RNA TUMOR VIRUSES

I. Introduction


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 (+buf)
   • B-type
   • budding
   • envelope, ASV & MuLV mutants
C. Definitions of biological behavior in vitro (Tables 2 & 3)

---transformation and focus assay
---permissive vs. non-permissive cells
---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)


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


---precedents for virion associated polymerases
---direct demonstration of viral DNA in infected cells:
  - nucleic acid hybridization (Neiman, Science 178:750, 1972)
  - transfection (Hill & Hilova, Virology 49:309, 1972)

---is reverse transcriptase a viral gene product?

---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 &

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

---how does reverse transcriptase work in vivo?

Forms of DNA: permuted linear with fragmented
plus strands

covalently closed circles in
nucleus (Guntaka et al., Nature
106:337, 1976)

D. Integration

---requirements and mechanisms

---the Fv-1 story: N tropic vs. B tropic viruses (Review:

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

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

---is an integrated template required? (Es)

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

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

E. Transcription of proviral DNA to RNA

F. Translation, assembly, budding of virus, transformation
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 (7mGpppGmCp---) (Furuichi & al., Nature 257:618, 1975); primer (see above, II C)

D. Genes and their definition

- **pol** (see above) - transmembrane - viral GTPase

- **gag** (see above) - ts mutants

- **gag**: 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)


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)
IV. Gene expression and regulation

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

- internal cistrons
- hybrid sequence, multiple enzymes, mRNA primes, etc.
- differences in expression

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)


C. The tentative model: precedents and problems

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


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:

- dissolution of cytoskeleton (microfilaments, microtubules)

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

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

VI. Endogenous viruses


B. Properties of endogenous viruses.

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


E. Do these viruses occur in nature?


Can endogenous viruses be used to study evolution?


What is the evidence for independently regulated oncogenes?


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?

Table 1

<table>
<thead>
<tr>
<th>Virus</th>
<th>ASV</th>
<th>ALV (or td ASV)</th>
<th>MSV</th>
<th>MuLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA subunit</td>
<td>3.3 x 10^6</td>
<td>2.8 x 10^6</td>
<td>1.9 x 10^6</td>
<td>3 x 10^6</td>
</tr>
<tr>
<td>Probable genes</td>
<td>gag</td>
<td>gag</td>
<td>gag (?)</td>
<td>gag</td>
</tr>
<tr>
<td></td>
<td>pol</td>
<td>pol</td>
<td>pol</td>
<td>pol</td>
</tr>
<tr>
<td></td>
<td>env</td>
<td>env</td>
<td>src (?)</td>
<td>env</td>
</tr>
<tr>
<td>Viral proteins</td>
<td>gp85, gp37</td>
<td>same as ASV</td>
<td>?</td>
<td>gp70, gp45</td>
</tr>
<tr>
<td></td>
<td>RT (α,β)</td>
<td>(Found as pseudotype of MLV)</td>
<td>p30, p6, p15</td>
<td>p15(E), p12, p10</td>
</tr>
<tr>
<td></td>
<td>p27, p19, p15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p12, p10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological effect</td>
<td>Sarcomas</td>
<td>Leukosis, other tumors</td>
<td>Sarcomas</td>
<td>Leukemias</td>
</tr>
<tr>
<td>in vivo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological Assay</td>
<td>Fibroblast</td>
<td>&quot;Plaques&quot; (some strains)</td>
<td>Fibroblast</td>
<td>Cell Fusion</td>
</tr>
<tr>
<td></td>
<td>Transformation</td>
<td></td>
<td>Transformation</td>
<td>(XC cells)</td>
</tr>
</tbody>
</table>

Table 2

Biology of principal avian viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Permissive (Avian) Cell</th>
<th>Non-Permissive (Mammalian) Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>nd ASV (or ALV) (src-)</td>
<td>T⁺ R⁺</td>
<td>T⁺ R⁻</td>
</tr>
<tr>
<td>td ASV (or ALV) (src-)</td>
<td>T⁻ R⁺</td>
<td>T⁻ R⁻</td>
</tr>
<tr>
<td>rd ASV (env⁻)</td>
<td>T⁺ R⁻</td>
<td>T⁺ R⁻</td>
</tr>
<tr>
<td>td ASV + rd ASV</td>
<td>T⁺ R⁺ (phenotypic mixing)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3

Biology of principal murine C-type viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Permissive (Murine) Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuLV (td)</td>
<td>T⁻ R⁺</td>
</tr>
<tr>
<td>MSV (rd)</td>
<td>T⁺ R⁻ (no particles)</td>
</tr>
<tr>
<td>MSV + MuLV</td>
<td>T⁺ R⁺ (phenotypic mixing)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Host Range</th>
<th>ASV env gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Type</td>
<td>A</td>
</tr>
<tr>
<td>C/A</td>
<td>-</td>
</tr>
<tr>
<td>C/B</td>
<td>+</td>
</tr>
<tr>
<td>C/C</td>
<td>+</td>
</tr>
<tr>
<td>C/D</td>
<td>+</td>
</tr>
<tr>
<td>C/E</td>
<td>+</td>
</tr>
<tr>
<td>C/O</td>
<td>+</td>
</tr>
</tbody>
</table>

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

### Table 5

<table>
<thead>
<tr>
<th>Host Range</th>
<th>MuLV Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell</td>
<td>N-tropic</td>
</tr>
<tr>
<td>NIH Swiss Mouse</td>
<td>+</td>
</tr>
<tr>
<td>BALB/c Mouse</td>
<td>-</td>
</tr>
<tr>
<td>Human Cells</td>
<td>-</td>
</tr>
<tr>
<td>&quot;resistance dominant&quot;</td>
<td></td>
</tr>
<tr>
<td>&quot;chromosome II&quot;</td>
<td>-</td>
</tr>
</tbody>
</table>
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)

Page 2 needs more input
Structure and Function of the Avian Sarcoma Virus Genome

Genes:
1mgppGppCp / ERNA' / gag / pol / env / src / (A)100-200A0U 3'

Mutations:

mRNA's:

Translation Products:

Proteins:
Core proteins
Antigens, assembly
RNA binding (p19)

Reverse transcription (α, β)
Envelope
Host range
Infectivity
Initiation + Maintenance of Transformation of Fibroblasts

Kilobases
5' 10 9 8 7 6 5 4 3 2 1 0 3'