

## Summary - Deciphering the Genetic Code

### Beginning of Modern Genetics:

- Mendel's laws of inheritance revealed probabilities of passing of genetic dominant & recessive traits.
  - ↳ Received little recognition during his own lifetime
  - ↳ Recognized only in the early 20<sup>th</sup> century.

### Avery's demonstration:

- Oswald Avery demonstrated that DNA produced inheritable changes
- This discovery was not well received.
- Not understood, how DNA using just 4 nucleotides stores the genetic information.

### Watson & Crick's model of DNA:

- Formed the famous double stranded model of DNA.
- Recognized that this model might allow replication.

### The "RNA Tie Club":

- To discover the genetic code which translates DNA to information to protein, George Gamow organized a 20 member "RNA Tie Club".
- Members wore ties representing symbol for one of the 20 amino acids.
- Scientist who discovered, was not the member of this club.

### Hurdles for Nirenburg

- Initial Goal was to determine whether DNA or RNA, was the template for the protein synthesis.
- Had no formal training in molecular genetics.
- No experience in the field & no staff at the outset.

### Experiments with Synthetic RNA:

- Nirenburg & Matthaei began experiments by studying long linear molecules of DNA & RNA.
- Chose a cell-free environment, created when cell walls are broken down, releasing the cell's contents
- Remaining cytoplasm can still synthesize protein when RNA is added
- This allows designing experiments to determine how RNA works free of complicated biological processes.
- Selected E. coli bacteria as their source of cytoplasm
- Added E. coli extracts to 20 test tubes, each containing a mixture of all 20 amino acids.
- In each test tube, one amino acid was radioactively tagged.
- Monitored radioactivity to follow the reactions.

### The Poly U Experiment:

- Matthaei added synthetic RNA made of only uracil to each of the 20 test tubes.
- Unusual Activity in one of the test tubes containing Phenylalanine
- Demonstrated that chain of uracil units in the hot tube instructed the addition of hot amino acid.
- Synthetic RNA made up of a chain of multiple units of uracil instructed a chain of amino acids to add PHE.
- Poly U served as a messenger directing protein synthesis.
- Proved that mRNA transcribes genetic information from DNA.
- Exactly how many U's required still remained a question.

Summary - Deciphering the Genetic Code

### Nirenburg in Moscow Then:

- Presented his successful poly-U experiment in Moscow.
- Unaware of outsider status
- Only 35 people attended
- Met Watson & presented the paper again → Reaction was incredible

### Rest of the Puzzles:

- After poly-U experiment, scientists raced to translate unique code words for each amino acids hoping to read entire genetic code of some living organisms one day.
- Researchers found coding unit for amino acids contain 3 nucleotides.
- Nirenburg & Philip Leder discovered a way to determine sequence of <sup>TTT</sup> codons in each triplet words for amino acids (1964)
- By 1966, Nirenburg deciphered all 64 RNA 3 letter code words for 20 codons.

### THE NOBLE PRIZE:

- In 1968, Nirenburg won the noble prize in physiology for his seminal work on genetic code
- Shared with Hargobind Khorana & Robert Holley.
- After this, Nirenburg turned his attention to microbiology.
- Spent rest of career to at NIH to save his time.

### Reactions:

- BIG DISCOVERY
- Raised ethical concerns about potential for genetic engineering.
- National Historic Chemical Landmarks.
- The American Chemical Society designated the Deciphering of the genetic code as a National Historic Chemical Landmark at NIH Bethesda.
- ACS is the world's largest scientific society with more than 1,54,000 members.

## Molecular Structure of Nucleic Acids

- ▶ Discussion on Structure of Nucleic acid proposed by Pauling & Corey.
  - Model consisted of 3 intertwined chains
  - Phosphates on the fibre axis.
  - Bases on the outside
- ▶ Reasons why Watson & Crick discarded the model.
  - No Acidic Hydrogen : In the absence of the acidic hydrogen, it becomes unclear about the forces holding these strands together, because the negative charged phosphate groups also repel each other.
  - Small distances : Some vanderwal distances appear to be too small that the structure did not seem feasible
- ▶ Comment on another 3 chain structure by Fraser
  - Phosphates are on the outside
  - Bases are in the inside
  - Bases are linked by hydrogen bonds. (Structure was ill-defined)
- ▶ Description of the structure of DNA given by Watson & Crick.
  - Structure has 2 helical chains coiled around the same axis.
  - Chemical Assumptions used : Each chain consists of phosphodiester groups joining  $\beta$ -D-deoxyribofuranose residues  $3'$ - $5'$  linkages.
  - The 2 chains (but not their bases) are related by a dyad perpendicular to the fibre axis.

- Both chains follow right handed helices
- Owing to the dyad, sequences of the atoms in the 2 chains run in opposite direction
- Comparison made to Furberg's Model : Each chain loosely resembles Furberg's model since bases are on the inside of the helix & phosphates are on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', since the sugar is roughly perpendicular to the attached base.
- Some specific dimensioning :
  - There is a residue on each chain after every  $3.4 \text{ \AA}$ . in the  $z$ -direction.
  - They assumed an angle of  $36^\circ$  between adjacent residues in the same chain
  - The direct consequence of this assumption is that the structure repeats after 10 residues on each chain i.e. after  $34 \text{ \AA}$ .
  - The distance of a phosphorous atom on the fibre axis is  $10 \text{ \AA}$ .
- As phosphorous atoms are on the outside, cations have easy access to the negative charged phosphates.
- The structure is open and the water content is assumably high.
- At lower water content, they expected the bases to tilt so that the structure could become more compact.

- Description of how the structure is held together
  - The 2 chains are held together by the purine & pyrimidine bases.
  - Planes of the bases are perpendicular to the fibre axis.
  - They are joined together in pairs, a single base from one chain being hydrogen bonded to a single base from the other chain, so that they 2 lie side-by-side with identical z-coordinates
  - One of the pair must be a purine & the other must be pyridine for bonding to occur.
- Hydrogen bonds are as follows :
- purine pos-1 to pyrimidine pos-1
  - purine pos-6 to pyrimidine pos-6.
- Only specific pairs of bases can bond together.
- If it is assumed that bases only occur in the structure with most plausible tautomeric forms (with keto rather than enol configurations), it was found that only specific pairs of bases can bond together.
- These specific pairs are
- (1) Adenine (purine) with Thymine (pyrimidine)
  - (2) Guanine (purine) with Cytosine (pyrimidine)
- For instance, if Adenine forms one member of a pair, on either chain, then the other member must be thymine.

- Consequences of the specific base-pairing.

- Sequence of bases on a single chain does not appear to be restricted in anyway.

- If sequence of bases on one chain is given, then the sequence of the other chain is automatically determined.

- Experimental support for specific base-pairing.

- It was experimentally found by Chargaff that the ratio of the amounts of adenine to Thymine, and the ratio of guanine to cytosine, are always very close to unity for DNA.

- This experimental observation directly supports the specific base pairing.

- This structure would not have been possible with a ribose sugar, instead of, the deoxyribose sugar, because the extra oxygen atom would make too close a van-der-waal's contact.

### What previous X-ray structure of the DNA tells?

- The previously published X-ray data on DNA was found to be insufficient for rigorous test of their structure.

- But the results found previously from the X-ray data, was compatible with the structure of the DNA proposed by Watson & Crick.

- Structure implies a copying mechanism.

Watson & Crick clearly indicated that as a consequence of the specific base-pairing, a possible copying mechanism for the genetic material emerges.

It has been written as

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a fully possible copying mechanism for the genetic material."

- Experimental Evidences for the polynucleotide chain being helical, and its existence in this form in the natural state.

- The structure of the DNA is same in all species in nucleoprotein, extracted or in cells, and in purified nucleate.
- Although the nitrogen base ratio differs considerably.
- The same linear group of polynucleotide material may pack together parallel in different ways to give crystalline, semicrystalline or paracrystalline material.
- In all these cases, the X-ray diffraction photograph consists of 2 regions, one determined largely by the regular spacing of the nucleotides along the chain, and other by the longer spacings of the chain configuration.
- The sequence of the nitrogenous bases along the chain are not made visible.

- Fibre Diagram of the Oriented paracrystalline DNA by Franklin & Goslin.
  - Astbury suggested that the strong  $3.4 \text{ \AA}$  reflection corresponds to the internucleotide repeat along the fibre axis.
  - The  $3.4 \text{ \AA}$  layer lines are not due to a repeat of the polynucleotide composition, but due to chain configuration repetition, which causes strong diffraction as nucleotide chains have higher density than the interstitial water.
  - The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to the fibre length.
- ▶ Diffraction by helices
  - Intensity distribution in the diffraction pattern of a series of points equally spaced along the helix is given by the square of Bessel function.
  - A uniform continuous helix gives a series of layers of spots corresponding to the helix pitch, the intensity distribution along the  $n^{\text{th}}$  layer line being proportional to the square of  $J_n$ , the  $n^{\text{th}}$  order Bessel function.
  - A straight line may be drawn through the innermost maxima of each Bessel function and the origin.
  - The angle which this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis.

- If a unit repeats  $n$  times along the axis helix, there will be a meridional reflexion ( $\text{J}_0^2$ ) on the  $n^{\text{th}}$  layer line.
- The helical configuration produces side-bands on this fundamental frequency, the effect being to reproduce the intensity distribution about the origin around the new origin, on the  $n^{\text{th}}$  layer line.

Effect of shape and size of the repeat unit on the diffraction pattern:

- If the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide.

- If the nucleotide consists of a series of points on a radius at right angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same.

## Summary of : Who discovered messenger RNA ?

- The paper discusses the history of the discovery of the m-RNA and about all the people involved in it.  
In this summary, we shall go over all the discoveries and breakthroughs that occurred in years that finally led to the discovery of the m-RNA.
- Genetic role of DNA:
  - Acceptance of genetic role of DNA began in 1944 with the publication of Avery, MacLeod & McCarty's first paper on the identification of the transforming principle in pneumococcal bacteria as DNA.
  - Became "working hypothesis" that DNA was the hereditary material remained unclear "how genes function"
- Watson & Crick Model of DNA:
  - ↳ Suggested that sequence of bases on DNA contain genetic information
  - ↳ New issue : How the information was turned into biological function.
- George Gamow's model
  - ↳ Proposed that proteins were synthesized on the DNA molecule itself.
- Reasons for dismissal of Gamow's model:
  - Crick dismissed this model; he was convinced that protein synthesis did not directly involve chromosomal DNA, but took place in cytoplasm & required RNA (form of RNA was not clear)
  - This was based on the work of Brachet and Casperson (1940's) who reported that
    - RNA was found in cytoplasm where protein synthesis took place
    - RNA levels increased in cells that were actively synthesizing proteins.

- Boivin's hypothesis on how RNA fitted into the gene function

↳ "The macromolecular desoxyribonucleic acids govern building of macromolecular ribonucleic acids, and, in turn, these control the production of cytoplasmic enzymes".

- This was the first hypothesis on RNA fitting into gene function.
- Boivin was one of the most visionary and earliest supporters of Avery's claim that DNA was the hereditary material.

- Dounce's Model :

- Proposed a theoretical model on how protein synthesis happened on RNA molecule but not DNA molecule.
- The model was wrong.

↳ Description of "Hypothesis of Collinearity" (Proposed by Crick) in the Dounce's model :

"Specific arrangement of amino acid residues in a given peptide chain is derived from the specific arrangement of nucleotide residues in a corresponding specific nucleic acid molecule."

- Dounce also refined his and Boivin's conception on link b/w nucleic acids and proteins → describing as "DNA - RNA - Protein"

- Drawbacks of Dounce's model :

- 1) Not specified the form, location or function of the RNA in this description.
- 2) Not based on transfer of genetic information b/w different molecules.
- 3) Each amino acid was assumed to have a physical link b/w DNA & RNA bases rather than the abstract informational link.

## Discovery of Ribosomal RNA :

- RNA rich structures called microsomal particles were found in the cytoplasm, it was only in 1958 that they were termed "ribosomes".
- Ribosomal RNA was the only form of RNA that had been clearly identified.
- It was quite possible to assume that this was the RNA intermediary b/w DNA and protein.

## Crick's Idea :

- Central Dogma : It was not possible that flow of information takes place from protein to DNA or RNA.
- Obligatory location for the cytoplasmic RNA template that his hypothesis required were the ribosomes.
- Assumed that ribosome consisted of a common protein structure together with a unique sequence of RNA, which acted as template for protein synthesis.
- This view was based on Hoagland & Zamecnik's discovery that during protein synthesis radiolabelled amino acids were initially found only in the ribosomes, strongly suggesting that amino acids had to be passed through the ribosome.
- Hypothesized the existence of "adaptor molecules": small, highly unstable, set of RNA molecules that would bring each amino-acid to ribosome so that it could make protein.
- Hoagland and Zamecnik were simultaneously identifying RNA species which eventually became to be transfer - RNA.
- So Crick proposed only 2 types of RNA: r-RNA & t-RNA and did not feel the need for m-RNA.

## Early Sightings:

- Many experiments in 1950's reported of a short lived RNA intermediary produced by genes → now we call them mRNA
- Either the speculating conclusions were not supported by the results or the results were reported erroneously -
  - Work done by Turner & Szafarz:
    - Attempted to identify differential turnover in different RNA fractions
    - Hampered by relatively primitive techniques
    - Hypothesized that
      - RNA was synthesized in the nucleus
      - Then passed, in form of small molecules, into cytoplasm
      - In cytoplasm, it was integrated with cytoplasmic particles of large dimensions.
  - In 1958, Turner showed that RNAase prevent synthesis of phage proteins following infection ~~from~~ of a bacterial cell.
  - Concluded that "RNA with rapid turnover is a specific product of the infection and plays a role in the synthesis of phage protein."
  - Work by First Monod's group & Arthur Pardee.
    - Showed that in mutant bacteria,  $\beta$ -galactosidase synthesis required the presence of RNA specific nucleotide uracil indicating that RNA synthesis was necessary for protein synthesis.
  - Work done by AL Hershey's group:
    - Shortly after infection with a phage, bacteria produced a form of RNA that was both synthesized at a high level and also broken down rapidly. No conclusions drawn.

- Work by Volkin & Astrachan (1956)
- Used radioactive phosphorus to show that when *E. coli* cells are infected with bacteriophage, radioactivity was found in an RNA fraction, the base composition of which was very different from the RNA normally produced by *E. coli*.

Interpretation: Transitory form of RNA was a precursor to DNA.

- Work by Volkin & Astrachan (1958)
- While radioactive RNA appeared rapidly in bacteria after phage infection, if the isotope was added later, then more radioactivity was found in RNA than DNA.

Interpretation: ~~This~~ RNA acts as a precursor to DNA.

- Work by Nomura, Hall & Spiegelman:
- Refined Volkin's & Astrachan's approach.
- Showed that after phage infection, 2 forms of RNA were synthesized, one was found in ribosomal fraction, the other in soluble RNA.
- Interpreted the soluble RNA fraction as either a precursor of ribosomal RNA (or its breakdown product) or as being involved in "the amino acid accepting function of normal soluble RNA."
- Can be related to Crick's adaptor molecule

### Imagining m-RNA

- Arthur Pardee, while working with Jacob Monod on the genetic basis of induction, in which bacteria forms  $\beta$ -galactosidase in a medium containing lactose.
- Mutant lac<sup>-</sup> bacteria could not grow into this medium unless they

acquired the  $\lambda^0$  gene (which codes for  $\beta$ -galactosidase).

- Purdee showed that when  $\lambda^0$  gene was transferred into a lac<sup>r</sup> individual,  $\beta$ -galactosidase synthesis began within minutes.
- This gave implication for the presence of messenger molecule.
- They could not say how exactly the process worked.
- Named this messenger molecule PaJaMo messenger molecule.
- Experiments also revealed that the gene did not produce any stable ~~mRNA~~ ribosome but only transitory messenger molecule 'X'.

### Idea for the mRNA:

- When Crick & Sydney were informed about the PaJaMo experiment they suddenly realized the presence of messenger RNA.
- This could explain the results by Volkin & Astrachan and many others that suggested that after phage infection, bacteria produced short lived form of RNA with same composition as phage DNA.

### Isolating mRNA

- Jacob & Brenner with the help of Matt Meselson and his ultracentrifuges isolated the mRNA.
  - They could also show that no new ribosomes were formed and the old ribosomes could synthesize proteins from the new mRNA.
- Isolation of m-RNA by Watson & Risdenrough:
- Work by Robert Risdenrough at Harvard convinced Jim Watson that protein synthesis took place through the action of transitory template RNA molecules.
  - In a long series of experiments, they could find reveal the presence of transitory RNA molecules.

### Description of mRNA by Jacob & Monod.

- Polynucleotide
- Varying Molecular Wt.
- Possessing high rates of turnover
- Base composition reflects that of DNA
- Temporarily associated with ribosomes

Work by Sol Spiegelman & Benjamin Hall (1960)

- Showed experimentally that the transitory m-RNA showed sequence complementary to the DNA.
- Route for DNA to RNA, as codified by Crick in 1957, was shown to be existent.

Nirenburg & Matthaei R. worked on various aspects in the discovery of m-RNA but <sup>not much</sup> nothing could be proved.

Conclusion:

We can't regard any person or group of persons for the discovery of m-RNA. Its discovery was a process which took decades.

Hence, no noble prize was given for the discovery of m-RNA.

## Summary of "Chemical Nature of the Substances inducing Transformation of Pneumococcal Types"

- The present study deals with the result of an attempt to determine the chemical nature of the substance including specific transformation of pneumococcal types
- DNA fraction from type III pneumococci which is capable of transforming unencapsulated & variants derived from type II to fully encapsulated type III cells.
- Highly purified & protein free material consisting largely of DNA, is capable of stimulating unencapsulated R variants of type II to produce a capsular polysaccharide identical in type specificity with that of cells from which the inducing substance was isolated.
- Inducing substance, on the basis of its chemical & physical properties, appears to be highly polymerized & viscous form of sodium desoxyribonucleate.
- On the other hand, type III capsular substance consists chiefly of a non-nitrogenous polysaccharide constituted of glucose-glucuronic acids unit linked in glycosidic union.
- It is evident that the inducing substance and the substance produced in turn are chemically distinct & biologically specific in their action and both are requisite in determining the type specificity of the cell of which they form a part.
- Experimental data presented in this paper strongly suggests that nucleic acids, atleast those of desoxyribose type, possess different specificities as evidenced by the selective action of the transformi

-ng principle.

- The paper also describes several methods for the isolation and purification of the active-transforming material.
- There were 4 major components in this study each of which provided significant challenge:

1 → Nutrient Broth:

- Individual lots of broth show marked & unpredictable variations in the property of supporting transformation.
- Procedure described by MacLeod & Mirick was used.

2 → Serum or Serous Fluid:

- Anti R pneumococcal rabbit serum was used because of the observation that reversion of an R pneumococcus to the homologous S form can be induced by growth in a medium containing anti R serum.
- Sera from various animal species, irrespective of their immune properties, contain an enzyme capable of destroying the transforming principle in potent extracts.
- This enzyme is inactivated by heating the serum at 60 - 65 °C, and sera heated at this temperature are often rendered effective in the transforming system.

3 → The R strain : R36 A.

The unencapsulated R strain used in the present study was derived from a virulent "S" culture of

pneumococcus type II.

- The designation to these variants as R forms has been used to refer mainly to the fact that on artificial media, the colony surface is "rough" in contrast to smooth.
- Methods for synthesis of this R<sub>36A</sub> variant has been described in the paper.

2 Facts that must be kept in Mind.

- (i) an R culture can undergo spontaneous dissociation and give rise to other variants which have lost the capacity to respond to the transforming stimulus.
- (ii) Pneumococcal cells contain an intracellular enzyme which when released destroys the activity of the transforming principle.
- Method of titration of transforming Activity has been shown in the paper.
  - Anti R properties of the serum in the medium cause the R cells to agglutinate during growth, and clumps of agglutinated cells settle to the bottom of the tube leaving a clear supernatant.
  - When transformation occurs, the encapsulated S cells not being affected by these antibodies, grow diffusely throughout the medium.

- The data obtained by chemical, enzymatic and serological analyses together with the results of preliminary studies by electrophoresis, ultracentrifugation, and ultraviolet spectroscopy indicate that, within the limits of the methods, the active fraction contains no demonstrable protein, unbound lipid or serologically reactive polysaccharide and consists principally if not solely of a highly polymerized, viscous form of DNA.
- Evidence is presented that chemically induced alterations in cellular structure and function is predictable, type-specific and transmissible in series.
- Various hypothesis that have been advanced concerning the nature of these changes are reviewed.
- The evidence presented in the paper supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of *Pneumococcus Type III*.

## The Composition of the Pentose Nucleic Acids of Yeast & Pancreas.

- The study consists of many experiments the results of which complement each other.

### (i) RNA of Yeast :

- Method of purification of RNA from Yeast has been described.
- % N = 15.3 ; % P = 8

### (ii) Pentose Nucleic Acid of Pig Pancreas :

- Follows from the procedure described by Levene & Jorpes.
- Free pentose Nucleic Acid gave no protein tests and contained only a small amount of DNA

$$\% \text{ N} = 15.4$$

$$\% \text{ P} = 7.9$$

### • Preparation of Ribose Nucleotides :

→ Methods of preparations of Adenylic acid, basic sodium guanylate & cytidylic acid have been explicitly mentioned in the paper.

→ Methods for the quantitative estimation of purines in nucleic acid & pyrimidines in nucleic acids have also been mentioned in the paper.

- Resistance of Pyrimidines to Acid Treatment:

- Liberation of pyrimidines from nucleic acids requires an extremely drastic treatment.

- Advantage can be taken of the fact that ease with which changes in the composition of the pyrimidine mixture can be followed with which changes in the composition of the pyrimidine mixture can be followed with the new chromatographic method.

- Experiments indicate the instability of cytosine, which was to a large extent converted to uracil, in acids other than formic acid.

- Thymine & Uracil resisted the acid treatment.

- Hydrolysis of Yeast RNA with strong HCl.

- ↳ There was enormous shift in the relative proportions of the 2 pyrimidines, although the total amount recovered was nearly the same.

- ↳ The molar cytosine to uracil ratio was found to be 0.3.

- Hydrolysis of Yeast RNA with Formic & HCl Acids

- This experiment excluded the possibility that the attacks on the nucleic acids by formic acid & HCl were directed against different groupings & were selective w.r.t. proportions of liberated pyrimidines.

- Composition of Yeast RNA:

## I Purines:

- Only purines encountered: Adenine & Guanine.
- Average Adenine % = 9.1 %
- Average Guanine % = 10.2 %

## II Pyrimidines:

- Cytosine & Uracil were the pyrimidines found in the experiment.
- Average Cytosine % = 6.7 %
- Average Uracil % = 2.3 %

## Composition of Pentose Nucleic Acid:

- Average Adenine % = 5.7 %
- Average Guanine % = 15.5 %
- Average Cytosine % = 5.5 %
- Average Uracil % = 1.2 %

Molar Guanine to Adenine ratio : 2.7.

A procedure, permitting the characterization of the nitrogenous constituents in very small quantities of nucleic acids and application of chromatography on filter paper to the identification of carbohydrate components of nucleic acids are likewise described.