THE UNIVERSITY OF WISCONSIN, MADISON

McARDLE LABORATORY FOR CANCER RESEARCH
(Director: Prof. H. C. PITOT)

SPONTANEOUS MUTATION OF RNA TUMOUR VIRUSES *

DAVID A. ZARLING

This editorial reviews the experimental evidence which shows that genes of transforming RNA tumour viruses spontaneously mutate with a very high rate. These genes include those for envelope glycoprotein, DNA polymerase, and transformation.

Genetic studies with RNA tumour viruses have demonstrated that the transforming members of this virus group have a gene that codes for a protein responsible for neoplastic transformation [7, 27, 33, 57]. Although normal avian [26, 50] and mammalian [4, 5, 8, 9] cells have RNA tumour virus-related nucleotide sequences in their DNA, nucleic acid hybridization experiments indicate that the complete RNA tumour virus sequences for transformation are not present in the genome of normal cells [37, 38]. It has not been ruled out that incomplete nucleotide sequences, partially homologous to the viral genes for either sarcomagenesis or leukemogenesis, are present in the DNA of normal cells. Complete genomes of transforming RNA tumour viruses are present in the DNA of virus-transformed sarcoma and leukemia cells and are infectious as viral DNA [23, 24, 25], whereas uninfected cells do not contain infectious viral DNA [10].

Uninfected cells may contain the complete nucleic acid sequences of non-transforming RNA tumour viruses and some normal cells have been found to release non-transforming RNA tumour viruses spontaneously. Most of the RNA tumour viruses observed in nature are non-pathological and can be found in normal tissues as well as tumour tissues, whereas transforming RNA tumour viruses are very rarely found [45].

It is not known how the transforming genes of oncogenic RNA tumour viruses are formed. One possibility is that they arise by recombination between transformation genes in tumour cells and non-pathological RNA tumour viruses, which often infect these cells. To date it has not been possible to demonstrate this type of phenomenon in spite of numerous efforts. Another possibility is that transforming viruses or transformation genes are formed spontaneously in normal cells by mutational processes. In order for transforming genes to be formed in a somatic cell during the life of a bird or mammal a very high rate of spontaneous mutation would be required. It is possible to infer from the knowledge that RNA tumour virus genes can have a

Key-words: Endogenous viral-related genes of normal cells; Leukemia and sarcoma virus mutants; Neoplastic transformation; RNA tumour viruses; Spontaneous mutation.

* *Supported by National Cancer Institute Fellowship CA-04019 and U.S. Public Health Service Program Project Grant CA-07175 from the National Cancer Institute.*

Received, September 22, 1975.

La Ricerca Clin. Lab. *6*, 13, 1976.

very high rate of spontaneous mutation that the endogenous viral-related genes in normal cells have a high rate of spontaneous mutation. Thus, studies on the rate of genetic change in RNA tumour virus genes brought into cells by infection (or in viral-related genes present in normal cells) provide an opportunity to understand spontaneous mutation in avian and mammalian cells and its relationship to developmental, evolutionary, and neoplastic processes.

GENETICS OF RNA TUMOUR VIRUSES

RNA tumour viruses are enveloped animal viruses that contain RNA and a DNA polymerase in their virion and replicate *via* a DNA intermediate. The RNA tumour virus genome (3 million molecular weight RNA) specifies the amino acid sequence of approximately 8 or 9 different proteins. These include 2 envelope glycoproteins, 4 or 5 internal structural proteins, a DNA polymerase, and probably a protein for transformation. Comprehensive reviews of virus replication and of the cellular and molecular biology of RNA tumour viruses have recently been published [18, 19, 45].

There is extensive genetic diversity in the envelope glycoproteins, internal structural proteins, and DNA polymerase in both the avian and mammalian RNA tumour viruses. Genetic diversity has also been observed in the ability of RNA tumour viruses to infect heterologous cells, to replicate in permissive cells, to establish and maintain transformation in fibroblastoid cells or stem cells of the reticuloendothelial system, to form neoplastic tumours, and in the types of tumours formed. Variation in RNA tumour viruses is a result of recombination between different RNA tumour viruses [28, 52] or between RNA tumour viruses and the viral-related genes in uninfected cells [16, 21, 22, 54] and of mutation that occurs during the replication of the viral genome [45].

Two types of spontaneously occurring RNA tumour virus mutants are known: conditional and non-conditional. 1) Conditional viral mutants have gene products that are functional only under certain physiological conditions or in certain species of cells. The only physiological condition studied so far is viral function at high or low temperatures. This type of viral mutant could have a single nucleotide change in a gene such that the protein produced would have a functional configuration only at certain temperatures. 2) Non-conditional viral mutants have a mutation in a gene that renders it inactive under all conditions. This type of viral mutant could have a deletion in a gene so that the messenger RNA or protein product would not be synthesized.

SPONTANEOUS RATE OF OCCURRENCE OF CONDITIONAL MUTATIONS

Spontaneous temperature-sensitive (*ts*) mutants were isolated from 10 subclones out of 300 examined of the Schmidt-Ruppin strain of Rous sarcoma virus (RSV) [44]. These *ts* mutants fell into 3 different categories. 1) The mutants were unable to transform cells; 2) the mutants were unable to produce progeny virus at high temperature; or 3) the progeny virus was not infectious at high temperature. The spontaneous rate of reversion for these *ts* SR-RSV mutants also appeared to be high.

Spontaneous temperature-sensitive mutants have also been isolated from the Moloney strain of murine sarcoma virus (MSV). WONG et al. [56] found many clones of Moloney MSV that contained heat-sensitive mutant virus. These mutants were found to have lesions in at least 2 different functions [55]. One of the mutants was defective in virus maturation and probably synthesized a protein which was conformationally non-functional at high temperature but was functional at low temperature. The other mutant had a temperature-sensitive defect in an early unidentified function.

A spontaneous cold-sensitive mutant of Moloney MSV was found among several clones examined from a non-mutagenized MSV stock [41]. The mutant was defective in

its ability to transform cells only at low temperatures. Cells transformed with this *ts* MSV rapidly lost their transformed phenotype when shifted to low temperature, but transformation was restored at high temperature; this indicates the existence of a temperature-sensitive protein required to maintain the transformed cell state. Thus, there is spontaneous mutation in the viral gene for transformation of fibroblastoid cells.

Other conditional mutants of RNA tumour viruses differ with respect to the type of morphological transformation they produce in fibroblastoid cells. RSV that produces round transformation in non-round cells (*morph*^{r} RSV) spontaneously mutates at a high rate to RSV that produces fusiform transformed cells (*morph*^{f} RSV). *Morph*^{f} RSV can also mutate to *morph*^{r} RSV [43]. In 15 subclones of *morph*^{r} RSV, there were 9 clones with various amounts of *morph*^{f} RSV mutants and 6 clones that contained no mutants [43]. In those clones that contained some mutants, up to 15 % of the virus in the clone was mutant. Thus, there is a high rate of spontaneous mutation in the gene for the transformed morphology.

Other RNA tumour virus mutants that have an altered host range occur spontaneously at a high rate. After passage of avian or mammalian RNA tumour viruses through heterologous cells, variant viruses can be selected from the viral population. The variant viruses often possess an altered host range with different efficiencies of plating (efficiencies of transformation) for the cells of the new host. For example, after injection of RSV into ducks, DURAN-REYNALS [13] obtained early and late malignant tumours. The early developing duck tumours produced an RSV that was not like the parental virus in that it was non-infectious for ducks but was infectious for chickens. The RSV recovered from the late developing duck tumours was highly infectious for ducks and chickens but also contained virus which was unlike the parental virus and produced different tumour cell morphologies, had new tissue affinities, and had altered viral antigens. Another variant of the Bryan strain of RSV, recovered from an RSV-induced duck tumour, was also found to have an expanded host range and was highly oncogenic in ducks, mice, and hamsters [30]. Clonal lines of avian sarcoma virus strain *Bratislava* 77 (B77) which had been recovered from B77 virus-transformed rat cells were found to have a higher efficiency of transformation in rat cells than the parental virus [1].

The results of studies in our laboratory [58] indicate that conditional host range mutants of B77 virus can originate by spontaneous mutation during viral replication in the cells of its natural host, the chicken. B77 virus mutants with high efficiencies of transformation in duck cells (relative to their ability to cause transformation in cultures of chicken cells) were selected from the wild-type viral stock (which has a low efficiency of transformation in duck cells) after infection of duck cells. In addition, B77 virus mutants were spontaneously formed that were unable to infect duck cells but were fully infectious in chicken cells.

The spontaneous rate of mutation of wild-type B77 virus to B77 virus with high efficiencies of transformation in duck cells was estimated from LURIA-DELBRÜCK-type fluctuation experiments [32]. In this experiment many individual clones of the wild-type B77 virus were assayed for the number of high efficiency duck transforming mutants in the clone. Some clones were observed to contain many mutants (up to 40 % high efficiency duck transforming mutants), whereas others contained only a few or no mutants (wild-type virus present only). Thus, the number of high efficiency duck transforming mutants exhibited significantly large fluctuations [32]. These large fluctuations in the distribution of mutants reflect the random occurrence of a mutation during the growth of the wild-type virus. A mutational event occurring early during the growth of a clone results in a clone that contains many mutants, whereas a mutational event occurring late during the growth of a clone contains only a few mutants. Clones without mutants consist of wild-type viruses in which a mutation

has not occurred. The number of clones without mutants compared to the total number of clones in a fluctuation experiment can be used to estimate the probability of a mutation occurring during the growth of a clone.

For the mutation of wild-type B77 virus to B77 virus with high efficiencies of transformation in duck cells, there was a probability of approximately 1 that a mutation would occur during 50 infected cell generations. From the fluctuation data it was also possible to estimate the rate of mutation of wild-type B77 virus to B77 virus which was unable to form foci in duck cells. In the fluctuation tests none of the parental virus was B77 virus with high efficiencies of transformation in duck cells. However, a few of the clones without high efficiency duck transforming mutants were shown to be B77 virus mutants which were unable to form foci in duck cells. Therefore the spontaneous mutation rate of wild-type B77 virus to B77 virus that was unable to form foci in duck cells was higher than the mutation rate of wild-type B77 virus to B77 virus with high efficiencies of transformation in duck cells.

We have examined the phase of the replicative cycle of B77 virus in which the host range mutants with high efficiencies of transformation in duck cells arose. Mutation could occur in an RNA tumour virus when the parental viral RNA was copied into DNA (RNA-to-DNA information transfer), prior to or during the process when it became integrated into the host cell DNA or when the integrated provirus was replicated with the host genome (DNA-to-DNA information transfer). Mutation could also occur during transcription of progeny viral RNA (DNA-to-RNA information transfer) with a mutant DNA made in the next round of infection by the mutant viral DNA [45].

If a mutant viral DNA intermediate were formed soon after infection with wild-type B77 virus, then B77 virus mutants would appear in the first progeny. However, our experiments showed that there was a significant lag in the time of appearance of B77 virus mutants compared with the initial appearance of wild-type progeny [58]. Therefore, a mutant provirus does not appear to be formed soon after infection.

Mutations that could have occurred during DNA-to-RNA information transfer can be blocked from forming mutant viruses by preventing any secondary infections during the growth of a clone. Blocking secondary infections was accomplished by performing fluctuation tests in which all mutants came from viral clones which originated from a single infected cell (thus, were cell clones as well as viral clones). Therefore, during the growth of these clones there were no uninfected cells for any mutant virus which arose during DNA-to-RNA information transfer to infect. When secondary RNA-to-DNA information transfers were blocked by blocking secondary infections, neither the rate of appearance of B77 virus mutants, nor the large fluctuations in their distribution were affected. These results indicate that spontaneous mutation in the B77 DNA did not occur primarily during the synthesis of viral DNA on an RNA template (RNA-to-DNA information transfer) nor during the transcription of progeny viral RNA from the DNA (DNA-to-RNA information transfer).

B77 virus mutation also required cell replication. Infected stationary cells did not produce mutants until several days after they were allowed to divide, whereas wild-type B77 virus was produced very rapidly after the cells were allowed to divide [58]. Thus, B77 virus mutation occurs during DNA-to-DNA information transfer. The molecular mechanism of these mutations is not known but may involve the state of the viral DNA intermediates, proviral DNA replication directly, and/or provirus repair or replacement. From the fluctuation experiments the rate of virus host range mutation has been estimated [58]. For example, in a typical fluctuation test 11 out of 13 wild-type B77 virus clones contained some mutants. Since these clones contained an average of about 50 transformed cells per clone, there was a probability of approximately 1 of a mutational event occurring in 50 cumulative cell replications. This spontaneous mutation rate for the B77 virus host range gene(s) is the highest known

for any animal virus character [14]. The mutation affecting the viral host range occurs in the B77 viral envelope gene (unpublished observations).

SPONTANEOUS RATE OF OCCURRENCE OF NON-CONDITIONAL MUTATIONS

In comparison with conditional-lethal RNA tumour virus mutants, non-conditional virus mutants are defective under all conditions. A point mutation in the viral genome would permit the synthesis of a mutant protein that could be functional only at certain temperatures or in certain species of cells. However, deletions in a portion of the viral genome could result in an absolute defect in the production or in the function of this protein.

Non-conditional mutants of RNA tumour viruses are spontaneously formed in the envelope glycoprotein, DNA polymerase, and transformation genes. The rate of this type of spontaneous mutation, which results in non-conditional defects, appears to be as high as for mutations producing conditional defects.

KAWAI and HANAFUSA [29] observed in cloned stocks of Schmidt-Ruppin RSV that mutant viruses lacking the envelope glycoprotein appear spontaneously. When these Schmidt-Ruppin RSV envelope mutants were recloned, 2 of the 17 viral clones examined were found to be lacking DNA polymerase as well as envelope glycoprotein. The properties of these double mutants in DNA polymerase and envelope glycoprotein are very similar to the Bryan strain of RSV and the Moloney strain of MSV.

A small proportion of the virus population in stocks of the envelope defective Bryan strain of RSV also has a mutation in the DNA polymerase gene [20]. Clones of viruses lacking DNA polymerase and envelope glycoprotein have also been isolated without the use of mutagens from the envelope defective Moloney strain of MSV [3, 17, 35, 40]. FRIIS et al. [15] have shown that the avian sarcoma virus envelope glycoprotein and DNA polymerase genes are closely linked. Since the non-conditional DNA polymerase mutations occurred in viruses that already lacked envelope glycoprotein, it is possible that deletions in the envelope gene might extend into the adjacent DNA polymerase gene. Double mutants in DNA polymerase and envelope glycoprotein can recombine with an avian leukosis virus containing a DNA polymerase gene to produce a virus defective in envelope glycoprotein only [29]. However, envelope glycoprotein mutants have never been observed to recombine with an avian leukosis virus containing an envelope glycoprotein gene [29]. It has been suggested that an envelope glycoprotein mutant cannot acquire the gene for envelope glycoprotein by recombination without losing the viral gene for fibroblastoid cell transformation because of a limitation in the total size of the genome necessary for virion formation [45].

Transformation-defective (*td*) RNA tumour viruses are spontaneously produced at a high rate. VOGT et al. [51, 53] observed that 6 out of 7 clones of RSV spontaneously gave rise to *td* RSV with frequencies ranging from 4 to 17 % of the virus in a clone. In other studies, 2 out of 6 subclones of Schmidt-Ruppin RSV contained variable amounts of *td* mutants [28]. Therefore, these mutants must have arisen during the growth of some of the clones. Clonal isolates of competent MSV are reported to form *td* MSV at a high frequency [2]. Some of these avian and murine *td* RNA tumour viruses have been shown to be deletion mutants and have an RNA genome 10 to 20 % smaller than the parental transforming sarcoma virus RNA [11, 12, 31, 34]. It is not known at which step in the life cycle of the virus that deletions are so frequently produced.

The mutation rate of a sarcoma virus to a leukemia virus or to a non-pathological virus has not been studied quantitatively. Some *td* viruses which are defective in their ability to transform fibroblastoid cells are leukemogenic [6]. Also, leukemia viruses contain viral nucleotide sequences that are not present in either sarcoma viruses or non-pathological RNA tumour viruses [37, 38]. Therefore the viral gene for transformation

of fibroblastoid cells is different from that for transformation of stem cells of the reticuloendothelial system, and these may mutate independently.

As discussed above, there is a high rate of spontaneously occurring conditional and non-conditional mutations in several RNA tumour virus genes. It is possible that a large portion of the genome of RNA tumour viruses is hypermutable, while other portions may have a lower rate of mutation.

TUMOUR VIRUS GENES PRESENT IN NORMAL CELLS — SIGNIFICANCE FOR NEOPLASIA

Genes and nucleotide sequences related to RNA tumour virus genes are present in the DNA of normal birds and mammals that are not spontaneously releasing virus [4, 5, 8, 9, 26, 36, 50]. It is not known whether the high spontaneous mutation rate observed in the genes of exogenously infecting RNA tumour viruses also occurs in the endogenous RNA tumour virus-related genes present in normal cells.

TEMIN [46, 47, 48] has hypothesized that RNA tumour viruses have evolved from cell genes on the basis of the relationships observed between RNA tumour virus genes or proteins and normal cellular genes or proteins. If this hypothesis is correct, then nucleic acid hybridization experiments indicate that the rate of mutation in the endogenous RNA tumour virus-related genes present in normal cells appears to have been much more rapid than the spontaneous rate of mutation in the unique host cell genes in fowl and mammals [45-49].

Normal uninfected cells may possess mutagenic mechanisms which could be responsible for producing a high rate of mutation in portions of the genome. It is now possible to test experimentally whether the endogenous RNA tumour virus-related genes present in normal cells are as hypermutable as the viral DNA intermediates of exogenously infecting RNA tumour viruses. A high rate of mutation has been observed in genes controlling the expression of or coding for the envelope glycoprotein of MLV in mice [39, 42]. The spontaneous mutation rate of the endogenous viral envelope glycoprotein gene present in normal chicken or mouse cells will be interesting to measure quantitatively.

Hypermutable genetic processes in normal avian and mammalian cells could lead to the modification of host genes or the formation of new genes. Nucleic acid hybridization experiments indicate that normal cells, in contrast to virus-transformed cells, do not contain complete RNA tumour virus genes for either sarcomagenesis or leukemogenesis [37, 38]. The mutagenic mechanism(s) responsible for a high rate of spontaneous mutation in viral genes could also be responsible for the formation of transforming genes in normal cells.

CONCLUSIONS

There is a very high spontaneous mutation rate for genes of RNA tumour viruses and perhaps for the endogenous RNA tumour virus-related genes present in normal cells.

SUMMARY

There are 2 categories of spontaneously occurring avian and mammalian RNA tumour virus mutants: conditional and non-conditional. 1) Conditional mutants are able to replicate in or transform cells only under certain physiological conditions or in certain cells. RNA tumour virus temperature-sensitive mutants, focus-morphology mutants, and host range mutants are spontaneously formed. Some of these conditional mutants probably arise by point mutations in the viral genome. 2) Non-conditional mutants have genetic lesions that render them inactive under all conditions. There are non-conditional spontaneous RNA tumour virus mutants that are missing either the

virion envelope glycoprotein or both the envelope glycoprotein and the virion DNA polymerase. These mutants cannot replicate or transform cells. Other spontaneous non-conditional mutants can replicate but are defective in their ability to transform fibroblastoid cells. These spontaneous transformation-defective mutants can have deletions in 10-20 % of the genomic RNA. Conditional mutants with an altered host range occur at a high rate of approximately 1 mutation/50 infected cell generations during DNA-to-DNA information transfer. This type of conditional mutation requires cell replication but does not occur frequently either during the original synthesis of viral DNA (RNA-to-DNA information transfer) or during the transcription of progeny viral RNA from the DNA (DNA-to-RNA information transfer). Temperature-sensitive and focus-morphology mutants also have a high rate of spontaneous formation. Non-conditional mutants missing the viral envelope glycoprotein, DNA polymerase, or transformation gene also appear to be spontaneously formed at a high rate. Normal avian and mammalian cells contain RNA tumour virus-related genes in their DNA. It is hypothesized that these endogenous RNA tumour virus-related genes in normal cells also have a high rate of spontaneous mutation and are involved in neoplastic processes.

REFERENCES

1) ALTANER C., TEMIN H. M.: Carcinogenesis by RNA Sarcoma Viruses. XII. A Quantitative Study of Infection of Rat Cells *in Vitro* by Avian Sarcoma Viruses - Virology *40*, 118, 1970.
2) BALL J. K., MCCARTER J. A., SUNDERLAND S. M.: Evidence for Helper Independent Murine Sarcoma Virus. I. Segregation of Replication-Defective and Transformation-Defective Viruses - Virology *56*, 268, 1973.
3) BASSIN R. H., PHILLIPS L. A., KRAMER M. J., HAAPALA D. K., PEEBLES P. T., NOMURA S., FISCHINGER P. J.: Transformation of Mouse 3T3 Cells by Murine Sarcoma Virus: Release of Virus-Like Particles in the Absence of Replicating Murine Leukemia Helper Virus - Proc. nat. Acad. Sci. (Wash.) *68*, 1520, 1971.
4) BENVENISTE R. E., TODARO G. J.: Evolution of Type C Viral Genes. I. Nucleic Acid from Baboon Type C Virus as a Measure of Divergence among Primate Species - Proc. nat. Acad. Sci. (Wash.) *71*, 4513, 1974.
5) BENVENISTE R. E., TODARO G. J.: Evolution of Type C Viral Genes: Inheritance of Exogenously Acquired Viral Genes - Nature (Lond.) *252*, 456, 1974.
6) BIGGS P. M., MILNE B. S., GRAF T., BAUER H.: Oncogenicity of Non-Transforming Mutants of Avian Sarcoma Viruses - J. gen. Virol. *18*, 399, 1973.
7) BIQUARD J., VIGIER P.: Isolément et étude d'un mutant conditionnel du virus de Rous a capacité transformante thermosensible - C.R. Acad. Sci. (Paris) *271*, 2430, 1970.
8) CALLAHAN R., BENVENISTE R. E., LIEBER M. M., TODARO G. J.: Nucleic Acid Homology of Murine Type-C Viral Genes - J. Virol. *14*, 1394, 1974.
9) CHATTOPADHYAY S. K., LOWY D. R., TEICH N. M., LEVINE A. S., ROWE W. P.: Qualitative and Quantitative Studies of AKR-Type Murine Leukemia Virus Sequences in Mouse DNA - Cold Spr. Harb. Symp. quant. Biol. *39*, 1085, 1974.
10) COOPER G. M., TEMIN H. M.: Infectious Rous Sarcoma Virus and Reticuloendotheliosis Virus DNAs - J. Virol. *14*, 1132, 1974.
11) DUESBERG P. H., VOGT P. K.: Differences between the Ribonucleic Acids of Transforming and Non-Transforming Avian Tumor Viruses - Proc. nat. Acad. Sci. (Wash.) *67*, 1673, 1970.
12) DUESBERG P. H., VOGT P. K.: RNA Species Obtained from Clonal Lines of Avian Sarcoma and from Avian Leukosis Virus - Virology *54*, 207, 1973.
13) DURAN-REYNALS F.: The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses - Cancer Res. *2*, 343, 1942.
14) FENNER F., MCAUSLAN B. R., MIMS C. A., SAMBROOK J., WHITE D. O.: In: The Biology of Animal Viruses. Academic Press, New York, 1974; 2nd Ed., p. 279.
15) FRIIS R. R., MASON W. S., CHEN Y. C., HALPERIN M. S.: A Replication Defective Mutant of Rous Sarcoma Virus which Fails to Make a Functional Reverse Transcriptase - Virology *64*, 49, 1975.
16) FUJITA D. J., CHEN Y. C., FRIIS R. R., VOGT P. K.: RNA Tumor Viruses of Pheasants: Characterization of Avian Leukosis Subgroups F and G - Virology *60*, 558, 1974.
17) GAZDAR A. F., PHILLIPS L. A., SARMA P. S., PEEBLES P. T., CHOPRA H. C.: Presence of Sarcoma Genome in a 'Non-Infectious' Mammalian Virus - Nature (New Biology) *234*, 69, 1971.
18) GILLESPIE D., SAXINGER W. C., GALLO R. C.: Information Transfer in Cells Infected by RNA Tumor Viruses and Extension to Human Neoplasia - Progr. nucl. Acid Res. mol. Biology *15*, 1, 1975.
19) GREEN M., GERARD G. F.: RNA-Directed DNA Polymerase Properties and Functions in Oncogenic RNA Viruses and Cells - Progr. nucl. Acid Res. mol. Biology *14*, 187, 1974.

20) HANAFUSA H., HANAFUSA T.: Noninfectious RSV Deficient in DNA Polymerase - Virology *43*, 313, 1971.
21) HANAFUSA T., HANAFUSA H.: Isolation of Leukosis-Type Virus from Pheasant Embryo Cells: Possible Presence of Viral Genes in Cells - Virology *51*, 247, 1973.
22) HAYWARD W. S., HANAFUSA H.: Recombination between Endogenous and Exogenous RNA Tumor Virus Genes as Analyzed by Nucleic Acid Hybridization - J. Virol. *15*, 1367, 1975.
23) HILL M., HILLOVA J.: Production virale dans les fibroblasts de poule traités par l'acide désoxyribonucléique de cellules XC de rat transformées par le virus de Rous - C.R. Acad. Sci. (Paris) *272*, 3094, 1971.
24) HILL M., HILLOVA J.: Virus Recovery in Chicken Cells Treated with Rous Sarcoma Cell DNA - Nature (New Biology) *237*, 35, 1972.
25) HILLOVA J., DONTCHEV D., MARIAGE R., PLICHON M.-P., HILL M.: Sarcoma and Transformation-Defective Viruses Produced with Infectious DNA(s) from Rous Sarcoma Virus (RSV)-Transformed Chicken Cells - Virology *62*, 197, 1974.
26) KANG C.-Y., TEMIN H. M.: Reticuloendotheliosis Virus Nucleic Acid Sequences in Cellular DNA - J. Virol. *14*, 1179, 1974.
27) KAWAI S., HANAFUSA H.: The Effects of Reciprocal Changes in Temperature on the Transformed State of Cells Infected with a Rous Sarcoma Virus Mutant - Virology *46*, 470, 1971.
28) KAWAI S., HANAFUSA H.: Genetic Recombination with Avian Tumor Virus - Virology *49*, 37, 1972.
29) KAWAI S., HANAFUSA H.: Isolation of Defective Mutant of Avian Sarcoma Virus - Proc. nat. Acad. Sci. (Wash.) *70*, 3493, 1973.
30) KUWATA T., KAWAKAMI H.: Isolation of a Variant Strain of Rous Sarcoma Virus Oncogenic for Ducks and Mammals - Arch. ges. Virusforsch. *32*, 1, 1970.
31) Lo A. C. H., BALL J. K.: Evidence for Helper-Independent Murine Sarcoma Virus. II. Differences between the Ribonucleic Acids of Clone-Purified Leukemia Virus, Helper-Independent and Helper-Dependent Sarcoma Viruses - Virology *59*, 545, 1974.
32) LURIA S. E., DELBRÜCK M.: Mutations of Bacteria from Virus Sensitivity to Virus Resistance - Genetics *28*, 491, 1943.
33) MARTIN G. S.: Rous Sarcoma Virus: A Function Required for Maintenance of the Transformed State - Nature (Lond.) *227*, 1021, 1970.
34) MARTIN G. S., DUESBERG P. H.: The Subunit in the RNA of Transforming Avian Tumor Viruses. I. Occurrence in Different Virus Strains. II. Spontaneous Loss Resulting in Non-Transforming Variants - Virology *47*, 494, 1972.
35) MAY J. T., SOMERS K. D., KIT S.: Defective Mouse Sarcoma Virus Deficient in DNA Polymerase Activity - J. gen. Virol. *16*, 223, 1972.
36) NEIMAN P. E.: Rous Sarcoma Virus Nucleotide Sequences in Cellular DNA: Measurement by RNA-DNA Hybridization - Science *178*, 750, 1973.
37) NEIMAN P. E., PURCHASE H. G., OKAZAKI W.: Chicken Leukosis Virus Genome Sequences in DNA from Normal Chick Cells and Virus-Induced Bursal Lymphomas - Cell *4*, 311, 1975.
38) NEIMAN P. E., WRIGHT S. E., MCMILLIN C., MACDONNELL D.: Nucleotide Sequence Relationships of Avian RNA Tumor Viruses: Measurement of the Deletion in a Transformation-Defective Mutant of Rous Sarcoma Virus - J. Virol. *13*, 837, 1974.
39) OBATA Y., IKEDA H., STOCKERT E., BOYSE E. A.: Relation of G_{IX} Antigen of Thymocytes to Envelope Glycoprotein of Murine Leukemia Virus - J. exp. Med. *141*, 188, 1975.
40) PEEBLES P. T., HAAPALA D. K., GADZAR A. F.: Deficiency of Viral RNA-Dependent DNA Polymerase in Noninfectious Virus-Like Particles Released from Murine Sarcoma Virus-Transformed Hamster Cells - J. Virol. *9*, 488, 1972.
41) SOMERS K. D., KIT S.: Temperature-Dependent Expression of Transformation by a Cold-Sensitive Mutant of Murine Sarcoma Virus - Proc. nat. Acad. Sci. (Wash.) *70*, 2206, 1973.
42) STOCKERT E., BOYSE E. A., OBATA Y., IKEDA H., SARKAR N. H., HORRMAN H. A.: New Mutant and Congenic Mouse Stocks Expressing the Murine Leukemia Virus-Associated Thymocyte Surface Antigen G_{IX} - J. exp. Med. *142*, 512, 1975.
43) TEMIN H. M.: The Control of Cellular Morphology in Embryonic Cells Infected with Rous Sarcoma Virus *in Vitro* - Virology *10*, 182, 1960.
44) TEMIN H. M.: The Role of the DNA Provirus in Carcinogenesis by RNA Tumor Viruses - In: SILVESTRI L. G. (Ed.): The Biology of Oncogenic Viruses. North-Holland Publ. Co., Amsterdam, 1971; p. 176.
45) TEMIN H. M.: The Cellular and Molecular Biology of RNA Tumor Viruses, Especially Avian Leukosis-Sarcoma Viruses, and Their Relatives - Advanc. Cancer Res. *19*, 47, 1974.
46) TEMIN H. M.: On the Origin of RNA Tumor Viruses - Ann. Rev. Genet. *8*, 155, 1974.
47) TEMIN H. M.: On the Origin of RNA Tumor Viruses - Harvey Lect. *69*, 173, 1974.

48) TEMIN H. M.: On the Origin of the Genes for Neoplasia. G.H.A. Clowes Memorial Lecture - Cancer Res. *34*, 2835, 1974.
49) TEMIN H. M.: Introduction to Virus-Caused Cancers - Cancer *34*, 1347, 1974.
50) TEREBA A., SKOOG L., VOGT P. K.: RNA Tumor Virus Specific Sequences in Nuclear DNA of Several Avian Species - Virology *65*, 524, 1975.
51) VOGT P. K.: Spontaneous Segregation of Nontransforming Viruses from Cloned Sarcoma Viruses - Virology *46*, 939, 1971.
52) VOGT P. K.: Genetically Stable Reassortment of Markers during Mixed Infection with Avian Tumor Viruses - Virology *46*, 947, 1971.
53) VOGT P. K., WYKE J. A., WEISS R. A., FRIIS R. R., KATZ E., LINIAL M.: Avian RNA Tumor Viruses: Mutants, Markers, and Genotypic Mixing - In: Molecular Studies in Viral Neoplasia. The Williams and Wilkins Co., Baltimore, 1974; p. 190.
54) WEISS R. A., MASON W. S., VOGT P. K.: Genetic Recombinants and Heterozygotes Derived from Endogenous and Exogenous Avian RNA Tumor Viruses - Virology *52*, 535, 1973.
55) WONG P. K. Y., MCCARTER J. A.: Studies of Two Temperature-Sensitive Mutants of Moloney Murine Leukemia Virus - Virology *58*, 396, 1974.
56) WONG P. K. Y., RUSS L. J., MCCARTER J. A.: Rapid, Selective Procedure for Isolation of Spontaneous Temperature-Sensitive Mutants of Moloney Leukemia Virus - Virology *51*, 424, 1973.
57) WYKE J. A., BELL J. G., BEAMAND J. A.: Genetic Recombination among Temperature-Sensitive Mutants of Rous Sarcoma Virus - Cold Spr. Harb. Symp. quant. Biol. *39*, 897, 1974.
58) ZARLING D. A., TEMIN H. M.: A High Spontaneous Mutation Rate of an Avian Sarcoma Virus - J. Virol. *17*, 74, 1976.

Requests for reprints should be addressed to:

DAVID A. ZARLING
McArdle Laboratory for Cancer Research,
The University of Wisconsin
450 North Randall Ave., Madison, Wis. 53706 - USA