Scolnick CBH line

RAI for p14 (not coat)
p52

Sum

Andro, estrogens, prog ⊖
Physiol doses
via cytoplasmic receptors - by in vivo & in vitro comp. studies
Specific for B vs C type (N or X tropic)
Need constitution level
(KiBALB not inducible)

Cut curves - sl ↑ in 30' (~2x)
10x by 24 HRS
2/3 max by 8 HRS

p14 RAI - ō ↑ at 30'
(cell extract) ↑ at 4 → 24 (10x)
ratio of p14/p52 is constant in induction

inhibitors - sl ↓ c̄ act. D.
no effect of cycloheX, ana C, HCl
cyclo does block induction of
p14 (as does act D)
(RNA synth inhib does not)

can protect receptor vs NEM or Iodoacetate by binding to Dex -

can distinguish hamster vs mouse receptor by differential protection by aldosterone -

now testing hamster-mouse hybrids for steroid ~~[illegible]~~ response -

iodoacetamide Rx of Lomm receptor complex will retain hormone but → 4x ↓ in binding to DNA (50% → 1%) ∃ loss of dex -

Non inducible clones appear at ~~to~~ 4×10^{-3} to 8×10^{-2} (by polym. assay) (vs $TG^S \rightarrow TG^R$ at 10^{-7})

Cl 101 but ↑ in MMTV RNA still seen c̄ lower basal level

Cl 14 Kinetics is same + also 10-20x

LB1 What controls constitutive levels? (not level of dex receptor - not amt of MMTV DNA)

LB112

see no ↑ in extracellular RNA/prot/polym. (NOT) despite 10 x ↑ in intracell RNA + prot -

(10) Thus far: hybrids BHK × C3H
loss of hamster chrom
MTV RNA (+), steroid resp (+)
receptor minime (+)
hamster (+)
Ex Mann (?) collectrol
glycerol grad

Chromatin – assay fractions for
teams by Coli polym, RPol II
get 20 X ↑ in template active chrom.

globin + MTV seqs in both active + inactive fx's

Simpson RS Virus – looks like paramyxos
RNP 130 Å
Virion 120-200 nm
SS RNA ? (? 28S by Simpson)
Proteins 7 (others see → 10)
HA –
NA –
Replication confined to cy to plasm
Act D –
Negative strand virus – RDRP (+)

Thesis – RDDP also → alt. pathway –

ts mutants of RSV → live vaccine –
(killed vaccine doesn't immunize) –

mutagenized ε 5FU ?
ts mutants established in ~~Hamster~~ BKR line + ground at NB temp
mutually → ts virus p̄ blept ↓
later → no spon. induction (for a time, mitomycin inducible)
but RS AG's present

cells slowed ~ 33rd passage –
antigen only by immunofluor.
senescence ~ 40th passage –
never viral gene exp. at NP, though
RNA + (mid-uptake + act D)
& No interference at NP temp

Prots $\bar{p}$ shift ↓ < 80K sh / 72K non glyc.
interference + (VSV ↓ 3 logs)
(phenotypic mixing)

Recover virus by cocult
or fusion (↑ rate)
or by IUDR ± DMSO
} both ↓ 1-2 logs at NP temp

DNA transfection per Hill + Hillova
c̄ Hep 2 cells – (by FA + virus prod)
abolished by DNAse (not by RNAse/T1)

amt of DNA req to → FA⊕ can differ
from amt to → virus by up to 1000 x

amnts similar to RCS

Can absorb transfection c̄ Act D + Mitol
(not c̄ aral)

* Can also recover infectious DNA from productively
+ chronically infected cells – Hep 2

DNA interv. ? Tech from encaph. virus / Measles / Influenza
in SCEPTS. (→ protein, no virus)

Palese FLU

Can only see cell segments of RNA in gels when infection performed at High MOI - WSN Vms 5pfu

1
2
3
4
5
6
7
8
9

other strains have similar but ~~[illegible]~~ signif differences

intensity varies c̄ MOI (↑ c̄ ↑ MOI)

BRATT ~~[illegible]~~ NDV L 175-200K

intra cell = virus prot.

HN 74K → Hemo adsorption

Fo → F 56-58K

NP 56K

46K ? function

M 41K

mRNA ~~[illegible]~~ = 35S c̄ polyA - assoc c̄ polysomes

→ L prot in vitro (no trypt. maps yet)

Prot. prop - polymerase (~~[illegible]~~

(Tsipis) NG, ⇒ 5FU, HONO ts mut 36 42.

A–E compl. groups –

A+E are RNA –, HA –, FFWI –

B not defective in measurable funct.

C+D RNA+ HA+, FFWI ±

M. Schlessinger SINDBIS

49S

5' capped ——— | ——— AAA

RNA – ts mut.

2–4 groups

RNA+ ts mut

3 compl. groups

26S (1.8 x 10^6)

E1+E2 are ~50K sp's

5' | C | PE2 | E1 gp | AAA

capped capsid prot spE2 at membrane

both good mRNA's in vitro – 26S → mainly capsid prot.

From 3' end c̄ SFDV: same 3' ends for 26+49S

Shuts off host prot. synthesis + can do shift up

p̄ shut off by ts mutants → disappearance

of viral prot synth: in mutant ⊖ for 26S

⊕ for 49S

200K prot still made

no peptide maps yet → ? replicase mRNA synd related prot.

26S has only a single nutration site –
ascites extracts (clockwise) – only ~ 2/3 of 49S translated –
2nd unit sites made avail by
synth of 26S RNA
of TMV
SV40

Models to explain complex patterns
include ? of cistron prot as
~~no~~ protease

Ellen Strauss – Sind bis mutant – maturation defective –
(in CEF) – abnl morph by EM –
ballooning – some c̄ multiple nucleocapsids –
particles are injectives (↑ specific infectivity
for particles c̄ mult. nucleocapsids) –
defect probably in capsid rather than
membr prot ~~[illegible]~~ –

Lazzarini – VSV – DI's – 1/3 – 1/2 full length RNA
new type – deleted viral RNA
covalently linked to complement
→ hairpin –

DI·011 (extreme) 10S + 19S
↓ ↓
90% ds SS RNA

Origin –
not extraction artefact
p̄ denat, seamness ~~[illegible]~~ in zero order Rxn –
(melts at 89° and 93°)
– 1000 bp's

RNA is VSV specific Can reach denaturable p̄ c̄ RNAse → Cot½ ↑ 10^{-3}

	RNP	RNA
VSV	3.5 μ	3.1 μ
DIVA	.8-.9 μ	.33

suggests in SS configuration in virion

Weiss - using XC plaque test to ~~[illegible]~~ test human sera + cells

only + Wooley isolate from Wo Mod. dams ~ 20 isolates from Alsatel fetuses?

Gallo cultures also had baboon virus -

Baltimore

RNA lytic viruses

III ds RNA

IV (+) Std — { genome = mRNA (picorna)
subgenome mRNA (toga viruses)

V (-) Std. all helical, enveloped viruses

a) 1 piece genome - rhabdo, para, pejeo

b) multiple - (arena, myxo, bunya)

3 circular RNPs
160 kinds
eg. Ukkinimi
Polymerase has strong affinity for MNA

Poly A/Polio (D. Spector)
No RNA synth in single step - ? temp for poly
? att for polym

In vitro (Kamarov) Hela system
Alls nascent cleavage patterns
Used VP1 deletion to look for ↓ size of products
no tryptic maps

In vivo -

1 : X 1½

obligate nascent cleavage

rapid ½ life (if not cleaved nascent

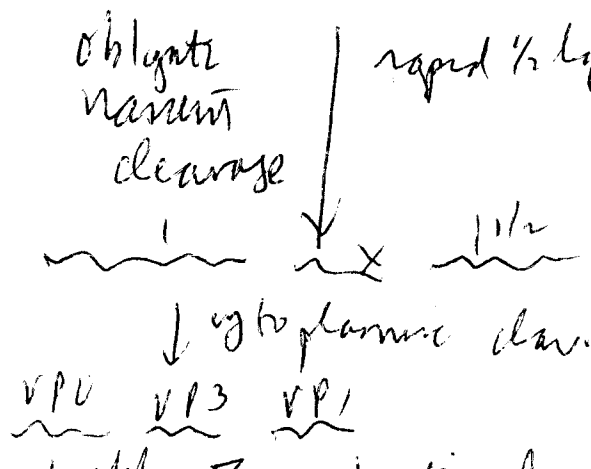
1 X 1½

↓ cytoplasmic clav.

VP0 VP3 VP1

↓ obligate maturation clav.

VP4 VP2



Mentu use salt shift
KCl 90 mM (for mit.)
↳ 150-160 (for elong)

Toga (see Schlesinger)

Badnack - N-termini of picornavirus proteins
are v. similar -
eg VP1 = gly
VP2 = ASP or ASX
VP3 = SER
VP4 = ?

? related to cleavage or assembly requirements

cf oncornav. p30's = pro

Lengyel - Interferon - gp c̄ nolecanol residues
1) antiviral
2) prime for interferon prod.
3) sensitize to ~~anti~~ tree effects of dsRNA

2 cells + ascites cells -
NDV to induce IF
Reo superinfection: SSRNA & dsRNA
+ prot synth all impaired

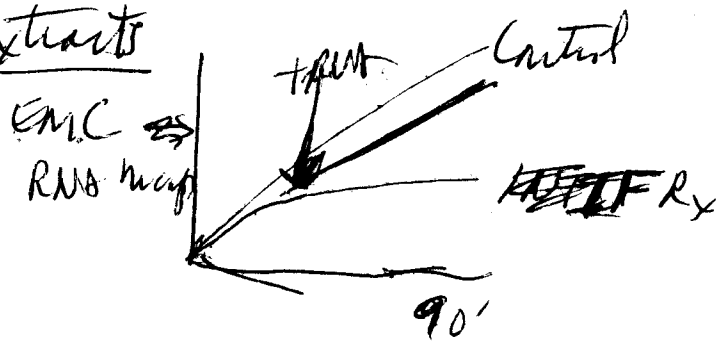
impairment of SS < ds

1) ~~of~~ reo prot synth is affected
when cell prot synth is not (Spec u/w)

2) IF does not protect cell vs cyto toxicity
of reo — it
localizes injection

3) virus ~~[illegible]~~
production may be ~~[illegible]~~ more affected (eg 90%)
than viral prot accum (40%)

cyto extracts



Extracts
pre incubated
+ dialyzed

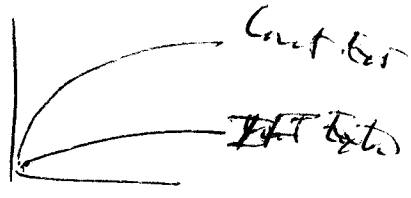
Dominant
inhibition by
mixing.
Inhib loosely
bound to
ribosomes
RNA is in large
polysomes +
not degraded
tRNA can
reverse effect

both initiation +
elong of stalled
chains ←

leu, lys, serine: impaired action of synthetase c̄ IF, due to tRNA inactivation, not to synthetase impairment — ? physiol. importance?

tRNA's stable if extracts not dialyzed vs sephadex — ?

if step omitted, stall effect c̄ EMC RNA



methylation of endog. RNA in extracts not altered ± IF but viral RNA methylation is (reo) markedly inhibited — not effect on enzyme, ~ SAM — RNA not degraded, in fact could be put back into control extract c̄ good results —

Samuels + Joklik — used host RNA from polysomes (: [illegible] + methylation + in vitro viral RNA (not methyl.) used dialyzed, preimuds ext. c̄ added tRNAs —

premethylated mRNA is protected