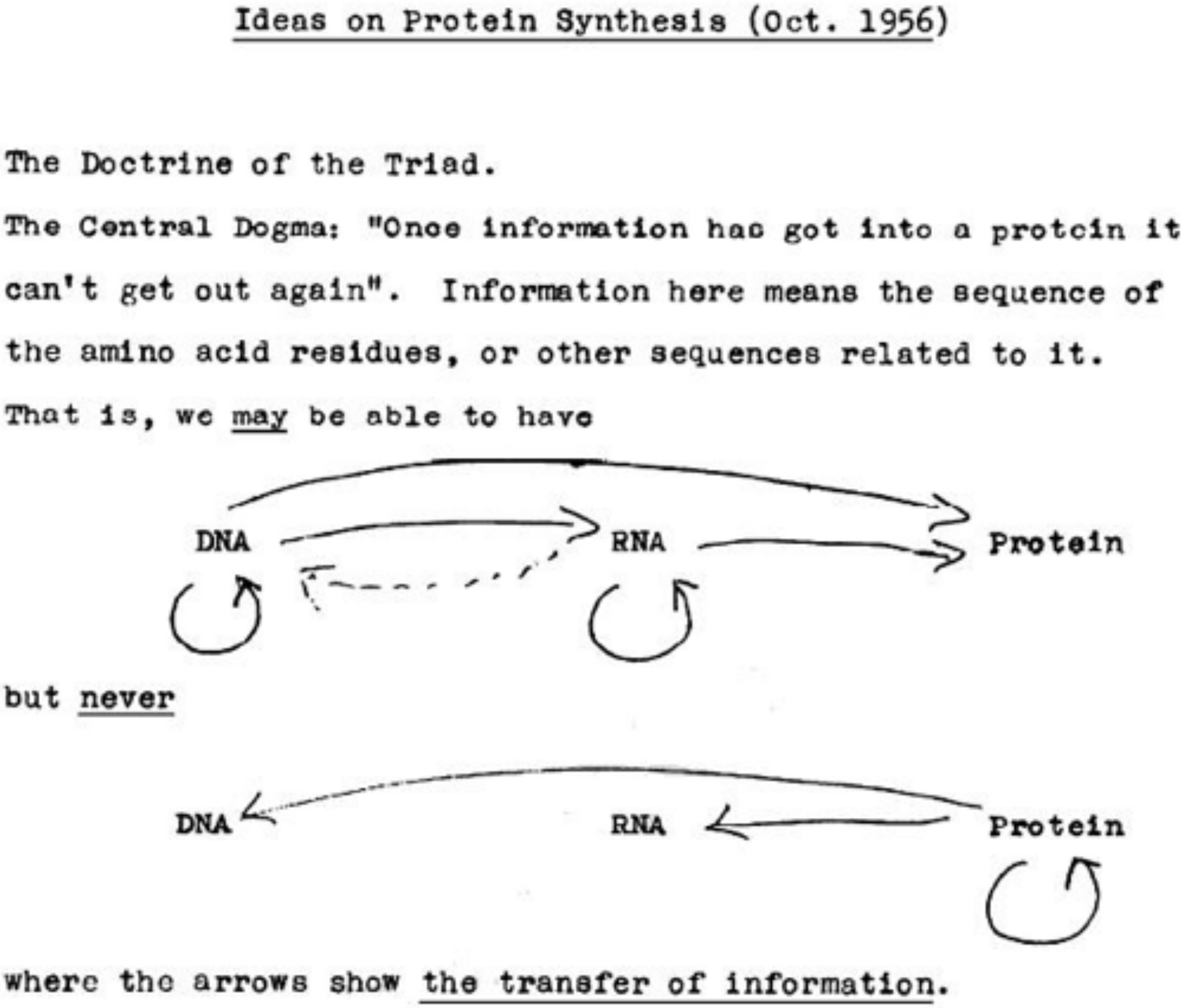


The Central Dogma

Do you what the word “Dogma” means?

Do we have dogmas in science?

The Central Dogma



Ideas on Protein Synthesis (Oct. 1956)

The Doctrine of the Triad.

The Central Dogma: "Once information has got into a protein it can't get out again". Information here means the sequence of the amino acid residues, or other sequences related to it.

That is, we may be able to have

DNA RNA Protein

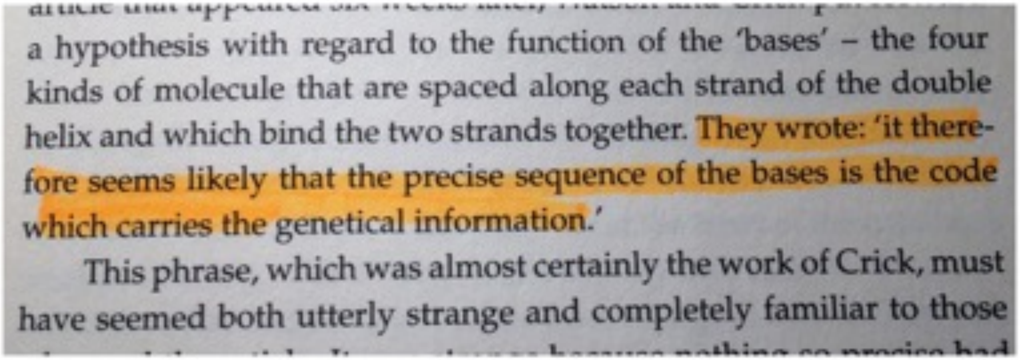
but never

DNA RNA Protein

where the arrows show the transfer of information.

The Central Dogma

Molecules as information and information flow

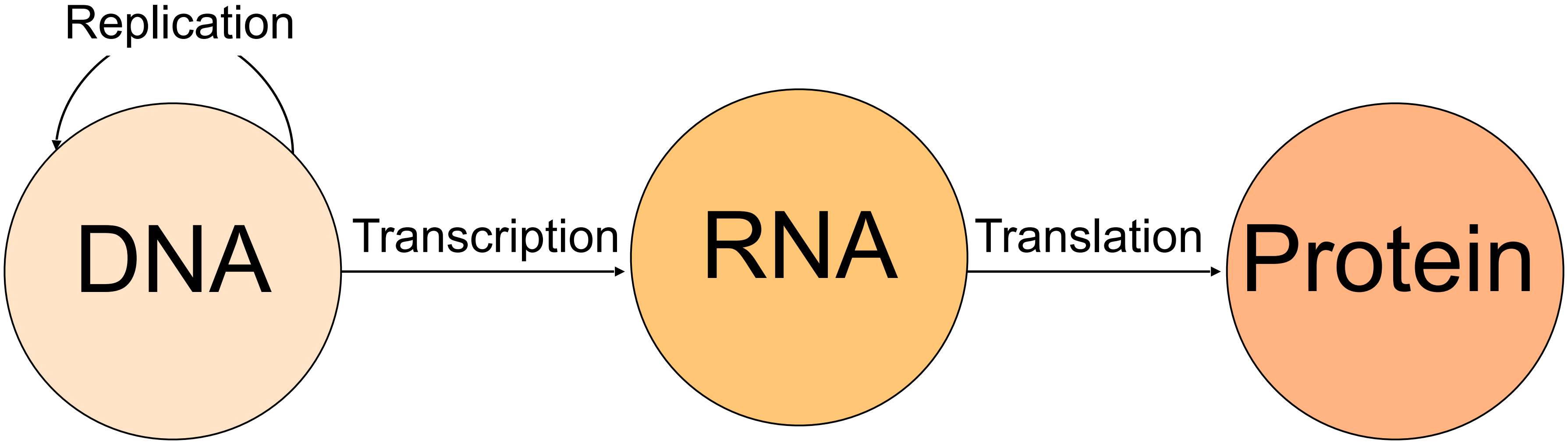


a hypothesis with regard to the function of the 'bases' – the four kinds of molecule that are spaced along each strand of the double helix and which bind the two strands together. They wrote: 'it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information.'

This phrase, which was almost certainly the work of Crick, must have seemed both utterly strange and completely familiar to those

Terms and processes

The flow of information from DNA to proteins go through an intermediate molecule and involves two processes.



Replication

DNA

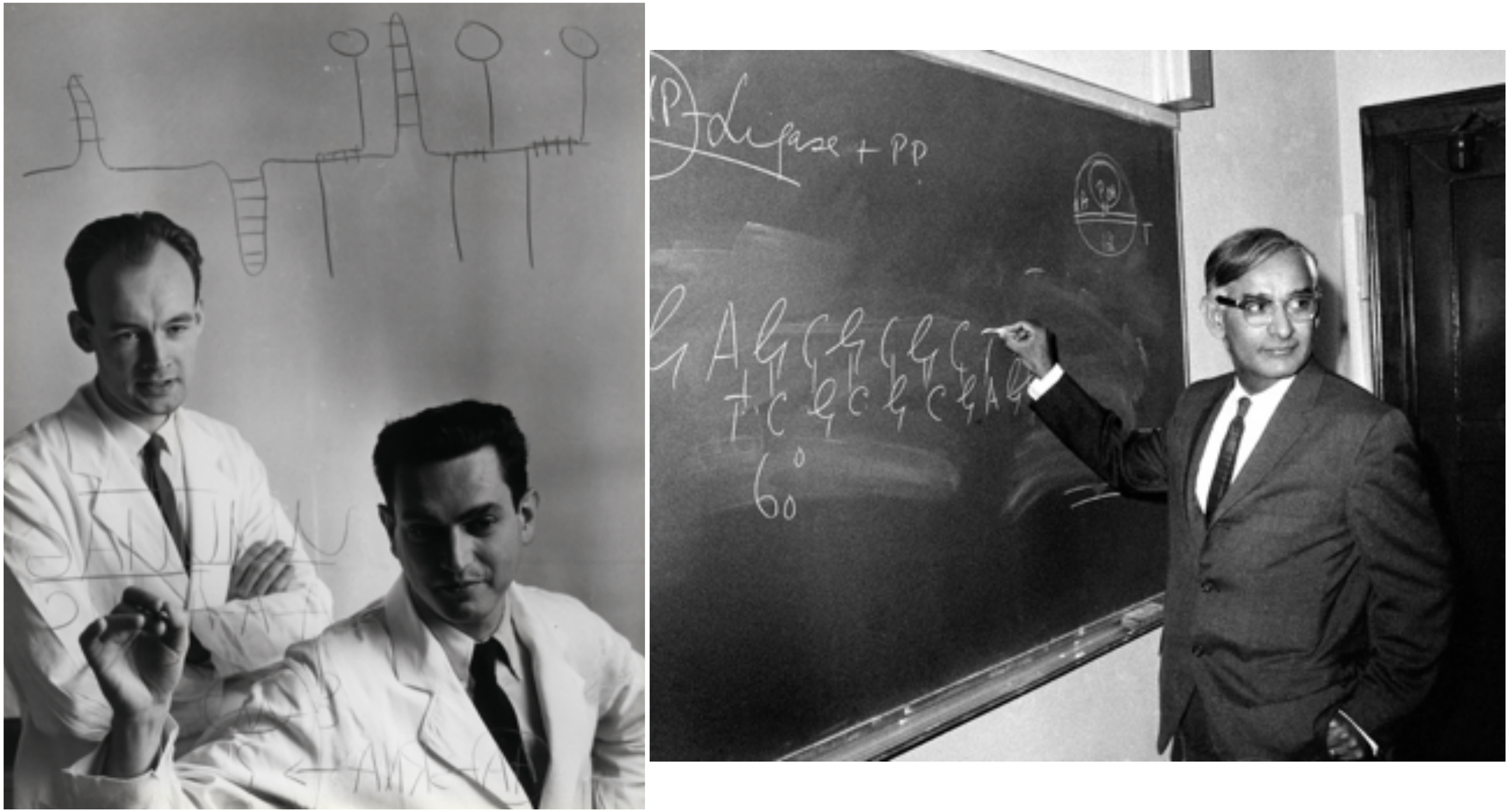
Transcription

RNA

Translation

Protein

The code



1965: Marshall Nirenberg and Har Gobind Khorana (and others)

The code revealing experiments

What scientific developments enabled discovering the genetic code?

What were the experiments based on?

The code revealing experiments

Major advances:

(1) Cell free protein synthesis
(2) Artificial (synthetic) m-RNA

The code revealing experiments

Decoding experiments

Stage 1

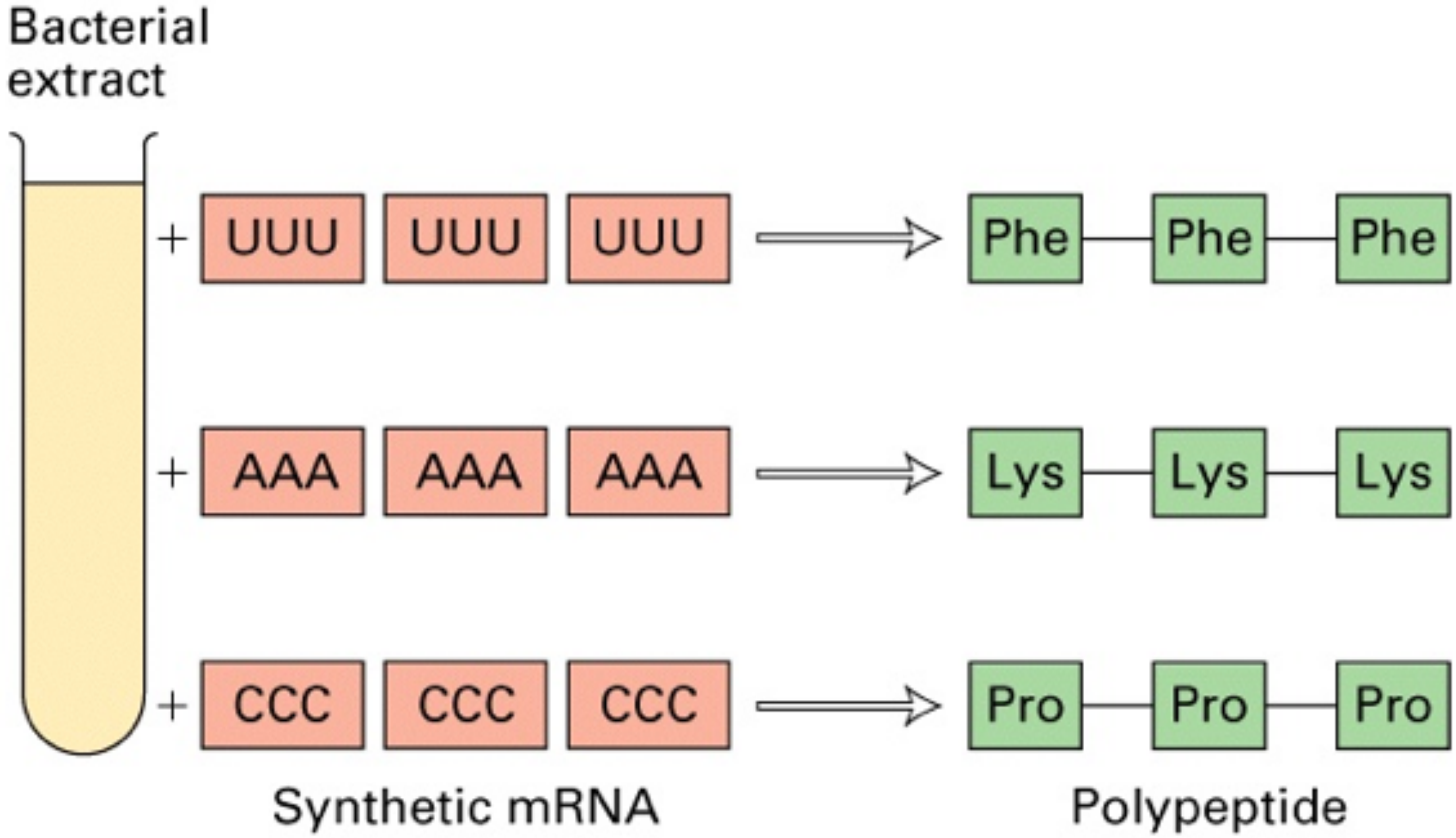
Mononucleotide polymers
Random copolymers

Stage 2

Ribosome binding
Assay

Mononucleotide polymers

Translating a mononucleotide polymer results in a a single amino acid polypeptide



Bacterial extract

+ UUU UUU UUU → Phe—Phe—Phe

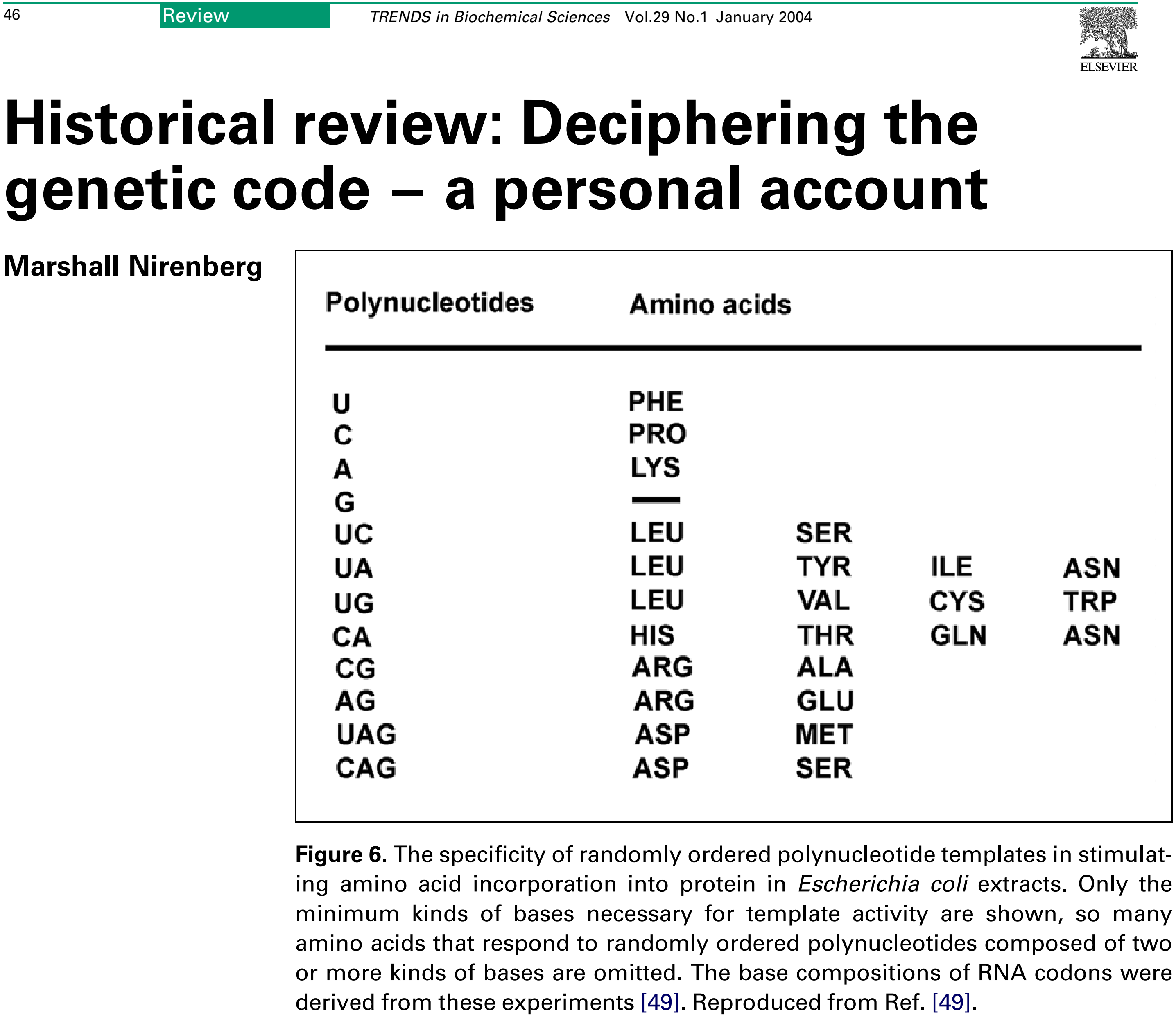
+ AAA AAA AAA → Lys—Lys—Lys

+ CCC CCC CCC → Pro—Pro—Pro

Synthetic mRNA

Polypeptide

Random copolymers



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ELSEVIER

Historical review: Deciphering the genetic code – a personal account

Marshall Nirenberg

Polynucleotides	Amino acids			
U	PHE			
C	PRO			
A	LYS			
G	—			
UC	LEU	SER		
UA	LEU	TYR	ILE	ASN
UG	LEU	VAL	CYS	TRP
CA	HIS	THR	GLN	ASN
CG	ARG	ALA		
AG	ARG	GLU		
UAG	ASP	MET		
CAG	ASP	SER		

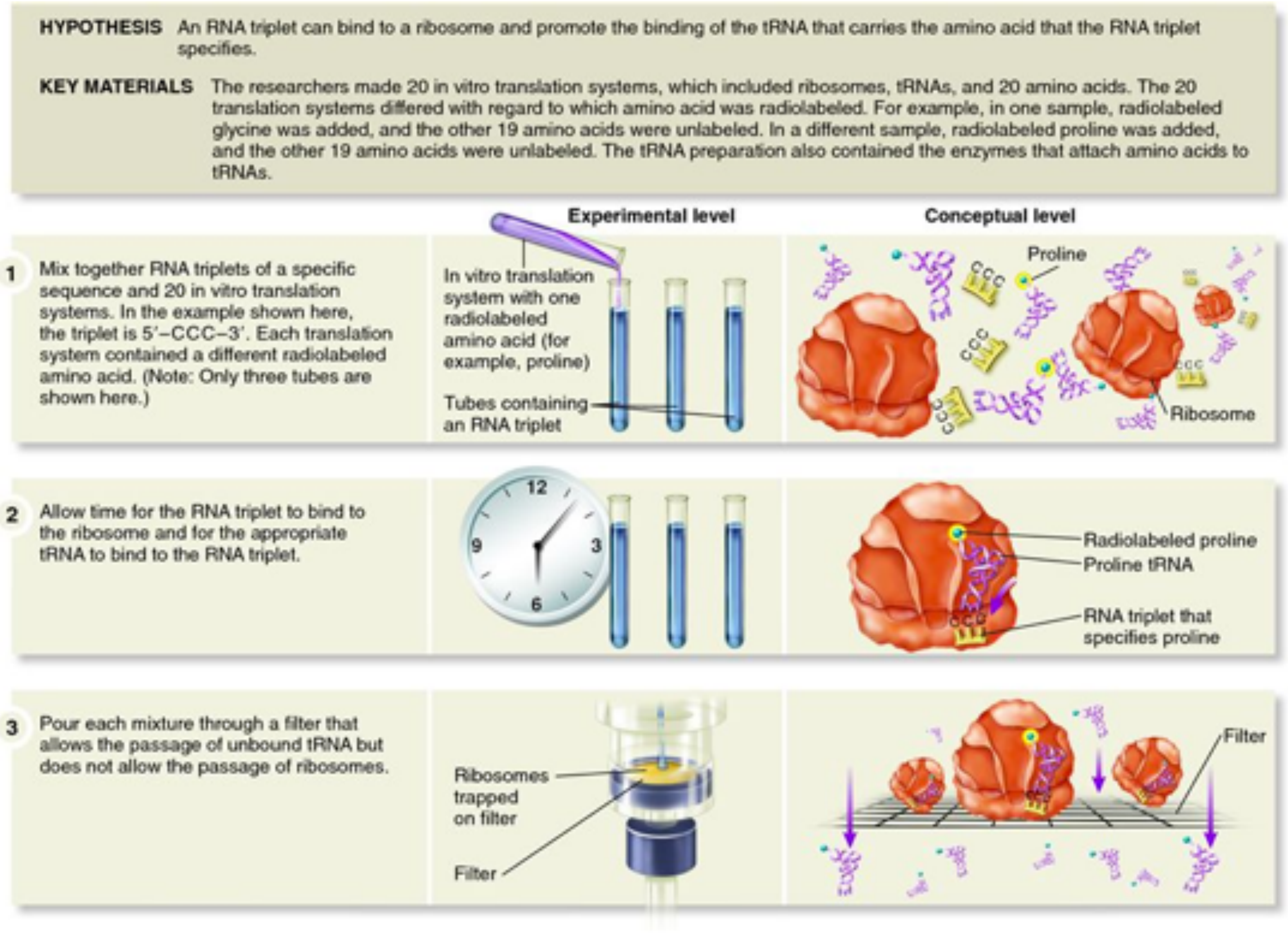
Figure 6. The specificity of randomly ordered polynucleotide templates in stimulating amino acid incorporation into protein in *Escherichia coli* extracts. Only the minimum kinds of bases necessary for template activity are shown, so many amino acids that respond to randomly ordered polynucleotides composed of two or more kinds of bases are omitted. The base compositions of RNA codons were derived from these experiments [49]. Reproduced from Ref. [49].

Random copolymers

Can random copolymers definitively identify the genetic code?

Ribosome binding assay

This technique revealed the language of the genetic material and the information hidden in it



HYPOTHESIS An RNA triplet can bind to a ribosome and promote the binding of the tRNA that carries the amino acid that the RNA triplet specifies.

KEY MATERIALS The researchers made 20 in vitro translation systems, which included ribosomes, tRNAs, and 20 amino acids. The 20 translation systems differed with regard to which amino acid was radiolabeled. For example, in one sample, radiolabeled glycine was added, and the other 19 amino acids were unlabeled. In a different sample, radiolabeled proline was added, and the other 19 amino acids were unlabeled. The tRNA preparation also contained the enzymes that attach amino acids to tRNAs.

1 Mix together RNA triplets of a specific sequence and 20 in vitro translation systems. In the example shown here, the triplet is 5′–CCC–3′. Each translation system contained a different radiolabeled amino acid. (Note: Only three tubes are shown here.)

2 Allow time for the RNA triplet to bind to the ribosome and for the appropriate tRNA to bind to the RNA triplet.

3 Pour each mixture through a filter that allows the passage of unbound tRNA but does not allow the passage of ribosomes.

What is the name of the protein coding unit of genetic information?

How many nucleotides it contains?

The genetic code

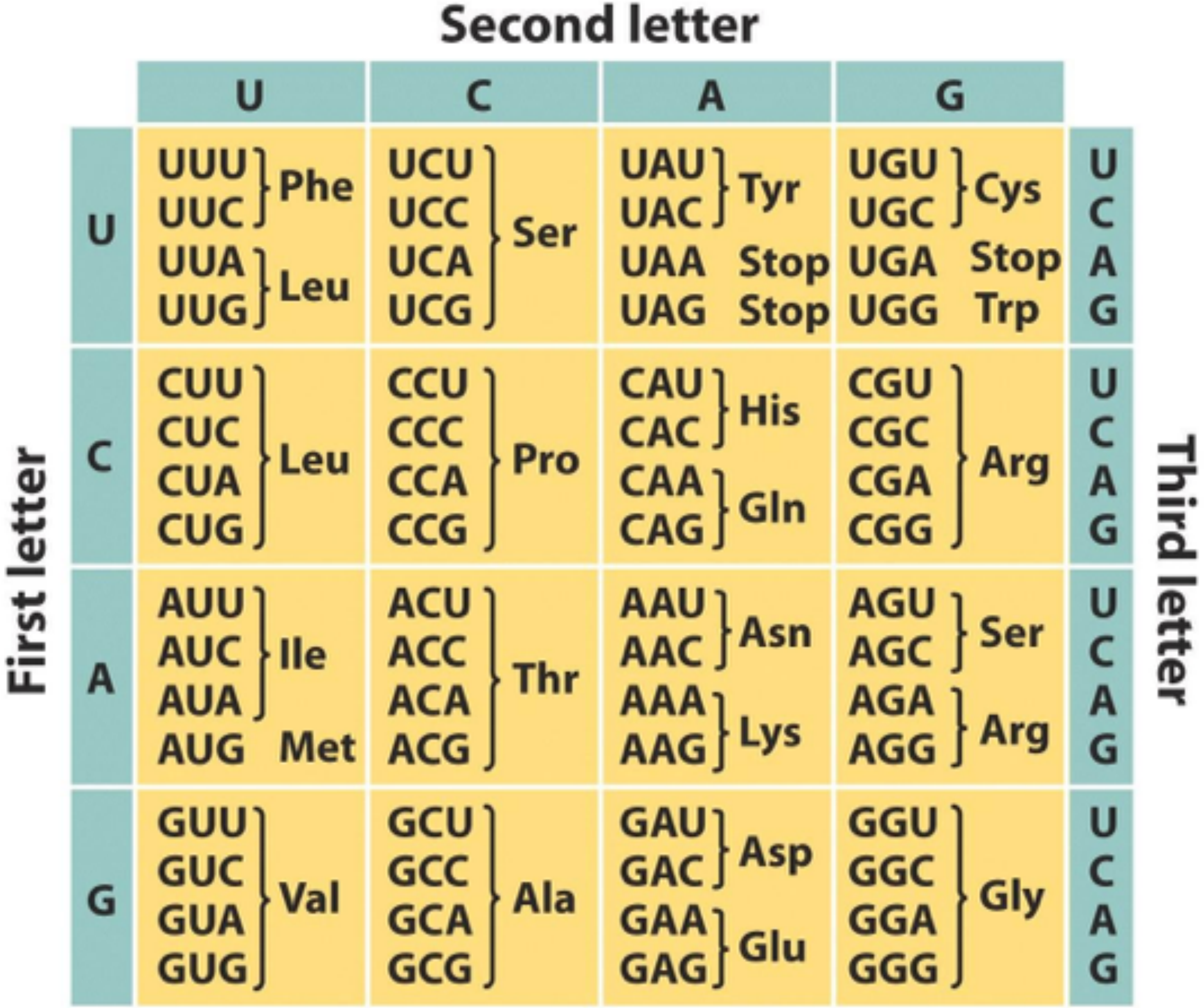
The genetic code is composed of 64 codons

- 61 amino acid coding codons
  - Start codon (AUG) methionine (Met)
  - 60 codons code for 19 other amino acids
- Three codons code for the stop of translation
  UAA
  UAG
  UGA

Characteristics of the genetic code

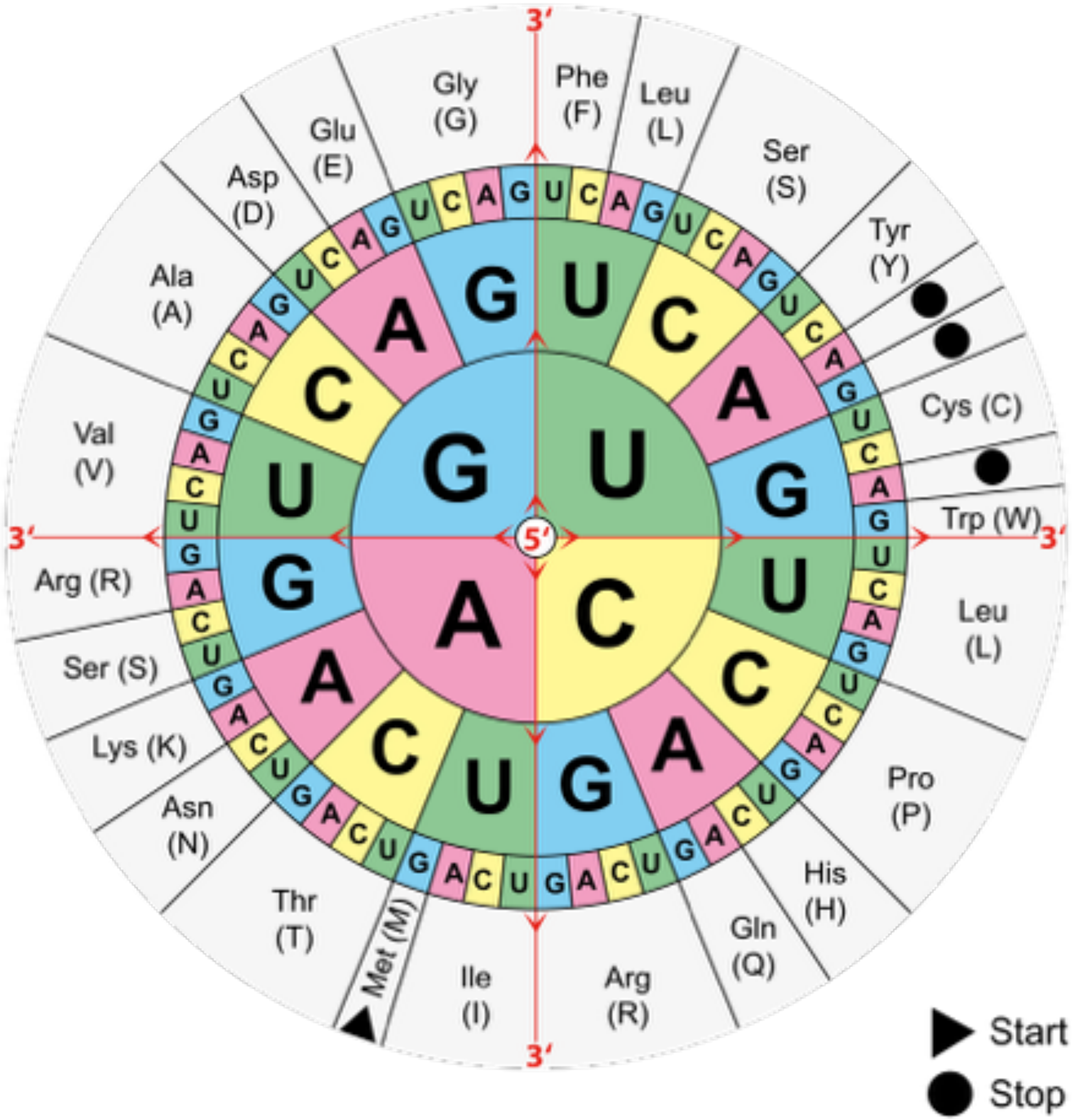
1. The genetic code is made of triplets of nucleotides (3nts) called codons.
2. The genetic code is continuous (no skipping).
3. The code is not overlapping. Every three nucleotides in a sequence code for one codon.
4. The genetic code is universal (almost).
5. The code has specific signals for start of translation and stop of translation.
6. The genetic code is “degenerate”.
7. The Wobble effect of the third base in the codon.

Characteristics of the genetic code



	Second letter				
First letter	U	C	A	G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC	UCC	UAC	UGC	C
	UUA Leu	UCA	UAA Stop	UGA Stop	A
	UUG	UCG	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Gln	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC	ACC	AAC	AGC	C
	AUA	ACA	AAA Lys	AGA Arg	A
	AUG Met	ACG	AAG	AGG	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC	GCC	GAC	GGC	C
	GUA	GCA	GAA Glu	GGA	A
	GUG	GCG	GAG	GGG	G

The genetic code



Expectations

- You are familiar, broadly, with the concepts covered.

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