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EMBO Heidelberg 4th Nov. 72

The Molecular Biology of Higher organisms

Choice of topics: mechanisms common to "all" plant or "all" animals
: personal selection.
make a few GENERAL theoretical suggestions.

Reason for success of classical Mol. Biol.

Theory { ① Firm theoretical substratum : chemistry, physical chemistry, physics.
② Lucky to have a broad theoretical framework fairly early
DNA → RNA → protein DOT, mRNA

Practice ↓ ③ choice of simple systems in spite of obvious disadvantages
eg. E Coli. Tevens phages etc.

④ use of modern physico-chemical techniques.
some simple, some requiring complex instrumentation.
eg. chromatography, ultracentrifuge, radioactive tracers, e/m, x-ray diff ETC

⑤ the use of Genetics as a tool (to be discussed later)

⑥ the use of cell-free systems : partly for "purity" (Hard often impure)
partly for ease of addition. Genetic
combined with studies on intact systems BIOCHEMISTRY for the kill, code

General approach reformulate problems, often in terms cellular, or, more often, molecular elements ie define sub-problems

Hope
for these the "classic" systems may not be the best
Thus we have the choice of the organism - discuss later.

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Summary of some of the problems

For each element have to consider ① Its Structure
② Its Function
③ Its Synthesis

~~Sa~~ ~~Sa~~ Start with Chromosomes

- ~~more~~ - bigger than prokaryotes : probably one chromosome all one molecule of DNA.
  - more complex than prokaryotes ~~ex~~ _ examples
    ie. DNA more varied : simple sequence, repititive, unique
    contains proteins histons 5 main types, some rather invariant
    non-histone (= acidic) proteins.
    cRNA?

~~a Gene~~ also: individual genes (= complementation group) rather big
Drosophila: a cv ~ 20,000 to 30,000 bp.
problem of structure bands : interbands
x-ray diff.
simple sequence = heterochromatic location.

Logic of controls
BudR

Function "HnRNA" large, ~~much rapidly degra~~ rapid turnover.
controls : little know acidic proteins? speculations.
Replication little known at molecular level : many starting points
(rate in xenopus)
Mitosis : folding + unfolding : movement (later) : centromere
(understudied)
Recombination : almost certainly breakage + repair.
mechanism? fungi?

Definition of fields. overcrowded - many simultaneous
underpopulated - not repeated
eg Sperry.
primite - one discovery can upset
whole viewpoint.
origin of life.

3/ Nucleoplasm : prob. no protein synthes.

Small RNA in nucleoplasm : several types, many copies.

HnRNA some of it ~~[illegible]~~ prob. a mRNA precursor.
where is mRNA portion?
other types of HnRNA? spectrum of turnover?
regulation at this level?
associated with protein? Georgiev's informofers

General idea : unusual for ~~[illegible]~~ RNA ~~[illegible]~~ to be naked.
"exceptions": tRNA : also DNA?

Secondary structure of NA important
obviously ~~[illegible]~~, tRNA, rRNA.
also true for mRNA (in eukaryotes?)? HnRNA?
DNA? change of pitch with base-ratio? (Brau)
— helix? ~~[illegible]~~ unwound stretches?

— poly A
~~export~~ — export to cytoplasm? — Genetics?
Biochemistry.

Protein synthesis (brief) ~~[illegible]~~ many problems same as in prokaryotes / need for better system, but Stahlein [illegible]
control at translation level? masked mRNA.
~~[illegible]~~ structure of ribosomes, of tRNA: "factors".

~~[illegible]~~ attachment to the endoplasmic reticulum : ~~secretion~~
Secretion (ie through membrane) : position of ribosomes
lead-piece on polypeptide chain?
rôle of polysaccharide?

Why rule out prokaryotes?
eg. structure of ribosomes.
may be nearer to special instruments available (e/m, x-rays) ^
which attack at molecular level.

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Branto, Chargeux & Luzzati.

Membranes now an agreed model

phospholipid-bilayer but not "denatured" protein : prob. true for all lipid membranes.

cell surface / membrane : instead "globular" proteins : inside, outside, right through.

lipid diffusion : in one layer : flip-flop : different lipids in each half ?

protein diffusion : slow but appreciable for individual proteins (outside only?)

"clumps" - pinacytosis. mechanism of this ?

also reverse pinacytosis. also relationship to other membranes in cells — eg. Golgi, vesicles, endoplasmic retic., nuclear membrane

also synthesis : are the lipids synthesized in different places.

function of proteins

"structural" eg. cell-cell recognition : antigen recognition ?

transport proteins eg. 'pumps'

[have any proteins going right through membrane be isolated in native form]

on inside : is there a subcortical gel ? in special cases ?

Fibrillar elements in cells

microtubules (several types)

actin, myosin + ?

neurofilaments.

characterisation of protein subunits (acrilamide gels, their fingerprints)

interactions ?

locations : membrane bound ? how

how do they get to locations ?

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Formation of tissues

cell-cell interaction surface proteins? polysaccharides?
specificity?

junctions : eg tight junctions.
eg nervous system but easier with other systems? / Insect cuticle, Hydrae, Arabaena.

positional information - gradients - waves : sources-and-sinks

"solid" structures eg cells walls, eg septum formation + ~~[illegible]~~ "wood" in plants
eg. collagen + bone in animals : many special examples.

Signals

hormones : lipid sol : go into cell (pumps?)
aminoids / lipid insol (eg insulin) act on outside : protein in mech. ? — CAMP
why used so often?

action? on chromosomes? plants : ?

"neurotransmitters : long range hypothalamic polypeptides.
short range eg acetyl choline etc.

how secreted (vesicles?)? how do they act.

misc : positional information mols
: mitotic control molecules

General idea : signal molecules not usually the same as "metabolic" molecules
(though often chemically related)

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Bn Techniques

obviously new new techniques

often can state a need (label cells which have recently fired a lot)

but ~~dif~~ v. difficult to predict.

new techniques can be simple

instrumentation : can be really new eg. high resolution e/m

or high source x-ray beam.

or can involve automation either (1) for old methods eg pipetting

(2) new problem eg. neuroanatomy

depends partly on type of engineering skills available in one place.

eg. ~~[illegible]~~ fine mechanical devices } ~~eg [illegible]~~

modern electronics. } prob. both.

~~in~~ do we include programming standard computers.

6 ?

includes organisms and cells in culture.

Choice of systems

- often difficult to select in advance (eg. Drosophila + giant chromosome) or Bacteria
- value of multiple systems to approach a problem eg. Code human rib TMV E. coli.

Some desirable properties: how big? can pipettes / electrodes } be put in?

other methods of addition – eg cell fusion, "transduction", eg viruses.

how short a life cycle?

can it all be done in the lab?

can one grow a lot of it? or use single cells? radioactive labelling?

biochemistry? can nucleus / enzymes } be easily extracted.

how "simple" is it? need for better classification of eukaryotes eg histones.

is it genetically easy? eg haploid, diploid etc.

~~addition~~ full genetic systems; but also partial eg Xenopus.

digression on genetics: always useful when there is ~~the~~

(a) good genetics
(b) the right conceptual scheme } to ~~test~~ distinguish between hypotheses.

Can it also be used to show (or hint at) the logic of the problem?

~~Systems.~~

Overall requirements: scientists are human – like to enjoy their work.

typically they would prefer – significance
– no very long investment (usually)
– techniques available or easily developed (")
– interesting results "certain"
– no one else working ("a few friends"

impossible! also difficult to select in advance

value of multiple systems to approach a problem eg. Genetic code