6/20/56
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J. Lederberg

McCollum Pratt Symp.

Tues. Beadle Ris | Benzer, Mitchell
Nanney Rhoades

Wed 9:00 ~~Ephrussi~~ Mazia Allfrey Sol | Hewitt, Rollins, Zam

Thurs. 9:00 Hartman Jacob Conrat | Chargaff Markham [illegible]
[illegible]

Fri 9:30 Art K, Ochoa Cohen | Stent, panel.

suggested notes J.J.

1. Bill Mac asked me here after the [illegible] Symp. where my function was to resist strenuously the idea that geneticists were making any progress at all. If I assume that my function here is to be a "wowser", I hope this won't be taken as a balanced presentation of my own views which are more optimistic + reasonable than may appear, and I also hope the copy won't read "indefensible" when I read "indefeasible" as happened at Detroit in discussing Dr. Horowitz' paper.

alt - modulator : [illegible] [illegible] + [illegible] it from doing anything
feed back problem of modulator = desc. [illegible].

(harder to talk about something is less + about — e.g. [illegible])
won't recommend until could study

but $Lp^{+}/Lp^{s}$ heterozygotes)
{ Hf bal
{ Lp bal linkage}.

Wr. Ochoa for you.

Beadle
①.

1

molecular ... chromosomes. W/C suggests mechanism

c/o does not cut through genes because they came out
In general different functions if separate and discrete
Quotes pseudoheterozygotes (cf. Ds; heterochromatin position effects!)

Confusion of classical tests of allelism
① close linkage of related functions ② "complementary alleles"

"M Mitchell phenomenon" a+-/-+b → tetrads c/ following
a+-b a++b A-+B A-+B. ∴ non complementary.

? Are complements always found for normal c/o of first markers?

explanations 2:0 mis-copying
often not acc. by c/o but there
is a significant correlation.
Assumes c/o is related by requirement for "pseudomutation"
proximity. [Why not c/o as replication phen.]
seems to leave out this hypothesis
"Pseudomutation may be deviations from randomness of c/o.

opposes Beadle's suggestions: gene = functional unit

mutational sites = sites
"heteroalleles" = nonalleles
homoalleles = "alleles"

what are means of defining these!

Jacob

Hfr Gal+ × F- Gal- should yield Gal+ HSR
and doesn't

[margin notes:]
won't found concerns on mechs vs struct
knows nothing about genes, only markers.
propose to leave out word "gene"
define allele s/reference to complementarity (a ~~genus~~!) What do we know about functions
6-strand c-over? ... markers not c/o. effectively close doubles.
= conversion
"
= Lindegren effect
= Longhouse effect
"

Yanofsky E' sec

B. hyp. [illegible] G — sec may inhibit defect

↓

↓

[illegible] E ∴ many genes strictly involved in synth each enzyme is direct consequence

"tenable to suppose that gene acts via RNA as template"
not yet v good evidence. + don't know whether
enzyme "is modified or is absent": (argument is circular)

also quotes blood Hb's — evidence on allelism not good.
but accepts it!
But Thalassemia is not allelic c sicklemia!

quotes Ford Symp. on 1:1 — but indefeasible

"genes always allelic" ∴ believes total spec enzyme assoc c single gene!

galactokinase — contra Su/E

eating vs breathing — cor. genes/cytoplasm & evolution
how do you tell.

End: primary genetic information in all living forms

by NA < DNA / RNA. — when RNA carries information

it is in host cell c DNA, protein. — Ditto for DNA

not necessary to assume c/o is ? mutagenic in higher org.

"Advanced genetics from an elementary standpoint
Limitations of genetic methodology  2

⊃ contains
⊂ is contained in.

(not necessary to assume it is [illegible] hexagonal)

But only when close markers are available can one detect the exceptions! May result either in exchange or non-exchange

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Ris

Cytochem results ① constancy of DNA - synth only in relation to chr reprod. ② histone also constant ③ protein + RNA - more in active cells, reversible.

EM - polytene in salivaries — fibrils 500 Å / 200 Å which are hollow.

no other structure. pres consists of NP. cf TMV

fibrils not affected by DNAse.

At prophase, fibrils move apart

Long. differentiation ? degree of [illegible] at diplotene!

Mazia — units are 4000 Å / 2000 Å — sites of c/o ?
Similar units of RNP are intermediate carriers of inform

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multi-strandedness of chromosome → problems of chr/ vs DNA

Importance of prophase as the bridge of fibril to chromosome

Bradley [illegible]: Disc 11:30 Am Tues.

Alpert - [Marshak - thinks too much explained away].

Ris - sequencing c/ Feulgen stain total amount is small but - sperm.

Butler - (Davison + _ ); nearly 1 base a al P. Harris 3 nas 6 protein [illegible]

Williams: — i Os phage is [illegible] Fe [illegible] of Maaloe on relative sty. of NA, protein.

empty or NA?

matter of fixation: i formalin no extraction?

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applaud (!!!

Benzer — "gene" is missing — gene-enzyme as a definition. function matter of taste positive test.

automotive analogy belonged to Detroit remark that seems

unit of recombination: [2 existence < map is cm / physical distance

Is a continuous series of intgs punctuated by experiments

evidence that there is a segment for cistron

T4 (Is there a linear cistron?)

trying to exhaust mutations in a segment.

| | (K-12) | S = "BB" |
|---|---|---|
| $r_I$ | r | r |
| $r_{II}$ | − | + |
| $r_{III}$ | + | + |

phenotypes

\+ + +

The groups are in general genes.

$r_{II}$ in two cistrons + no exceptions to square.

A = 4%

B = 2%

Exceptions: apparent deletions stable to reversion
are always linear Benz[?] segments?

[can also be crossover suppressor or asynapsis] [sure]

(importance of exhaustive analyses —
had its origins possibly in Stadler's study

[Prefer Sager's equiv. unit? / cit? / fit? / bit]

Concentrated on deletion class ?. 11000 to start
150 included & tested in pairs

one bears most fragment, 2 phenotypes (Delamnia's)

many singles, infer 0's.
1; not yet "run into the ground";
about 12 sites so far.

[importance of formalizing / linear alleles only / complex loci / confusing]

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overlapping deletes found in some cases. Can be used to subdivide.

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no exceptions to linearity yet.
mutons & rectons = or ? (nucleotide not excluded.

[probably pairing rather than deletion in ? of Lac,]

S.B.

generate mutants from del x +? same bypgenes ċ indicators (same agonist + bypassing?)

H.M.

no evidence of -- as complements to ++.

heat shocks < (in proto q) conversion, no effect on rec of exterior markers.

" of conidia > conversion; negligible effect on c/o. to as high as 4%.

ABC
abc
AbC

expects conversion in -×.

(simulation of reciprocal crossover).

non reciprocal crossing over?

Roman confirmed Lindegren on + --- asci and identity of the (-).

Sol discrimination Lindegren

glossary
policy

Nanney — definitions: [illegible]: prob. of [illegible]
discrepancy gametes [illegible].

general "homeostatic principle".
mutation s.l. — any change s.s. — via [illegible], [illegible] gross.
"hereditary modification" is broader.

cytoplasmic = extranuclear [illegible] chromosomal.
① ② steady state concept
nucleus [illegible] concept
most observations in sexual heredity fit ①

but not satisf. for differentiation he says. But gross partitioning not hold up. ∴ suggested that cytoplasm ? cellular heredity.
then new work (Briggs & King, etc.). But: ⓐ speed ⓑ direction in time ⓒ regular predictable pattern ⓓ integration ⓔ
most data are ambiguous.

why not apply steady state concept to the nucleus

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Rhoades — point mutations
extragenic: minute def; rearrangements c/PE; PE; c/o
c/o of components of a "compound locus".
intragenic: "true gene mutations"

[illegible] [illegible]
[illegible] this typo!
need a dictionary; [illegible] these
glossaries [illegible] paper

Send [illegible]
For
[illegible].

7 – Rhoades – not summative – extra

why not grade by unit test

Dan — look for such conjugates in flagella?
implant cysteine in vivo to induce mitosis — cytochem evidence of -SH accumul.

Mazia

spindles.

Digitonin as solubilizer — sea urchin eggs.
did not work in KCl solution!

ATP — M/100 (natural level = $5 \times 10^{-3}$).
98% protein: but not RNP as formerly thought.
~10% of total cell protein

nucleotide of spindle found not RNA — no pyrimidine. May be ADP or ATP conjugate, i.e. nucleotide-protein (= actin)
back to Rapkine!

Multipolar mitosis — centers may influence orientation of chains — binding agent may be diffusing from them.

core extract + crystals of cysteine gave model asters ? and spindle.

Where does this protein come from?
soluble proteins in s.u. eggs by u/c. techniques
— a component disappears during cleavage division.

Hoffman-Berling: glycerolysed + ATP:
elongation of spindle & shortening of chromosomal fibres.

Allfrey
nuclei
mouse thymus. .25M sucrose $CaCl_2$ — low speed blender + diff cent.
25% contamination c whole cells. Study AA incorporation.

chloramphenicol, FPA had little or no effect on alanine incorporation.
Ribonuclease had no effect on M — uptake.

cortisone abolished uptake!!! 5-6-Cl BzRib

into flu B effects: inhib of uptake + effects mimics.
$Na^+$ is required contra $K^+$!!...

opt sucrose is .25M   10% deviation → 30% inhibition!
secular variations from animal to animal (hormonal?)

F&T, heating, breaking in blender destroy activity. Old nuclei do not last overnight.

Specificity:

a) of d, l-alanine* ~~dl~~ flooded with unlabelled d, l. etc.
b) Labelled well. Old nuclei do not dose*.

["not exchange" — not proven unless substantial replacement had occurred! ~~not~~ asked by Vassar. ~~not satisfactory~~ reply.]

30 mg nuclei .6γ alanine* / hour.
∴ replacement is 8γ NP/hour

DNA removal with lysolase, phosphatases (many plateaus) cells are unaffected.

[Radioautography is homogenous — Now closely examined "from whole slide" — was pt/pt autography.]

Supplementary DNA. (DNAse not removed at first but this not so successful)

But 4X increase by "adding DNA" [~~Specificity of DNA~~?]

add "denatured DNA" — just as effective

"spermine acid" had same activity.

"deoxyribose phosphate

DNAse treated — core / dialysate } both fractions +

RNA also +

RNA mononucleotides etc ca. 9.

dinucleotides: AA, AU, AC, GC, c. 9.

Sol) template hypothesis — "modern theology" → DNA RNA protein

now? either DNA or RNA

azathymine ?

Block DNA synthesis UV, azathymine, thymineless stages — no effect on enz. synthesis

Block RNA synthesis — uracil-mutants; analogues. inhibits enzyme synth.

Chantrenne: RNAS accelerates when catalase is induced

Vollum - T2 inf cells - spec RNAS?

? Is RNA a template? Need a subst. expt.

"I would have preferred to be injurious than courageous" (Gale)
ingenuity no longer paying off.

cell parts expts:

Gale: -RNA fractured cells, restore by intact RNA. species-sp!

Sol's lab: needed steps: activated a.a. needed as preliminaries. + would be foolish to expect a water-soluble prepn to work. thought to use protoplasts

Properties:

dissolved by lipase; collected all DNA = and RNA synthesis prot.

osmotic conditions

what is membrane?
dimensions?

1 per protoplast

"fairly good" quant recovery

properties: enzyme synthesis under right conditions.

Enzymes had little effect as a rule, but a few preps did slow effects. Age matters. All protoplasts are unresolvable > 30 mins c. q. Resolve while making

(Is this in Ford paper?)
> 99% DNA removed. no effect on enzyme synth
and some stimulation.
∴ might make "strong statement".

But acid soluble DNA still present (60% remains!)
System was also very labile. ∴ studied broken protoplasts.
Many methods tried: importance of initial stabilizing medium.
sucrose, phosphate e.g.; used a K, succinate system
then osmotic shock.

900 uM/mg P/hour.
what is enzyme activity?

1:3 substrate vacuole!
1:5 " matrix!

Many pellets thus prepared are designated.
dil c 4.2–4.7-fold. (from what?)
resuspended loses 85% of DNA

RM = reading mixture. Incubate substrates in a phosphate
HDP
lose DNA.

what are "substrates" morphologically??

adding RNA causes factor fractivity
and soluble NA now absent.

∴ Substrates s/DNA make Enzyme
— RNA donot " ".

Is RNA being resynth. in inducting medium:
from 4 – 118 γ/10ml.
<1 – 44.

40 min lag + linear resynthesis.

What is "RNA" – acid pptble Disle reaction
hydrolysis → UV absorpt. exp.

Examined other capacities & got considerable
resynth of RNA DNA + some protein
resyntheses. 5–6 x increase in all components
make more DNA than RNA, prot.
10 x. interest. only culture.
but RNAse-treated are inactive.

~~RNA~~

nucleoside diphosphates

Put back RNA. — Ochoa substrates restore EF ability.
enzyme stops effect.

not specificity.

Ochoa: ~~[illegible]~~

But not all RNA
was removed.
>50% removed e.g.
how much till diphosph.
DNA is nonsense DNA.

if so, is
the template only
protein
"what is RNA" —

Did you say – RNA cells can
be restored by nucleoside diph.
do they make enzyme?

poss. regeneration of limits?

What are substrates made of?
relate to Allfrey –
restoration by NA small fragments
of low specificity.

Hamnett

Radiation effects on TP. Target theory may apply ($S^R \to S^S$).

1. Quantitation problems. Reactants a → b. b is competent only about 15 mins. a is impure, containing unknown ineffective molecules. Synchronous development of competence in exptl. cultures. Level from 0 to $10^5$ to 100 / $10^6$ bacteria. Later waves may also occur. Can add DNA from start and get higher assays. Linear assay possible with ②.

Relationship of competence to division cycle.

Thomas found X eff. depends on number of competent bacteria. Limiting reaction evidently bimolecular. Had to study kinetics at high DNA, plateaus are formed.

$10^5$

$10^4$

log log plates

What are reasons for nonlinearity? heterogeneity of DNA? Extra DNA gave 2 ← gives early plateau, decreased by propn of unmarked particles. 1st plateau is f(total DNA). Break due to multi-sorption. At high mult., inhibition is partly removed, may be max. of effective sorptions.

1st plateau is region where doubles begin to occur detectably.

must be sensitive + resistant particles

?- genic imbalance? - transformed cell may be killed or nontransformable.

extra DNA in phage may have a similar function

[polyspermy?]

Irradiated DNA —

~~ts~~ liver assay at 3-4% survival.
~~native DNA~~ mass of DNA determines liver region.

indirect effects problem: — irradiation in dry state, where protective substances have no effect, & sens. volumes are smallest. i.e. sens to indirect effects.

Kinetics of inactivation:

(≈ 7×10^5).

k = 7 kr

direct eff.

log S

10%

dose

[Marmur + Fluke (2 × 6 × 10^6)]

only 1/10 app. chem. m.w.

(radit) radar < central. ? Residual activity possibilities.

Saving DNA not heterogeneous in class.

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Destructive particles? Fractionate DNA < high guanine high T.P. / low guanine low act.

no difference.

urea no effect

more sensitive units are present in same preparations.

why are small aggregates > sensitive

postulates a sensitive region for S^R locus (guess 3×10^4 mol.)

target prevents incorporation.

S

terminal

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Aggregates: ? energy transfer or cross-links which inactivate.

Hotchkiss: assume × hypothesis.
Quantitative factors
Capsule system – originally by dilution. Inaccuracies: sediment too soon or too late e.g. Mountain, entirely must be specified.
If sediment is unsuspended, may let remain add'l. × s.
pres. matter of selective conditions. etc. So moved to drug resistance.

Rollin doesn't see plateaus — level of plateau depends on proportion of $S^r/S^s$ DNA.

50 fold range.

put A's on word
DNA/cell
DNA/maximum max. ×
"molecules"/DNA/cell.

Benedict – chromatographs of DNA anion exch.

Ecteola cellulose –
Variety of fractions tested

In comp. syst., ever get initial plateau?

Least elutable DNA is most active
2M NaCl MNH$_3$. ∴ heterogeneous

sp. separation?

Austrian: SI → × SIII, → S$_1$,
SI, III → × R → III, I, I–III
phen III

H. Taylor – Quant. assay.

DNA is not exhaustive!

What is being measured in essays?
∴ not a stoichiometry + assumption of a time slice of a bimolecular reaction needs kinetic justification

Zamenhof: The transforming principle.

① test for activity of DNA. assoc. physical analyses.

② composition – modified by Bu uracil

Effect of BU uptake: sp. mut. rate not changed, cells still alive. But do get two colony types, incl. "pinpoint colonies," but these → large by mutation. + can establish pure lines. ~10% of such mutations.

large colonies also gave 2 colony types. "Unstabilized" the strains, every colony started to be altered.

③ Nature of heterogeneity?

④ No fractionation so far.

⑤ auxiliary system.

[ — x in synthetic medium?

$2 \times 10^{-9}$ γ/cell per marker = 5x content of cell.

1 cell has 250 molecules. @? groups of characters not DNA ⓑ 1 mol > 1 determinant (i.e. linkage).

attempt to decrease size of DNA → inact.

( synergises problem

$10^{-4}$ γ DNAse destroys 100γ/ml DNA in 20 minutes

drying, dialysis also ", act disappears by prechopping.

[ assay methods for these procedures

deamination "" 5, 20', $NH_2$ –

dialysis: loss of mut stability + … trunity.

M. … :

~~diaminofurint~~ $Me_2SO_4$ reactions c guanine. also esterifies $PO_4^{3-}$.

diaminofurint – mech?

∴ DNA is target of strong mutagens

UV treated, heat treated all unstabilized.

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assay systems for DNA

Burn up again.

mutant broth + DNA competent

but cells ... genes is a protein making

pn. Hem. coli? Neisseria

importance of

SR

3

1% 0

UV

differences →

1 capsule c

3 = SR

2 capsuled

4. SR from type 6.

1 + 2 similar

A to pm W. — Bell incorporated after cessation of growth + no increase in DNA/cell. ∴ exchange without resynthesis of DNA

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Goodgal: ΔDNA 300 mols/nucleus ~ 1 cell's worth

(is uptake homogeneous? Yes!) — in 0 - 90% range

DNA?
comp cells.

S cells do not X
do not ~~t~~ DNA.

∴ 1 gene per cell.

~~II~~ Assumed M = $6 \times 10^6$ (i.e., $10^{-17} N_A$)

~~count~~ cent - ~~diff~~ values = ($5.5 \times 10^6$ ~~same~~ for S, Ery th.

No separation obtained except by UV

Lerner ind. may suggest a subunit

E

S

Loodgal coli enzyme + ATP + Mg + light
restored UV'd TP from 3 - 30%

says Hemophilus, pneumo. do not photo-reactivate per se.

Lerner - ? as phage. — no.

Benedict, Thomas early fractions have M ~ 400,00 even
octanucleotides (pairing minimum)

Leidy virus specific (à la Schaeffer)

$10^{-6}$ $10^{-7}$ $10^{-3}$

influenzae
induced parainfluenzae
suis

$S^R$ → X

homo
hetero ratio is constant for a given pop. tried of strain of donor.

cf. Salmonella + coli X Shigella

"Crossing-over"

15 secs in heterozygotes

4 steps in histidine

V. occ. recurrence. 1 stabilities for reversion.

also differs in recomb frequencies!

some mutants give confusing overlaps. histidine.

Kalckar

Only 2-point in

only comment is that detailed data have yet to be published & therefore critical comments not yet available. There are difficulties in analyses esp. 2 point tests

Non-aggression pact

Hewitt - general review of label works re DNA effects

Hartman : — classifies recombination by vector
DNA, phage, cell conjugation.

a. DNA b. Transduction; lysogeny c. Cell conjugation.
Types: generalized vs. prophage-limited.
stable vs. abortive

Induced lysogenies are → ✕ in Salmonella! generalized.

$S^R$ → ✕ not transduced in TMV?

- Zinder etc. expts.
Britain's hyp. — attachment specificity?

Jacob: gal by heterologous phage → ✕ $ly^+$ : induction can be lethal
metabolic state of recipients : need phenomic expression : or effect
of host - background on eff. → ✕

chemistry of exogenotes — analogy; no direct evidence.
P32 decay : exog ≪ prophage.

lys.

This is Salmonella.

at least several gene loci long. — "20 - 30 $\geq$ loci" long?
(not sites?)

incorporation step - possibilities: "unilinear" $Fla^+$ → ✕ $Fla^-$.

Jacob: eclipse to lysis. cyclic behavior
temperate quality.

prophage = phage not in noninfectious state.
replication coordinated w/ bact. div.
expression: → phage + immunity.
induction UV → → phage.
etc.
immunity usually specific for related
superinf. phage does not interact genetically except on induction.

? chemistry of prophage — Hershey + Chase
injected DNA, but no data on whether info. is transferred.

How many prophage/cell. Double infection, markers in output have input ratio. Induced bacteria superinfected behave as if 3 per bacterium. ~ 3 nuclei.

Jacob – also competition between related prophages, usually no mixed lysogenics for +, mutants of same phage.

E. coli K-12 L+T. EML – $\lambda^+$. can cross $\lambda^+ \times \lambda^s$.
1st expts by L's + E.W.

Clowes "never rejects Xyl, Mal, etc!

14 phages of cross-immunity. 7 are inducible A
7 are noninducible. B

A are ~~distal to λ.~~ between Gal and R
all show zygotic induction, degree varies in same order as time of entrance.

B: all are distal to R – most are linked to

T L Az T1 Lac Gal λ, Xyl Mal S

no zygotic induction c̄ these phages.
each phage has fixed site. two general regions.

one of the B's ⟶×

3 prophages can be ⟶× by one other.

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site

phage crosses among A's.
3 c mutants

1 2 3
C

specific immunity site = localization-determining sites, near C1-2

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caroscean: depth taxon.

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Myers Lewis.

F-Conrat
8/15 lesion mixed from (mixed RNA) · proteins.
none mixed from mixed virus.

W+
C

Schramm

L. Pauling

Davidson

J. Monod.

You
+me

host modification in chemical terms.
any pure clones in TMV
Volkin asked
any effort to stabilize RNA with normal plant proteins
answered in course.

Is the ~~prophage~~ bacterium
Lwoff — an ensemble of prophages?

Hotchkiss / Zinder(?) / Chargaff
Doty(?)

Buy Ch+ Dav. | Are there conservative exchange reactions?

Doty — plan of mountains.

adv. of section is to show the clouds.

Chargaff: language difficulties — dictionaries lacking
a coil ≠ mol helix

MC, HMC
N-Me-Adenine.

base composition: 4 bases in RNA — AGUC
4 " DNA AGUT

plant viruses may be atypical. In animal RNA closer association c/ protein.

"NA can carry information" >>> Is protein unimportant?

This is the fundamental question & cancels synapsis

poss. as nucleotide seq. Any finite sequence represented more than once. Is message carried many times?

maybe other than 3-5 linkages; i.e. as folds.
not excluded that NA is large cycle

$\frac{A+T}{G+C}$ varies from 0.4 – 2.0
Tb sea urchin

Gamma = 6 bits

But A ≈ T  $\frac{A+T}{G+C} \rightarrow 1.$
G ≈ C

Meaning of regularities.

- Chargaff - unitary complements well preserved.

now? sequential analyses.

crudely Pu-Py-relations in trinucleotides.

can fractionate to small extent DNA's for different G/A ratios:

~~If~~ Is 5MC / C random. ~~but~~ No occurs fractionally.

clearest material genetic content of T2?

RNA. - no regularities, but no precise premises at first.

crude nucleoproteins: the relation A + C = G + U was preserved, ~~i.e.~~, but no others.

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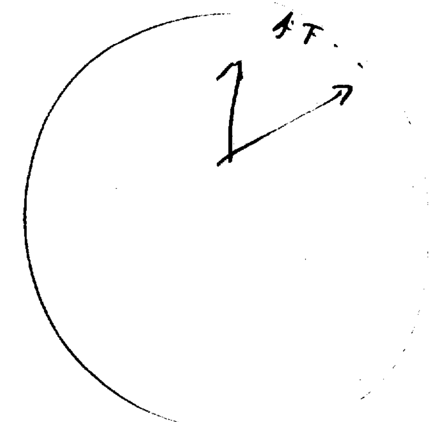
Smith. - no good evidence on branching of RNA.

Sequence problems.

Whitfield - remove phosph.

oxidise

remove purine ... expensive pure material.



Chargaff
5MC

Ochoa
re pri. 1

Smith —

agar uptake in B. cereus cells.
not homogeneously. cyclic end turnover faster.

Crick 5MC does not go in at random. Agree that naive repl. mechanism is ruled out & maybe unit of agglomeration is not nucleotide but an oligonucleotide unit.

5MC is preferred next to guanine. Suggests repl principle.

~~6-amino~~-N-methyladenine, =X replaces thymine
He suggests that XT replaces TT pairs but gives tremendous (flexibility, too much.)

Chargaff complains

Toulouse & Zam.

Ochoa — whole cell RNA & enzyme RNA.

Bendich — recorded deviations from 1:1

Crick: must have Pur: Pyr (large: small)

X-ray picks out the regularities. and for given room, G-C A T fit best.

maybe some unpaired chains in some preps.

If all 4 bases are in any chain, only W/C model so far fits the X-ray.

Not yet certified professional judgment on X-ray concordance
but no misconceptions yet.

felicitous

coiling is paranemic not plectonemic. No sales of model yet, at least
(Why read SF if you can read PNAS!)

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nucleoprotamine: prot fills in the "major" groove.
1 side ~~[illegible]~~ per 3½ Å. not straight up.
as 3d chain, basic groups matching P.
nonpolar spacings - loops of 2 or more
∴ nonpolar AA should occur in pairs.
much analytic detail fits that.

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nucleohistone

Kalckar what he's publishing

Mad concentration of analytical work on biol. significant material!

Subanalysis factory?

JW

4 AGUC.

synth poly RNA, 1 or AU
all gave = bad photos
poly A - good clear photo

and RNA ~ DNA s regular base pairing
except A:A

Largest issues are

conc. on microorganisms based on feasibility of manipulation methods; but through the eggs.

(1) Availability of test systems for DNA, RNA.

(2) Concentration of analytic effort on bio assay systems.

→ specificity absolutely essential.

Phage
Transformations (or as I like to call them DNA-mediated).

Rich – mixing poly A poly U interact at 1: ratio
as measured by specific absorption. as well as sedimentation

possibility of base pairs as well as others.

Franklin

density diagrams
of TMV
and TMV.



∴ not core but RNA is
wound into peripheral protein!

– probably single strand if fully ordered, not ex. RNA.

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Low: many helices.
globular proteins any here?

Art Kornberg. jigsaw puzzles
– ingenuity, taking courage, [illegible]
Enzymatic synthesis of nucleotides.

phosphoribosylamine $\xrightarrow[C_1 \ldots]{}$ → ~~[illegible]~~ inosine → adenosine / → xanthosine

asp → orotic acid (free base) → (phosphoribosyl PP) orotidylic acid → uridine phosph
↓
cytidine

| P.C. no free purines or pyrimidines | O → uridine typical
L. arabinosus works through uracil → uridine phosph.

"salvage pathways" HX, G, A or U $\underset{PRPP}{\rightleftarrows}$ nucleotide

Nucleotide $\xleftarrow{ATP}$ Nucleoside + P ⇄ R-1-P + base

Nucleotide –P / –PP } needed for further synthesis; Kalckar reaction;

ATP + BP → ADP + BPP → BP + (BPP)

AcH + Triose ph → deoxyribose phosphate $\xrightarrow{\text{phosphorylase}}$ nucleosides
but where free purines?

? reduction of ribotides.

Deoxy UP $\xrightarrow{\text{Coli}}$ Th-PPP
{ tetrahydrofolate / serine } = ~~[illegible]~~ THFA
$CH_2OH$

of coenzyme synthesis
nucleotidyl-residue reaction.

Nic - R - P + ARPPP → Nic - R - P R A + PP
etc for FAD.

pyridoxid.
ARPPP + $RCH_2COOH$ → $ARPOOCCH_2R$.
ditto a.a. → adenyl a.a. Hoagland.

---

? polynucleotide

ATP + PPPpolyN → ATPPpolyN + PP

n polyNP + ATP → polyNPPA + PP

---

C, ATP → NA. Then Azotobacter / Littauer purified a coli enzyme
NRPP → "RNA".

---

DNA:

deoxynucleoside PPP + ATP + primer $\xrightarrow[P]{\text{enzyme } S}$ "DNA"
thymidine + ATP → TRP → TRPP
TDP is effective as TTP.
mixtures of base TP more active than single All 4 together do react
somewhat of added primer (about 1%).

primer

DNA - primer ~~heated~~ rich in

crude coli fraction ↑, rich in DNA Recently purified. Bollum to nuclear fraction + ...

DNA + (1, 2 / d / a b) → other 3 deoxynucleotides.

now need all four components

ATP not needed. Some enzyme probably phosphorylase not needed.

Noworthy net synthesis: 30-50% increase.

Thy RPPP is precursor.

Chargaff analysed:
coli } +
Hemophilus }
T2 ½

which specificity of primer DNA is primer

could biol activity increase if pure DNA were used?

Ochoa + Heppel.

Azotobacter enzyme → nucleoside PP → RNA

"polynucleotide phosphorylase"

has made ADP → poly A, G, U, C etc. also
AU, AGUC

Potter had shown the other phosphates in cells.

? structure + nature of polynucleotide — structure is RNA (Rich)

poly AU.

$A\cancel{B}P\overset{*}{P}\overset{*}{} + U\cancel{B}P$ 1:1

↓

Hmy $AU_n$ about 1:1

products of RNAse. [phosphodiester attacks pyr nucl. ∴ yields pyr ends.]

U 38 AU .35 AAU .20

AAAU 4.9 AAAAU 2.1

50% of #X in UX sequence is A,

takes advantage of P* transfer from A to U at splitting: finally it is spread A, U, in polymer.

? sequence is "random"

[meaning of random. random like polystyrene bonds.]

? Intracellular route. Means of orientation? $K_S$ is however very low. $K_m$ $10^{-2}$; these conc < intracellular. Maybe local sites of NDP formation. The enzyme is unspecific — e.g. poly HX. also unspecific in phosphorolysis: also destroy RNA ... rates vary. dihydrouridine OP ... as 5'BzCh DP. also ... -5-PP

Now general path is clear, but specificity is still dubious. (try various RNA)

Mechanism: primer? Most preps may have some nucleotide. possibly not.

~~Mode~~ NMP as start of chain? no
✓
But AMP + UDP → no A in endgroups.

? one or several enzymes.

Is RNA specific
exc. in plant viruses

Changed standard spec.

---

Potter: ATP → DNA   ADP → RNA.

growth control. [ Thus ATP inhibits RNA formation.

contra biol stability of DNA.
/ irreversibility of Hornby rx
Soleplard.

homogenate labelled RNA does exchange activity } no net
liver " " does not } change

$$\rightarrow UDP \rightarrow UTP \rightarrow UDPG$$

UDP ⇅ RNA; UTP ↓ DNA (2H ← red. potential)

---

Hornby contrasts:

| | RNA pyrophosph inhibits | DNA but not inhib. |
|---|---|---|
| Reversibility | + | (−) |
| Km | $10^{-2}$ | $10^{-5}$ |
| diversity of N | 1 | 4 |
| primer | − ? | + |
| extent | ∞ | > 1½ |
| specificity | ... | ATP not usable. |

Cohen – inoculatable systems. posit strongly model.

RNA asymmetry only as enzyme stage?

reduce almost to E coli and phage

T evens: HMC and GHMC
1. may be unwise to extrapolate
2. inf cells stop new enzyme synth. — do ghosts?
3.

prot
DNA
phage.

– Stent: transfer DNA inf to protein as 1/several poss.

early S35 loss not major in T4 sloan.
? repair mechanisms

5MT inhibits DNA synth. only if added at 0 lysis.

if added later, → DNA s/ further protein synth.

Rate of DNA synth ∝ time of add chlo.

? role of protein: inform or ~~eng~~ logistic support.

Some small RNA is also turned over

Is B growing, thymine, thymidine ⇸ DNA.

pres. thymidylic acid.

Depleted B. thymidine → DNA.

(consistent c Kornberg scheme)

ribose nucleotides → deoxyribotide

thymidine analogues won't inhibit B.
do inhibit virus synthesis

---

Do thymineless in wild L15?

Amino uracil → methyl purine incorp.

BrUracil inserts itself

turnover is manifestly rather than whole RNA
only fractions was [illegible]
Volkin
Shape of pulse curves

Nov + Sz. mutrate in wt; adenosine vs. aluminise.
∴ metabolic control of genetic material
Zamenhof Brill effect – "exchange in DNA"
Adelberg T⁻h⁻ → T⁻h⁺ 100× highest in deficient medium.
control of nucleotide pool a key to mutagenesis.

Volkin spec in granule fractions. In pulse expt showed RNA, not DNA turnover

adr Stent ~~Seymour~~:

What's the suicide curve of T2 grown on hot bacteria, cold medium or reverse. (i.e., what is distribution of the bacterial DNA in progeny phage. ?)

t

Stent: rotares—
unwinding by breaks + reunions about every 5th.
separation c/replication → dispersive replication:

other schemes are conservative. [illegible] base pairing.

c/o affects dispersion depending on mechanism.

| how does protamine dissociate?
| C-O can still be a copy mechanism.
| does this mean second step is still semi dispersive? has 4 understand 2d expt.

1 : 200 :
par₁ → prog₁
grow hot phage₁ → prog₁

D/o conservation.

~~what RNA DNA?~~ W + M rabbe
mountains
v Potter

$prog_1 \to prog_2$ still 50%
→ prog.

Hershey thinks quest muffin.

Cy: individual autoradiography
GS: $P_{32}$ inactivation method prog₁ — inactivation assay, ability to ~~[illegible]~~ to transfer $P_{32}$ to progeny.

Cy } big pieces    Gunther { ½ is distributed to 10–20% units
                                         { ½ small pieces.

i.e., same dispersion of parental atoms.

of Cy: Both methods on prog₂ < Gunther same fraction of big, small pieces

bacterial nuclei — hot cells at 1 division
assumes nucleus is unit    all cells divi...

Nozzi

Obligate mating for replication:

15 nucleus one unit
are cells 1 nucleus?
B.?
[Magi]

---

Ey: ...measure 15 c/month.

stain size does not change from prog. → prog 2
semiconservative replication: 2 → 3.

Labeling precipitation?
stars are in H⁺ fraction & 90%.
(Total activity?) →

---

problem of bi helix — may be segments of open loops.

Baltimore Symposium Notes.

[Bridges] genes as units of co; no co in genes. (Private discussion: are there discontinuities e.g. in Drosophila data). Review Muller + Raffel.

Benzer made most of the necessary replies.

"Mary Mitchell phenomenon" — glad someone mentioned Lindegren; for awhile it sounded like the new edition of the Soviet Encyclopedia on Stalin.

"classical crossing over" — implication of breakage model — possibility of copy choice still remains.

"not necessary to assume c/o is ever intragenic. [G. Detroit Symp. — Science as genius of confusion punctuated by short recesses in the lab.] — so many fam. faces.

[Ris] — emphasized building from chromosome fibril to chromosome.

[Benzer] — "Advanced Genetics from an Elementary Standpoint." — main objection as a many times damned neologist: simple Anglo Saxon terms are preferable: around our lab, 'fried sit, mit and pfit (scit), salt malt and pfalt.

Serious semantic problem + necessity of purgatory glossaries since cit. defns. too often abandoned.

automotive analogy belonged to Detroit.

Meaning of cit, e.g. in McClintock's material.

Comment on content: possible relative reliability of asymptotic enumeration and mapping. discontinuity in Drosophila data? cf Muller + Raffel.

[Navney] — steady state concept in nucleus. — B+K; L+1.

[Rhoades] — why not go whole hog — or is Goldschmidt too close too?

not a semantic question: only way to satisfy "point mutation" is nucleotide substitution.

---

[Hartman] Reliability of 2-point data? 3-pt. better but few results yet reviewed critically by geneticists. Questions both on the enzymes + on mapping.

[Jacob] — no comment now would take all day. Lewis's red. ad absurdum: whole genome as ensemble of prophages

F. Carrat — (a) no pure clones in TMV (b) stabilize RNA c plant proteins — converse of Volkin? (c) host modification of fn

[Chargaff] — marker vs gene.

(Call Dr. Edman)

10 AM Sat.
Busy — 6:50 P.M.

2 — #29

suggestive comments

Wednesday.

Mazia — flagella as promising contractile units

is there cytochemical evidence of SH accumulation? Implant cysteine?

Allfrey "Exchange reaction" — whatever that means: is there any real evidence for this, if so this might be more interesting than synthesis. Did he answer Mason's objection?

DNA removal inhibited uptake — but pieces of RNA, DNA worked. Any evidence here of DNA resynthesis à la Sol?

RNA removal studied? Did he mention?

Sol Review specificity of RNA removal
morphology of [illegible], inactive [illegible]?

Hotchkiss - Pollin - Zamenhof — relationship of competence waves to fission cycle

importance of clarifying quantitative aspects for evaluating e.g. Bendich results e.g.

discrepancy in kinetics illustrates problem. What are units of activity.

role of synapsis if, after all uni-molecular subst. is source of mutation.

auxiliary conditions in Hemophilus? Clarify growth in defined media.

Species interactions — have some examples in Salmonella, which may be connected with phage adaptation. The datum is usually.

| | | |
|---|---|---|
| A → × B | 1 | 1 < 3 < 4 |
| $B_A$ → × A | 2 | |
| $B_A$ → × B | 3 | 3 > 1 may select a more compatible segment |
| B → × B | 4 | recombination best explanation. |

Segregation only a transient condition.

Zamenhof may be implying a dis-integrated system of DNA "molecules," — this is what Hamilton had in mind citing genetic recombination.

Biol. function of "DNA" as emphasized here. Review other situations < Boivin, Demerec

... few papers had anything to do with chemistry of heredity. / a science ... may not ... here
have to say what anyone discloses — elitist approach.

Most genetic discussions were matters of definition; Dr. Benzer's remarks were especially refreshing. We are looking for discontinuities, either of the molecular order which we must assume as the ultimate chemical unit if DNA is accepted as the basic hereditary material. This is not the occasion to review the evidence for this thesis; several authors have cautioned that it may ultimately be not the whole story but at the present time, nothing else is in the picture (except RNA/virus).

Dr Benzer — his euphonious terms have the objection only of being Greek derivatives; in our lab we had tried unit, mut and ... ; muton and cistron are fated for a longer life. At this point would adopt Dr. Lederberg's suggestion: we should have absolutely no discussion of terminology, but in a mathematical sense should insist that each author start by defining his terms. Our next papers in ... Genetics starts with a glossary & until we seriously adopt a really useful dictionary, this may not be so outlandish an idea.

Of course we may also need existence theorems. One of Benzer's contributions is that cistrons do exist as recon segments. — ~~Del. reliability of exhaustive~~

Drosophila has not necessarily been pushed to extremes; Benzer's exhaustive analysis of a segment had its ancestry in Muller & Raffel's analysis (Hartman) of the scute-achaete region, a job that itself needs extension. Del. reliability of exhaustive enumeration + 2-point tests in any material.

On Dr. Rhoades' talk, Dr Spiegelman asked whether this was a semantic problem; of course Rhoades was asking the material question, are any mutations substitutions of nucleotides, which are the closest possible approximation to point mutations. — Sign of McClintock for pos. effect

Benzer to a point of Chargaff's query, which is really an old question in the relationship of mutations to genes. Conceivably a mutation is a single nucleotide substitution, but it would be a meaningless case if adenylic acid were a transforming agent. The meaning of the code is not in the letters or syllables, but the words and sentences. The specificity of transforming agents can best be visualized in terms of synaptic abilities; it would be worth knowing if [ribose] oligonucleotides were mutagenic in the pneumococcus.

(Lederberg) ⟹

Fin

Meeting reminiscent of the Detroit Symposium — where Benzer would have been even more topical — seeming sometimes to remind me that the sc life these days is a lineal sequence of confusion punctuated by short spits in the laboratory.
+ informational exchange = recons

somewhat platitudinous

Gales material here and Dr. Spiegelman's here had emphasized the ... of this ingenious frontal attack (have to watch out for such words, at Detroit indefeasible became indefensible.) What does this attack mean?

(1) We biologists need all the help we can get from the chemists & physicists; disappointing that the most advantage is not taken of biologically active systems e.g. in the physico-chemistry of pneumococcal rather than calf thymus DNA. Agreed this may need some tactics that would be ...

(2) We biologists have to be more courageous about doing the impossible in bioassay — Dr Spiegelman was not denying he had been making gold.

~~E.g. Mazia's model expt c cysteine should not be too hard to duplicate since [illegible] The RNA systems have turned out to be astonishingly [illegible]~~

The transformists have reported encouraging progress in the quantitation of the two presently available systems; ~~[illegible]~~

There are good reasons for wanting to find simpler systems, though no doubt Dr. Spiegelman could make protoplasts in Hemophilus or pneumococcus if he put his brain at it. The two systems now in use are relatively uneconomical both from the viewpoint of availability of mutant markers + physiological complexity & there is little aid in analysis of these genes by breeding. Reports of DNA-mediated transfer in [illegible] have appeared from time to time but no reproducible recipes are now in print. As the crucial problem may well be the <u>penetration</u> of DNA, we may be more optimistic about the use of protoplasts, such as Dr. Spiegelman has described. Unfortunately, our trials <u>so far</u> with penicillin-induced protoplasts of E coli have been entirely unrewarding.

The direct approach at the replacement of cellular organelles also has its place in the justification of models; e.g., Dr Mazia assures me it would be feasible to implant cysteine <u>in ovo</u> to try to set up [illegible] in the [illegible]

I would like to take the privilege of asking some questions to clear up some points on which I was confused.

Would Dr. Sp. clarify the specificity of RNA substitution in his protoplast system?

This was reminiscent of Dr. Allfrey's ~~[illegible]~~ denucleated nuclei. Any chance of DNA resynthesis here?