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Molecular Genetics – the Ideas

Nature of the ideas: easy, many, not deep: easily related to everyday life i.e. can make models.

School-children can understand.

nevertheless ideas (as well as techniques) important in development.

reason for this: basic theory of chemistry and physics.

physics not known is (mainly) irrelevant.

chemistry calculations difficult in practice.

Schroedinger book "What is life?" Influence

Ito point. Stability, chemical bonds, genes.

aspects of chemistry & physics used:

obviously in techniques e.g. X-ray diffraction

radioactivity

(ultracentrifuge, electrophoresis, etc)

but especially: bond-distances and angles.

Hydrogen bonds, van der Waals interactions.

stereochemistry: the one handedness – nucleic acid

– protein,

– sugars etc.

shows shape is important. e.g. many enzyme reactions

thus why stereochemistry is important.

few basic principles: the helix: equivalence (virus point groups)

almost equivalence ~ coiled coils (viruses)

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the basic ideas DNA ⇝ RNA ⇝ protein.
their success; except r-RNA.

subsid. idea: nucleic acid replication due to base-pairing
~~the~~ also probably involved in protein synthesis
(though that necessarily more complicated)

the two linear languages : the 4 letter one
the 20 letter one.

related by the code

ie only sequence matters; units held together stably by chemical (ie strong) bonds.

① how does this give the ~~[illegible]~~ great variety required?

proteins: the one-dimensional nature of the chemical structure
(remember cross-links)

the three-D nature of the physical structure.

analogy: the structure of a paragraph of English.
(contrast Chinese)

② why two languages?

the importance of natural selection — must basic idea in all biology.
(the idea: random mutations)

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the requirements for natural selection

① geometrical replication – fairly exact

② meaningful random mutations necessary
i.e. "mistakes" – "side-reaction"
thermal vibration. | gives opportunity to improve.

③ "versatility"

[④ requirement for an "association" i.e. a cell]
Gene + products must stay associated.

thus nucleic acid: → from 2 (+3) stranded structure.
idea for replication, but very
inversatile. (probably no third base pair possible)

~~protein~~
~~[illegible]~~

protein: main structure is α-helix
single-stranded. marginally stable in
water (hydrophobic group tuck inside)

20 ~~[illegible]~~ diff residues, with a variety of
possible chemical functions.

BUT probably bad for replication.

Hard not clear that an elaborate system
could not do it. May be due to "origins" i.e. historical accident.

~~notice the coupling system is elabor~~

4

Are two distinct languages necessary on theoretical grounds i.e. to avoid confusion between the master copy (the gene) and the machine tools (many copies required) ~~[illegible]~~?

notice process is unidirectional explain

the central dogma (explain)

irreversibility due to energy put into it.

(also helps to increase accuracy?)

partly explains why ~~inherited~~ acquired characteristics not inherited.

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maths in biology ~~[illegible]~~ for special purposes

e.g. x-ray diffraction

is any theory too difficult for experimentalists? } theory of ~~[illegible]~~ unwinding ~~[illegible]~~ helices (the pure theoretician)

recently, the economics of the cell.

lack of application of information theory in molecular genetics yet.

probably due to very low noise of the channel.

in fact mutation rate too low

(natural selection prob. operated to reduce this)

repair mechanisms?

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most important overall conclusion.

in ~~biology~~ genetics individual atoms matter, but only in

special places.

obvious for many years that genetic information at or near atomic level.

example of sperm: size: half information:

human complexity: therefore ---

how does atom produce its effect?

take sickle cells case

explain disease (two genes) 1 aa. change.

thus 1 (×2) "atoms" wrong. how does this kill.

fertilized egg. cell division: many 10^{12-13} cells.

cells in bone-marrow: each many copies of

(stable) m-RNA: each make many, wrong copies of proteins.

10^6 cells/second ~~10^6~~ 10^7 molecules Hb?

(life 3 months?)

Molecular Genetics

finite { stress the genetic message is a linear one, its written in a polymer there is a lot of it; and its written at the atomic level.

Problem for the future: control: embryology (shape & form) origins. HNS.

Remarks on methods

Some experiments done on intact cells; some on purified parts of broken cells; some are both techniques.

methods must (So can be repeated many times) be rapid, and sensitive i.e. work on small quantities

techniques for Separation: ultra centrifuge
electrophoresis
Chromatography + columns
— paper.
(counter current)

Nuclear physical-chemistry.

techniques for detection and recognition
— U.V. absorption
— radioactivity.

Subject has advanced much because methods so powerful (in addition to a good active theory)

1/

The Structure of DNA by X-rays

work of Wilkins + Franklin

Nature of Structure — S

(also calf-thymus)

preparation of DNA : break open cells / : selective precipitation.

can be got fairly pure, but "damaged" fibrous mass.

technique of pulling fibres — to get alignment. an art

control of humidity (also the salt, and pH)

taking x-ray photograph. Longish exposures. need fair amount of material (contrast e/m)

what do x-rays see? electron density : not base sequence.

repeating structure gives discrete "spots". Examples : S

note a fibre diagram; crystallites, etc. S

To solve structure: Fourier transform : amplitudes but not phase

(pseudo-echo symmetric). Transform of a helix. ~~bases~~ stacks of bases

backbone S

Model building : distances and angles known.

"rotating" and "physical contact" are not known, but obey rules.

Good for for all models

S

base pair in a crystal. ethyl guanine | other crystals.

methyl cytosine.

[AT pair different]

Meselson - Stahl expt

ultra centrifuge (commercially available) - two types) swinging bucket

various ways to use : boundary sedimentation.

Sucrose gradient "

density gradient centrifugation (Vinograd)

explain : method.

use of heavy isotopes. ie pair N_{14} + N_{15}

describe experiment : grow many generations on N_{15}

time zero : change to N_{14}.

later time, break open + put into cell.

DNA "chromosome" broken into pieces, but still high MW.

later do not expect S

Results S

Show a duplex.

Subsidiary experiments show not end to end

and almost certainly a two chain duplex, not a four.

3

KORNBERG — JOSSE

~~Ans~~. Nearest neighbour experiment. use of radioactive isotopes

diff. DNAs have diff average composition.

sequence not random.

design of experiment. ——— S

result. ——— S (control — can be done twice)

shows chains run in opposite directions..

4

Fine Genetic Mapping

Results by genetic methods show

ⓐ genes arranged in a linear (circular) array i.e. not 2D

ⓑ units of a gene in linear

ⓒ many distinct sites inside a gene.

BENZER

my example: the r_{II} locus of phage T4. explain growth cycle

r_{II} mutants.

"Spontaneous"

chemical – base analogues

[acridines]

	B	K
mutant	grow but look different	don't grow
wild	grow	grow.

How ⓐ can we pick up mutants

ⓑ selection technique

method of genetic cross: using selection technique.

map distance.

deletion mapping.

result map unit many sites. ——·—— S

If lots of time do cis–trans test.

to show two genes, or cistrons.

Colinearity of gene and protein — State result

Yanofsky's results : A protein of tryptophane synthetase of E. Coli.

Work of Sarabhai, Stretton & Brenner, and Bolle.

Head protein of phage T4.

major protein of ~~head~~ phage. major late protein.

∴ most radioactive label goes into this protein

∴ no need to purify.

Special class of mutants "amber"

	Host A	Host B
Amber mutant.	mutant chain terminated	"wild" chain finished.

Special gene & which does this

Then can be grown in Host B
but tested in host A.

Series of amber mutant, picked up by hand work. map — S

~~Host~~

Chemical test in host A.

Label in vivo all with one radioactive amino acid

Break open. (remove DNA) digested with trypsin

do one-D paper electrophoresis to see which peptides there. Repeat with ① diff. amino acids for ② chymotrypsin ③ diff. amber. Result — S