Biochemistry 201

Tumor Virus Minicourse

Harold E. Varmus, M.D.

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?

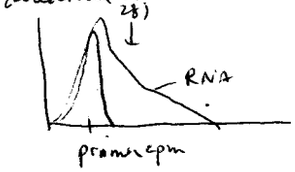
(1) physical structure, three enzymatic activities, templates, and primers

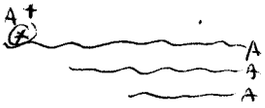
(2) utility for molecular biologists

Lecture (2)

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

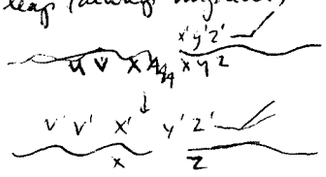
short Rxn - dCTP to label 1° → tRNA
stop labeling virus - = Spot 1
= $tRNA^{Trp}$ - sequence changing
1 per subunit





- short stop DNA - sequence (note ≅ terminal T loop)

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"

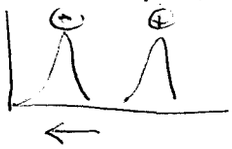
---terminal redundancy

---making full length cDNA (Rothenberg & Baltimore, J. Virol. 21:168, 1977)
- optimizing conditions
- ? note problem with not made in stationary cells.

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

---how does reverse transcriptase work in vivo?

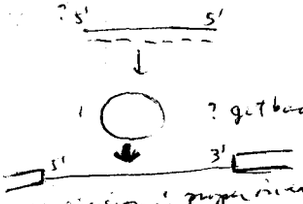
synthesis in 1st 6 hours - Few copies - detect with hybridization
⊕ in enucleated cells. -
primer for 1st strand prob $tRNA^{Trp}$
? 2nd



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

Form I - Classical Denvirt. as in SV40
EM ⊖ purification - 10^6 fold
vDNA is infectious

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)



No excision ∴ proper orient ? cell site } of genome

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)
OMIT

N-tropic MuLV + VSV in NIH Swiss → VSV(VSV) MSV(MuLV/N)
replicate in BALB/c as well as NIH Swiss ← αVSV

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

Molecular and N-tropic in BALB/c
vDNA ↓ } ? mech.
int ↓ } ? rel. —
RNA ↓

impaired integration (Jolicoeur & Baltimore, PNAS 73:X, 1976)
223C

NOTE MSV acquires tropism ∴ target prob viral protein ? RTase ? permitting for entry, for uncoatinization, for C → N transport

E. Transcription of proviral DNA to RNA

OMIT

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

AMV prot. nuclei
RNA* + cDNA

Lamanatin inhibit

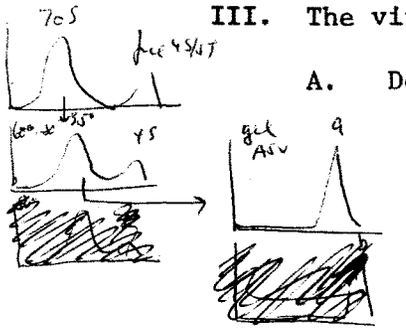
---host RNA polymerase II is responsible (Rymo et al., PNAS 71:2782, 1974)

---what is/are primary transcript(s)? (see below, IV)
Consider further chapter discussing genes + their order

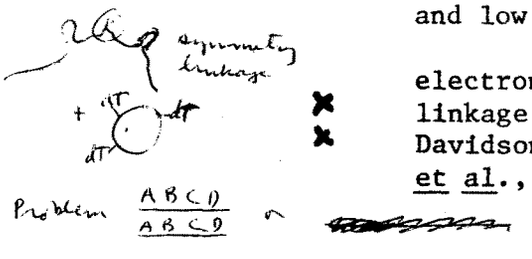
F. Translation, assembly, budding of virus, transformation

⊛ ← Summary: ✘ ✘ ✘

III. The viral genome

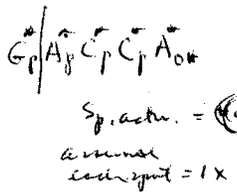


A. Definition of its structure: physicochemical analysis: subunits and low molecular weight RNA's

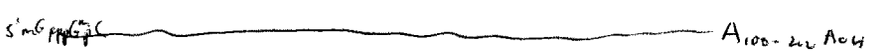


electron microscopy shows 5'-5' linkage of subunits (Bender & Davidson, Cell 7:595, 1976; Kung et al., J. Virol. 16:397, 1975)

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)

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

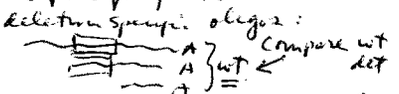
---env: deletion mutants (Kawai & Hanafusa, PNAS 70:3493, 1973) host range determinants (Table 4)

---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)

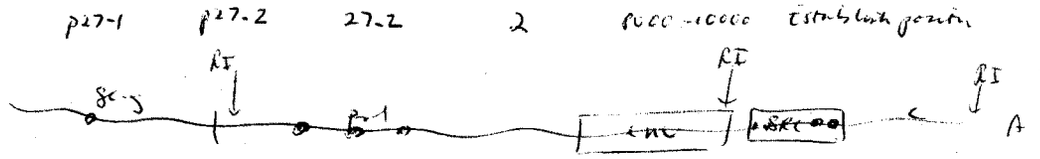
---oligonucleotide mapping (with deletions mutants, recombinants) (Duesberg et al., ICN-UCLA Symposium IV, p. 107, 1976; Wang et al., PNAS 73:3952, 1976)



---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	500-2000	Confirm position
env	C	B	C	1	2500-5200	
pol	ts	wt	wt	1 or 2	6000-8000	Deduced position
gag	p27-1	p27-2	27-2	2	9000-10000	Establish position



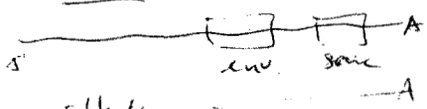
IV. Gene expression and regulation

gag - 3000/v.
env - 500/v
pol 50/v
src - ??

A. Problems in permissive cells: differing amounts of gene products
single initiation per mRNA in eukary. internal cistrons
∴ reg. requires multigl. mRNAs – ∴ multi. promoters, WT. processing of RNA (Sv40) or of protein (eg. polio)

B. Experimental approaches

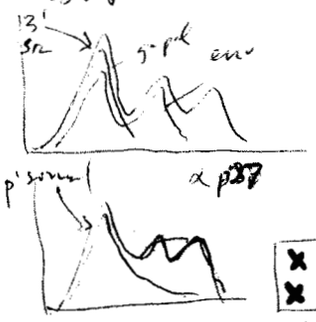
Probes



---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)

OMT (Keith covered it)

V. Transforming genes

Review

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

OMIT [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^- (no particles)
td ASV (or ALV) (src^-)	T^- R^+	T- R- (no particles)
rd ASV (env^-)	T^+ R^- (non-infectious particles)	T^+ R^- (no particles)
td ASV + rd ASV	T^+ R^+ (phenotypic mixing)	

T = transformation
R = replicati

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

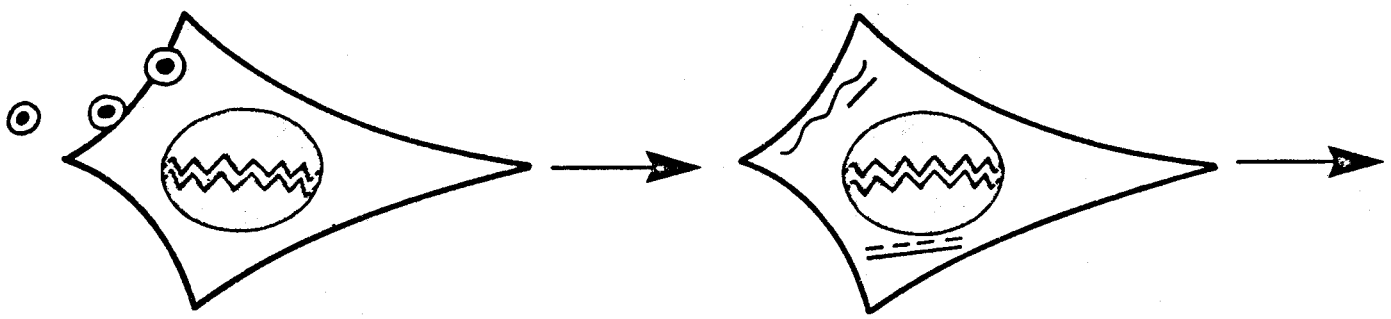
Table 5

	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

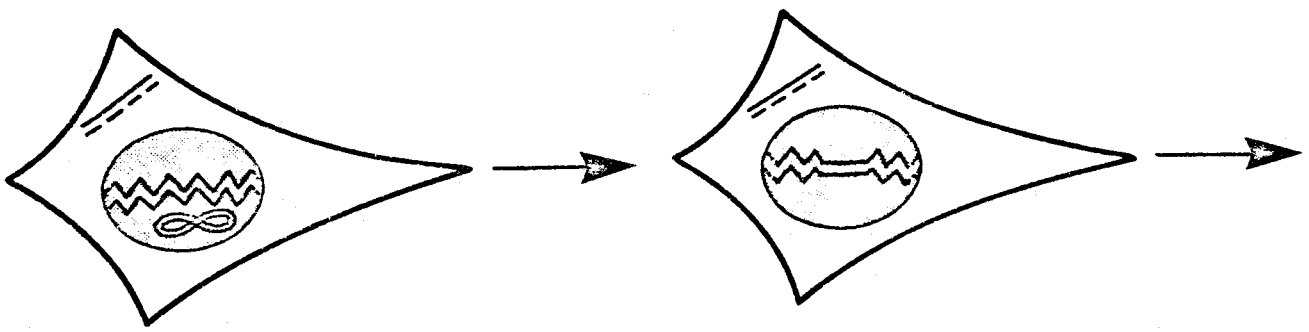
Fv-1 chromosome IV

INFECTION OF A PERMISSIVE HOST BY AVIAN SARCOMA VIRUS



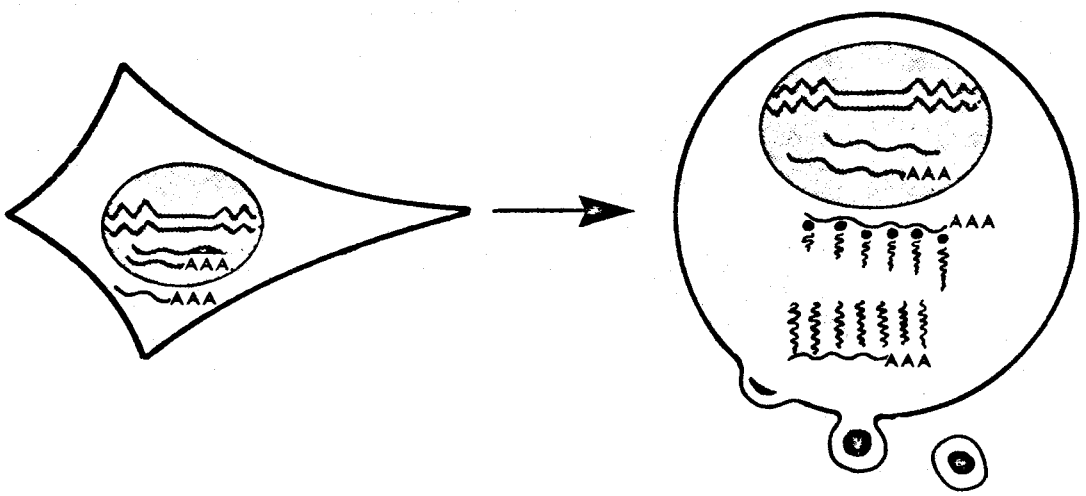
ABSORPTION AND PENETRATION

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



TRANSPORT OF VIRAL DNA TO THE NUCLEUS

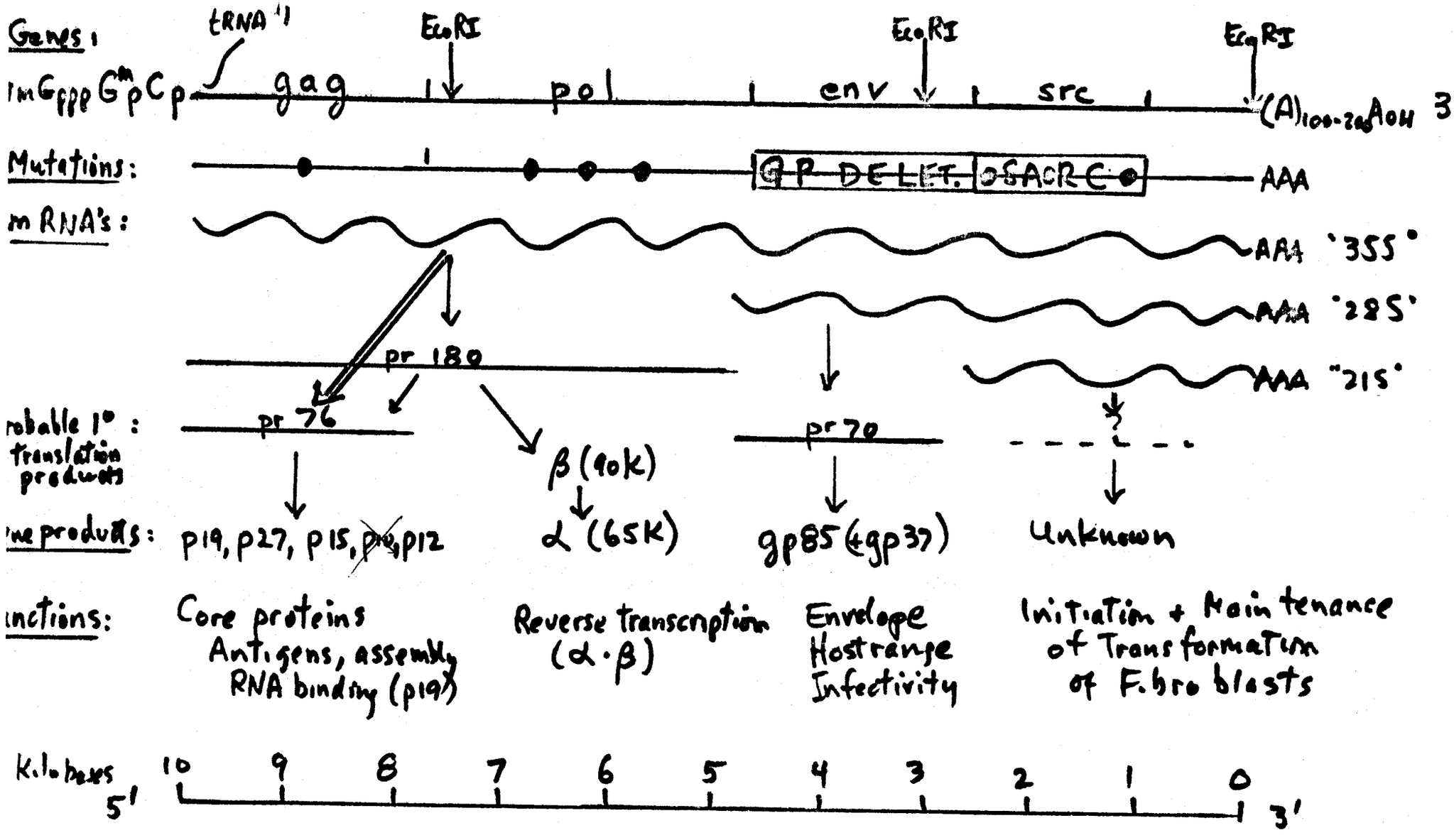
INTEGRATION OF VIRAL DNA CIRCLES INTO THE HOST GENOME (9—24 hours)



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 week of DNA synth



STRUCTURE AND FUNCTION OF THE AVIAN SARCOMA VIRUS GENOME