LIFE CYCLE OF RNA ONCOGENIC VIRUSES

A. M. Wu
Dept. of Molecular Biology
Bionetic Research Foundation
7300 Pearl Street
Bethesda, Maryland 20014

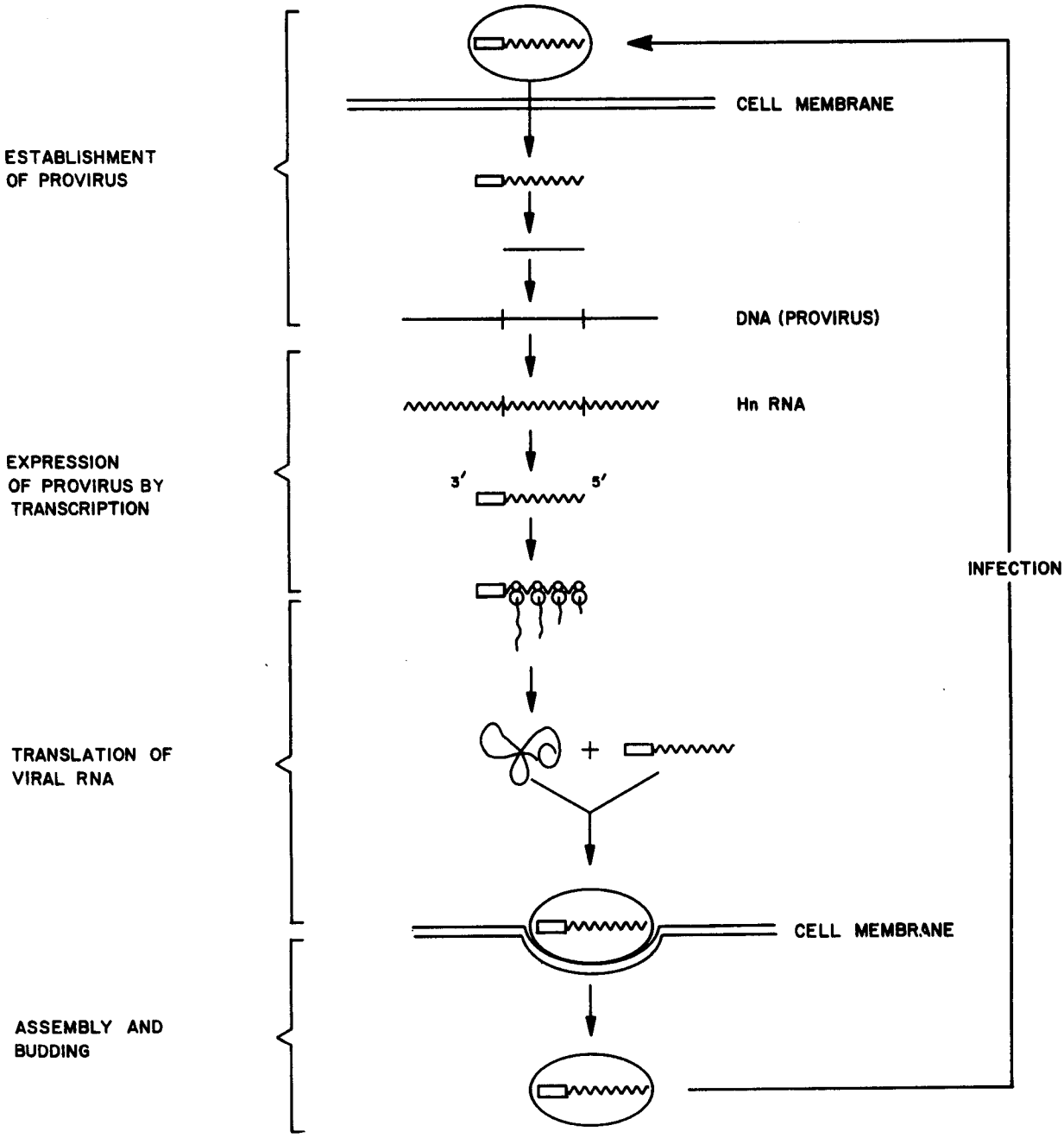
R. C. Gallo
Laboratory of Tumor Cell Biology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

In view of the increasing evidence suggesting that human cancer cells contain molecular footprints related to murine and primate type-C RNA tumor viruses (1–7), it is appropriate in this symposium to discuss a simple scheme for the life cycle of RNA tumor viruses. It is hoped that this scheme will be useful in searching for virus related proteins, nucleic acids and particulates in human leukemic cells, and in providing a working hypothesis for further studies on the mechanism of viral replication. The scheme is illustrated in the attached figure.

Virus particles

The RNA viruses contain a single stranded RNA with a molecular weight of 10^7 daltons which normally sediments at 70S and some other smaller RNA molecules which sediment at 35S, 28S, 18S and 4–5S. (For a review see ref. No. 8). The RNA contain a sequence of poly (A) with a size of about 200 nucleotides (9–11) which is probably located at the 3′ end of the RNA chain (12, 13). In avian RNA viruses, the 70S RNA can be dissociated to subunits of 30 to 40S (14, 15, 16). However, in mammalian RNA tumor viruses, the subunit structure of the 70S RNA is not well characterized (17, 18). The RNA is located in the core of the viruses (19–21). Some other important molecules such as reverse transcriptases (22, 23) and group specific (gs) antigens (24) are also located in the viral core. Generally, the cores are enveloped in a lipoprotein membrane (25). The average density of whole virus particles is about 1.16 gm/cm^3 measured in a sucrose density gradient while that of the cores is about 1.25 gm/cm^3 (26, 27). (For a recent review see ref. 28.)

DIAGRAMMATICAL REPRESENTATION OF LIVE CYCLE OF RNA TUMOR VIRUSES



SYMBOLS :

- : POLY (A)
- : VIRAL RNA WITH POLY (A)
- : MONOSOMAL RIBOSOME
- : POLYPEPTIDE
- : DNA
- Hn : HETEROGENOUS RNA

Possible States of Provirus Gene Expression in Balb/C 3T3 Cells

Classification of Cells	State of Expression	Cell State[1]	Induction of Infectious Viruses	Detection of Virus-Like		Examples
				Reverse Transcriptase	Nucleic Acid	
Uninfected	Unexpressed	N_T	Yes	Not Tested	Not Tested	Uninfected 3T3 CLS,
Non-Producer	Partial	T	Yes	Yes	Yes	Balb/K 3T3[3] Balb/M 3T3[4]
Producer						
A) Defective Particles	Partial	T	Yes	Yes	Yes	S^+L^-[5]
B) Infectious Particles Transforming	Full	T	N.A.[2]	Yes	Yes	MuSV (MuLV) Infection
Non-Transforming	Full	NT	N.A.	Yes	Yes	MuLV Infection

[1] N.T. - Not Transformed; T – Transformed

[2] N.A. - Not Applicable

[3] Balb/C K3T3 – Non-Producer of Balb/3T3 Infected with Kirsten Murine Sarcoma Viruses.

[4] Balb/C M3T3 – Non-Producer of Balb/3T3 Infected with Moloney Murine Sarcoma Viruses.

[5] S^+L^- - Any Cells Containing Sarcoma Virus Genetic Information, but not Leukemia Virus Genetic Information. In Case of Murine S^+L^- Cells, they are Producing Defective Particles.

Establishment of Provirus

Upon infection, the envelopes of the viruses which are presumably responsible for attachment and penetration of virus to cells are decoated. The location of the decoating is debatable (29, 30) and its mechanism is not known. The genetic information residue in the exposed RNA is then transcribed into DNA form. This transcriptional step is mainly catalyzed by RNA directed DNA polymerase (conventionally called reverse transcriptase or RNA dependent DNA polymerase) (31–34; for recent review see reference No. 35). Some other enzymes such as ribonuclease H (viral and/or cellular) (34–39), exonuclease and DNA ligase (41) might also participate in this transition. The subcellular location of this transition is still unclear (29, 30, 42). Two lines of information suggest that this "reverse" transcriptional step is essential for viral transformation and/or viral infection. These are: (a) A mutant of Rous sarcoma viruses (RSV) designated as RSV (0) which is noninfectious (43), also lacks RNA directed DNA polymerases (43); (b) A treatment of virus particles with various derivatives of rifamycin SV, inhibitors of reverse transcriptase, resulted in a loss of viral infectivity in culture (45) and in animals (46). The size of DNA product inside the infected cells is not known, although the size of DNA product in most *in vitro* systems has been found to be 4–5S (47–49). The poly (A) sequence of RNA probably is not copied because it was reported that in an *in vitro* endogenous reaction system, the DNA product did not contain a sequence of poly (T) (50). The DNA product *in vivo* is termed the *provirus*. It is not known whether the provirus exists in the infected cell in a free form as an episome, in an integrated part of some episome or in an integrated part of the host chromosomal structure. In avian systems, some preliminary experiments suggested that proviruses are in integrated forms (51). Nothing is known about the mechanism of integration.

State of the provirus

The genetic information of the provirus may stay unexpressed, partially expressed or fully expressed. Examples of these are shown in Table 1. In uninfected murine fibroblasts there is no sign of infection or transformation. However, intact viruses (termed "endogenous virus") can be produced from some of these cells on treatment with a halogenated deoxyuridine (52, 53). For example, two types of infectous viruses (Balb virus –1 and Balb virus –2) are induced from Balb/3T3 cells by IdU (54). It appears than that the genetic information of the provirus in these cells is not expressed. Alternatively, some expression of the proviral information may occur, and not be detectable due to lack of known characteristics. Normally murine sarcoma viruses are always associated with murine leukemia virus but murine leukemia viruses can be obtained without association of sarcoma viruses. Therefore, the outcome of viral infection by the murine sarcoma leukemia complex depends on the composition of sarcoma viruses and leukemia viruses in the inoculants. If cells are infected with a sarcoma-leukemia virus complex which is dominated by leukemia viruses almost all of the infected cells are transformed and are able to produce both sarcoma and leukemia infectious particles (55). However, if cells are infected with sarcoma-leukemia virus complex, dominated by sarcoma viruses, again most of the infected cells are transformed and are producing infectious particles but some of the transformed cells are

either nonproducers (56, 57) or S^+L^- (58, 59). S^+L^- is defined as any cells containing sarcoma virus genetic information but not that of leukemia virus. In case of murine S^+L^- cells, they are producing defective particles. These virally transformed nonproducer and S^+L^- cells, can be considered examples of cells exhibiting partial expression of the proviral genome. In infected but not virus producing cells, although no release of virus particles has yet been detected (56, 57), nucleic acid sequences (RNA) homologous to nucleic acid sequences (DNA products) of infected virus and virus-like reverse transcriptase have been observed. The role of virus-like RNA is understandable, however, the significance of reverse transcriptase in nonproducing cells deserves some thought. The possibility that the presence of reverse transcriptase is due to "leakiness" in gene regulation with no apparent function of the enzyme cannot be ruled out. Alternatively, reverse transcriptase might be involved in gene amplification of proviral genomes. Whatever the role of reverse transcriptase is, the presence of virus-like reverse transcriptase in a nonproducing cell provides us with a "footprint" for the presence of viral information, in the cells in question. This concept of possible presence of viral information in nonproducers is, in fact, substantiated by the fact that the expression of genetic information required for virus production can be induced either by helper virus or by chemical agents such as 5-iodo-2-deoxyuridine (IdU) or 5-bromo-2-deoxyuridine (BrdU) (52, 53, 60). Human leukemic cells can be considered as a type of nonproducers because they are transformed cells which do not produce virus particles, at least not in most circumstances as far as we can tell, but they contain nucleic acids homologous to those of the primate and murine RNA tumor viruses (3, 4, 5) and their reverse transcriptases (1, 2) share common antigenitity with primate and murine RNA tumor viruses (6, 7). Obviously, only the induction of infectious virus particles from human leukemic cells is the proof of infection. The other example of partial expression of the provirus is the S^+L^- cells (58). These cells are producing virus particles but the particles are defective in their content of both viral nucleic acid (61) and of viral reverse transcriptase (62). Just like the nonproducers, S^+L^- cells also contain (a) nucleic acid sequences homologous to viral nucleic acid sequences (61) and (b) virus-like reverse transcriptase (63). Furthermore, infectious particles (both of sarcoma and leukemia nature) can be induced by helper virus (58, 64) or by the chemical agents (IdU or BrdU) from S^+L^- cells (58). Apparently, both nonproducer and S^+L^- cells contain sufficient information for viral replication, but the degree of expression is regulated. Finally in case of virus producing cells after infection, they can be divided into transformed and nontransformed cells. In general, the sarcoma-leukemia virus complexes cause transformation both in animals and in culture, while the leukemia viruses cause transformation in animals but not in cultured fibroblasts.

Expression of Provirus

The RNA of RNA tumor viruses is similar to cellular m-RNA in at least two aspects. Both contain a sequence of poly (A) (9–11) which is probably at the 3′ end (13, 14; 65–67) and both can be used as for cell free protein synthesis (68, 69). With this information in mind, we previously proposed that the process of expression of proviral genome upon induction is probably similar or identical to processing of cellular

m-RNA (66, 70). Heterogenous large RNA molecules would be synthesized first, reactions catalyzed by host RNA polymerases of host origin (since no novel RNA polymerases in the virus infected cells has yet been found [Sethi and Gallo, unpublished data]). When synthesized, the heterogenous RNA molecules are much larger than that of m-RNA (71), but they are cleared and degraded to a smaller size accompanied by an addition of a sequence of poly (A). This final RNA is the mature viral mRNA, similar to cellular m-RNA (65, 67); the poly (A) sequence of viral RNA is probably added to 3′ end sequentially rather than segmentally. It takes about 10 minutes to complete the process from synthesis of heterogenous RNA to the addition of poly (A) sequence for cellular m-RNA (67, 71) and poly (A) synthesis can be preferentially inhibited by cordycepin (3′-deoxyadenosine) (66). The necessity of poly (A) synthesis in viral replication was suggested by the fact that virus production induced by IdU from BALB/K3T3 cells can be inhibited by a relatively low concentration of cordycepin (70, 72). The enzymes required for poly (A) synthesis and for degradation of heterogenous RNA are not known.

Little is known about synthesis of virus specific proteins *in vivo*. Recently, it has been shown that *in vitro* the viral RNA can be used as templates in a cell free protein synthesis system (69, 73). In all of these reports, the results are rather preliminary either due to lack of species specificity (only works well with an *E. coli* cell free system), or due to the fact that the protein products are not specific. However, these *in vitro* findings suggest that the viral RNA, after transportation, can serve as templates for the synthesis of virus specific proteins by host translational machinery. Recently, virus-specific mRNA and nascent polypeptides was demonstrated in the polyribosomes of transformed cells replicating murine sarcoma-leukemia viruses (74). This finding further supports the proposal of using translational machinery for viral replication. Involvement of a unique species of tRNA, quantitative change in pre-existing tRNA or appearance of specific modifying enzymes (such as tRNA-methyl-transferases) are possible regulations at this step. Recently, we observed that dexamethasone and many other glucogenic corticosteroids were able to stimulate virus production 3–25 fold from nonproducer (Balb/K3T3 cells) induced by IdU (72). Similar stimulation of virus production was observed in virus producing cells (75). Studies on the effective time course for the hormone effect indicated that these steroids acted after poly (A) synthesis, since a low concentration of cordycepin could nullify the stimulatory activity of the hormone (72, 75).

After virus specific proteins are made, the packaging of virus particles is then feasible. Again, little is known about assembly of virus particles. It is possible that the same viral RNA which were used as a m-RNA in translation were then packed and released as virus particles (74). The final step in the release of virus particles is generally characterized by budding. The budding can occur either intracytoplasmically and/or extracellularly (76). With avian viruses, soon after budding, the size of RNA is about 35S. Somehow the 35S RNA molecules are converted to 70S RNA at the relative high culturing temperature (37–40 °C) (77). The relationship of infectivity before and after conversion of RNA to 70S is not clear. It is not known whether there is such a conversion in the mamalian system.

Conclusion

The above scheme for the life cycle of type-C RNA tumor viruses is a simplified, and perhaps biased summary of observations regarding their replication mixed with some speculation for the sake of completion. The portions derived from speculations may be useful as working hypotheses for studying the mechanism of viral replication in animal systems, and for evaluating results of studies looking for molecular "footprints" of these viruses human cancer cells. The analogy of human leukemis cells and murine nonproducer cells is of particular interest. In fact, it has been shown that the virus related nucleic acids in human leukemic cells have a higher degree of homology with sarcoma viruses than with those of leukemia viruses (both from mouse and monkey) (5). Since the murine nonproducers also contain the sarcoma genome, this observation may be of particular relevance for understanding putative viral oncogenesis of human leukemia.

References

1. Gallo, R. C., Yang, S. S., and Ting, R. C., (1970) *Nature, 228*: 927.
2. Sarngadharan, M. G., Sarin, P. S., Reitz, M. S., and Gallo, R. C., (1972) *Nature New Biology, 240*: 67.
3. Baxt, W., Hehlmann, R., and Spiegelman, S., (1972) *Nature New Biology, 240*: 72.
4. Baxt, W., and Spiegelman, S., (1972) *Proc. Nat. Acad. Sci., USA, 69*: 3737.
5. Gallo, R. C., Miller, N., Saxinger, W. C., and Gillespie, D., (1973) *Proc. Nat. Acad. Sci., USA, 70*: 3219.
6. Todaro, G. J., and Gallo, R. C., (1972) In, the IV Lepetit Colloquium "Possible Episomes in Eukaryotes", L. Silvestri (Ed.), North Holland Publishers, Amsterdam.
7. Todaro, G. J., and Gallo, R. C., (1973) *Nature, 244*: 206.
8. Bader, J. P., (1969) In, Proc. 3rd Int. Symp. on Comparative leukemia Research, R. M., Dutches (ed.) S. Karger Publishing Co., Amsterdam, Vol. 36: 140.
9. Lai, M. M. C. and Duesberg, P. H., (1972) *Nature, 235*: 383.
10. Gillespie, D., Marshall, S., and Gallo, R. C., (1972) *Nature New Biology, 236*: 227.
11. Green, M., and Cartas, M., (1970) *Proc. Nat. Acad. Sci., USA, 69*: 791.
12. Erikson, R. L., (1973) *Nature*, in press.
13. Perry, R. P., (1973) Personal communication.
14. Duesberg, P. H., (1968) *Proc. Nat. Acad. Sci., USA, 60*: 1511.
15. Biswal, N., and Benyesh-Melnick, M., (1969) *Proc. Nat. Acad. Sci., USA, 64*: 1372.
16. Erikson, R. L., (1969) *Virology, 37*: 124.
17. McCain B., Biswal, N., and Benyesh-Melnick, M., (1973) *J. Gen. Virol., 18*: 69.
18. Bader, J. P., and Steck, T. L., (1969) *J. of Virol., 4*: 454.
19. Epstein, M. A., (1958) *Nature, 181*: 1808.
20. Padgett, F., Kearns-Preston, V., Voelz, H., and Levine, A. S., (1960) *J. Natl. Cancer, Inst., 36*: 465.
21. Wu, A. M., Ting, R. C., Yang, S. S., Paran, M., and Gallo, R. C. (1973) *Bibl. Haemat, 39*: 506.

22. Gerwin, B., Todaro, G. J., Zeva, V., Scolnick, E., and Aaronson, S. A., (1970) *Nature, 228*: 435.
23. Coffin, J. M., and Temin, H. M., (1971) *J. Virol., 7*: 625.
24. Bauer, H., and Schaeffer, W., (1966) *Virol., 29*: 494.
25. Duesberg, P. H., Martin, G. S., and Vogt, P. K. (1970) *Virol., 41*: 631.
26. Bader, J. P., Brown, N. R., and Bader, A. V., (1970) *Virol., 41*: 718.
27. Duesberg, P. H., (1970) *Curr. Top. Microbiol. Immunol., 51*: 79.
28. Gallo, R. C. and Ting, R. C. (1972) *CRC Lab. Sci.*, p. 403.
29. Dales, S. and Hanafusa, H., (1973) *Virology, 50*: 440.
30. Hatanaka, M., Kakefuda, T., Gilden, R. V., and Callon, E. A. O., (1971) *Proc. Nat. Acad. Sci., USA, 68*: 1844.
31. Baltimore, D., (1970) *Nature, 226*: 1209.
32. Temin, H. M. and Mazutani, S., (1970) *Nature, 226*: 1211.
33. Gallo, R. C., (1971) *Nature, 234*: 194.
34. Spiegelman, S., Burny, A., Das, M. R., Keydar, J., Schlom, J., Trarnicek, M., and Watson, K., (1970) *Nature, 227*: 1029.
35. Sarin, P. S., and Gallo, R. C., *In*, International Review of Science, Series in Biochemistry, Vol. 6, Nucleic Acids, K. Burton (ed.) Butterworth Medical and Technical Publishing Co., Oxford, Chap. 8.
36. Mölling, K., Bolognesi, D. P., Bauer, H., Busen, W., Plassmann, H. W., and Hausen, P., (1971) *Nature New Biology, 234*: 240.
37. Baltimore, D., and Smoler, D. F., (1972) *J. Biol. Chem. 247*: 7282
38. Keller, W., and Crouch, R., (1972) *Proc. Nat. Acad. Sci., USA, 69*: 3360.
39. Leis, J. P., Berkower, I., and Hurwitz, J., (1973) *Proc. Nat. Acad. Sci., USA, 70*: 466.
40. Grandgenett, D. P., Gerard, G. F., and Green, M., (1972) *J. Virol., 10*: 1136.
41. Mizutani, S., Temin, H. M., Kodama, M., and Wells, R. D., (1971) *Nature New Biology, 230*: 232.
42. Kara, J., Mach, O., and Cerna, H., (1971) *Biochem. Biophy. Res. Commun., 44*: 162.
43. Hanafusa, H., and Hanafusa, T., (1971) *Virology 43*: 313.
44. Hanafusa, H., Baltimore, D., Smoler, D., Watson, K. F., Yaniv, A. and Spiegelman, S., (1972) *Science, 177*: 1188.
45. Ting, R. C., Yang, S. S., and Gallo, R. C., (1972) *Nature New Biol., 236*: 163.
46. Wu, A. M., Ting, R. C., and Gallo, R. C., (1973) *Proc. Nat. Acad. Sci., USA, 70*: 1298.
47. Hurwitz, J., and Leis, J. P., (1971) *J. Virol., 9*: 116.
48. Leis, J. P., and Hurwitz, J., (1971) *J. Virol., 9*: 130.
49. Duesberg, P., and Canaani, E., (1970) *Virology, 42*: 783.
50. Reitz, M., Gillespie, D., Saxinger, W. C., Robert, M., and Gallo, R. C., (1972) *Biochem. Biophys. Res. Commun., 49*: 1216.
51. Markham, P. D., and Baluda, M. A., (1973) in press.
52. Lowy, D. R., Rowe, W. P., Teich, N. and Hartley, J. W. (1971) *Science, 174*: 155.
53. Aaronson, S. A., Todaro, G. J. and Scolnik, E. M. (1971) *Science, 174*: 157.
54. Aaronson, S.A., and Stephenson, J.R. (1973) *Proc. Nat. Acad. Sci., USA, 70:* 2055.
55. Todaro, G. J., and Aaronson, S. A., (1969) *Virology, 33:* 175.

56. Aaronson, S. A., and Rowe, W. P., (1970) *Virology, 42*: 9.
57. Aaronson, S. A., and Weaver, C. (1971) *J. Gen. Virol., 13*: 245.
58. Bassin, R. H., Tuttle, N., and Fischinger, P. J., (1970) *Int. J. Cancer, 6*: 95.
59. Aaronson, S. A., Bassin, R. H., and Weaver, C., (1972) *J. Virol., 9*: 701.
60. Aaronson, S. A. (1971) *Proc. Nat. Acad. Sci., USA, 68*: 3069.
61. Phillips. L. A., Haapala, D. K., Hollis, J., Wu, A., Bassin, R. H., and Fischinger, P. J. *Proc. Nat. Acad. Sci.*, in press.
62. Bassin, R. H. and Gerwin, B., Personal communication.
63. Gerwin, B., and Bassin, R. H., Personal communication.
64. Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles P. T., Moura, S., and Fischinger, P. J. (1971) *Proc. Nat. Acad. Sci., USA, 68*: 1520.
65. Sheldon, R., Kates, J., Kelley, D. E., and Perry, R. P., (1972) *Biochem. 11*: 3829.
66. Darnell, J. E., Philipson, L., Wall, R., and Adesnik, M., (1971) *Science, 174*: 507.
67. Adesnik, M., Salditt, M., Thomas, W., and Darnell, J. E., (1972) *J. Mol. Biol., 71*: 21.
68. Lucas-Lenard, J., and Lipmann, F., (1971) *Annu. Rev. Biochem., 40*: 409.
69. Siegert, W., Kotlings, R. N. H., Bauer, H. and Hofschneider, P. H., (1972) *Proc. Nat. Acad. Sci., USA, 69*: 888.
70. Wu, A. M., Ting, R. C., Paran, M., and Gallo, R. C. (1972) *Proc. Nat. Acad. Sci., USA, 69*: 3820.
71. Penman, S., Roshash, M., and Penman, M., (1970) *Proc. Nat. Acad. Sci., USA, 67*: 1878.
72. Paran, M., Gallo, R. C., Richardson, L. S., and Wu, A. M., (1973) *Proc. Nat. Acad. Sci., USA*, in press.
73. Duesberg, P. H., (1973) Personal communication.
74. Vecchio, G., Tsuchida, N., Shanmugam, G., and Green, M., (1973) *Proc. Nat. Acad. Sci., USA, 70*: 2064.
75. Wu, A. M., Richardson, L. S., Paran, M., and Gallo, R. C., (1973) Submitted for publication.
76. Bernhard, W., (1958) *Cancer Res., 18*: 491.
77. Canaani, E., Helm, K. V. D., and Duesberg, P., (1973) *Proc. Nat. Acad. Sci., USA, 70*: 401.