

RNA TUMOR VIRUSES

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enu
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+IC
g²⁵

I. Introduction

General Reviews: "The molecular biology of tumor viruses",
Ed. J. Tooze, Cold Spring Harbor Laboratory (1973).

Bishop, J.M. and Varmus, H.E., "The molecular
biology of RNA tumor viruses", Cancer: A Com-
prehensive Treatise 2:3-48 (1975).

Bader, J.P., "Reproduction of RNA tumor viruses",
Compr. Virol. 4:253-332 (1975).

Hanafusa, H., "Avian RNA tumor viruses", Cancer:
A Comprehensive Treatise 2:49-90 (1975).

Lieber, M. M. and Todaro, G.M., "Mammalian type C
RNA viruses", Cancer: A Comprehensive Treatise
2:91-130 (1975).

Moore, D.H., "Mammary tumor virus", Cancer: A
Comprehensive Treatise 2:131-167 (1975).

Temin, H.M., "Mechanism of cell transformation by
RNA tumor viruses", Ann. Rev. Microbiol. 25:609-
648 (1971).

Cold Spring Harbor Symposium Quant. Biol. 39,
Vol 2 (1975).

ICN-UCLA Symposium on Molecular & Cellular
Biology, Vol. IV (1975).

- A. A brief history of RNA tumor virology: Ellerman and Bang & ALV
Rous and RSV (ASV)
Bittner and MMTV
Gross and MuLV
(Gross, Oncogenic Viruses, Pergamon Press, 1970)

- B. Morphology and chemistry of virus particles (Table 1)
 - C-type (+ bud)
 - B-type
 - budding
- RNA TV's all similar, despite wide distribution + wide biological potential*
emphasis on ASV fast mutants

Plan
Stress: RNA TV's as tools for
understanding: (of Cancer program)
onogenesis
gene expression/reg
homeo actin
evolution
information transfer
recombination

Make up:
Begin i some facts →
then exp't to exp'line.
- replication
- mapping genome
- regulation of viral gene exp.
- Transformation
- endogenous viruses
- human agents.

C. Definitions of biological behavior in vitro (Tables 2 & 3)

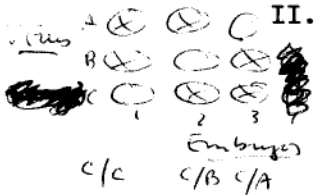
---transformation and focus assay * Temin & Rubin

---permissive vs. non-permissive cells (cf SV40)
 high effic. low effic.

---replication-defective and transformation-defective viruses ●*

note trans. function apparently not req. for replication (SV40)

II. Replication



A. Absorption and penetration: surface determinants of host range - cell receptors and virus envelope glycoproteins (Tables 4 & 5)

Suscept. dominant - phenotypic mixing → virus infections
 ... cell gene env - noninfectious - host antigen ENV

B. The basis for the provirus hypothesis (Temin, H.M., Science 192:1075, 1976; Temin, PNAS 69:1016, 1972).

---Inhibitor experiments

---BUdR sensitizes the viral genome to light (Boettiger & Temin, Nature 226:1211, 1970).

ssRNA → dsDNA → int.

↓
 dsRNA → int.

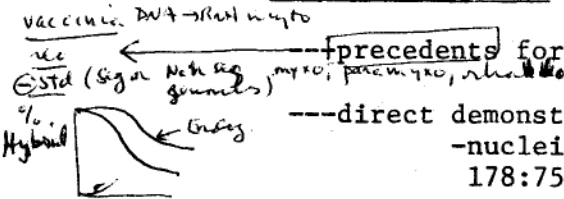
C. Reverse transcriptase (Reviews: Temin, H.M. and Baltimore, D., Adv. Virus Res. 17:129, 1972; Sarngadharan, M.G., Alloudean, H.S. and Gallo, R.C., Methods in Cancer Research 7:426, 1976; Green & Gerard, Progress in Nucleic Acid Res. 14:188, 1974; also forthcoming reviews by Verma, Taylor, and Weinberg in BBA Reviews in Cancer, 1977).

1st 12 hrs. DNA inhib. works later act. trans. works

? RNA → DNA where substrate enzyme - cyclohex → about int.

Temin's exp't ASV + d.c.t.

Baltimore's exp't. vs. MLV



---precedents for virion associated polymerases

---direct demonstration of viral DNA in infected cells:
 -nucleic acid hybridization (Neiman, Science 178:750, 1972)

-transfection (Hill & Hillova, Virology 49:309, 1972)

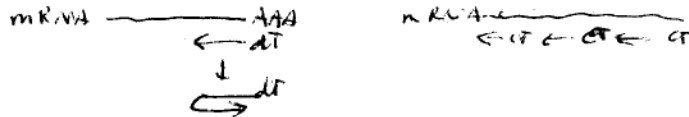
---is reverse transcriptase a viral gene product?

-early coordinate ts mutants (Verma, Mason, Drost & Baltimore, Nature 251:27, 1974) and deletion mutants (Hanafusa et al., Science 177:1188, 1972)

---how does reverse transcriptase work in vitro?

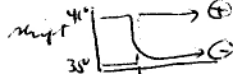
(1) physical structure, three enzymatic activities, templates, and primers

(2) utility for molecular biologists



NP cells 5%
 X C DNA → infection of cells
 ts HSV/Hamster → recovery of ts mutant

Mutants: Mutagenesis studies
 Screen (no substrate)
 Exp't: early coord. effect on T & R



- mutants 10h
 - Pol ts in vitro
 - ↓ DNA synth. in vivo

readily purified: Δβ ASV; 70K MLV

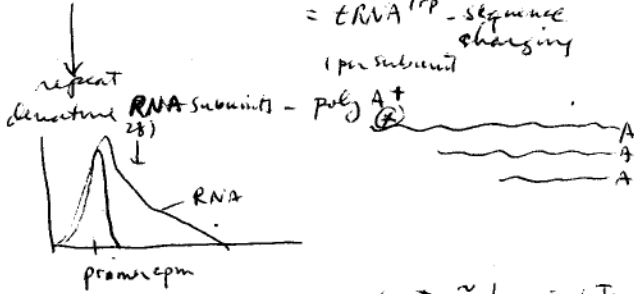
3 acts: RNA → DNA (A-R-C: d G (vs cell DNA priming)
 DNA → DNA) gap fill - no exc. no displacement
 RNase H

primers from DNA or DNA template
 → exc., jumping

Lecture 2

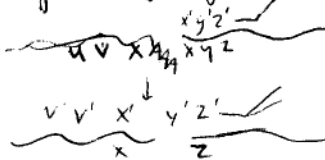
Early - repetitive copying
short pieces
1° product - 100+ bases

short Run - dCTP to label 1° → tRNA
replicates virus - = Spot 1
= tRNA^{Trp} - sequence charging
(per subunit)



short stop DNA - sequence (note ≈ terminal Triose)

Focus on leap (always implicit)



(3) problems posed by the natural template: primer
(Taylor et al., ICN-UCLA Symposium IV, p. 161, 1976; Haseltine & Baltimore, ICN-UCLA Symposium IV, p. 175, 1976)

---location of the primer near the 5' end

---"short stop" DNA (for sequence, see Shine et al., and Maxam et al., PNAS, in press 1977)

---the "transcriptional leap"

---terminal redundancy

---making full length cDNA (Rothenberg & Baltimore, J. Virol. 21:168, 1977)

- optimizing conditions
- ? note full length not made in stationary cells

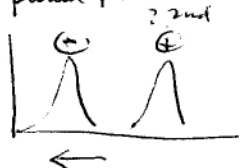
---initiating the second ("plus") strand made, but how? - not beginning - ? template as primer

---how does reverse transcriptase work in vivo?

synthesis in 1st class. - few copies - detect with hybridization

⊕ in enucleated cells. -
primer for 1st strand prob + RNA^{3'-P}

synthesis in the cytoplasm (Varmus et al., PNAS 71:3874, 1974)



Form I - Classical linear as in SV40

EM p purification - 10⁶ fold

vDNA is infectious

Forms of DNA: permuted linear with fragmented plus strands

covalently closed circles in nucleus (Guntaka et al., Nature 253:507, 1975 and J. Mol. Biol. 106:337, 1976)

D. Integration

---requirements and mechanisms

---the Fv-1 story: N tropic vs. B tropic viruses (Review: Lilly & Pincus, Adv. Canc. Res. 17:231, 1973)

OMITTED

(Table 5)

N-tropic MuLV + VSV in NIH Swiss →

VSV pseudotypes reveal intracellular block VSV (VSV) MSV (MuLV/V) (Huang et al., J. Virol. 12:659, 1973)

Muller & anal. N-tropic in B cell

vDNA not ↓ RT ↓

replicate in B cell as well as NIH Swiss

impaired integration (Jolicové & Baltimore, PNAS 73:8, 1976)

NOTE MSV requires tropism of its helper target prot. viral protein? RTase? primary frames, for activation, for C → N transport

E. Transcription of proviral DNA to RNA

---is an integrated template required? < EB Fv-1

---host RNA polymerase II is responsible (Rymo et al., PNAS 71:2782, 1974)

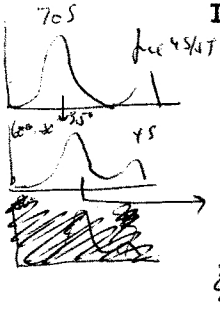
---what is/are primary transcript(s)? (see below, IV)

consider further after discussing genes + their order

F. Translation, assembly, budding of virus, transformation

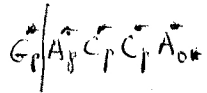
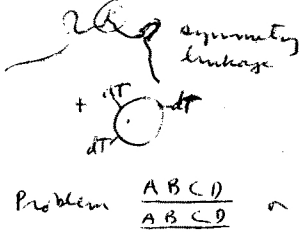
⊕ ← **Summary**: x x x

III. The viral genome



A. Definition of its structure: physicochemical analysis: subunits and low molecular weight RNA's

electron microscopy shows 5'-5' linkage of subunits (Bender & Davidson, Cell 7:595, 1976; Kung et al., J. Virol. 16:397, 1975)



B. The subunits are identical: T1 oligonucleotide fingerprinting

(Beeman et al., PNAS 71:4254, 1974; Billeter et al., PNAS 71:3560, 1974)

2-Digestion
isolate - count - complex \leftarrow RNAse
OH

$$Sp. actn. = \frac{\text{cpm}/\mu\text{mole}}{\text{Total cpm}/\text{Total nucleot.}} \sim 10000$$

annual
count rate = 1x

C. Additional structural features: poly(A) at 3' end (Wang & Duesberg, J. Virol. 14:1515, 1974); capped 5' end (7mGpppG^mpCp---) (Furuichi et al., Nature 257:618, 1975); primer (see above, II C)

D. Genes and their definition

---pol (see above) - ts mutants - also lack major structural proteins

xx ---gag: ts mutants (Hunter et al., Virology 69:35, 1976) and translation in vitro (see below, IV B)

---env: deletion mutants (Kawai & Hanafusa, PNAS 70:3493, 1973) host range determinants (Table 4)

---src: ts and deletion mutants (Martin, Nature 277:1021, 1970; Vogt, Virology 46:939, 1971)

$a = b + x$ RNA 16%
ASV \rightarrow ts ASV

E. Mapping

---genetic recombination (Kawai & Hanafusa, Virology 49:37, 1972; Bernstein et al., Virology 70:206, 1976)

---oligonucleotide mapping (with deletions mutants, recombinants) (Duesberg et al., ICN-UCLA Symposium IV, p. 107, 1976; Wang et al., PNAS 73:3952, 1976)

---heteroduplex mapping (Junghans et al., PNAS, in press)

---restriction endonuclease mapping (Shank et al., unpublished)

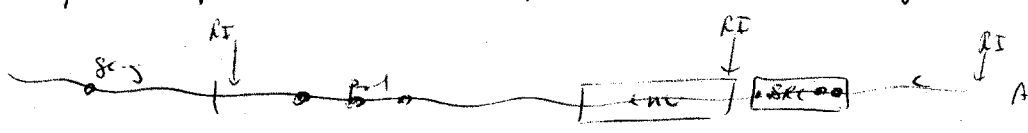
high rate recomb - no good map
ts T vs ts T \rightarrow no wt
ts T vs ts T \rightarrow wt - gaps = distance

distance from poly A to deletion specific oligos:
A } compare wt
A } let
A }

- Add dels to map + src ts

distance to primer specific oligo

	ES	P1	P2	C	x	RAV-B	Recombs	Spe TS	Distance	Conclusion
src	+	-	-	-	-	-	+	1	500-600	Common source
env	C	B	B	B	B	B	C	1	2500-5000	
pol	ts	wt	wt	wt	wt	wt	wt	1 or 2	6000-8000	Deduced source
gag	p27-1	p27-2	p27-2	p27-2	p27-2	p27-2	27-2	2	8000-10000	Established source



IV. Gene expression and regulation

gas - 3000/μ
env - 500/μ
pol - 50/μ
src - ?

A. Problems in permissive cells: differing amounts of gene products

single initiation per mRNA in internal cistrons
in reg. requires multiple initiations - small promoters w/ processing of RNA (Src)
or protein (cytoplasm)

B. Experimental approaches

SV40
TMV
Sindbis

---size and sequence composition of viral RNA's (Bishop et al., ICN-UCLA Symposium VI, p. 1, 1976; Weiss, Baker et al., unpublished)

---immunoprecipitation of polyribosomes (Mueller-Lantzsch and Fan, Cell 9:579, 1976)

---analysis of products of translation in vivo and in vitro (Vogt et al., J. Mol. Biol. 96:471, 1975; Pawson et al., J. Virol. 19:950, 1976; Oppermann et al., unpublished)

C. The tentative model: precedents and problems

primary process vs. co-transcripts
SV40 RNA read through RNA No 28S Nucleoarginine

D. Blocks to viral gene expression in non-permissive cells

E. The mechanisms of reversion (Frankel et al., Science 191:1264, 1976; Deng et al., Virology 76:313, 1977; 65:522, 1974)

F. A model for study of hormone action: glucocorticoids regulate the rate of synthesis of MMTV RNA (Young et al., J. Virol. 21:139, 1977; Ringold et al., submitted to PNAS)

OMIT (Keith covered it)

V. Transforming genes

Review

A. Definition and functions of the avian src gene

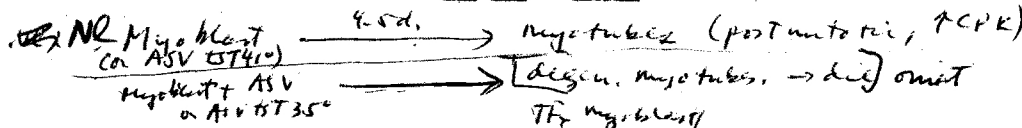
not req. for reg. ? gene product defunct growth

B. Manifestations of transformation of fibroblasts by the src gene:

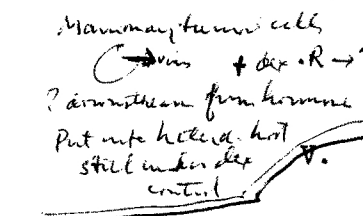
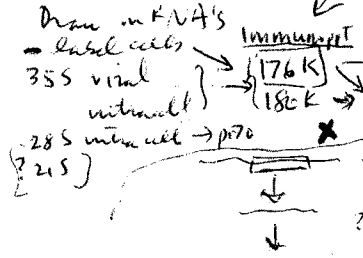
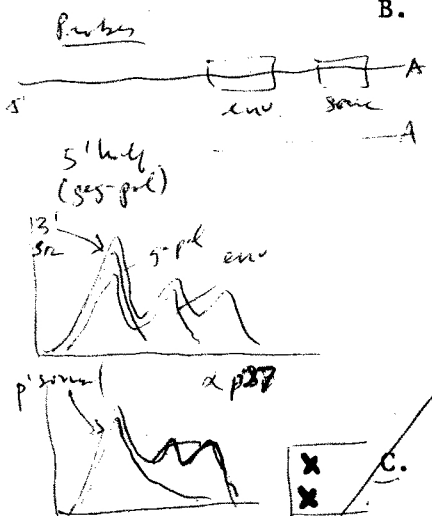
-growth, morphological and surface changes

-dissolution of cytoskeleton (microfilaments, microtubules) (Wang & Goldberg, PNAS 73:4065, 1976; Edelman & Yahara PNAS 73:2047, 1976; Ash et al., PNAS 73:3603, 1976)

C. The transforming gene prevents differentiation of myoblasts (Holtzer et al., PNAS 72:4051, 1975)



late differentiation (30° → differentiation) ∴ ASV src helped to diff. cell; delay diff. of precursors



cytoskeleton + changing perception of "cytosol" + membrane fluidity

Ash: fibroblast morph & myosin ConA recept. X

to SR-A/PK: morph & actin & tubulin X

? effect on cytoskeleton: note T.G. exp. - nuclear not necessary

3

n(CERC) } in animal, RNA to *cDNA
td ASV CERC }
ASV CEF (+) } ? mechanism of release
no virus (A in mRNA's

D. The transforming gene activates transcription of embryonic globin genes (Groudine & Weintraub, PNAS 72:4464, 1975)

Abelson transformant (B) by myeloblasts, fetal liver cultures, in vitro

empirical { src → sarcoma, rare form of human cancer
Many other types of microorganisms mediated tumor viruses

MC29 " myeloblast precursor" }
AEV " erythroid " } murine cultures
note Fe exp: myeloblasts phase 1/2 → magnetic separation

E. Are there other transforming genes (e.g., for leukemia, carcinoma, etc.) (Rosenberg & Baltimore, ICN-UCLA Symposium IV, p. 311, 1976; Graf et al., ICN-UCLA Symposium IV, p. 321, 1976).

No genetic definition

VI. Endogenous viruses

A. The evidence for the oncogene-virogene hypothesis (Todaro & Huebner PNAS 69:1009, 1972) (1) NE cells have real Ag's, viral DNA

independent, regulated

good evidence for (2) Virus released or induced of I.V.R induction from clones of mouse cells.

B. Properties of endogenous viruses

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C. Mapping the chromosomal site of an endogenous murine leukemia virus (Chattopadhyay et al., PNAS 72:906, 1975)

removes pleurocytoma, overcomes organotropism of MLV in infected new hosts (→ genetic trans.)

D. Creating an endogenous virus by infection of pre-implantation embryos (Jaenisch et al., ICN-UCLA Symposium IV, p. 283, 1976; PNAS 73:1260, 1976).

Does this occur in nature?

E. Trans-species spread of endogenous viruses: the RD-114 story (MacAllister et al., Nature N.B. 235:3, 1972; Benveniste & Todaro Nature 252:456, 1974) RD the baboon sarcoma → fetal cat brain → fetal cat cells in cult → RNA TV - first vs human (K0 cells) + fetal cat

similar virus induced from cat, grown up in human (K0) Ag + RNA related - RD 114 seq. sub. in cats from Med. housing - baboon seq. independent

Can endogenous viruses be used to study evolution?

The baboon virus and the origin of man (Benveniste & Todaro, PNAS 71:4513, 1974; Nature 261:101, 1976) unique seq overall diverged in accord. e evolv. time, unrelated to geograph. DNA seq related to BEV diverged as f (time + geography) - man on Asian scale

What is the evidence for independently regulated oncogenes?

The origin of the avian src gene (Stehelin et al., Nature 260:170, 1976; Padgett et al., Cell, in press, 1977; Spector et al., unpublished; Varmus et al., ICN-UCLA Symposium IV, p. 339, 1976) Same in all cell - but is it linked to virogene? (NO) is its expression altered in spont. & chemically induced tumors? (NO)

F. Tested for virus prod (→ leak) + viral RNA in lymphoid cell. i.e. retroviral genes (dominant)

Virus considered absent but ? male (of no apparent) BEV, RAV-O

VII. Do human RNA tumor viruses exist?

A. Defining approaches in the context of RNA tumor viruses of animals. → endogenous (? induce -VIRUS) defect virus, selected animal virus reagents (±) → partial expression (comp. sensitive) cells; defect virus, selected animal virus reagents (±) → virus prod. by tumor cells.

B. What is HL-23 virus, where did it come from and what has it done?

(Gallagher & Gallo, Science 197:350, 1974; Reitz et al., PNAS 73:2113, 1976; Okabe et al., Nature 260:264, 1976; Wong-Staal et al., Nature 262:190, 1976)

HL-23 E AML
↓
budding viruses
↓
propagated in culture
↓
2 components BEV (±) (WTA) + (WTA) SSV (±) -

Table 1

	<u>ASV</u>	<u>ALV (or td ASV)</u>	<u>MSV</u>	<u>MuLV</u>
<u>RNA subunit</u>	3.3 x 10 ⁶	2.8 x 10 ⁶	1.9 x 10 ⁶	3 x 10 ⁶
<u>Probable genes</u>	gag pol env src	gag pol env	gag src (?)	gag pol env
<u>Viral proteins</u>	gp85, gp37 RT (α•β) p27, p19, p15 p12, p10	same as ASV	? (Found as pseudotype of MLV)	gp70, gp45 RT (1 subunit) p30, p19 , p15 p15(E), p12, p10
<u>Biological effect in vivo</u>	Sarcomas	Leukosis, other tumors	Sarcomas	Leukemias
<u>Biological Assay</u>	Fibroblast Transformation	"Plaques" (some strains)	Fibroblast Transformation	Cell Fusion (XC cells)

Table 2

Biology of principal avian viruses

<u>Virus</u>	<u>Permissive (Avian) Cell</u>	<u>Non-Permissive (Mammalian) Cell</u>
nd ASV	T ⁺ R ⁺	T ⁺ R ⁻
td ASV (or ALV) (src ⁻)	T ⁻ R ⁺	T ⁻ R ⁻
rd ASV (env ⁻)	T ⁺ R ⁻ (non-infectious particles)	T ⁺ R ⁻
td ASV + rd ASV	T ⁺ R ⁺ (phenotypic mixing)	

T = transformation
R = replication

Table 3

Biology of principal murine C-type viruses

<u>Virus</u>	<u>Permissive (Murine) Cell</u>
MuLV (td)	T ⁻ R ⁺
MSV (rd)	T ⁺ R ⁻ (no particles)
MSV + MuLV	T ⁺ R ⁺ (phenotypic mixing)

ASV = avian sarcoma virus; ALV = avian leukosis virus
MSV = murine sarcoma virus; MuLV = murine leukemia virus
nd = non defective
td = transformation defective
rd = replication defective

Table 4

<u>Host Range</u>	<u>ASV env gene</u>				
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>
<u>Chicken Type</u>					
C/A	-	+	+	+	+
C/B	+	-	+	+	+
C/C	+	+	-	+	+
C/D	+	+	+	-	+
C/E	+	+	+	+	-
C/O	+	+	+	+	+

e.g. C/A = chicken "bars" subgroup A virus
C/O = "bars" nothing
+ = susceptible

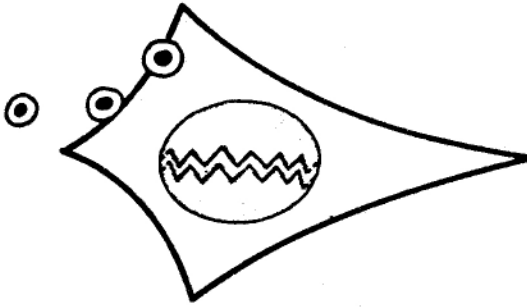
Table 5

<u>Host Range</u>	<u>Cell</u>	<u>MuLV Strains</u>		
		<u>N-tropic</u>	<u>B-tropic</u>	<u>Xenotropic</u>
Fv-1 ⁿⁿ	NIH Swiss Mouse	+	-	-
Fv-1 ^{bb}	BALB/c Mouse	-	+	-
	Human Cells	-	-	+
Fv-1 ^{nb}		-	-	-

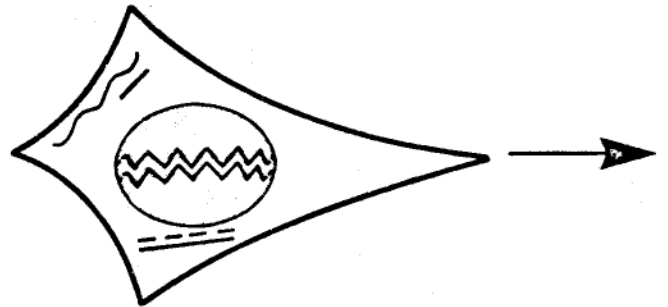
∴ resistance dominant

Fv-1 chromosome IV

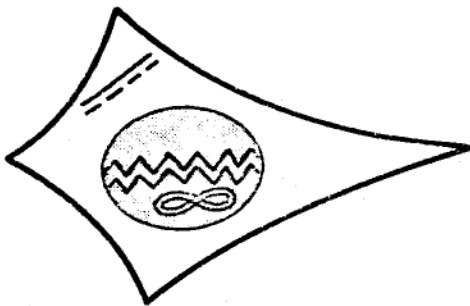
INFECTION OF A PERMISSIVE HOST BY AVIAN SARCOMA VIRUS



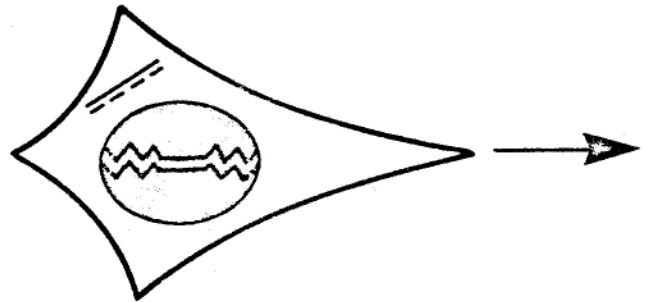
ABSORPTION AND PENETRATION



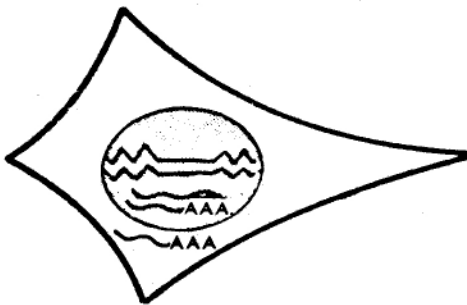
SYNTHESIS OF RNA—DNA HYBRIDS
AND DOUBLE-STRANDED VIRAL DNA
IN CYTOPLASM (0—6 hours)



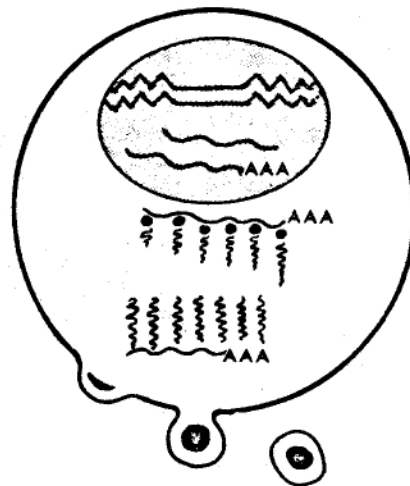
TRANSPORT OF VIRAL DNA
TO THE NUCLEUS



INTEGRATION OF VIRAL DNA CIRCLES
INTO THE HOST GENOME (9—24 hours)

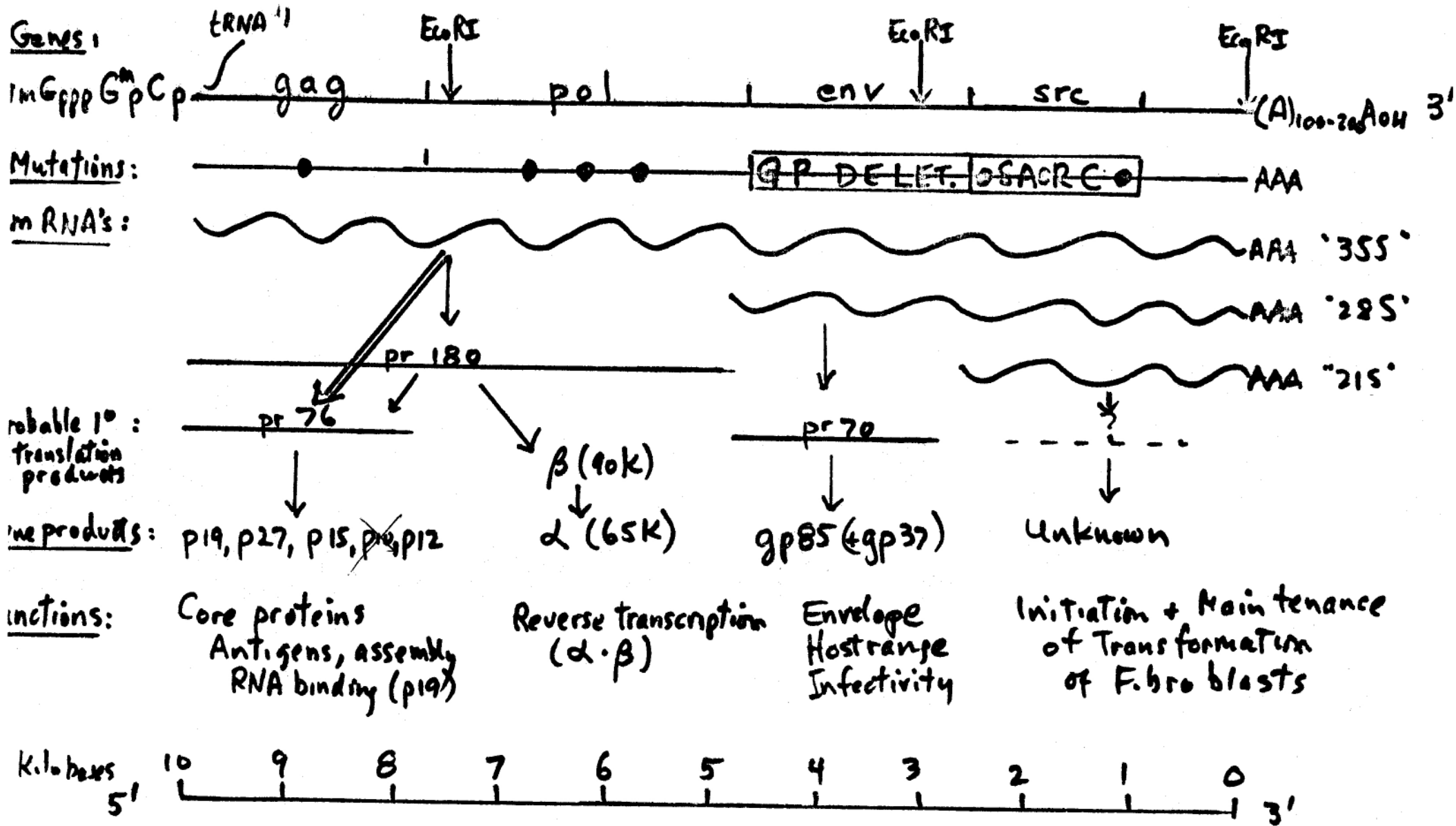


SYNTHESIS OF VIRUS-SPECIFIC RNA,
ADDITION OF POLYADENYLATE,
TRANSPORT TO CYTOPLASM
(after 18 hours)



SYNTHESIS OF VIRAL PROTEIN IN POLYSOMES,
CELL TRANSFORMATION, VIRAL ASSEMBLY
AND RELEASE (after 24 hours)

also page 2 needs of DNA synth



STRUCTURE AND FUNCTION OF THE AVIAN SARCOMA VIRUS GENOME