

Molecular Basis of Inheritance

DNA - carrier of genetic information in all cellular organisms.

Few viruses (TMV, polio, retroviruses) have RNA as genetic material.

Key Milestones

- 1869 Friedrich Miescher discovers 'nuclein'
- 1928 Griffith's transformation experiment
- 1944 Avery, MacLeod, McCarty - DNA = TP
- 1952 Hershey-Chase - DNA = genetic material
- 1953 Watson-Crick double helix
- 1958 Meselson-Stahl - semi-conservative
- 1961-66 Genetic code cracked (Nirenberg, Khorana)
- 1990-2003 Human Genome Project
- 2010 1st synthetic genome (Venter)

Indian contribution - Har Gobind Khorana (Nobel 1968)
- synthesised oligonucleotides, cracked the code.

Griffith's & Avery's Experiments

Griffith (1928) - Transformation

Worked on *Streptococcus pneumoniae* :

S (smooth, virulent) - causes pneumonia in mice

R (rough, non-virulent) - mice survive

Experiments :

(a) Live R injected \rightarrow mouse lives

(b) Live S injected \rightarrow mouse dies

(c) Heat-killed S injected \rightarrow mouse lives

(d) Heat-killed S + Live R \rightarrow mouse DIES,
live S recovered from dead mouse.

Conclusion : R strain TRANSFORMED into S.

Some 'TRANSFORMING PRINCIPLE' from killed S.

Avery + MacLeod + McCarty (1944)

Purified 'transforming principle' and showed :

Protease + RNase \rightarrow transformation still works

DNase \rightarrow transformation BLOCKED

Hence : DNA = transforming material = genetic material

Hershey-Chase (1952) - confirmed DNA

Bacteriophage with ^{35}S protein + ^{32}P DNA - only ^{32}P ent

Nucleotide = Base + Sugar + Phosphate

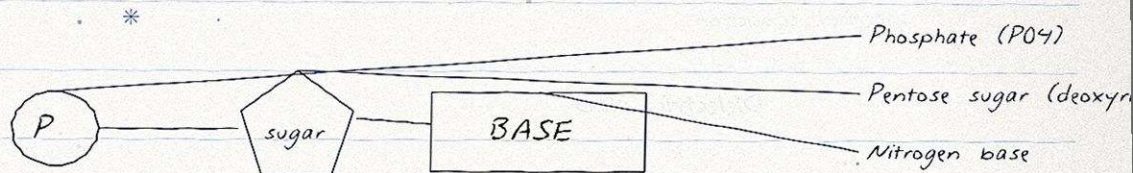


Fig. A single nucleotide unit

Nitrogen Bases

Purines (2 fused rings) : Adenine (A), Guanine (G)

Pyrimidines (1 ring) : Cytosine (C), Thymine (T)

[Uracil (U) - in RNA only]

Sugar Difference

DNA - deoxyribose (2'-OH replaced by H)

RNA - ribose

Chargaff's Rules

%A = %T ; %G = %C ; Purines = Pyrimidines

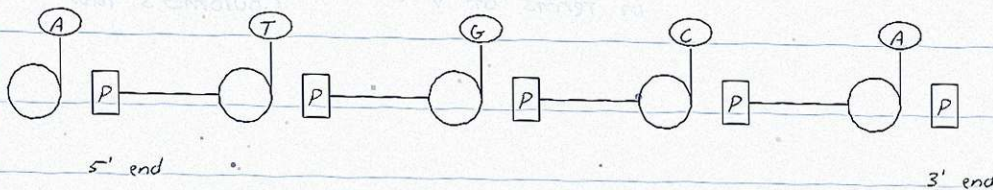
DNA - Polynucleotide Chain

Polymerisation

Two nucleotides join through PHOSPHODIESTER bond between 3' OH of one sugar and 5' phosphate of next.

Backbone = sugar-phosphate-sugar-phosphate...

Fig. Strand polarity (5'-3')



Double Helix Features

- (i) Two strands - antiparallel (5'-3' & 3'-5')
- (ii) Right-handed helix
- (iii) Diameter 20 Å
- (iv) Pitch 3.4 nm ; 10 bp per turn
- (v) Adjacent base distance = 0.34 nm
- (vi) Bases held by H-bonds : A=T (2), G=C (3)
- (vii) Bases inside, sugar-P backbone ~~inside~~ outside
- (viii) Major + minor grooves alternate.

Double Helix (Watson-Crick, 1953)

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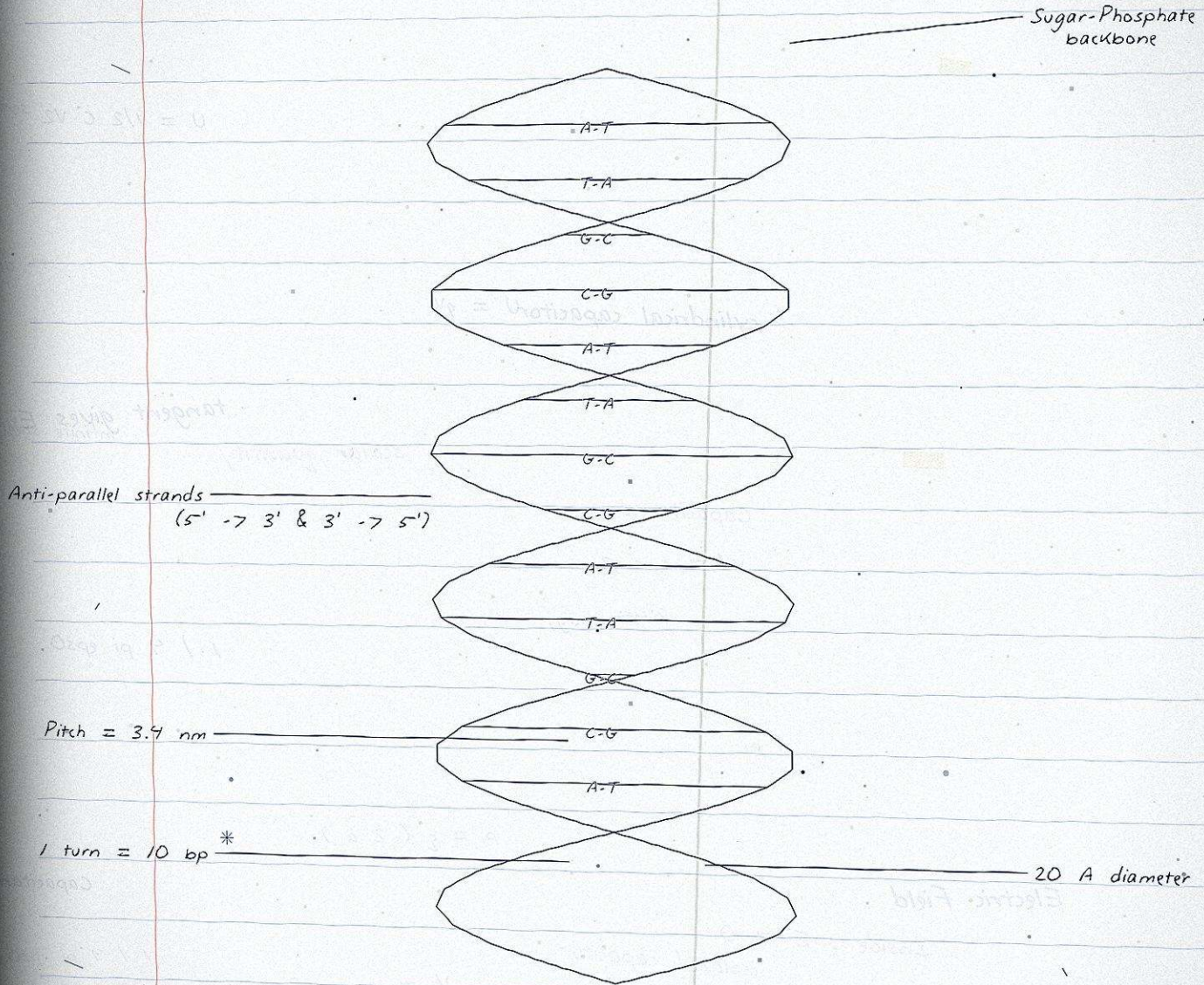


Fig. B-form double-helical DNA

Total length of human DNA 2.2 m (3.3 x 10⁹ bp)
 Distance between adj. base pairs = 0.34 nm
 Other forms : A-DNA (dehydrated), Z-DNA (zigzag).

Packaging of DNA in Nucleus

Human DNA = 2.2 m total length per cell

But nucleus = 10 micrometer wide! \rightarrow needs packaging

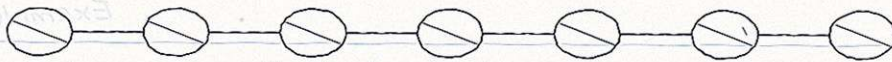
In Prokaryotes

Single circular DNA + polyamines (no histones).

Compacted into NUCLEOID by supercoiling.

In Eukaryotes - Levels of Packing

(i) Naked DNA - 2 nm



(ii) Nucleosome ("beads on string") - 11 nm



(iii) Chromatin fiber (30 nm)

(iv) Looped domains (300 nm)

(v) Metaphase chromosome (1400 nm)

Compaction ratio 10 4 fold !

Histones & Nucleosomes

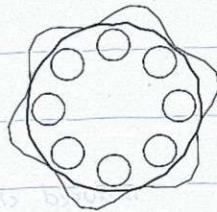
Histones - basic proteins rich in Lys + Arg.

Five types : H1, H2A, H2B, H3, H4.

Histone Octamer

8 histones = 2 copies each of H2A, H2B, H3, H4 form a positively charged HISTONE OCTAMER core.

DNA wrapped (1.65 turns)



Histone octamer

Each nucleosome - * 200 bp of DNA

Core wraps about 1.65 turns of DNA.

H1 (linker) - binds outside, holds DNA in place.

Chromatin Types

Euchromatin - loosely packed, transcriptionally active

Heterochromatin - ~~active~~ densely packed, inactive

Non-Histone Proteins (NHC)

DNA Replication

Process by which each DNA strand serves as a **TEMPLATE** for synthesis of a new complementary strand.
Result : two identical daughter DNA molecules.

Three Possible Models

- (a) **CONSERVATIVE** - parent intact + new helix
- (b) **SEMI-CONSERVATIVE** - 1 old + 1 new strand each
- (c) **DISPERSIVE** - random mixing of old and new

Meselson-Stahl (1958)

Used ^{15}N (heavy isotope) labeled E. coli \rightarrow light ^{14}N .

Density-gradient centrifugation showed :

Gen 0 : all heavy ($^{15}N/^{15}N$)

Gen 1 : all hybrid ($^{15}N/^{14}N$) - intermediate

Gen 2 : 50% hybrid + 50% light ($^{14}N/^{14}N$)

CONCLUSION - DNA replicates **SEMI-CONSERVATIVELY**.

Parent (2 old) *

↓

Daughter (1 old + 1 new)

Replication Fork & Enzymes

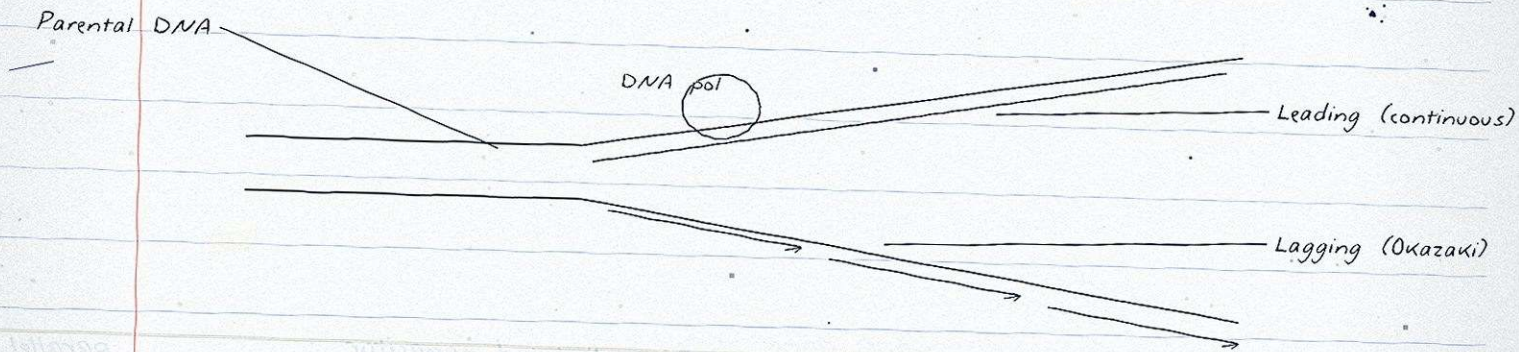


Fig. Bidirectional replication fork

Enzymes & Their Roles

- Helicase - unwinds DNA helix
- SSB proteins - stabilise single strands
- Primase - lays down short RNA primers
- DNA pol-III - main replicating enzyme (5'-3')
- Ligase - joins Okazaki fragments

Transcription

Process of copying genetic information from DNA into RNA, catalysed by RNA polymerase.

Only ONE strand of DNA is transcribed (template / antisense)

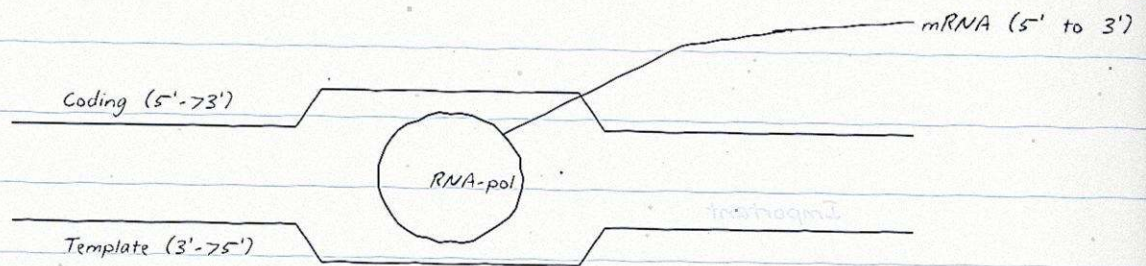


Fig. Transcription bubble

Three Stages

1. INITIATION - RNA pol binds at promoter
2. ELONGATION - ribonucleotides added 5'-3'
3. TERMINATION - RNA pol reaches terminator

Three RNA Polymerases (Eukaryotes)

RNA pol I - rRNA (28S, 18S, 5.8S)

RNA pol II - mRNA (hnRNA)

RNA pol III - tRNA + 5S rRNA + snRNA

Prokaryotes - ONE RNA pol does all 3.

Post-Transcriptional Processing

Eukaryotic primary transcript (hnRNA) is modified before becoming mature mRNA.

(i) 5' Capping

7-methyl guanosine (m7G) added at 5' end.

Function - protection + ribosome binding signal.

(ii) 3' Tailing

Poly-A tail (200 adenines) added at 3' end.

Function - stability + export from nucleus.

(iii) SPLICING - removes INTRONS

Introns - intervening non-coding sequences.

Exons - coding sequences (kept in mRNA)

Introns are ~~kept~~ removed; exons spliced together.

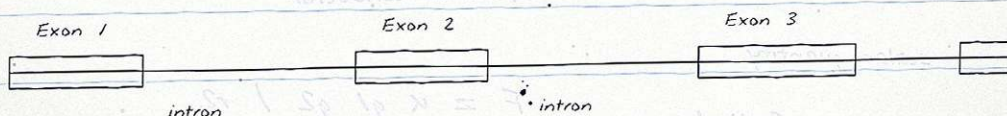


Fig. Split gene (eukaryotes only)

Spliceosome

Genetic Code

Sequence of 3 nucleotides on mRNA = CODON

Each codon specifies ONE amino acid.

Salient Features

- ① Triplet ($4^3 = 64$ possible codons)
- ② Unambiguous & specific (one codon = one aa)
- ③ Universal (same in all organisms)
- ④ Degenerate (>1 codon / aa, max 6)
- ⑤ Non-overlapping + commaless
- ⑥ Polarity (read 5' to 3' on mRNA)
- ⑦ Start codon = AUG (Methionine)
- ⑧ Stop codons = UAA, UAG, UGA

Mutations & the Code

Point mutations - change in one nucleotide.

Frame-shift - insertion / deletion changes reading frame.

Sickle cell - GAG ~~becomes~~ mutated to GUG (Glu \rightarrow Val).

tRNA - Adapter Molecule

tRNA carries amino acid at 3' (CCA) end and anticodon at loop end.

Anticodon-codon pairing aligns aa in correct order.

Charging tRNA

tRNA & Ribosome

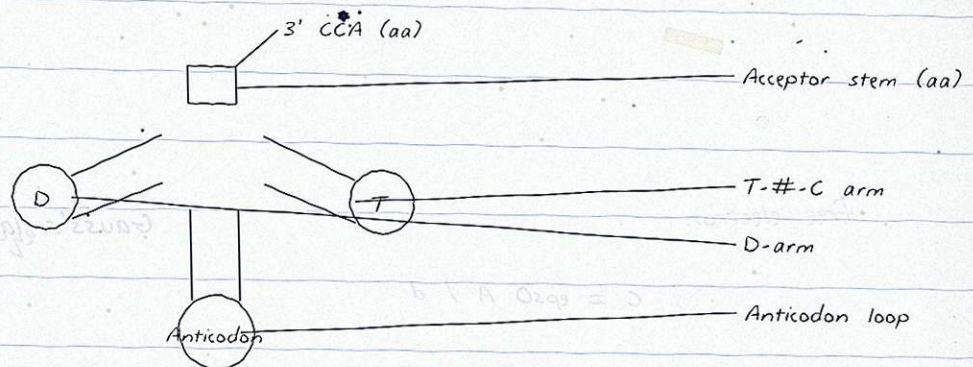


Fig. Cloverleaf model of tRNA

Ribosome - Workbench

Two subunits - small (40S) + large (60S) - eukaryotic
(70S in prokaryotes : 30S + 50S)

Has 3 sites for tRNA : A (aminoacyl), P (peptidyl),
E (exit).

Composed of rRNA (60 %) + ribosomal proteins.

rRNA is the catalyst (peptidyl transferase = ribozyme).

Translation - Protein Synthesis

Stage 1 - INITIATION

Small subunit + Met-tRNA(i) find AUG start.

Large subunit joins \rightarrow intact ribosome ready.

Stage 2 - ELONGATION

(a) tRNA + aa enters A site - matches codon

(b) Peptide bond forms (in P site) \rightarrow ribozyme

(c) Ribosome translocates \rightarrow tRNA shifts P \rightarrow E (exits)

(d) Repeat until stop codon

Stage 3 - TERMINATION

Stop codon (UAA / UAG / UGA) reached.

Release factor enters A site \rightarrow polypeptide released.

Ribosome subunits dissociate.

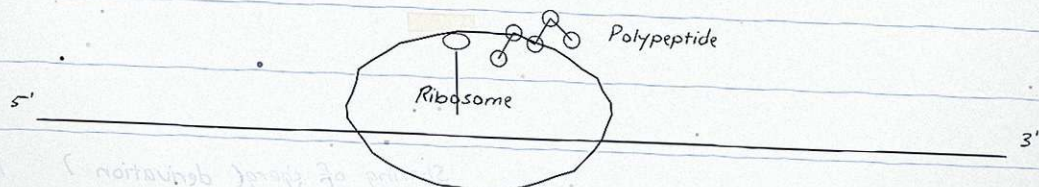
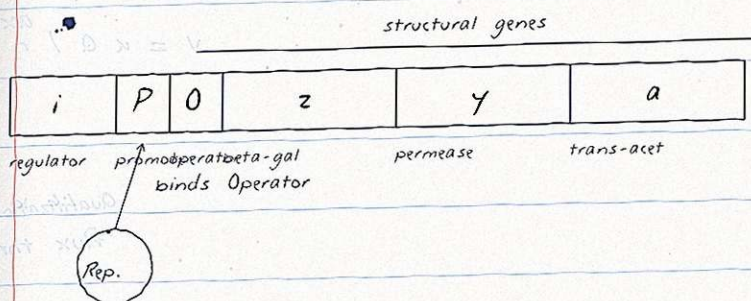


Fig. Ribosome translating mRNA

Lac Operon (Jacob & Monod 1961)

OPERON - set of genes regulated together.

Lac operon - **INDUCIBLE** operon for lactose metabolism in *E. coli*.



OFF (no lactose)

Active repressor binds operator \rightarrow RNA pol blocked.

ON (lactose present)

Lactose \rightarrow allolactose \rightarrow binds repressor.

Repressor inactivated \rightarrow ~~blocked~~ operator free.

RNA pol transcribes $z, y, a \rightarrow$ proteins made.

Catabolite Repression

Glucose preferred over lactose.

High glucose \rightarrow low cAMP \rightarrow CAP-cAMP binds weakly

\rightarrow low transcription rate.

Trp Operon

Human Genome Project (HGP)

Mega-project (1990-2003) by 6-nation consortium.

Goal - sequence ALL 3.3×10^9 bp of human genome

Cost ~~\$30 million~~ \$3 billion.

Goals

- ① Identify all 30,000 genes
- ② Sequence 3 billion bp
- ③ Store in databases
- ④ Improve data analysis tools
- ⑤ Address ethical/legal/social issues
- ⑥ Transfer to private sector

Salient Features

- (a) Total 3.3×10^9 bp - human genome size
- (b) Avg gene size 3000 bp
- (c) Total genes 30,000
- (d) Less than 2 % of genome codes for proteins
- (e) Repeated sequences make up large portion
- (f) Chr 1 - most genes (2968) ; Y - fewest (231)
- (g) Many SNPs (1.4 million) identified.

Applications

Disease gene discovery, personalised medicine, pharmacogenomics, ancestry, evolution studies.

DNA Fingerprinting

- Identifies individuals based on **UNIQUE** patterns in their DNA. Developed by Alec Jeffreys (1985).
Indian pioneer - Laji Singh (CCMB Hyderabad).

Principle - VNTRs

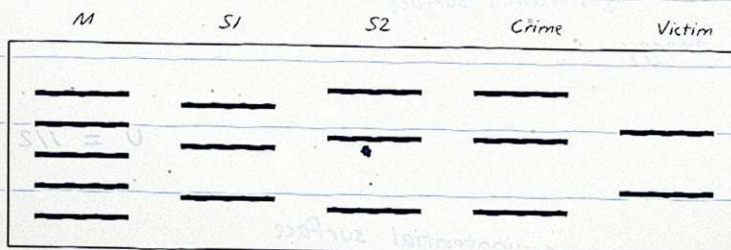
Variable Number Tandem Repeats = short sequences (11-60 bp) repeated tandemly in 'satellite DNA'.

Number of repeats varies between individuals.

Identical twins have **IDENTICAL** fingerprints.

Six Steps

- Isolation of DNA (from any tissue)
- Restriction enzyme digestion
- Gel electrophoresis (separate by size)
- Southern blotting (transfer to nylon)
- Hybridisation with VNTR probe
- Autoradiography (visualise bands)



Crime = S2 (match?)

DNA Fingerprinting - Applications

Forensic

Crime detection - matching crime sample with suspects.
Identification of victims (mass disasters).

Paternity / Maternity

Disputed parentage - child's bands should match alternately with mother and father.

Wildlife Conservation

Identify illegal trade of body parts (ivory, horns).
Track endangered species, breeding programmes.

Plant Breeding

Identification of new varieties + genetic diversity.

Anthropology + Evolution

Tracing human migrations, evolution of species.
Linking populations to ancestral groups.

Medical Diagnostics

Detect mutations causing genetic diseases.

Indian achievements - ~~vaccine~~ 7100,000 cases solved using

PCR + DNAFP

RNA - Types & Functions

Three Main Types

- ① mRNA - messenger; carries genetic code (5%)
- ② tRNA - transfer; brings amino acids (15%)
- ③ rRNA - ribosomal; structural (80%)

Other RNAs

snRNA - small nuclear; splicing

miRNA - micro; regulation

siRNA - silencing via RNAi

hnRNA - primary nuclear transcript (pre-mRNA)

RNA as First Genetic Material

Evidence - RNA can catalyse + carry info.

Hence - RNA WORLD hypothesis.

~~DNA~~ RNA evolved from DNA / vice versa? RNA first.

Ribozymes

RNA molecules with catalytic activity (Cech, Altman).

Eg. Group I introns self-splice; ribosome peptide bond formation done by rRNA.

Why DNA Took Over

DNA more stable (2'-H instead of 2'-OH).

Double-stranded -> error correction possible.

Translation Inhibitors & Antibiotics

Why Antibiotics Work

Most antibiotics target the BACTERIAL ribosome (70S) without affecting our cytosolic 80S ribosome.

Common Examples

Antibiotic	Effect
Streptomycin	binds 30S - misreading codons
Tetracycline	blocks A site (tRNA entry)
Chloramphenicol	inhibits peptidyl transferase
Erythromycin	blocks translocation
Puromycin	premature chain release
Rifampicin	inhibits RNA polymerase (transcription)

Selective Toxicity

Eukaryotic ribosome (80S) has structural differences from prokaryotic (70S) - so antibiotics don't harm us.

Mitochondrial Ribosomes

Are 70S (prokaryotic) - some antibiotics (like aminoglycosides) can damage them → side effects of these drugs.

Resistance Mechanisms

Recap & Numbers

Key Numbers

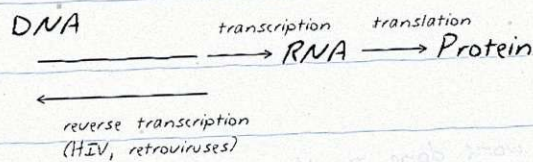
Human genome size	3.3×10^9 bp
Total length of DNA	2.2 m / cell
Number of genes	30,000
Coding %	< 2 %
Chromosome with most genes	1 (2968)
Chromosome with fewest	Y (231)
Diameter of B-DNA	20 Å
Pitch (1 turn)	3.4 nm
Base pairs / turn	10
Distance / bp	0.34 nm
Sense codon	61
Stop codons	3 (UAA, UAG, UGA)
Start codon	1 (AUG)
HGP duration	1990-2003
DNA fingerprinting (Jeffreys)	1985
Watson-Crick model	1953
DNA as transforming material	Avery 1944
Hershey-Chase exp	1952

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Central Dogma + Mutations

Central Dogma (Crick, 1958)

Information flow :



Mutations - Types

(a) Substitution - one base replaced (point mut)

Silent - no aa change

Missense - different aa (sickle cell)

* Nonsense - premature stop

(b) Insertion / Deletion - frame shift

(c) Chromosomal (translocation, inversion, deletion)

Causes of Mutation

Physical - UV, X-rays, gamma rays

Chemical - base analogs (5-BU), alkylating agents

Biological - transposons, viruses

Significance

Source of variation - raw material for evolution.