Proc. Nat. Acad. Sci. USA
Vol. 71, No. 8, pp. 3152–3156, August 1974

Differences Between the Integration of Avian Myeloblastosis Virus DNA in Leukemic Cells and of Endogenous Viral DNA in Normal Chicken Cells

(DNA·DNA reassociation kinetics/RNA·DNA hybridization/RNA tumor viruses/proviral integration site)

R. M. EVANS, M. A. BALUDA, AND M. SHOYAB

The University of California School of Medicine, Department of Microbiology and Immunology, Los Angeles, Calif. 90024

Communicated by Paul D. Boyer, June 10, 1974

ABSTRACT **The nature of integrated viral DNA in normal and leukemic chicken cells has been studied by sequential nucleic acid hybridization procedures that localize the viral specific DNA in cellular DNA regions differing in reiteration frequency. First, DNA·DNA reassociation was employed to fractionate cellular DNA sequences according to their reiteration frequencies. Next, the DNA in each fraction was denatured, immobilized on nitrocellulose filters, and then hybridized with viral [^{3}H]RNA. In normal cells, endogenous viral DNA appears to be associated with cell sequences reiterated 1200 times, and each integration unit appears to have a maximal size approximately equivalent to the 35S RNA subunit of the virion. In infected cells, additional viral sequences are found which reassociate as if they integrated adjacent to unique cellular DNA, or in tandem with endogenous viral DNA.**

The replication of avian myeloblastosis virus (AMV) and other RNA tumor viruses proceeds through a DNA intermediate (i.e., the provirus) (1). This viral specific DNA becomes covalently linked to the cellular genome in transformed chicken cells (2, 3). Normal chicken cells also contain, in lesser amounts, DNA sequences that represent endogenous oncornavirus information partially homologous to AMV RNA (4–6). The observation that some normal chicken embryo cells can be induced to release subgroup E avian leukosis virus suggests that the DNA detected by molecular hybridization in these cells codes for an entire endogenous RNA virus (7). Qualitative and quantitative differences between viral DNA in normal and transformed cells indicate that transformed cells acquire viral specific sequences in addition to those present prior to infection (3, 6, 8, 9, 25).

The integration of viral sequences in bacterial and mammalian DNAs indicates the general nature of this phenomenon (2, 3, 10–18). In *Escherichia coli* both site-specific and random integration have been detected (10, 11). In mammalian cells, the DNA of simian virus 40 appears to be associated with the nonreiterated fraction of cell DNA, while herpes simplex 2 virus DNA seems to be integrated in the reiterated portion of the host DNA (16, 18).

The localization of genes in eukaryotic cells is difficult because of genomic complexity and scarcity of characterized cellular markers. However, potential markers exist in the 20–40% of eukaryotic DNA composed of sequences reiterated 10^1–10^6 times per cell genome (19). The localization of the proviral DNA in relation to these sequences in chicken DNA was carried out in the following manner. First, DNA isolated from normal or leukemic cells was fragmented so that appreciable viral and cellular information would exist on the same fragment. These fragments were denatured and fractionated on hydroxyapatite according to their reassociation rates. Finally, the concentration of viral DNA sequences present in each single-stranded DNA fraction was determined by immobilization of the DNA on nitrocellulose filters and hybridization with an excess of ^{3}H-labeled 70S AMV RNA.

Usually, reassociation experiments are carried out with DNA fragmented to a very small size (e.g., 10^5 daltons) to permit the sequences to renature at their own rate without being influenced by adjacent sequences having a different reiteration frequency. However, in these experiments, large fragments of DNA were employed in order to determine the effect of adjacent cellular sequences on the reassociation kinetics of viral DNA sequences (20).

Results indicate that endogenous viral sequences in normal cells are integrated adjacent to reiterated cell DNA and that each integrated proviral unit is approximately equivalent to the 35S RNA subunit of the virion. Results with DNA from leukemic chickens indicate that additional proviral information, specifically associated with the infecting virus, may integrate as single 35S subunits adjacent to unique cellular DNA sequences or as multiple 35S subunits end-to-end.

METHODS

70S AMV [^{3}H]RNA. Virus released from myeloblasts grown in the presence of [^{3}H]nucleotide precursors was purified, labeled RNA was extracted, and only 70S RNA was finally used, except in one experiment in which purified 35S RNA was used (4).

Preparation of DNA. Normal chick embryo cells and erythrocytes or peripheral blood myeloblasts from leukemic chicks were gently lysed with a Dounce homogenizer in RSB buffer (0.01 M NaCl + 0.01 M Tris·HCl (pH 7.4) + 3 mM $MgCl_2$) containing 0.5% Nonidet. Nuclei were pelleted by centrifugation at 600 × *g* for 3 min at 4°. The nuclei were washed three times in RSB buffer and finally resuspended in 50 ml of SSC (0.15 M NaCl + 0.015 M Na citrate) and 0.2% sodium dodecyl sulfate. RNAse A and RNAse T_1 previously heated at 100° for 5 min was added at 100 μg/ml

Abbreviations: AMV, avian myeloblastosis virus; C_0t, product of DNA nucleotide concentration and time; SSC, standard saline-citrate solution (0.15 M sodium chloride–0.015 M sodium citrate, pH 7); 0.1 × SSC means that the concentration of the solution used is 0.1 times that of the standard saline-citrate solution.

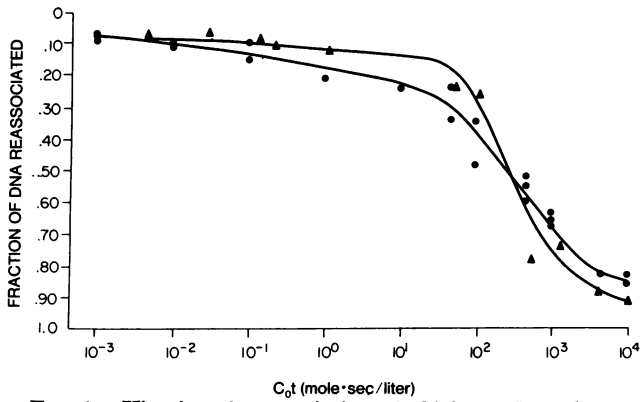

FIG. 1. Kinetics of reassociation of chicken DNA: Chicken DNA sheared (●, 1.3 × 10^6 daltons) or sonicated (▲, 0.25 × 10^6 daltons) was denatured and permitted to renature at 200 μg/ml in 0.4 M sodium phosphate buffer (PB) (pH 6.8) for C_0t values above 10^1. For the lower C_0t values (10^{-3} to 10^1) lower DNA (8–120 μg/ml) and lower salt (0.04–0.16 M) concentrations were used. Samples were withdrawn at different time intervals, diluted to 0.02 M PB with chilled (0°) double-distilled water and kept on ice until passed through a column of packed hydroxyapatite at 60°. Samples were added to the column in small volumes to decrease possible renaturation prior to adsorption to the hydroxyapatite. The column was washed with the same buffer, then single-stranded DNA was eluted with 5 bed volumes of 0.17 M PB and double-stranded DNA with an equal amount of 0.4 M PB. The concentration of DNA in single-stranded and in double-stranded fractions was calculated from the absorbance at 260 nm after making a correction for the hyperchromic shift of denatured DNA.

and 50 units/ml, respectively, and the mixture was incubated for 12 hr at 37°. Predigested Pronase was then added to a concentration of 2 mg/ml and incubated for 12 hr at 37°. This solution was extracted with chloroform–isoamyl alcohol (24:1) and dialyzed through four changes of SSC (21). DNA made in this manner had an average molecular weight greater than 12 × 10^6. This preparation was then mechanically sheared in a Waring blender at 0° for 5 min yielding 15–18S fragments. Size was determined by velocity sedimentation through linear 5–20% alkaline sucrose gradients (0.3 M NaCl, 1 mM ethylenediaminetetraacetate, 0.3 N NaOH) centrifuged at 30,000 rpm at 4° for 16 hr in a SW-40 rotor. DNA from phage φX174 (molecular weight of 1.6 × 10^6) was used as a marker. 6–8S DNA fragments were obtained by ultrasonication in a Branson sonifier for 5 min at room temperature.

DNA Reassociation. Fragmented DNA was boiled for 5 min and immediately added to sodium phosphate buffer (pH 6.8) at 60°. The reaction mixture was then incubated at 60°, and aliquots were removed upon attaining the desired C_0t (where C_0 is the initial concentration of deoxynucleotides in moles/liter and t is the time in seconds) (19). All C_0t values have been standardized to correct for variation in salt concentrations needed to slow or accelerate individual reactions (22, 23). Each point on the C_0t curves represents an unfractionated sample of at least 500 μg of DNA. Reactions were stopped by adding the renatured aliquot to chilled double-distilled sterile water. The sample was then added to a column of packed hydroxyapatite (DNA-grade Bio-Gel HTP, Bio-Rad) at 60°. Single-stranded DNA was eluted by extensive washing with 0.17 M phosphate buffer and double-stranded DNA was eluted with 0.4 M phosphate buffer. The A_{260} was read and each fraction was then dialyzed several times against 0.1 × SSC.

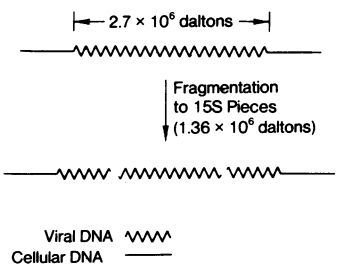

FIG. 2. Schematic representation of the fragmentation of integrated proviral DNA and adjacent cellular DNA. The proviral unit is postulated to be equivalent to the 35S RNA subunit of a C-type RNA tumor virus.

DNA·RNA Hybridization. After dialysis, DNA samples representing the single-stranded components of each C_0t value were denatured and trapped on nitrocellulose Millipore filters as previously described (4). A zero C_0t control is represented by denaturing DNA from normal or leukemic chicks, immediately quenching it in an ice bath and then trapping on filters as above. All filters were hybridized with 70S AMV [^{3}H]RNA as reported (4). DNA isolated from mouse embryos was used as a control to measure nonspecific binding of 70S RNA to the filters.

RESULTS

Reassociation Kinetics of Fragmented Chicken DNA. The reassociation kinetics of denatured chicken DNA, fragmented to average sizes of 7S and 15S (average 0.23 × 10^6 and 1.36 × 10^6 daltons, respectively), were determined by hydroxyapatite column chromatography. The rate of reassociation of a particular sequence depends upon the repetition of that sequence in the genome. As shown in Fig. 1 with fragments of 7 S, approximately 15% of the DNA representing reiterated sequences renatures rapidly, while the remaining 85% renatures as unique sequences. Increased renaturation was observed with the larger fragments due to the inclusion of unique cellular sequences in the fragments that contain rapidly renaturing DNA. Fragmented normal or leukemic cell DNA of similar size reassociates in an identical manner.

Hybridization of Different Reiteration Families of Chicken DNA with AMV RNA. Fig. 2 presents the most simplistic model of integration involving a single 35S viral RNA subunit per integration site. Only the region of cellular DNA adjacent to the proviral DNA is considered in the diagram. For the present purpose, interstitial cellular DNA stretches will be assumed to be much larger than the integrated DNA. DNA fragmented into pieces 1.36 × 10^6 daltons long is approximately equivalent to one-half of the molecular weight of a 35S subunit of viral RNA. The random fragmentation of cellular DNA into such pieces will generate two populations of fragments containing viral DNA. One population is composed of end fragments that contain both viral and adjacent cellular sequences, and the other population is composed of internal fragments that consist entirely of viral sequences. With DNA fragments of this size, end fragments on the average will comprise 50% of a 35S subunit and internal fragments will comprise the remaining 50%. If, instead of

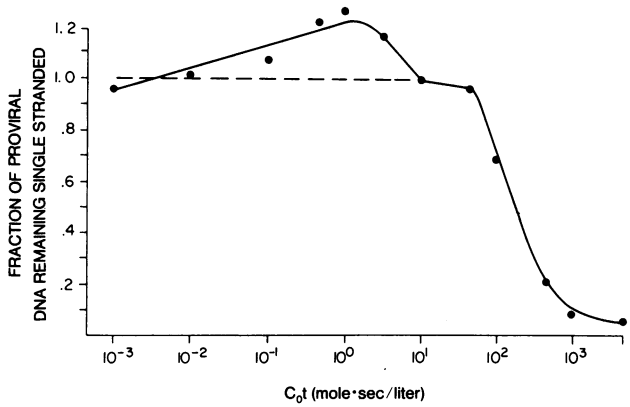

FIG. 3. Kinetics of renaturation of viral sequences from sonicated leukemic myeloblast DNA. Single-stranded DNA was prepared as described in Fig. 1. These samples were dialyzed against 0.1 × SSC (0.015 M NaCl–0.0015 M sodium citrate) to lower their salt concentration, then denatured and trapped on nitrocellulose filters as described (4). Hybridization was performed with purified 35S AMV [^{3}H]RNA. The fraction of proviral DNA remaining single-stranded was calculated from the ratio of viral RNA cpm hybridized per 100 μg of single-stranded DNA to cpm hybridized per 100 μg of a zero C_0t control corrected for total input DNA from Fig. 1. The control hybridized 650 cpm/100 μg.

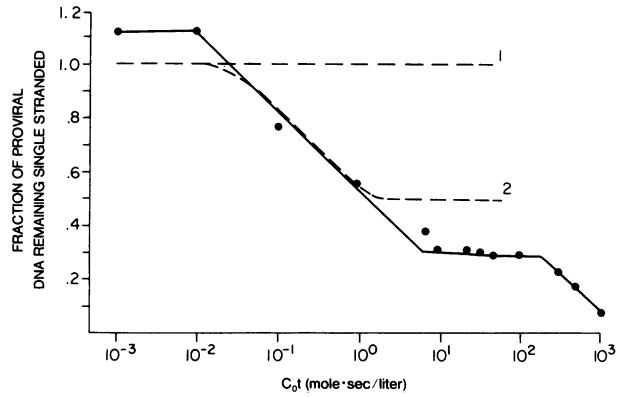

FIG. 4. Kinetics of renaturation of viral sequences from normal cellular DNA. Single-stranded DNA was prepared as described in Fig. 1 and processed as in Fig. 3. The control hybridized 1096 ± 96 cpm/100 μg (mean ± standard deviation). If the precision of the hybridization is 10%, then the range of frequency estimation will be 1000–1900 per cell for the rapidly depleted viral sequences. The theoretical curves represent expected results if proviral DNA was integrated adjacent to unique host sequences (*upper broken line*) or host sequences reiterated 1200 times (*lower broken line*).

2.7×10^6 daltons (35S subunit), the unit of integration were 10.8×10^6 daltons, equivalent to 4 subunits possibly representing 70S species, the end fragments would represent only 12% of the proviral unit, while the internal fragments would compose 88% of the viral information.

The proviral DNA sequences associated with the end fragments will be influenced by the adjacent cell DNA only if the adjacent cellular DNA region is more reiterated in the genome than the proviral DNA sequences. In such a case, the extent to which the viral DNA reassociation is accelerated will then depend upon the reiteration of the adjacent cellular DNA. Finally, the reassociation rate of the internal fragments that consist entirely of viral DNA will be determined by their own reiteration frequency, which may or may not correspond to the number of viral genome equivalents per cell genome.

The reassociation of viral sequences was followed by measuring the amount of viral DNA that remained single-stranded after various time intervals of DNA·DNA renaturation. The viral specific DNA sequences were detected by hybridization in viral RNA excess of those DNA sequences that had not yet reassociated by a given C_0t.

The use of DNA sonicated into fragments of 7S permits the viral sequences present in transformed cells to reanneal at a rate reflecting their concentration in the cell. A difference in rate of reassociation between these fragments and fragments of higher molecular weight (15 S) should reflect the influence of adjacent host cell DNA sequences. Fig. 3 shows the results obtained with leukemic cell DNA fragmented into segments of 2.5×10^5 daltons (7 S). As expected, the bulk of the viral sequences renature with a low reiteration frequency (2–4) roughly proportional to the number of viral genome equivalents per haploid cell genome determined by other hybridization methods (3, 5, 7, 23). An unexplained enrichment, up to 20%, of proviral DNA occurs over the range of C_0t values from 0.1 to 4 but this does not affect the observation that viral sequences do not renature before a C_0t of 1, indicating the absence of highly reiterated viral DNA.

When normal cell DNA fragmented to a size of 1.36×10^6 daltons was examined, a distinct perturbation in the reassociation kinetics of virus-specific DNA sequences was observed (Fig. 4). The larger size of the DNA fragments caused a major portion of the viral sequences to renature rapidly. The observed drop beginning at a C_0t of 0.01 indicates the renaturation of sequences reiterated approximately 1200 times. Since rapid renaturation did not occur with sonicated DNA (Fig. 3), the larger 15S DNA fragments must have contained reiterated cellular sequences as well as viral sequences. The second order kinetics of the accelerated renaturation suggest that the reiterated cellular sequences covalently linked to viral DNA belong to a family of sequences that renature at a $C_0t_{1/2}$ of 0.28 and correspond to a reiteration frequency of 1200. These findings indicate that the proviral sequences are integrated next to reiterated cell DNA and that there is a restriction (i.e., to a given class of reiterated cell sequences) in the site at which viral information may be located. If DNA sequences complementary to the viral RNA had been integrated into the unique regions of the cellular genome, they would have reassociated at a much lower rate and a theoretical curve such as that shown by the *broken line* (*curve 1*) in Fig. 4 would be expected.

Further analysis of Fig. 4 yields information on the size of the integration unit. As seen between C_0ts 10–500, the curve plateaus out at approximately one-third of the initial total radioactivity, corresponding to a loss of two-thirds of the viral information from the single-stranded DNA. The fraction of integrated viral sequences that will remain attached and reassociate with adjacent cellular DNA will depend upon the size of the integration unit, as illustrated in Fig. 2. If the proviral DNA in normal cells exists as integrated segments equivalent to the 35S viral RNA subunit, a theoretical curve can be estimated from the reassociation of 15S DNA fragments (Fig. 4, *curve 2*). The results are in good agreement with such a model.

Kinetics of Reassociation of Proviral DNA Sequences in Fragmented Leukemic DNA. Leukemic cell DNA contains DNA sequences complementary to AMV in addition to the endogenous oncornavirus DNA sequences present in normal cells (6). Fig. 5 shows two theoretical curves based on the possible locations of the new viral DNA sequences. *Curve 1* represents the results expected if all the proviral subunits were integrated as single 35S DNA equivalents next to reiterated cell DNA in a fashion identical to the integration of the endogenous viral sequences in normal DNA (Fig. 4). Theoretical *curve 2* presents two possibilities: (*i*) The endogenous DNA in normal cells is present at the same place in leukemic cell DNA and the additional viral DNA synthesized after infection with AMV is integrated within unique cell DNA, or, (*ii*) The additional viral DNA in leukemic cells is integrated either in tandem or within the preexisting integration site of the endogenous viral DNA. The plateaus are calculated on the basis of 18S DNA fragments corresponding to a molecular weight of 2.1×10^6.

The results (*solid line*) show an approximate fit to *curve 2*. There is an early drop similar to that seen with normal DNA, but the reaction appears to occur faster since it reaches a plateau sooner. The rapidly renaturing DNA has a minimal $C_0t_{1/2}$ of 0.07 and a size-corrected maximal reiteration frequency of 3800 (22). This suggests that some viral DNA sequences are located in similarly reiterated DNA regions in the genomes of leukemic and normal cells. The increased rate might be due to the effect of larger pieces accelerating the renaturation process. However, the much higher fraction of viral sequences that remains in single-stranded DNA at high C_0ts indicates that the additional information present in leukemic cells may be associated with unique cell DNA. Alternatively, if the viral DNA sequences acquired during infection were inserted within the endogenous viral DNA, similar results would be expected.

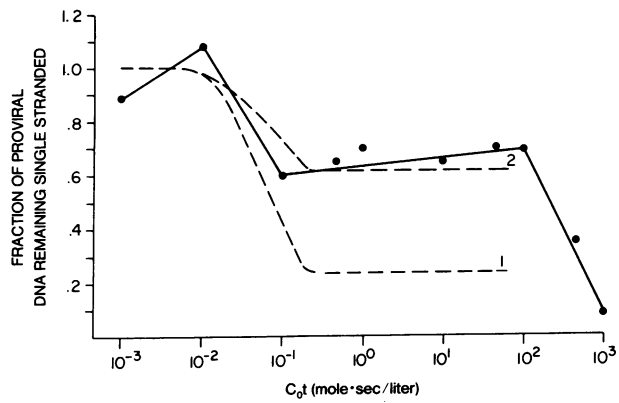

FIG. 5. Kinetics of renaturation of viral DNA from infected erythrocytes obtained from leukemic chickens. The experiment was carried out as in Fig. 3 with DNA fragments of 2.1×10^6 daltons. Zero C_0t controls hybridized 2538 ± 177 cpm/100 μg. According to the assumptions in Fig. 4, the range of frequency estimation will be 2700–6000 for the rapidly depleted sequences. Lower theoretical curve (*1*) (*broken line*) describes expected results if all endogenous and exogenous sequences were integrated as individual 35S subunits adjacent to reiterated cell DNA. Upper theoretical curve (*2*) describes results expected if exogenous sequences integrate adjacent to unique cell sequences or in tandem with endogenous viral sequences.

DISCUSSION

Endogenous avian oncornavirus DNA sequences in normal chicken cell DNA appear to be integrated next to moderately repetitive cellular DNA. The effect of host cell DNA sequences adjacent to integrated proviral DNA is demonstrated by comparing the rate of proviral DNA reassociation at two fragment sizes. The rapid renaturation observed in this experiment with high-molecular-weight fragments indicates viral sequences are associated with cell sequences repeated approximately 1200 times. This dramatic acceleration of renaturation is lost if sonicated, low-molecular-weight, DNA is used. After sonication, most of the viral DNA consists of subgenomic fragments unattached to neighboring chicken DNA.

The amount of proviral DNA whose reassociation kinetics are influenced by adjacent reiterated cellular DNA sequences depends upon the size of the proviral DNA per integration site and upon the size of the DNA fragments. If we do not consider the anomalous effect which large size fragments might have on DNA·DNA renaturation nor the progressive thermal degradation which probably occurs during the renaturation process, the size of the viral DNA per integration site can be calculated from the fragment size and from the fraction of viral DNA that remains single-stranded at the plateau between C_0ts of 5 and 200, i.e., [1/(1 − fraction at plateau)] × (fragment size) = (size of proviral unit). From the data (Fig. 3, *solid line*), the size of an integrated proviral DNA unit in normal cells is calculated to be 2.0×10^6 daltons, which approximates one DNA equivalent of the 35S (2.7×10^6 daltons) subunit of AMV RNA.

The presence of endogenous viral specific DNA in uninfected chicken cells complicates the analysis of the reassociation data obtained with DNA from leukemic cells infected by AMV. The results show a composite effect of both types of proviral DNA and their interpretation must be qualified by the following considerations. Infection with AMV results in a two- to six-fold increase in virus-specific DNA per cell (24). If the endogenous DNA sequences do not shift in position and are not replaced by AMV, the difference in renaturation patterns of normal and leukemic DNAs can be attributed to the new DNA sequences synthesized after infection with AMV.

The fraction of viral DNA remaining single-stranded obtained with leukemic DNA between C_0ts 10^{-1} and 10^2 is much higher than would be expected if the exogenous sequences had become linked to repetitive DNA in a manner similar to their endogenous counterparts. Again, from the fraction of viral DNA remaining denatured at the plateau, the size of the integration unit can be calculated to be 5.3×10^6 daltons. This is equivalent to two 35S RNA subunits, suggesting that the newly synthesized viral DNA sequences may integrate in tandem with preexisting endogenous DNA sequences. An alternative explanation is that the new DNA sequences integrate in the unique region of the host DNA. Both alternatives would yield similar results with the size of DNA fragments used.

These findings also suggest a potential method for localizing genes within the DNA of eukaryotes by the use of sequential molecular hybridization, providing a purified population of complementary RNA or DNA molecules is available.

This investigation was supported by Public Health Research Grant CA-10197 from the National Cancer Institute and by Research Contract N01-CP-33283 from the Virus Cancer Pro-

gram of the National Cancer Institute. R.M.E. is a predoctoral student supported by Public Health Service Training Grant 5T01-A1-00249. We would like to thank Dr. Randolph Wall for critical review of the manuscript.

1. Temin, H. (1972) "RNA tumor viruses—background and foreground," *Proc. Nat. Acad. Sci. USA* **69**, 1016–1020.
2. Markham, P. D. & Baluda, M. A. (1973) "The integrated state of oncornavirus DNA in normal chicken cells and in cells transformed by avian myeloblastosis virus," *J. Virol.* **12**, 721–732.
3. Varmus, H. E., Vogt, P. K. & Bishop, J. M. (1973) "Integration of deoxyribonucleic acid specific for Rous sarcoma virus after infection of permissive and nonpermissive hosts," *Proc. Nat. Acad. Sci. USA* **70**, 3067–3371.
4. Baluda, M. A. (1972) "Widespread presence, in chickens, of DNA complementary to the RNA genome of avian leukosis viruses," *Proc. Nat. Acad. Sci. USA* **69**, 576–580.
5. Neiman, P. E. (1973) "Measurement of endogenous leukosis virus nucleotide sequences in the DNA of normal avian embryos by RNA-DNA hybridization," *Virology* **53**, 196–204.
6. Shoyab, M., Baluda, M. A. & Evans, R. M. (1974) "Acquisition of new DNA sequences after infection of chicken cells with avian myeloblastosis virus," *J. Virol.* **13**, 331–339.
7. Weiss, R. A., Friis, R. R., Katz, E. & Vogt, P. K. (1971) "Induction of avian tumor viruses in normal cells by physical and chemical carcinogens," *Virology* **46**, 920–938.
8. Neiman, P. E. (1972) "Rous sarcoma virus nucleotide sequences in cellular DNA: Measurement by RNA-DNA hybridization," *Science* **178**, 750–753.
9. Baxt, W. G. & Spiegelman, S. (1972) "Nuclear DNA sequences present in human leukemic cells and absent in normal leukocytes," *Proc. Nat. Acad. Sci. USA* **69**, 3737–3741.
10. Signer, E. R. (1968) "Lysogeny: the integration problem," *Annu. Rev. Microbiol.* **22**, 451–498.
11. Ikeda, H. & Tomizawa, J. (1969) "Prophage P: an extrachromosomal replication unit," *Cold Spring Harbor Symp. Quant. Biol.* **33**, 791–798.
12. Sambrook, J., Wesphal, H., Srinivasan, P. R. & Dulbecco, R. (1968) "The integrated state of viral DNA in SV40 transformed cells," *Proc. Nat. Acad. Sci. USA* **60**, 1288–1295.
13. Hirai, K. & Defendi, V. (1972) "Integration of SV-40 DNA into DNA of permissive monkey kidney cells," *J. Virol.* **9**, 705–707.
14. Babink, L. A. & Hudson, J. B. (1972) "Integration of polyoma virus DNA into mammalian genomes," *Biochem. Biophys. Res. Commun.* **47**, 111–118.
15. Burlingham, B. T. & Doerfler, W. (1971) "Three size classes of intracellular adenovirus DNA," *J. Virol.* **7**, 707–719.
16. Frenkel, N., Roizman, B., Cassai, E. & Nahmease, A. (1972) "A DNA fragment of Herpes simplex 2 and its transcription in human cervical cancer tissue," *Proc. Nat. Acad. Sci. USA* **69**, 3784–3789.
17. Gelb, L. D., Milstein, J. B., Martin, M. A. & Aaronson, S. A. (1973) "Characterization of murine leukemic virus specific DNA present in normal mouse cells," *Nature New Biol.* **244**, 76–79.
18. Gelb, L. D. & Martin, M. A. (1973) "Simian virus 40 DNA integration with the genome of virus-transformed mammalian cells," *Virology* **51**, 351–357.
19. Britten, R. J. & Kohne, D. E. (1968) "Repeated sequences in DNA," *Science* **161**, 529–540.
20. Davidson, E. H., Hough, B. R., Amenson, C. S. & Britten, R. J. (1973) "General interspersion of repetitive with nonrepetitive sequence elements in the DNA of *Xenopus*," *J. Mol. Biol.* **77**, 1–23.
21. Weintraub, H., Campbell, C. & Holtzer, H. (1972) "Identification of a developmental program using bromodeoxyuridine," *J. Mol. Biol.* **70**, 337–350.
22. Wetmur, J. G. & Davidson, N. (1968) "Kinetics of renaturation of DNA," *J. Mol. Biol.* **31**, 349–370.
23. Britten, R. J. & Smith, J. (1970) "A bovine genome," *Year B. Carnegie Inst.* **68**, 378–386.
24. Ali, M. & Baluda, M. A. (1974) "Synthesis of avian oncornavirus DNA in infected chicken cells," *J. Virol.* **13**, 1005–1013.
25. Shoyab, M., Evans, R. M. & Baluda, M. A. (1974) "Presence in leukemic cells of avian myeloblastosis virus specific DNA sequences absent in normal chicken cells," *J. Virol.* **14**, 47–49.