COMPARISON OF THE RNAs OF MURINE AND HUMAN CORONAVIRUSES

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INTRODUCTION

Coronaviruses cause acute and/or persistent disease in many species of animals. One factor that determines target organ and lethality of coronavirus infection is the strain of virus (McIntosh, 1972; Robb et al., 1980). For example most strains of mouse hepatitis virus (MHV), such as A59 and MHV3, were isolated from the livers of infected mice and are primarily hepatotropic (McIntosh, 1974). In contrast the JHM strain of MHV is primarily neurotropic (McIntosh 1974; Bailey et al., 1949). Most MHV strains are closely related serologically (McIntosh, 1974).

Human coronaviruses (HCV), such as the 229E strain, are primarily respiratory viruses (Hamre and Procknow, 1966; McIntosh, 1974). Recently, however, there has been a report of the recovery of putative human coronaviruses from the brain tissue of two multiple sclerosis patients (Burks et al., 1980). At least some human coronavirus strains cross react immunologically with neurotropic murine and porcine coronaviruses (McIntosh, 1974).

Biochemical studies to date have shown that various murine and human coronavirus strains, although biologically distinct, have similar size genome RNAs and structural proteins (Bond et al, 1979;

Robb and Bond, 1979, Hierholzer, 1976, McNaughton and Madge, (1978). We have used molecular hybridization, oligonucleotide fingerprinting, and agarose gel electrophoresis to further compare the genome and intracellular RNAs of MHV strains A59, MHV3 and JHM and HCV strain 229E.

MATERIALS AND METHODS

Cells and Viruses

MHV strains A59 (Sturman and Takemoto, 1972), MHV3 (Dick et al 1956) and JHM (Bailey et al., 1949) were grown in DBT or 17CL-1 mouse cell lines (Sturman and Takemoto, 1972). Human coronavirus strain 229E (Hamre and Procknow, 1966) was grown in human embryonic lung cell line, L-132 (Davis and Bolin, 1960). All viruses were plaque purified twice and stocks grown at a multiplicity of infection (m.o.i.) of approximately 10^{-4} plaque units (pfu) per cell.

RNA Preparation

Preparation of genome RNA. Cells were infected at m.o.i. of 0.1 to 1 to pfu/cell and in some experiments were labeled with ^{32}P -inorganic phosphate or ^3H -uridine. MHV was grown at 37°C and harvested at 18-20 hours post infection. 229E was grown at 32°C and harvested at 40 hours post infection. In most experiments, virus was purified from the medium as previously described (Kennedy and Johnson-Lussenberg, 1975-76; Robb et al 1979). RNA was extracted from virions by proteinase K treatment in the presence of 1%SDS, followed by phenol extraction (Weiss et al., 1977) and further purified by sedimentation on a sucrose gradient. Genome RNA sedimented as a uniform peak at about 57S. In some experiments RNA for hybridization was extracted from virus pelleted from the medium, and used without further purification.

Preparation of intracellular RNA. Infected cells were labeled with ^3H -uridine in the presence of Actinomycin D (Act D) from 4-9 hours post infection for MHV or 16-24 hours post infection for 229E. Cells were lysed by pipeting in the presence of 1% NP40 in RSB (0.01MTris, pH7.4; 0.01M NaCl; 0.005M MgCl_2), the nuclei pelleted and RNA extracted from the cytoplasm as described above for virion RNA. In most experiments poly(A)-containing RNA was selected by chromatography on oligo (dT)-cellulose columns (Aviv and Leder, 1972).

RNA Analysis

Oligonucleotide fingerprinting. High specific activity ^{32}P -labeled purified genome RNA was digested with T1 ribonuclease and

the resulting oligonucleotides analyzed by two dimensional polyacrylamide gel electrophoresis as previously described (Stern and Kennedy, 1980; Lee et al 1979).

Agarose gel electrophoresis. ^3H -uridine labeled RNA was subjected to electrophoresis in 1% agarose gels containing methyl mercury hydroxide as denaturant (Bailey and Davidson, 1976) and gels were flouorographed (Chamberlain 1979). Unlabeled RNA was electrophoresed in these gels and then used in RNA blots as described below.

Probe Synthesis

Preparation and characterization of cDNA rep. Purified A59 genome RNA was used as a template to synthesize high specific activity ^{32}P - or ^3H -labeled cDNA using avian myeloblastosis virus polymerase and oligomers of calf thymus DNA as primer. Since the calf thymus DNA hybridizes and primes DNA synthesis at random places along the genome RNA, this DNA should be equally representative of the entire genome and thus is designated cDNA_{rep} (Taylor et al., 1976). For liquid hybridization experiments where a single stranded cDNA probe is desirable, cDNA_{rep} was chromatographed on an hydroxylapatite column to remove double stranded DNA (Leong et al., 1972). cDNA_{rep} synthesized by this technique is both specific for viral nucleotide sequences and equally representative of the majority of the genome (see Results section).

Preparation of cDNA_{3'}. High specific activity ^{32}P -labeled cDNA was synthesized as above only using oligo(dT)₁₂₋₁₈ as primer (Tal et al 1977). The oligo(dT) hybridizes to poly(A) at the 3' end of the genome RNA and thus primes DNA synthesis only at the 3' end of the genome. This cDNA was hybridized to poly(A) and subsequently passed through an oligo(dT) cellulose column to eliminate DNA not covalently linked to oligo(dT). Since this cDNA was not greater than 1000 nucleotides in length (data not shown) it should represent not more than 5% of the 3' end of the genome and thus is designated cDNA_{3'}.

Molecular Hybridization

Hybridization in solution was carried out at 68°C in 0.6M NaCl and analyzed by S1 nuclease digestion (Leong et al., 1972).

RNA Blots. RNA was electrophoresed in agarose gels as above, blotted to diazobenzylloxymethyl (DBM) cellulose, and then hybridized with ^{32}P -labeled cDNA and autoradiographed (Alwine et al, 1977).

RESULTS

Homology among the Genomes of A59, MHV3, JHM and 229E

Molecular hybridization. All three MHV strains and 229E contain plus stranded, polyadenylated RNA genomes of about 6×10^6 daltons (MacNaughton, 1978). We have used molecular hybridization as a measure of nucleotide sequence homologies among these genomes. For this purpose we synthesized a virus-specific complementary DNA (cDNA) probe, cDNA_{rep}, containing sequences homologous to most or all of the A59 genome (see Materials and Methods for details in preparation). To validate the specificity of cDNA_{rep}, we measured the rates of hybridization of 32 P-labeled cDNA_{rep} with cytoplasmic RNA extracted from A59-infected and uninfected cells (Fig.1). In this Crt analysis (Leong et al., 1972) cDNA_{rep} did not hybridize with uninfected cell RNA even at very high Crt values where hybridization went to completion with infected cell RNA. Thus cDNA_{rep} is sufficiently free of host sequences to serve as probe for virus-specific RNA. Furthermore, cDNA_{rep} contains sequences equally representative of at least 65% of the A59 genome (data not shown).

Figure 1. Kinetics of hybridization of cDNA_{rep} with cytoplasmic RNA from A59-infected and uninfected cells. Various amounts (80μg to 8 ng) of RNA were hybridized with 4000 cpm (0.5 ng) of 32 P-labeled cDNA_{rep} to the indicated Crt values. Hybridization was assayed by digestion with S1 nuclease. (O) infected cell RNA (Δ) uninfected cell RNA.

The A59 cDNA_{rep} was hybridized to completion with RNA in vast excess, to genome RNA and intracellular RNAs of all four viruses. These hybridizations were done in solution, under stringent conditions and assayed by S1 nuclease digestion (see Materials and Methods for details). The results are summarized in Table 1. All three MHV genomes contain extensive sequence homology. The A59 and MHV3 genomes contain more homology than the A59 and JHM genomes. We could detect no sequence homology between A59 cDNA_{rep} and the 229E genome under these hybridization conditions. Under the less stringent hybridization conditions used for RNA blotting there is some cross reactivity between 229E RNA and A59 cDNA_{rep} (see below).

Oligonucleotide fingerprints. Genome RNAs were also compared by oligonucleotide fingerprinting analysis. Figure 2 shows the results of two dimensional gel electrophoresis of T1 ribonuclease digests of ³²P-labeled genome RNAs. The oligonucleotides that are large enough to be significant in the comparison of the RNAs are found in the top third of the fingerprints. In agreement with the hybridization results, oligonucleotide fingerprinting suggests that all three MHV genomes are related. The most closely related are the A59 and MHV3 genomes having most of the significant oligonucleotides in common. The JHM and MHV3 genomes are the next most closely related pair and the JHM and A59 genomes are more distantly related. By this technique, the 229E genome shows little if any homology with the MHV genomes.

Comparison of Murine and Human Coronavirus Intracellular RNAs

Agarose gel electrophoresis. Murine and avian coronaviruses generate multiple subgenomic polyadenylated intracellular putative mRNAs of the same polarity as genome RNA (Stern and Kennedy, 1980; Leibowitz et al, manuscript in preparation). We have found that human coronavirus 229E generates a similar set of intracellular subgenomic RNAs. RNA was extracted from the cytoplasm of coronavirus infected cells that had been labeled with ³H-uridine for several hours, late in infection, in the presence of Act D to inhibit host cell RNA synthesis. Poly(A)- containing RNA was selected and electrophoresed in agarose gels as shown in Figure 3. Lane a shows the position of purified A59 genome RNA on these gels. Each MHV strain generates six subgenomic RNAs ranging in molecular weight from 0.62 to 3.6 x 10⁶ daltons (Figure 3, Table 2). This is in basic agreement with Leibowitz et al., (manuscript in preparation) and the RNAs have been numbered as in that paper. For all three strains, the RNAs are of the same sizes and present in approximately equal relative proportions with the possible exception that in the case of JHM, RNAs 4 and 5 may be less abundant relative to the other RNAs. The extra band between RNAs 2 and 3 observed in MHV3 RNA, as well as other extra bands are occasionally observed. We do not know the significance of these bands. Lanes f and g show a

Table 1. Homology Among the A59, MHV3, JHM, and 229E Coronavirus Genomes

<u>Source of RNA</u>	<u>Percent Hybridization of</u> <u>A59 cDNA_{rep}</u> ^a
A59	100
MHV3	87
JHM	74
229E	0

- a. A59 cDNA_{rep} was hybridized to completion with a vast excess of RNA from virions or infected cells. These values are averages of 2-3 experiments and have been normalized to 100% hybridization of A59 cDNA_{rep} with its homologous A59 RNA. The actual values for hybridization of A59 cDNA_{rep} with A59 RNA ranged from 85-100%.

comparison of the human and murine coronavirus subgenomic RNAs. Again in the case of 229E, there are six size classes of RNAs (numbered in analogy to the MHV RNAs) but each is of lower molecular weight than its MHV counterpart (Figure 3, Table 2). There is little genome sized RNA detected in the infected cell with any of these viruses.

RNA Blots

Blotting with cDNA_{rep} We also used molecular hybridization to study coronavirus intracellular RNAs. Hybridization with cDNA probes is a very sensitive technique that may be used to detect small amounts of virus-specific intracellular RNA in the presence of a vast excess of cellular RNA, without the use of Act D. This technique of "RNA blotting" is illustrated in Figures 4 and 5. RNA extracted from A59-infected DBT cells late in infection, RNA extracted from uninfected DBT cells, and A59 virion RNA were electrophoresed in an agarose gel, blotted onto DBM paper and hybridized with cDNA_{rep} (Alwine et al., 1977). As shown in Figures 4 and 5, there is no hybridization detected with RNA from uninfected cells. As expected virion RNA contains one major species of about 6×10^6 daltons. All six subgenomic RNAs are detected in RNA from infected

Figure 2. Oligonucleotide fingerprints of coronavirus genomes. High specific activity ^{32}P -labeled purified coronavirus genome RNAs were incubated with T1 ribonuclease and analyzed by two dimensional polyacrylamide gel electrophoresis. The asterisk shows the position of the xylene cyanol blue dye marker.

Figure 3. Agarose gel electrophoresis of ^3H -uridine labeled virus-specific intracellular RNA. ^3H -uridine labeled RNAs, extracted from virus particles (lane a) or infected cells labeled in the presence of Act D late in infection (lanes b-g) were chromatographed on oligo (dT)-cellulose columns and electrophoresed in 1% agarose gels containing 10 mM methyl mercury hydroxide.

- lane a. A59 genome RNA
 b. A59-infected cell cytoplasmic RNA
 c. A59-infected cell cytoplasmic RNA
 d. MHV3-infected cell cytoplasmic RNA
 e. JHM-infected cell cytoplasmic RNA
 f. MHV3-infected cell cytoplasmic RNA
 g. 229E-infected cell cytoplasmic RNA

Table 2. Molecular Weights of Coronavirus Subgenomic RNAs

RNA	Molecular Weight ($\times 10^{-6}$ Daltons) ^a	
	Murine	Human
1 (GENOME)	6.0	(6.0) ^b
2	3.6	2.8
3	3.0	2.1
4	1.3	1.25
5	1.2	1.0
6	0.90	0.84
7	0.62	0.60

- a. The murine coronavirus RNAs were coelectrophoresed with murine ribosomal 45S, 32S, 28S and 18S RNA standards. A plot of logarithm of the molecular weights of these RNAs vs. distance migrated formed a straight line. The sizes of the viral RNAs were determined from this calibration curve. The size of the human RNAs were determined by coelectrophoresis with the murine coronavirus RNAs.
- b. We did not analyze the 229E genome by gel electrophoresis. However, MacNaughton (1978), by gel electrophoresis, and we, by sucrose velocity sedimentation, have found the 229E and MHV genomes to be the same size.

cells, although RNAs 2 and 3 are difficult to see over background. The same pattern is obtained with RNA that was synthesized in the presence or absence of Act D (Figure 5A). All six RNAs are detected with A59 cDNA_{rep} when either MHV3 or JHM intracellular RNAs are analyzed (data not shown). As with directly labeled RNA, there is little if any genome sized RNA detected in the infected cell.

Preliminary blotting experiments suggest that A59 cDNA_{rep} can be used to detect 229E intracellular RNA in a blot, but only when RNA is present at a level ten fold higher than that needed to detect MHV RNA. Hybridization conditions used in a blot (41°C, 50% formamide) are less stringent than those for solution hybridization (as described above) and thus would be expected to allow the detection of more poorly matched hybrids, not detected by solution hybridization (see Discussion).

The 3' ends of virus-specific intracellular RNAs. cDNA probes, specific for certain regions of the viral genome can be used to analyze the genetic content of virus-specific intracellular RNAs. The subgenomic RNAs of an avian coronavirus, infectious bronchitis

Figure 4. Hybridization of virus-specific RNA in gels. Unlabeled RNAs were electrophoresed as in Figure 3, transferred to DBM paper and hybridized with approximately 2×10^6 cpm of ^{32}P -labeled cDNA_{rep}.

- lane a. 2 μg A59-infected cell cytoplasmic RNA
- b. 6 μg A59-infected cell cytoplasmic RNA
- c. 20 μg A59-infected cell cytoplasmic RNA
- d. 60 μg A59-infected cell cytoplasmic RNA
- e. 2ng A59 genome RNA
- f. 20ng A59 genome RNA
- g. 6 μg uninfected cell cytoplasmic RNA
- h. 60 μg uninfected cell cytoplasmic RNA

virus (IBV) and MHV strains A59 and JHM form nested sets. That is, all the RNAs overlap in sequence so that each RNA contains all the sequences of the next smaller one and in addition extra sequences

Figure 5. Hybridization of infected cell RNAs with cDNA_{rep} and cDNA_{3'}. RNAs were electrophoresed in agarose gels, transferred to DBM paper and hybridized with cDNA_{rep} (panel A). The probe was removed and the blot hybridized with cDNA_{3'} (Panel B).

- lane a. 2 μ g A59-infected cell cytoplasmic RNA
- b. 6 μ g A59-infected cell cytoplasmic RNA
- c. 20 μ g A59-infected cell cytoplasmic RNA
- d. 6 μ g A59-infected cell cytoplasmic RNA, synthesized in the presence of Act D.

(Stern and Kennedy, 1980; Leibowitz et al., manuscript in preparation). In the case of IBV, all subgenomic RNAs contain sequences homologous to the 3' end of genome RNA and extend various distances toward the 5' end (Stern and Kennedy, personal communication). To test this for MHV, we synthesized cDNA₃₁, a probe complementary to the 3' end of A59 genome RNA (see Materials and Methods for details). This probe was hybridized to MHV intracellular RNA in a blot (Figure 5). As with cDNA_{rep}, RNAs 2-6 are detected with cDNA₃₁, suggesting that all RNAs contain sequences homologous to the 3' end of genome RNA. The larger RNAs (2 and 3) are more difficult to detect with cDNA₃₁ than with cDNA_{rep}. This is probably because, as suggested by the direct labeling experiment above (Figure 3), RNAs 2 and 3 are less abundant than RNAs 4-7 and hybridization with cDNA₃₁ should be a measure of the number of RNA molecules in each band rather than the total amount of RNA in each band.

DISCUSSION

The A59 and MHV3 strains of MHV are primarily hepatropic while the JHM strain is highly neurotropic (McIntosh 1974). We have shown here by oligonucleotide fingerprinting and by molecular hybridization that JHM has diverged from the other two strains in its nucleotide sequence as well as in its biology. The oligonucleotide fingerprints revealed less homology among the three MHV strains than the hybridization experiments. This would be expected because the former technique is more likely to reflect small change, even one base, in RNA sequence.

All three strains of MHV generate six subgenomic intracellular putative mRNAs. It is unlikely that any of these are defective viral genomes as all virus was plaque purified and stocks were grown at low m.o.i. There are no obvious strain dependent differences in the subgenomic RNAs that could reflect the different tropism of JHM.

We have found no homology between A59 cDNA_{rep} and 229E RNA when measured by a stringent solution hybridization assay. However, when hybridization criteria are lowered as in an RNA blot, a low level of homology is detected. Thus human and murine coronaviruses are probably related, but quite diverged from each other. Recently a similar phenomenon has been observed for murine and simian papova virus genomes (Hawley et al, 1980). Homology among these genomes may be demonstrated only under relaxed hybridization conditions.

229E is an unusual strain of HCV in that it is tissue culture adapted and does not grow in mouse brains. Most other strains of HCV, exemplified by OC43, do not grow in tissue culture and must be propagated in human tracheal organ culture or in suckling mouse brains (McIntosh, 1974). The putative human coronaviruses isolated from multiple sclerosis brain tissue are more closely related to

OC43 than 229E (Burks et al 1980). We plan to analyze OC43 RNA for possible sequence homology with MHV and 229E.

Both avian and murine coronavirus putative mRNAs have been shown to overlap in sequence and to contain the same 3' sequences (Stern and Kennedy, 1980, personal communication; Liebowitz et al. manuscript in preparation). This is the case for several other classes of animal viruses (Cancedda et al 1975; Oppermann et al 1977; Deininger et al., 1979). In most cases each subgenomic RNA serves as mRNA for the its 5' gene. We and others (Leibowitz and Weiss, this volume; Sidell et al., 1980) have started to analyze the mRNA capacities of the MHV intracellular RNAs. In cell-free translation experiments RNAs 6 and 7 have been shown to code for the 25,000 dalton virion glycoprotein and nucleocapsid structural proteins respectively.

We have synthesized and characterized MHV-specific cDNA probes. The use of high specific activity labeled probes either in solution hybridization or in RNA blots is sensitive enough to detect < 1 copy/cell of virus-specific genome RNA (Parker and Stark, 1979; Spector et al., 1978). These probes will be especially useful in the analysis of virus-specific RNA in situations where viral RNA may be present in very small quantities such as during persistent infection in cultured cells or in animals. Furthermore, cDNAs representing specific portions of the genome will be useful in analyzing the genetic content of virus-specific RNAs.

Acknowledgements. Susan R. Weiss would like to thank Dr. J. Michael Bishop, in whose lab some of this work was carried out.

REFERENCES

- Alwine, J.C., Kemp, D.J. and Stark, G.R. 1977, Method for detection of specific RNAs in agarose gels by transfer to diazobenzyl-oxymethyl paper and hybridization with DNA probes. *Proc. Nat. Acad. Sci.* 74: 5350-5354.
- Aviv, H. and Leder, P. 1972, Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid cellulose. *Proc. Nat. Acad. Sci.* 69: 1408-1412.
- Bailey, O.T., Pappenheimer, A.M., Sergeant, F., Cheever, M.D., and Daniels, J.B. 1949, A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. *J. Expt. Med.* 90: 195-212.
- Bailey, J. and Davidson, N. 1976, Methyl mercury as a reversible denaturing agent for agarose-gel electrophoresis. *Analyt. Biochem.* 70: 75-85.
- Bond, C.W., Liebowitz, J.L., and Robb, J.A. 1979, Pathogenic murine coronaviruses, II. Characterization of virus-specific proteins

- of murine coronavirus. *Virology* 94: 371-384
- Burks, J., DeVald, B.L., Jankovsky, L.D., Gerdes, J.C. 1980, Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science* 209: 933-934.
- Cancedda, R., Villa-Kamaroff, L., Lodish, H.F., and Schlesinger, M. 1974, Initiation sites for translation of sindbis virus 42S and 26S messenger RNAs. *Cell* 6: 215-222
- Chamberlain, J.P. 1979, Fluorographic detection of radioactivity in polyacrylamide gels with the water soluble fluor, sodium salicylate. *Analyt. Biochem.* 48: 132-135.
- Deininger, P., Esty, A., LaPorte, P., and Friedman, T. 1979, Nucleotide sequence and genetic organization of the polyoma late region: features common to the polyoma early region and SV40. *Cell* 18: 771-779.
- Dick, G.W.A., Niven, J.S.F., Gledhill, A.W. 1956, A virus related to that causing hepatitis in mice (MHV). *Brit. J. Expt. Path.* 37: 90.
- Hamre, D. and Procknow, J.J. 1966, A new virus isolated from the human respiratory track. *Proc. Soc. Expt. Biol. Med.* 121: 190-193.
- Hierholzer, J.C. 1976, Purification and biophysical properties of human coronavirus 229E. *Virology* 75: 155-165.
- Howley, P.M., Israel, M.A., Law, M.F., Martin, M.A. 1980, *Proc. Nat. Acad. Sci.* 254: 4876-4883.
- Kennedy, D.A. and Johnson-Lussenberg, C.M. 1975-1976. Isolation and morphology of the internal component of human coronavirus; 229E. *Intervirology* 6: 197-206.
- Lee, Y.F., Kitamura, N., Nomato, A. and Wimmer, E. 1979, Sequence studies of poliovirus type 1, type 2, and two type 1 defective interfering particles RNAs. *J. Gen. Virol.* 44: 311-322.
- Leong, J.A., Garapin, A.C., Jackson, N., Fanshier, L., Levinson, W., and Bishop, J.M. 1972, Virus-specific ribonucleic acid in cells producing Rous sarcoma virus: Detection and characterization. *J. Virol.* 9: 891-902.
- McNaughton, M.R. 1978, The genomes of three coronaviruses. *FEBS Letters* 94: 191: 194.
- McNaughton, M.R. and Madge, M.H. 1978, The genome of human coronavirus 229E. *J. Gen. Virol.* 39: 497-504.
- McIntosh, K. 1974, Coronaviruses: A comparative review. *Current Topics in Microbiology and Immunology.* 63: 85-129
- Opperman, H., Bishop, J.M., Varmus, H.E. and Levintow, L. 1977, A joint product of the genes gag and pol of avian sarcoma virus: a possible precursor of reverse transcriptase. *Cell* 12: 993-1005.
- Parker, B. and Stark, G. 1979. Regulation of simian virus 40 transcription: Sensitive analysis of the RNA species present early in infection by virus or viral DNA. *J. Virol.* 31: 360-369.
- Robb, J.A. and Bond, C.W. 1980. Coronaviridae. *Comprehensive Virology* 14: 193-245.

- Robb, J.A., Bond, C.W. and Leibowitz, J.L. 1979, Pathogenic murine coronaviruses. I. Characterization of biological behavior in vitro and virus specific intracellular RNA of strongly neurotropic JHMV and weakly neurotropic A59V viruses. *Virology* 94: 385-399.
- Siddell, S.G., Wege, H., Barthel, A. and ter Meulen V. 1980. Cell-free synthesis of structural protein pp60.
- Spector, D.H., Smith, K., Padgett, T., McCombe, P., Roulland-Dussoix, D., Moscovici, C., Varmus, H.E., and Bishop, J.M. 1978. Uninfected cells contain RNA related to the transforming gene of avian sarcoma viruses. *Cell* 13: 371-379.
- Stern, D. and Kennedy, S.I.T. 1980, The coronavirus multiplication strategy. I. Identification and characterization of virus specific RNA. *J. Virol.* 34: 665-674.
- Sturman, L.S. and Takemoto, K.K. 1972, Enhanced growth of a murine coronavirus in transformed mouse cells. *Infection and Immunity* 6: 501-507.
- Tal, J., Kung, H.J., Varmus, H.E. and Bishop, JM 1977. Characterization of DNA complementary to nucleotide sequences adjacent to poly(A) at the 3' terminus of the avian sarcoma virus genome. *Virology* 78: 183-197.
- Taylor, J.M., Illmensee, R., and Sommers, S. 1976, Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. *Biochim. Biophys. Acta.* 442: 324-330.
- Weiss, S.R., Varmus, H.E. and Bishop, J.M. 1977, The size and genetic composition of virus-specific RNAs in the cytoplasm of cells producing avian sarcoma-leukosis viruses. *Cell* 12: 983-992.