Biochemistry 201

Tumor Virus Minicourse

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RNA TUMOR VIRUSES

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 Comprehensive 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, Permogon Press, 1970)

B. Morphology and chemistry of virus particles (Table 1)
- C-type (+ bud)
- B-type
- budding

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

---transformation and focus assay

---permissive vs. non-permissive cells (cf SV40)

---replication-defective and transformation-defective viruses

II. Replication

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

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).

C. Reverse transcriptase (Reviews: Temin, H.M. and Baltimore, D., Adv. Virus Res. 17:129, 1972; Sarngadharan, M.G., Allaudeen, 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).

---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

(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)

---initiating the second ("plus") strand

---how does reverse transcriptase work in vivo?

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

Forms of DNA: permuted linear with fragmented plus strands

✘ covalently closed circles in nucleus (Guntaka etal., 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) (Table 5)

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

impaired integration (Jolicoeur & Baltimore, PNAS 73:X, 1976)

E. Transcription of proviral DNA to RNA

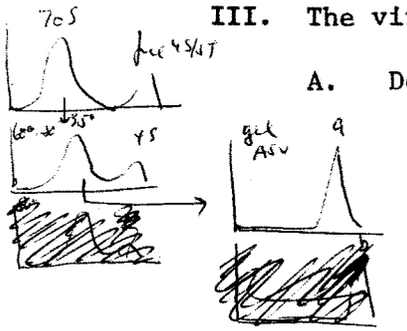
---is an integrated template required? EB / Fv-1

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

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

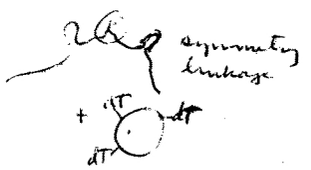
F. Translation, assembly, budding of virus, transformation

Summary: ✘ ✘ ✘

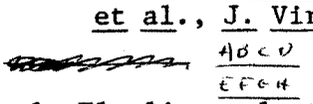


III. The viral genome

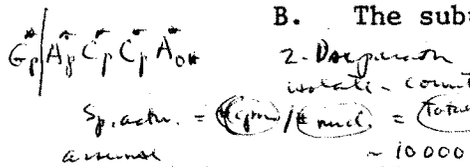
A. Definition of its structure: physicochemical analysis: subunits and low molecular weight RNA's



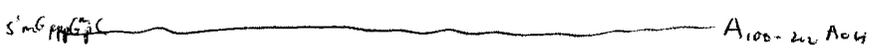
electron microscopy shows 5'-5' linkage of subunits (Bender & Davidson, Cell 7:595, 1976; Kung et al., J. Virol. 16:397, 1975)



B. The subunits are identical: T1 oligonucleotide fingerprinting (Beeman et al., PNAS 71:4254, 1974; Billeter et al., PNAS 71:3560, 1974)



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



D. Genes and their definition

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

---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)

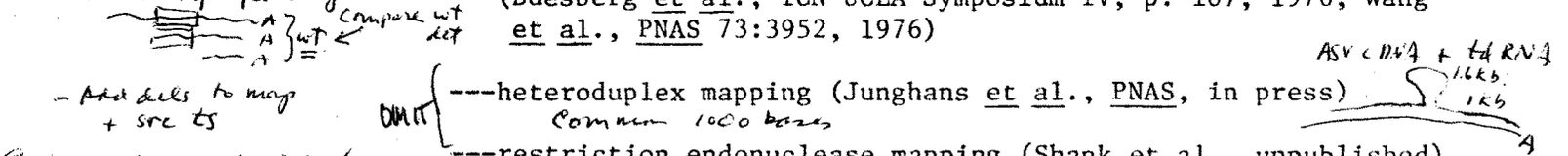
E. Mapping

---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)

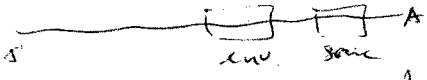


	ts pol Pr C	× RAV-B	→ Recombs	Spots	Distance	Conclusion
src	+	–	+	1	500–1000	Confirm position
env	C	B	C	1	2500–5000	
pol	ts	wt	wt	1 or 2	6000–8000	Deduced Pol A.
gag	p27-1	p27-2	27-2	2	9000–10000	Established position

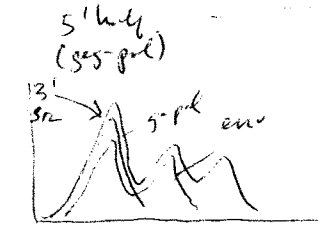
IV. Gene expression and regulation

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

B. Experimental approaches



---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

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)

V. Transforming genes

A. Definition and functions of the avian src gene

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)

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

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).

VI. Endogenous viruses

A. The evidence for the oncogene-virogene hypothesis (Todaro & Huebner PNAS 69:1009, 1972)

B. Properties of endogenous viruses

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

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).

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)

F. 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)

G. 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)

VII. Do human RNA tumor viruses exist?

A. Defining approaches in the context of RNA tumor viruses of animals.

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)

Table 1

	ASV	ALV (or td ASV)	MSV	MuLV
RNA subunit	3.3×10^6	2.8×10^6	1.9×10^6	3×10^6
Probable genes	gag pol env src	gag pol env	gag src (?)	gag pol env
Viral proteins	gp85, gp37 RT ($\alpha \cdot \beta$) 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
Biological effect in vivo	Sarcomas	Leukosis, other tumors	Sarcomas	Leukemias
Biological Assay	Fibroblast Transformation	"Plaques" (some strains)	Fibroblast Transformation	Cell Fusion (XC cells)

Table 2

Biology of principal avian viruses

Virus	Permissive (Avian) Cell	Non-Permissive (Mammalian) Cell
nd ASV	T^+ R^+	T^+ R^-
td ASV (or ALV) (src^-)	T^- R^+	T- R- (no particles)
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

Virus	Permissive (Murine) Cell
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

Host Range	ASV env gene				
Chicken Type	A	B	C	D	E
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

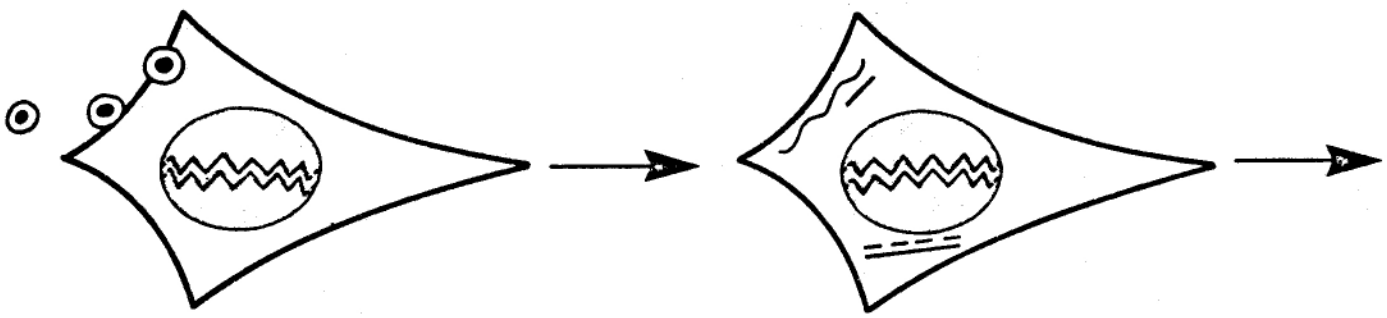
Table 5

	Host Range	MuLV Strains		
	Cell	N-tropic	B-tropic	Xenotropic
$Fv\text{-}1^{nn}$	NIH Swiss Mouse	+	-	-
$Fv\text{-}1^{bb}$	BALB/c Mouse	-	+	-
	Human Cells	-	-	+
$Fv\text{-}1^{nb}$		—	—	—

∴ resistance dominant

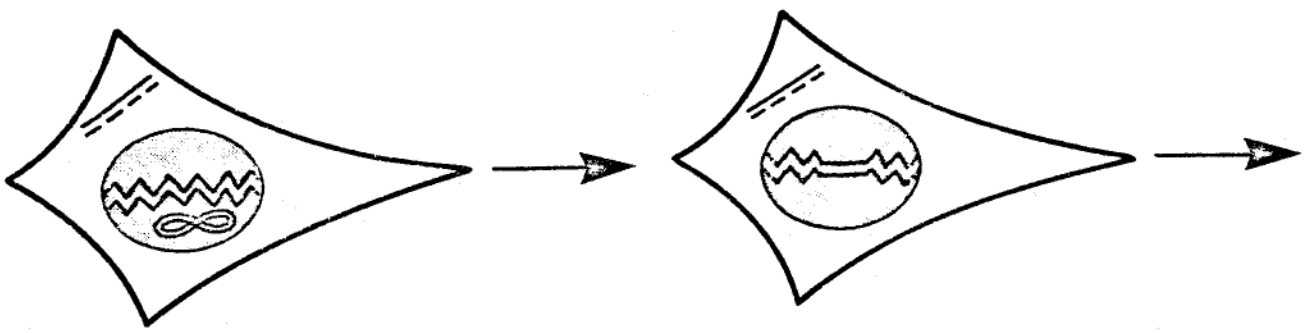
Fv-1 chromosome IV

INFECTION OF A PERMISSIVE HOST BY AVIAN SARCOMA VIRUS



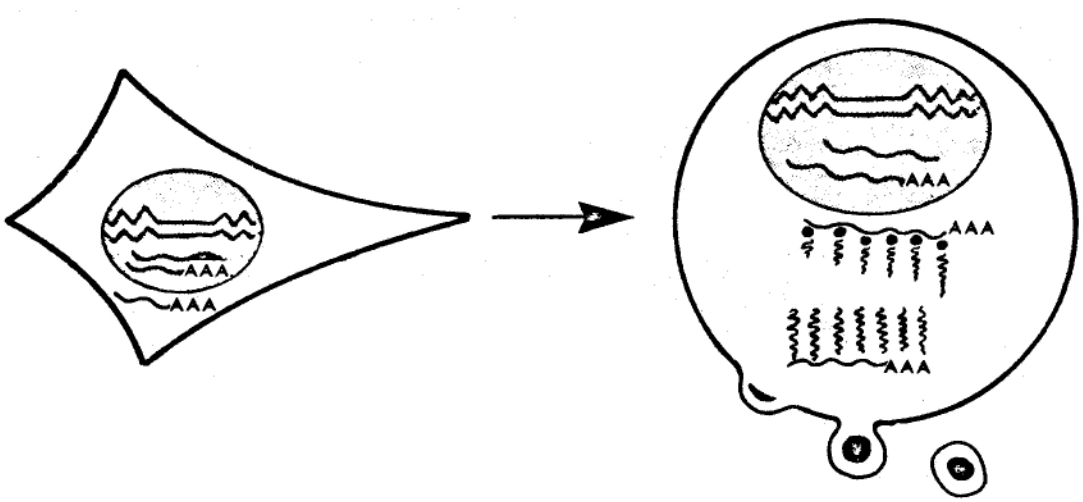
ABSORPTION AND PENETRATION

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



TRANSPORT OF VIRAL DNA
TO THE NUCLEUS

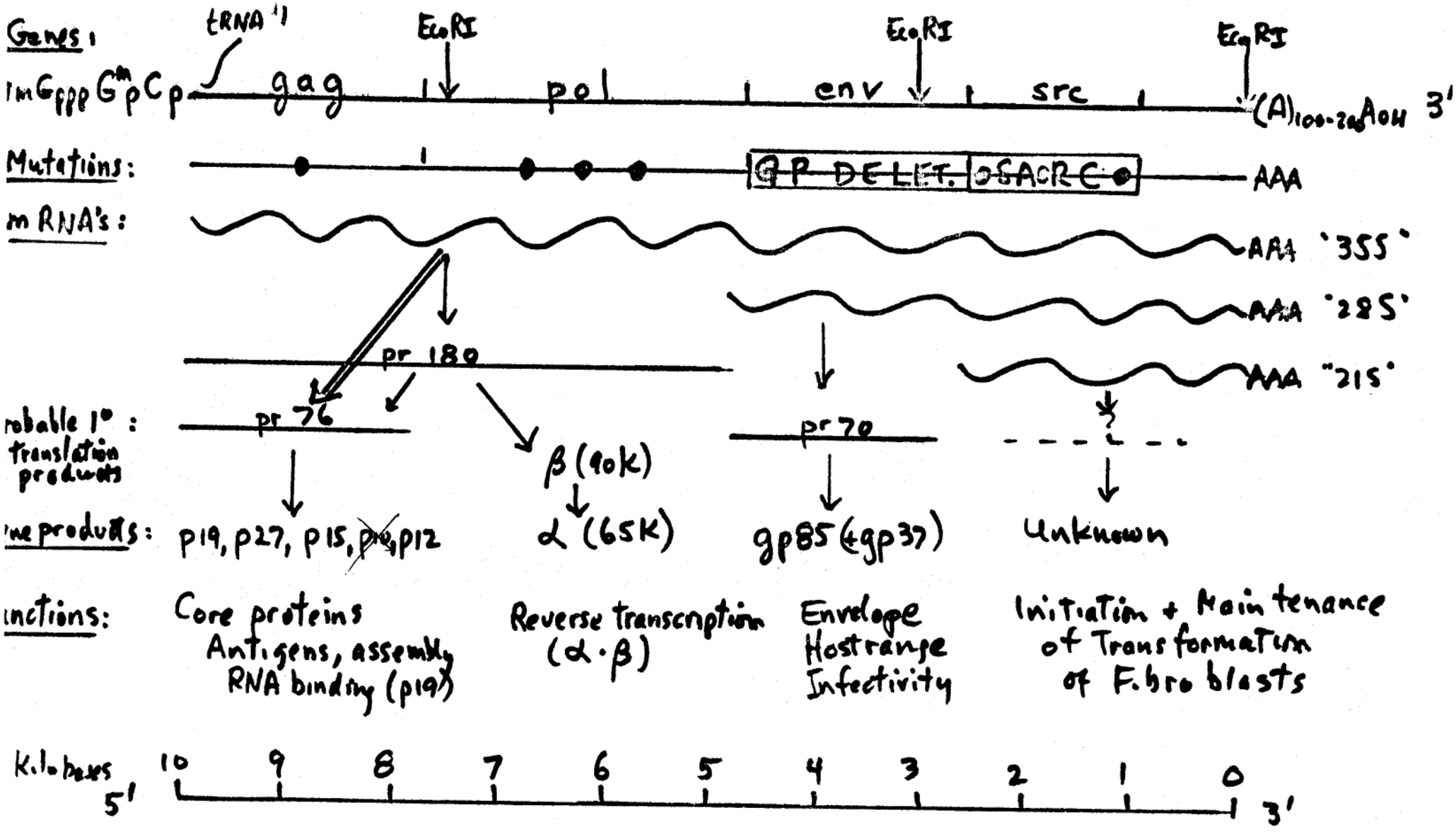
INTEGRATION OF VIRAL DNA CIRCLES
INTO THE HOST GENOME (9—24 hours)



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 much of DNA synth



STRUCTURE AND FUNCTION OF THE AVIAN SARCOMA VIRUS GENOME