JOURNAL OF VIROLOGY, Oct. 1979, p. 208-219
0022-538X/79/10-0208/12$02.00/0

Vol. 32, No. 1

Oligoribonucleotide Map and Protein of CMII: Detection of Conserved and Nonconserved Genetic Elements in Avian Acute Leukemia Viruses CMII, MC29, and MH2

KLAUS BISTER,[1] HANS-CHRISTOPH LÖLIGER,[2] AND PETER H. DUESBERG[1]*

Department of Molecular Biology, University of California, Berkeley, California 94720,[1] and Institut für Kleintierzucht, Celle, Germany[2]

Received for publication 20 April 1979

RNA and protein of the defective avian acute leukemia virus CMII, which causes myelocytomas in chickens, and of CMII-associated helper virus (CMIIAV) were investigated. The RNA of CMII measured 6 kilobases (kb) and that of CMIIAV measured 8.5 kb. By comparing more than 20 mapped oligonucleotides of CMII RNA with mapped and nonmapped oligonucleotides of acute leukemia viruses MC29 and MH2 and with mapped oligonucleotides of CMIIAV and other nondefective avian tumor viruses, three segments were distinguished in the oligonucleotide map of CMII RNA: (i) a 5′ group-specific segment of 1.5 kb which was conserved among CMII, MC29, and MH2 and also homologous with *gag*-related oligonucleotides of CMIIAV and other helper viruses (hence, group specific); (ii) an internal segment of 2 kb which was conserved specifically among CMII, MC29, and MH2 and whose presence in CMII lends new support to the view that this class of genetic elements is essential for oncogenicity, because it was absent from an otherwise isogenic, nontransforming helper, CMIIAV; and (iii) a 3′ group-specific segment of 2.5 kb which shared 13 of 14 oligonucleotides with CMIIAV and included *env* oligonucleotides of other nondefective viruses of the avian tumor virus group (hence, group specific). This segment and analogous map segments of MC29 and MH2 were not conserved at the level of shared oligonucleotides. CMII-transformed cells contained a nonstructural, *gag* gene-related protein of 90,000 daltons, distinguished by its size from 110,000-dalton MC29 and 100,000-dalton MH2 counterparts. The *gag* relatedness and similarity to the 110,000-dalton MC29 counterpart indicated that the 90,000-dalton CMII protein is translated from the 5′ and internal segments of CMII RNA. The existence of conserved 5′ and internal RNA segments and conserved nonstructural protein products in CMII, MC29, and MH2 indicates that these viruses belong to a related group, termed here the MC29 group. Viruses of the MC29 group differ from one another mainly in their 3′ RNA segments and in minor variations of their conserved RNA segments as well as by strain-specific size markers of their *gag*-related proteins. Because (i) the conserved 5′ *gag*-related and internal RNA segments and their *gag*-related, nonvirion protein products correlate with the conserved oncogenic spectra of the MC29 group of viruses and because (ii) the internal RNA sequences and nonvirion proteins are not found in nondefective viruses, we propose that the conserved RNA and protein elements are necessary for oncogenicity and probably are the *onc* gene and *onc* gene products of the MC29 group of viruses.

CMII is an avian acute leukemia virus (18) that causes myelocytomatosis in chickens (24, 25) and also transforms fibroblasts in tissue culture (17). Avian acute leukemia viruses carry one known genetic function, termed the *onc* gene for oncogenic cell transformation (18). The *onc* gene of these viruses has not been defined genetically because defectiveness in all three replicative genes of RNA tumor viruses (3-5, 17, 20, 21) has hindered recombination and deletion analyses relying on additional genetic markers. Such deletion analyses were essential in defining the genetic elements of nondefective Rous sarcoma virus (RSV) (31-33). However, biochemical analyses of the RNAs of several strains of avian acute leukemia viruses MC29 (10, 26; P. H. Duesberg, K. Bister, and C. Moscovici, Virology, in press) and MH2 (10a, 13) have distinguished two sets of sequences about 40% of which are specific and 60% of which are related

to genes of helper independent avian tumor viruses and termed group specific. In MC29 the specific sequences map between 2.5 and 4.5 on a map of 5.7 kilobases (kb) from the 3′ end of the RNA and are flanked by a 5′ and a 3′ group-specific RNA segment. The 5′ group-specific and the internal MC29-specific segment code together for a nonstructural protein of 110,000 daltons (3, 26). Three of the eight large RNase T_1-resistant oligonucleotides that define the specific segment of MC29 RNA have also been found, although not mapped, in MH2 RNA (10a, 13) and this RNA also codes for a nonstructural, *gag* gene-related protein which measures 100,000 daltons (20, 30a).

By analogy with the transforming *src* gene of RSV, the specific sequences of MC29 and MH2 were hypothesized to be necessary for or identical with the *onc* genes of these viruses because they are, like *src* (31), unrelated to viral replicative genes (10, 26). Similarity between the specific nucleotide sequences and the oncogenic spectra of MC29 and MH2 supported this hypothesis (10a, 13).

To extend and refine the notion of a related class of specific sequences, defined as being unrelated to nondefective helper viruses, in different strains of avian acute leukemia viruses, we have identified and analyzed RNA and protein of CMII and also of its associated helper virus CMIIAV for comparisons with MC29 and MH2. CMII was selected because its oncogenic spectrum is distinct from, but overlaps with, those of MC29 and MH2 (16, 17, 24, 25). Because of the close relationships observed among the three acute leukemia viruses (see below), RNAs were compared at the level of shared RNase T_1-resistant oligonucleotides. This method gives upper estimates of specific sequences because it responds to single base changes. Consequently, sequences referred to below as conserved are very closely related. Sequences referred to as nonconserved at the oligonucleotide level are known to be at least distantly related if compared by the less sensitive method of RNA-complementary DNA hybridization used in earlier studies (10, 11, 26, 30). Related sequences of acute leukemia viruses and nondefective helper viruses, which were identified by hybridization or by shared oligonucleotides, were termed group specific, following the published definition (11, 26).

Here we have analyzed the composition and map order of the RNase T_1-resistant oligonucleotides of CMII RNA and have identified a 90,000-dalton *gag*-related, nonstructural CMII protein. By comparing mapped oligonucleotides of CMII with mapped and unmapped oligonucleotides of acute leukemia viruses MC29 and MH2 and with mapped oligonucleotides of CMIIAV and other nondefective avian tumor viruses, the oligonucleotide map of CMII RNA was divided into three segments: (i) a 5′ group-specific segment that was conserved among CMII, MC29, and MH2, and also homologous with *gag*-related sequences of helper viruses; (ii) an internal segment that was conserved specifically among CMII, MC29, and MH2; and (iii) a 3′ group-specific segment nonconserved among CMII, MC29, and MH2 at the oligonucleotide level, but homologous in 13 of 14 oligonucleotides with CMIIAV and including *env* oligonucleotides of other nondefective viruses of the avian tumor virus group. The *gag* relationship and analogy with the 110,000-dalton MC29 counterpart suggested that the 90,000-dalton CMII protein is translated from the 5′ and internal segments of CMII RNA.

The conserved nature of their 5′ and internal RNA segments and of the nonstructural, *gag* gene-related proteins encoded by these sequences indicates that CMII, MC29, and MH2 are a closely related group, termed the MC29 group. The members of the MC29 group differ mainly in their 3′ RNA segments and in minor variations of their conserved RNA segments and by strain-specific size markers of the *gag*-related proteins, 90,000 (CMII), 100,000 (MH2), and 110,000 (MC29) daltons.

MATERIALS AND METHODS

Cells and viruses. Avian myelocytomatosis virus strain CMII was originally isolated in West Germany in 1964 (24, 25). It was propagated here in quail cell cultures either together with its original helper virus as CMII(CMIIAV) complex or after superinfection with ring-necked pheasant virus (RPV) of subgroup F as CMII(CMIIAV + RPV) complex. The MC29 quail nonproducer cell line Q8 and the MC29(RPV)-infected quail cells obtained by superinfection of Q8 with RPV have been described before (3). MH2(MH2AV)-infected quail cells have also been described previously (13). A laboratory strain of MC29(MCAV-A and B) was obtained from C. Moscovici (Duesberg et al., Virology, in press).

Preparation, gel electrophoresis, and oligonucleotide analyses of radioactive viral RNA. CMII(CMIIAV) or CMII(CMIIAV + RPV)-infected quail cells were labeled with [^{3}H]uridine or with $H_3{}^{32}PO_4$, and virus was purified essentially as described before (10, 12, 31). Preparation of viral 50 to 70S RNA and conditions of electrophoresis in 2.1% polyacrylamide gels followed published procedures (10, 12, 31). The preparative separation of the 6-kb CMII RNA and the 8.5-kb helper virus RNA species of the CMII-helper virus complex was achieved by two different procedures. (i) Viral 50 to 70S [^{32}P]RNA purified by glycerol gradient sedimentation (10) was heat denatured and subjected to electrophoresis in a 2.1% polyacrylamide gel (10, 12). The radioactivity in 1-mm gel slices was determined by Cerenkov radiation, and

RNA peaks were eluted from 7 to 12 pooled gel slices by stirring for 12 h at room temperature in 7.5 ml of 0.4 M LiCl-0.01 M Tris (pH 7.4)-2.5 mM EDTA-0.2% sodium dodecyl sulfate (SDS) containing 100 μg of yeast RNA. After pelleting of the polyacrylamide at 15,000 × *g*, the RNA was ethanol-precipitated after the addition of NaCl to 0.1 M and freed of soluble polyacrylamide by sucrose gradient sedimentation (10). (ii) Viral 50 to 70S RNA was sedimented in a 15 to 30% sucrose gradient in 0.05 M NaCl-0.01 M Tris (pH 7.4)-5 mM EDTA-0.2% SDS by centrifugation in a Beckman SW41 rotor for 210 min at 40,000 rpm and 20°C. The peak of labeled RNA was separated in a leading half, termed the 60 to 70S pool, and a trailing half, termed the 50S pool. Both pools of [^{32}P]RNA were subjected to electrophoresis in 1.7% agarose (Bio-Rad Laboratories, Richmond, Calif.) gels (0.6 by 10 cm) with other conditions as described previously (12). Electrophoresis was continued at 10 V/cm until a bromophenol blue marker had migrated 10 cm (~2.5 h). The radioactivity in 1-mm gel slices was determined by Cerenkov radiation, and the leading half of the 50S RNA pool was eluted from gel slices as described above (see Fig. 2). After pelleting of the agarose at 15,000 × *g*, the RNA was ethanol-precipitated for further analysis. Contamination with soluble agarose was negligible.

For the preparation of polyadenylic acid [poly(A)]-tagged RNA fragments of discrete size classes, monomer RNA species prepared by method i were alkali-fragmented by incubating two samples for 1 and 4 min, respectively, in 0.05 M Na_2CO_3 at pH 11 and 50°C. Fragments were combined, and poly(A)-tagged species were selected by binding to oligodeoxythymidylic acid-cellulose (31). Fractionating into different size classes was by sedimentation in a 10 to 25% sucrose gradient in 0.01 M NaCl-0.01 M Tris (pH 7.4)-1 mM EDTA-0.1% SDS in a Beckman SW41 rotor for 7 h at 40,000 rpm and 20°C. Alternatively, native 50S RNA prepared by method ii was heat-dissociated, and poly(A)-containing RNA fragments present as nicked RNA subunits in the 50S complex, as well as intact 6-kb RNA, were selected and fractionated into size classes as described above. Preexisting nicks in 50S RNA complexes obtained from virus harvested at 6- to 8-h intervals (7, 9) obviated the need for chemical degradation. Fingerprinting of RNase T_1-resistant oligonucleotides of [^{32}P]RNAs prepared by methods i and ii was by electrophoresis at pH 3.5 and homochromatography on DEAE-cellulose as described before (31, 32), with the modification that Cellogel strips (Kalex Scientific Co., New York, N.Y) were used for the electrophoresis.

Radiolabeling of cells, immune precipitation of intracellular viral proteins, and polyacrylamide gel electrophoresis. Labeling of cells with [^{35}S]methionine, preparation of detergent extracts, and immune precipitation with antisera directed against whole Nonidet P-40 (Shell Chemical Co.)-disrupted virus (Prague strain of RSV-C) or against the major nonglycosylated (*gag*-related) structural protein p27 (anti-p27 serum) were carried out essentially as described before (3). The anti-p27 serum used also contained weak activity against the *gag* protein p19 (M. Hayman, personal communication). The antiserum against the viral glycoproteins (anti-gp85 serum) was prepared by D. Bolognesi and was obtained from G. S. Martin. The immune complexes were precipitated by goat antiserum to rabbit immunoglobulin (Antibodies, Inc., Davis, Calif.) as a second antibody (3). Alternatively, the protein A-containing bacterium *Staphylococcus aureus* was used as an immune complex adsorbent (22). Briefly, 250 μl of clarified cellular detergent extract was incubated with 1 to 2 μl of the specific antiserum, and after 60 min at 0°C 20 to 40 μl of a freshly prepared 10% suspension of the formaldehyde-fixed *S. aureus* in lysis buffer (0.5% Nonidet P-40-0.5% sodium deoxycholate-50 mM NaCl-25 mM Tris, pH 8.0) was added. After 30 min at 0°C, the bacteria were pelleted at 1,200 × *g* for 10 min and washed three times in lysis buffer. The pellet was resuspended in 50 μl of sample buffer (10% glycerol-5% β-mercaptoethanol-3% SDS-0.005% bromophenol blue-0.06 M Tris, pH 6.8) and heated to 100°C for 2 min. After pelleting of the bacteria at 2,000 × *g* for 15 min, the supernatant was used for electrophoresis. Immune precipitates were analyzed by electrophoresis on 6 to 18% linear polyacrylamide gradient slab gels, using the discontinuous SDS-Tris-glycine system (23) as described previously (3). Electrophoresis was at 18 mA for 6 h. Gels were stained with 0.5% Coomassie blue in 50% methanol-10% acetic acid (vol/vol) for 20 min and destained overnight in 30% methanol-10% acetic acid. They were then dehydrated with two changes of dimethyl sulfoxide for 1 h, treated with 20% 2,5-diphenyloxazole in dimethyl sulfoxide (wt/vol) for 2 to 3 h, rehydrated with water for 1 h, and dried under vacuum at room temperature. Kodak X-Omat XR-5 X-ray film was used for fluorography (6).

RESULTS

Identification of CMII and CMIIAV RNA. Two pseudotypes of CMII were propagated on quail cells for analyses of viral components: one was a CMII(CMIIAV) complex derived from the original isolate after several passages in chicken; the other was the same virus complex superinfected with RPV of subgroup F. Both CMII-infected quail cultures displayed a transformed phenotype that was similar in morphology to that described for MC29-infected quail cells (3, 5). After labeling cultures with [^{3}H]uridine, virus was purified and 50 to 70S viral RNA was prepared by published procedures (3, 10, 31). Virus yield, measured by production of [^{3}H]uridine-labeled virus with a buoyant density of 1.16 to 1.19 g/ml (3, 10), was about 10 times higher in the culture superinfected with RPV than in the culture infected with CMII(CMIIAV) alone. The 50 to 70S [^{3}H]RNAs of CMII pseudotypes were mixed with [^{14}C]RNA standards of MC29 pseudotypes, and after heat dissociation of 50 to 70S RNA dimers two distinct RNA monomer species were resolved by electrophoresis in 2.1% polyacrylamide gels (Fig. 1A and B). One of these coelectrophoresed with the 8.5-kb RPV RNA standard of MC29(RPV), and the other had an electrophoretic mobility that was slightly lower than that of the 5.7-kb MC29 RNA stan-

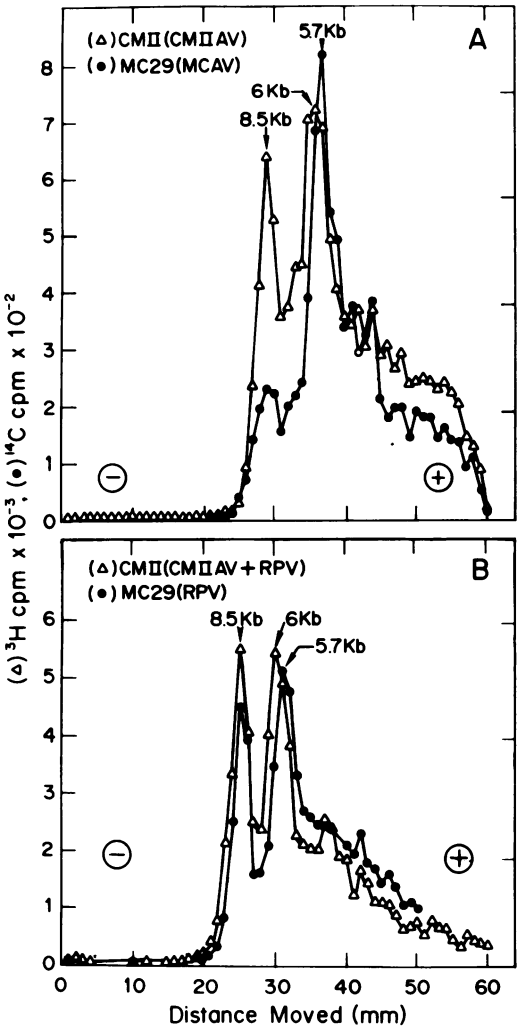

FIG. 1. *Simultaneous electrophoresis of CMII and MC29 RNAs. (A) A mixture of 50 to 70S [^{3}H]RNA of CMII(CMIIAV) propagated in quail cells and [^{14}C]RNA of MC29(MCAV-A and B) (Duesberg et al., Virology, in press) was heated to 100°C for 45 s and subjected to electrophoresis in a 2.1% polyacrylamide gel for 5 h at 8 V/cm as described (12). (B) Electrophoresis as above of [^{3}H]RNA of CMII(CMIIAV + RPV) propagated in quail cells together with [^{14}C]RNA of MC29(RPV) (10).*

dard (10). The 8.5-kb RNA components of CMII pseudotypes are thought to be the RNAs of the original CMIIAV helper virus of CMII in Fig. 1A and a mixture of CMIIAV with RPV in Fig. 1B. By analogy with the RNAs of MC29 and MH2 pseudotypes studied previously, the smaller RNA component of CMII pseudotypes is thought to be the genome of the defective CMII virus. Based on its electrophoretic mobility relative to those of the 5.7-kb MC29 and 8.5-kb RPV RNA standards, its molecular weight was estimated to be 6 kb (2). Hence, the apparent molecular weight of CMII is different from those of MC29 and MH2 RNAs, which are indistinguishable if compared electrophoretically (10a, 11, 13, 14). The ratio of 6-kb CMII to 8.5-kb helper virus RNA was higher in the CMII(CMIIAV) complex (Fig. 1A) than in the complex superinfected with RPV (Fig. 1B). However, because the yield of CMII virus was at least 10 times higher after superinfection with RPV, this pseudotype was used for many analyses.

Specific and group-specific oligonucleotides of CMII and CMIIAV RNAs and comparison with those of MC29 and MH2. We have used shared RNase T_1-resistant oligonucleotides to investigate whether the RNA of CMII shares specific sequences with the RNAs of acute leukemia viruses MC29 and MH2 and with nondefective viruses of the avian tumor virus group, particularly the helper virus CMIIAV. CMII RNA was either prepared electrophoretically from acrylamide gels as a 6-kb RNA monomer (Fig. 1) or as a native 50S RNA dimer complex. By the second method, the native 50 to 70S RNA complex of CMII(CMIIAV + RPV) RNA was fractionated first by sedimentation into a leading half, termed the 60 to 70S pool, and a trailing half, termed the 50S pool, and each pool was further fractionated by electrophoresis in 1.7% agarose gel. It can be seen in Fig. 2 that the peak of the 50S RNA pool had a higher electrophoretic mobility than that of the 60 to 70S pool. RNA from the leading two-thirds of the 50S peak was eluted as described for the preparation of RNA from acrylamide (10, 26). After ethanol precipitation, it could be used directly for fingerprinting, thus eliminating the sedimentation step necessary to remove soluble acrylamide from RNA prepared by the former method. CMII RNA prepared by either method was <20% contaminated by degraded helper virus RNA migrating with denatured 6-kb RNA or by forward trailing helper virus RNA complexes migrating with native 50S CMII RNA complex as judged from the relative molarity of helper virus oligonucleotides in fingerprints of CMII RNA (see below, Fig. 3). The yield of 6-kb CMII RNA prepared as monomer was about 10 to 25% of the total 50 to 70S RNA applied to a gel, varying with the integrity of the RNA and the ratio of 6- to 8.5-kb species in the starting material. The yield of 50S CMII RNA complex prepared from agarose gels was 30 to 50%. The difference is due to the presence of nicked RNA species in the 50S RNA complex (7, 9) that are not recovered if 6-kb RNA monomer species are prepared. Therefore, it was advantageous to use

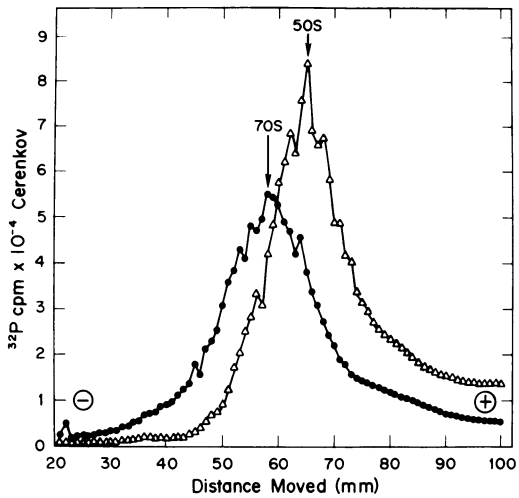

FIG. 2. *Preparative separation of native 50 and 70S RNAs of CMII(CMIIAV + RPV) [^{32}P]RNA by electrophoresis. CMII(CMIIAV + RPV) 50 to 70S [^{32}P]RNA (12.5 × 10^6 cpm) was purified by sedimentation in a 15 to 30% sucrose gradient in 0.05 M NaCl-0.01 M Tris (pH 7.4)-5 mM EDTA-0.2% SDS by centrifugation in a Beckman SW41 rotor for 210 min at 40,000 rpm and 20°C (not shown). The leading half (●) and the trailing half (△) of the 50 to 70S RNA peak were pooled and subjected to parallel electrophoreses in separate 1.7% agarose gels as described in Materials and Methods. Electrophoresis was at 10 V/cm until a bromophenol blue marker had migrated 10 cm in both gels (2.5 h). The radioactivity in 1-mm gel slices was determined by Cerenkov radiation. Both gels were plotted on the same graph.*

50S RNA for oligonucleotide mapping based on analyses of poly(A)-tagged RNA fragments (see below). Since about 30% of heat-dissociated 50S CMII RNA consisted of poly(A)-tagged monomer species which had a size distribution suitable for mapping, about 10 to 15% (30% of 30 to 50%) of the starting 50 to 70S RNA was available for mapping. By contrast, only 1 to 2.5% of the starting 50 to 70S RNA is available for mapping if intact 6-kb RNA (recovered at 10 to 25% yield from 50 to 70S RNA) is alkali-degraded and poly(A)-tagged fragments are selected at a 10% yield (31).

The RNase T_1-resistant oligonucleotides of CMII RNA prepared as 50S RNA from CMII(CMIIAV) were autoradiographed after two-dimensional fingerprinting as shown in Fig. 3A. The same fingerprint was obtained when CMII RNA was prepared as a 6-kb electrophoretic species from CMII(CMIIAV + RPV) (not shown). On the basis of their autoradiographic intensities and quantitations (not shown; 1, 31), over 20 oligonucleotides of 6-kb CMII RNA were present at equimolar ratios and therefore were probably from the same RNA species. The composition of these RNase T_1-resistant oligonucleotides, in terms of their RNase A-resistant fragments, is reported in Table 1. The Table reflects a summary of identical data obtained from CMII RNA prepared from CMII(CMIIAV) and CMII(CMIIAV + RPV). Minor oligonucleotides, also detectable in the fingerprints of CMII RNA, had homologous counterparts in helper virus RNA (see below, Fig. 3B). They are labeled with three-digit numbers in Fig. 3A and are thought to reflect the expected contamination of CMII RNA by helper virus RNA.

A fingerprint of 8.5-kb CMIIAV RNA, prepared electrophoretically from a 50 to 70S CMII(CMIIAV) RNA mixture (Fig. 1), is shown in Fig. 3B. On the basis of their autoradiographic intensities, all unique, large oligonucleotides were present at equimolar ratios and hence were probably from the same RNA species. CMIIAV oligonucleotides shared with CMII RNA were labeled with the same single- and double-digit numbers as their CMII counterparts. CMIIAV-specific oligonucleotides were identified by three-digit numbers (Fig. 3B). RNase A-resistant fragments of these oligonucleotides are reported in Table 1. Coincident compositions of some of these CMIIAV oligonucleotides were derived from two other sources, either as shared homologs from CMII RNA (see Fig. 3A and Table 1) or as CMIIAV-specific oligonucleotides after subtracting RPV oligonucleotides (10) from a mixture obtained from 8.5-kb RPV and CMIIAV RNAs prepared from CMII(CMIIAV + RPV) RNA (not shown).

A comparison shows that CMII RNA shared 18 of 25 oligonucleotides with CMIIAV RNA and 13 with MC29 RNA, some of which are also shared with MH2 RNA. Six oligonucleotides shared between CMII and CMIIAV (no. 7, 14, 16, 22, 24, and C oligonucleotides) were also shared between CMII and the other acute leukemia viruses (Table 1).

Comparing the oligonucleotide maps of CMII, CMIIAV, and MC29. To determine whether CMII oligonucleotides related to nondefective helper viruses and to acute leukemia viruses MC29 and MH2 occupy specific map locations, oligonucleotide maps of CMII and CMIIAV RNAs were prepared. The location of a given oligonucleotide relative to the 3′ poly(A) end of the RNA was deduced from the size of the smallest poly(A)-tagged RNA fragment from which it could be isolated to construct the oligonucleotide maps shown in Fig. 5. Poly(A)-tagged CMII RNA fragments were prepared either from electrophoretically prepared and alkali-degraded 6-kb RNA (Fig. 1) or from heat-dissociated 50S CMII RNA (see above, Fig. 2).

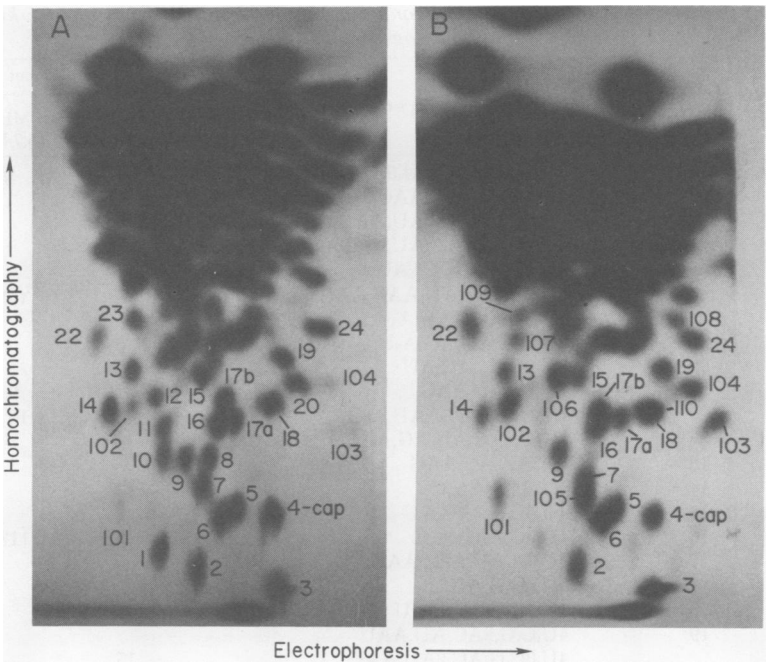

FIG. 3. *Autoradiographs of RNAse T_1-digested 6-kb (A) and 8.5-kb (B) [^{32}P]RNA species of CMII(CMIIAV) after two-dimensional electrophoresis-homochromatography (fingerprint analysis). (A) The 50 to 70S RNA of CMII(CMIIAV) was sedimented as described in the legend of Fig. 2. The peak of labeled RNA was then separated into a leading (60 to 70S) half and a trailing (50S) half. Further purification of the 50S component by agarose gel electrophoresis could here be omitted because, as a result of the initial excess of 6-kb RNA in the total 50 to 70S RNA complex (Fig. 1A), the 50S RNA recovered was <25% contaminated with helper virus RNA. The 50S RNA (~7×10^5 cpm) was digested with RNAse T_1, and the digest was resolved by electrophoresis and then homochromatography on DEAE-cellulose as described (31, 32). (B) The 6-kb and 8.5-kb RNA species of heat-dissociated 50 to 70S CMII(CMIIAV) [^{32}P]RNA (~4.5×10^6 cpm) were separated by electrophoresis in polyacrylamide. RNA peaks were identified by Cerenkov radiation and were eluted from pooled gel slices as described in Materials and Methods. The 8.5-kb RNA was then subjected to fingerprint analysis as for Fig. 3A. The large oligonucleotides were numbered and their RNase A-resistant fragments are listed in Table 1. Identical oligonucleotides found in the T_1 digests of the 6-kb and the 8.5-kb RNAs were given the same number in both fingerprints. Oligonucleotides found in 8.5-kb helper virus RNA that are not shared with 6-kb CMII RNA were given numbers of the 100 series.*

Fragments were fractionated according to size by sucrose gradient sedimentation, and discrete size classes were fingerprinted (Fig. 4). Fragments obtained by both methods gave the same results. The oligonucleotide map constructed from the data shown in Fig. 4 and other data not shown is depicted in Fig. 5A. The oligonucleotide map of MC29 RNA derived recently (26; Duesberg et al., Virology, in press) is placed next to it for direct comparison. On the basis of oligonucleotide homology with acute leukemia viruses MC29 and MH2, and with the helper viruses CMIIAV (Fig. 5B) and other nondefective avian tumor viruses, the oligonucleotide map of CMII could be divided roughly into three major segments. (i) A 5′ group-specific segment was defined by a 5′ cap and four internal oligonucleotides between 4.5 and 6 kb from the 3′ poly(A) coordinate. It shared three of its internal oligonucleotides with an equivalent segment of MC29 RNA. At least two of these oligonucleotides had unmapped homologs in MH2 RNA (Table 1; 13). In addition, these four internal oligonucleotides (no. 14, 16, 17b, and 22) were also shared with nondefective CMIIAV (Fig. 5B) and other helper virus RNAs (Table 1), and one (no. 16) was identical with a *gag* gene oligonucleotide of nondefective Prague RSV (10, 26). Because of its relationship to nondefective viruses of the avian tumor virus group, this segment was termed group specific, like its counterpart in MC29 (26). The cap oligonucleotide of CMII was not the same as that of the MC29 RNA used here as standard. However, other MC29 variants have since been isolated which have the same class I cap oligonucleotide as CMII (Duesberg et al., Virology, in press). (ii) An internal CMII-, MC29-, and MH2-specific segment was defined

TABLE 1. *Composition of RNase T_1-resistant oligonucleotides of 6-kb CMII RNA and 8.5-kb CMIIAV RNA and homologs in other viruses*

Spot no.		RNase A digestion products[a]	Spot no.		
CMII 6 kb	CMIIAV 8.5 kb		MC29 5.7 kb[b]	MH2 5.7 kb[c]	RPV 8.5 kb[d]
1		2U,3C,G,2AC,AU,3AAC	1	4a	
2	2	4U,4C,G,3AC,2AAC,2AAU			
3	3	8U,6C,G,2AC,2AU,AAC,AAU,A_3U			
4-cap	4-cap	4U,4C,G,3AC,3AU,m^7GpppGmC[e]			
5	5	6U,8C,G,2AC,2AAU			
6	6	5U,9C,2AC,AU,AAC,AAG		(5)	
7	7	3U,5C,AC,AAU,A_3C,A_4G	5		
8		2U,8C,3AU,AG,A_3U	6		
9	9	4U,6C,G,3AC,AU			
10		3U,8C,AC,AAG	7b	9	
11		U,3C,AC,AG,AAU,A_3C	8b	10	
12		U,4C,2AC,AU,AG,AAC	12		
13	13	2U,5C,3AC,AAG			18
14	14	4C,G,2AC,A_4C	13		
15	15	4U,5C,G,3AC,AU			8?[f]
16	16	4U,6C,AAG,A_4C	9	11?[f]	5
17a	17a	2U,3C,AU,AG,2AAU			
17b	17b	5U,8C,G,AU			
18	18	4U,4C,G,2AU,A_4U			
19	19	4U,C,G,2AC,AU,AAU			13?[f]
20		4U,3C,G,AC,2AU,AAU	15		
22	22	4C,3AC,AG	26	26	23
23		U,5C,2AC,AAG	120		
24	24	4U,3C,G,AU,AAU	18		
C	C	G,AC,AU,AAU,A_3C	C	C	C
	101	C,G,AC,A_4C,A_5C			
	102a	4C,2AC,AU,AG,AAC			
	102b	4C,2AC,AU,A_4G			
	103	4U,2C,G,3AU,AAU			
	104	7U,5C,G,AU			12
	105	5U,9C,G,AC,AU,A_3C			
	106	3U,4C,G,2AC,AU,A_5N			
	107	2C,AU,AG,A_5N			
	108	U,C,G,2AU,AAU			
	109	2C,AC,AG,A_3U			
	110	3U,3C,AC,AU,AG			

[a] Elution of RNase T_1-resistant oligonucleotides from DEAE cellulose, RNase A digestion and electrophoretic analysis of the digests have been described (31).

[b] MC29 oligonucleotides have been described (10, 26; Duesberg et al., Virology, in press).

[c] MH2 oligonucleotides have been described (10a, 11, 13).

[d] Numbered as previously reported (10).

[e] Cap oligonucleotide of class I (34).

[f] Tentative identification.

by six oligonucleotides mapping between 2.5 and 4.5 kb and shared all six oligonucleotides with an equivalent segment of MC29 RNA (26; Duesberg et al., Virology, in press). Three of these oligonucleotides had unmapped homologs in MH2 RNA (Table 1; 13). No oligonucleotide of this RNA segment was related to any known nondefective avian tumor virus. Thus, this segment appears specific for avian acute leukemia viruses CMII, MC29, and MH2. (iii) A 3′ group-specific segment extended to 2.5 kb from the 3′ poly(A) end and was defined by 14 oligonucleotides including the highly conserved C oligonucleotide (10, 11, 31) near the poly(A) end. It shared 13 of these with CMIIAV and other nondefective avian tumor viruses (Table 1, Fig. 5B), thus identifying this segment as group specific. CMII oligonucleotides no. 9 and 24 of this segment had *env* gene homologs in Schmidt-Ruppin RSV (no. 4) (32) and in Prague RSV (no. 18) (33), respectively. This suggests that the 3′ segment of CMII, like its MC29 equivalent (26; Duesberg et al., Virology, in press), contains *env* gene-related sequences.

CMII and CMIIAV are isogenic in group-specific sequences. Several cloned stocks of

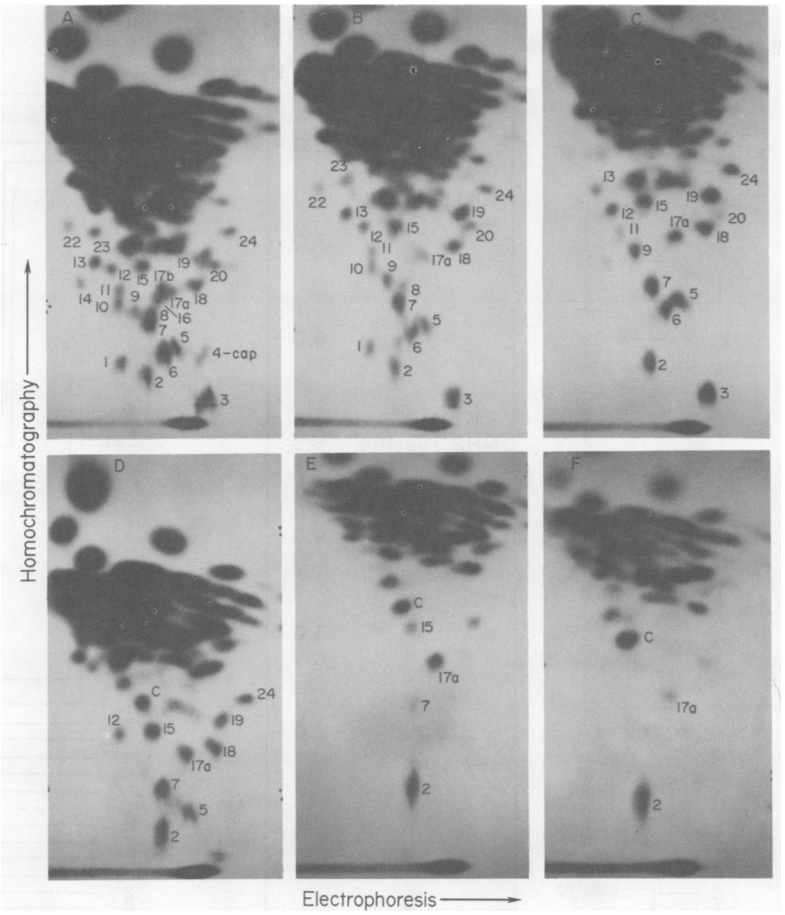

FIG. 4. *Autoradiographs of RNase T_1-digested poly(A)-containing fragments prepared from 6-kb CMII RNA after fingerprint analysis. The 6-kb [^{32}P]RNA of CMII was prepared electrophoretically from 50 to 70S CMII(CMIIAV + RPV) RNA (~32 × 10^6 cpm) as described for Fig. 1 and 3 and was alkali-fragmented. Poly(A)-containing fragments were isolated by binding to oligodeoxythymidylic acid-cellulose (Materials and Methods). They were then fractionated according to size by sedimentation in a 10 to 25% sucrose gradient in 0.01 M NaCl-0.01 M Tris (pH 7.4)-1 mM EDTA-0.1% SDS in an SW41 Beckman rotor for 7 h at 40,000 rpm and 20°C. Six RNA pools with average sedimentation coefficients of 28S (A), 26S (B), 21S (C), 18S (D), 13S (E), and 6S (F) were collected and analyzed by fingerprint analysis as described in Fig. 3. Oligonucleotides with identical composition, as determined by RNase A digestion, are given the same number in all fingerprints. Alternatively, fingerprint analysis of poly(A)-tagged fragments of 6-kb CMII RNA was carried out by heat dissociation of the 50S component of CMII(CMIIAV + RPV) [^{32}P]RNA prepared as described in Fig. 2, followed by selection of poly(A)-containing RNA fragments and fractionation in different size classes, as described above. The same oligonucleotide map was obtained by both methods.*

MC29 and MH2 have been compared to cloned stocks of MC29-associated (MCAV) and MH2-associated viruses (MH2AV) for oligonucleotide homology (10a, 11, 13; Duesberg et al., Virology, in press). Although homology was observed in all of these cases, their related, group-specific sequences, defined primarily by RNA-complementary DNA hybridization, did not share significantly more homologous oligonucleotides than the group-specific sequences of MC29 or MH2 and those of several other nondefective viruses. Hence, a given cloned helper virus, presumed to be derived from the original isolate, did not appear more closely related to the respective acute leukemia virus than many other helper viruses studied. In contrast, a much closer relationship appears to exist between the group-specific sequences of CMII and CMIIAV. All five 5′ group-specific and 13 of the 14 3′ group-specific oligonucleotides of CMII have CMIIAV homologs in equivalent map positions (Fig. 5B). The only difference, i.e., between oligonucleotide no. 102a and 12, may be due to a point mutation in one of the RNAs, because the composition of

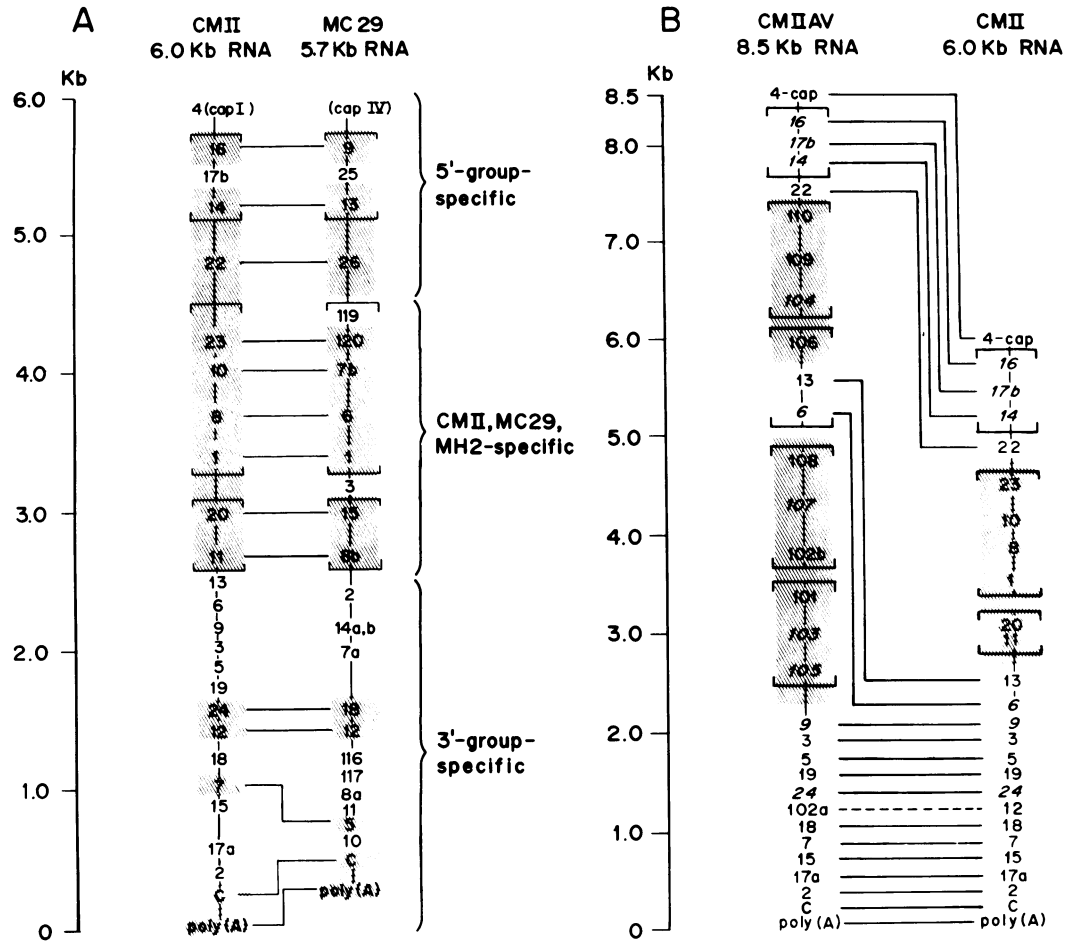

FIG. 5. *Comparison of the oligonucleotide maps of (A) 6-kb CMII RNA and 5.7-kb MC29 RNA and (B) 6-kb CMII RNA and 8.5-kb CMIIAV RNA. (A) Oligonucleotides found in 6-kb CMII RNA were ordered according to the size of the smallest poly(A)-tagged RNA fragment from which they can be isolated (Fig. 4). When the relative order of oligonucleotides is uncertain, they are shown in parentheses. The resulting oligonucleotide map of CMII is compared with the map of 5.7-kb MC29 RNA prepared from MC29(RPV) RNA (11, 26; Duesberg et al., Virology, in press). Oligonucleotides identical in composition (Table 1) are linked by solid lines, and RNA sequences shared between the two viruses are in a hatched background. Division of the MC29 and CMII oligonucleotide maps into 3' group-specific, MC29-, CMII-, and MH2-specific, and 5' group-specific segments is indicated in the margin. (B) The oligonucleotide maps of 6-kb CMII and 8.5-kb CMIIAV RNAs (prepared as described for CMII RNA [data not shown]) are compared. Oligonucleotides identical in composition are linked by solid lines, and two closely related ones are linked by a dotted line. The CMII-specific RNA sequences are shown on a hatched background in the CMII map, and CMIIAV RNA sequences not present in CMII RNA are shown on a hatched background in the CMIIAV map. Oligonucleotide markers for the gag, pol, and env genes of avian tumor viruses (see text and references 32 and 33) are depicted in italics.*

these two oligonucleotides appears to be very similar (Table 1).

Two contiguous segments of CMIIAV oligonucleotides are not present in CMII RNA. These include a conserved *pol* oligonucleotide, no. 107 (no. 20b in PR RSV) (33), and a conserved *env* oligonucleotide, no. 105 (no. 6 in PR RSV) (33). Two oligonucleotides shared between CMIIAV and CMII, i.e., no. 13 and 6, map in between the two RNA segments only present in CMIIAV RNA. One of them, no. 6, is closely related to a highly conserved *pol* oligonucleotide, no. 4, of PR RSV (33). Hence, the RNA of replication-defective CMII may be viewed as the product of two deletions of the CMIIAV RNA and the insertion of the CMII-specific sequences in place

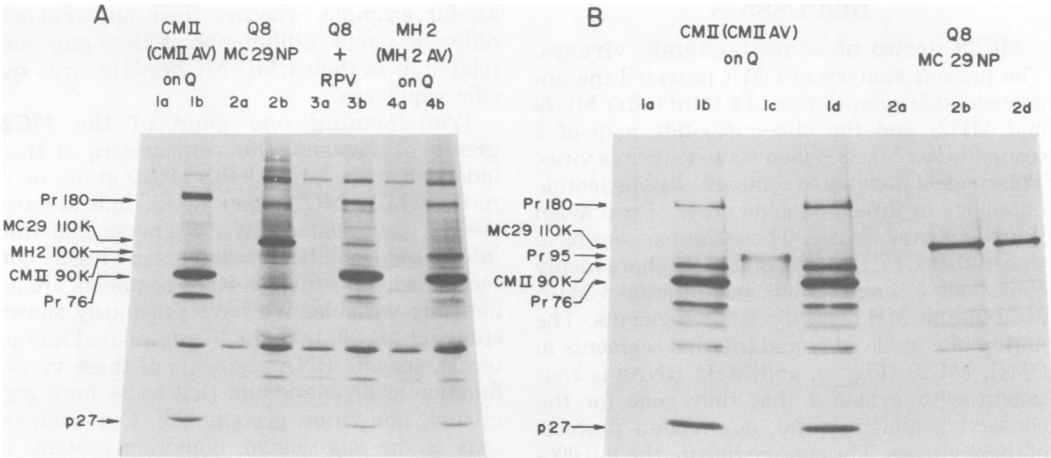

FIG. 6. *(A) Virus-specific protein synthesis in (1) CMII(CMIIAV)-infected quail cells, (2) MC29-nonproducer clone Q8, (3) MC29-nonproducer clone Q8 superinfected with RPV, and (4) MH2(MH2AV)-infected quail cells. Cells were pulse-labeled for 60 min with [^{35}S]methionine (50 μCi/ml), and detergent extracts were prepared. Immune precipitations were carried out with (a) normal rabbit serum and (b) anti-whole virus serum, with goat anti-rabbit immunoglobulin G as the second antibody. The immune precipitates were analyzed by electrophoresis on a 6 to 18% gradient SDS-polyacrylamide gel (3). Exposure time of the fluorograph was 24 h. (B) Analysis of virus-specific protein synthesis with specific antisera. (1) CMII(CMIIAV)-infected quail cells and (2) MC29-nonproducer Q8 cells were labeled and extracted as in Fig. 6A. Immune precipitates were formed with (a) normal rabbit serum, (b) anti-whole virus serum, (c) anti-glycoprotein serum, and (d) anti-p27 serum. Staphylococcus aureus was used as immune complex adsorbent. Electrophoresis was as in Fig. 6A. Exposure time of the fluorograph was 15 h.*

of the deletion near the 5′ end in CMIIAV RNA.

Identification of a nonstructural, *gag* gene-related 90,000-dalton CMII protein. Because of the close relationship between the 5′ *gag*-related and internal CMII-MC29-specific RNA segments of CMII and MC29, it may be argued that the two viruses are variants differing mainly in their 3′ group-specific segments. Studies with MC29 have shown that the 5′ *gag*-related and internal MC29-specific RNA sequences are translated into a 110,000-dalton *gag* gene-related nonstructural protein. An analogous 100,000-dalton protein was found in MH2-infected cells. In addition to their size, these proteins have other strain-specific, diagnostic markers, including serological properties (3, 19, 20, 26, 30a).

A *gag* gene-related protein of the same size class was also found in cells transformed by CMII. Figure 6A compares by electrophoresis the proteins precipitated with antibody against disrupted Rous virus from CMII-infected quail cells to those from MC29- and MH2-infected quail cells. It can be seen that a 90,000-dalton protein was present in CMII-cells that is smaller than the 110,000-dalton protein of MC29 (3, 26) and the 100,000-dalton protein of MH2 (20, 30a). This protein was not observed in cells infected only with nontransforming helper viruses, nor was it detected as a major component in virus particles (not shown; 3, 19, 26). The CMII(CMIIAV)-producing cells used in these experiments also contained helper virus coded Pr180 *gag-pol*, Pr76 *gag*, and p27 *gag* proteins, as did MC29 and MH2 virus-producing cells analyzed in parallel (Fig. 6) (3, 15, 19, 27, 28). The *gag* gene relationship of the 90,000-dalton CMII protein is demonstrated in Fig. 6B. Like the MC29 110,000-dalton protein, the CMII 90,000-dalton polypeptide was immunoprecipitated by antisera against whole virus or *gag*-specific antiserum raised against the *gag* proteins p27 and p19. It was not precipitated by antiserum against the viral glycoproteins, which only precipitates the precursor Pr95 of the *env* proteins (19); neither was it precipitated by normal rabbit serum. Other protein bands observed on the gels include host-cell proteins nonspecifically precipitated by normal as well as immune serum and intermediate products of the processing of the Pr76 *gag* precursor (3, 15, 19).

We conclude that the *gag*-related protein of CMII is analogous to the 110,000-dalton MC29 and 100,000-dalton MH2 proteins, but distinct by a strain-specific size marker. It appears probable that the 90,000-dalton CMII protein is also a nonstructural protein like its MC29 and MH2 counterparts, because such proteins are not found in viruses or in cells infected by nondefective avian tumor viruses.

DISCUSSION

MC29 group of acute leukemia viruses. The present analysis of CMII provided the oligonucleotide composition of a third (after MC29 and MH2) and the oligonucleotide map of a second (after MC29) avian acute leukemia virus. This made it possible to compare oligonucleotide homology of three and gene order of two avian acute leukemia viruses. These comparisons have shown that CMII, MC29, and MH2 share highly conserved 5′ *gag*-related, and internal CMII-, MC29-, and MH2-specific RNA segments. The notion of conserved 5′ and internal segments in CMII, MC29 (Fig. 5), and MH2 RNAs is consistent with evidence that they code for the conserved, nonstructural, *gag*-related proteins of these viruses. The gene coding for the 110,000-dalton MC29 protein was shown previously to map in the 5′ group-specific and internal-specific segment of MC29 RNA (26). Our finding of *gag*-related oligonucleotides in the 5′ segment of CMII RNA and of *gag*-related determinants in the 90,000-dalton CMII protein indicates that this protein also maps in the 5′ half of CMII RNA. The presence of the CMII-*gag* oligonucleotide no. 16 in MH2 and MC29 suggests that the 100,000-dalton MH2 protein also maps in the 5′ half of MH2 RNA. Hence, all three *gag*-related proteins form a conserved class sharing *gag*-related and presumably non-*gag*-related determinants, RNA map location, and nonstructural, possibly transforming function (26; see below). This is consistent with recent evidence that the 90,000-dalton CMII and 110,000-dalton MC29 proteins share *gag*-related as well as specific tryptic peptides (M. Hayman, personal communication). The conserved nature of their 5′ and internal RNA segments and of the *gag* gene-related proteins encoded by these sequences indicates that CMII, MC29, and MH2 are a closely related group, which, based on the prototype MC29, is termed the MC29 group of acute leukemia viruses. These viruses have also been grouped together on the basis of shared oncogenic properties (16). However, because there are significant overlaps among the oncogenic spectra of the viruses of the MC29 group and other avian acute leukemia viruses, such as avian erythroblastosis virus, and even avian sarcoma viruses (29), a biochemical definition as proposed here appears preferable to one based on biological markers.

The viruses of the MC29 group differ from one another mainly in their 3′ RNA segments and only in minor variations of their conserved 5′ and internal RNA segments. Further work will be necessary to explain such aspects of the nature of the strain-specific markers of *gag*-related, nonstructural proteins of the MC29 group as, for example, whether their apparent size differences reflect differences in their *gag* gene-related or in their CMII-MC29-MH2 virus-specific sequences.

Transforming *onc* gene of the MC29 group of viruses. Our comparisons of three independent isolates of the MC29 group of viruses, CMII, MC29, and MH2, indicate that their 5′ *gag*-related RNA segments and their internal, specific RNA segments are highly conserved, whereas their 3′ RNA segments are genetically variable. We have previously shown, studying MC29, that the 5′ *gag*-related and internal, specific RNA segments of these viruses function as a genetic unit that codes for a *gag*-related, non-virion protein (26). Our comparisons of the *gag*-related, nonvirion proteins of MC29, CMII, and MH2 indicate that these proteins are physically and serologically conserved, which is in accord with the conserved nature of their RNA templates. It follows that the conserved viral genetic unit that consists of the 5′ *gag*-related and internal RNA section and that codes for a *gag*-related, nonvirion protein correlates with the closely related oncogenic spectra of these viruses (16). Therefore, it is probable that this genetic unit is necessary for transformation and is possibly the *onc* gene of these viruses. This is further supported by the fact that in the case of CMII, part of the genetic unit, i.e., the internal, specific RNA sequence, is the only contiguous RNA sequence that is not shared with nontransforming CMIIAV.

The genetic variability among the 3′ group-specific RNA segments of the MC29 group of viruses (see also Duesberg et al., Virology, in press) suggests that these RNA segments might play an indirect or no role in cell transformation. This view is consistent with the observation that in CMII, the 3′ RNA segment is isogenic with that of the nontransforming helper virus CMIIAV. The presence of oligonucleotides related to the *env* genes of nondefective viruses in the 3′ RNA segments of CMII (Fig. 5) and MC29 (26; Duesberg et al., Virology, in press) provides a genetic basis for explaining the variability of these RNA segments by recombination with the *env* genes of helper viruses.

ACKNOWLEDGMENTS

We thank Mike Hayman for communicating to us his results on CMII and MC29 proteins prior to publication. We are grateful to Lorrine Chao for technical assistance.

This work was supported by Public Health Service grant CA-11426 from the National Cancer Institute.

LITERATURE CITED

1. **Beemon, K., P. H. Duesberg, and P. Vogt.** 1974. Evidence for crossing-over between avian tumor viruses based on analysis of viral RNAs. Proc. Natl. Acad. Sci. U.S.A. 71:4254-4258.
2. **Bishop, D. H. L., J. R. Claybrook, and S. Spiegelman.**

1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. Mol. Biol. **26**:373-387.

3. **Bister, K., M. J. Hayman, and P. K. Vogt.** 1977. Defectiveness of avian myelocytomatosis virus MC29: isolation of long-term nonproducer cultures and analysis of virus-specific polypeptide synthesis. Virology **82**:431-448.
4. **Bister, K., and P. K. Vogt.** 1978. Genetic analysis of the defectiveness in strain MC29 avian leukosis virus. Virology **88**:213-221.
5. **Bister, K., P. K. Vogt, and M. J. Hayman.** 1978. MC29, a highly defective avian acute leukemia virus, p. 57-66. *In* S. Barlati and C. de Giuli-Morghen (ed.), Avian RNA tumor viruses. Piccin Ed., Padova, Italy.
6. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83-88.
7. **Canaani, E., K. v.d. Helm, and P. Duesberg.** 1973. Evidence for 30-40S RNA as precursor of the 60-70S RNA of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **70**:401-405.
8. **Coffin, J. M., M. Champion, and F. Chabot.** 1978. Nucleotide sequence relationships between the genomes of an endogenous and an exogenous avian tumor virus. J. Virol. **28**:972-991.
9. **Delius, H., P. H. Duesberg, and W. F. Mangel.** 1974. Electron microscope measurements of Rous sarcoma virus RNA. Cold Spring Harbor Symp. Quant. Biol. **39**: 835-843.
10. **Duesberg, P. H., K. Bister, and P. K. Vogt.** 1977. The RNA of avian acute leukemia virus MC29. Proc. Natl. Acad. Sci. U.S.A. **74**:4320-4324.

10a. **Duesberg, P. H., P. Mellon, A. Pawson, K. Bister, and P. K. Vogt.** 1979. Anatomy of the RNA and gene products of MC29 and MH2, two defective avian tumor viruses causing acute leukemia and carcinoma: evidence for a new class of transforming genes, p. 241-260. *In* R. Neth and K. Mannweiler (ed.), Modern trends in human leukemia, III. Springer-Verlag, Heidelberg.

11. **Duesberg, P., P. Mellon, T. Pawson, G. S. Martin, K. Bister, and P. Vogt.** 1978. Defining transforming (*onc*) genes and gene products of avian acute leukemia and carcinoma viruses, p. 245-266. *In* J. G. Stevens, G. J. Todaro, and C. F. Fox (ed.), Persistent viruses. Academic Press Inc., New York.
12. **Duesberg, P. H., and P. K. Vogt.** 1973. RNA species obtained from clonal lines of avian sarcoma and from avian leukosis virus. Virology **54**:207-219.
13. **Duesberg, P. H., and P. K. Vogt.** 1979. Avian acute leukemia viruses MC29 and MH2 share specific RNA sequences: evidence for a second class of transforming genes. Proc. Natl. Acad. Sci. U.S.A. **76**:1633-1637.
14. **Duesberg, P. H., P. K. Vogt, K. Bister, D. Troxler, and E. M. Scolnick.** 1978. Oncogenic (*onc*) genes of sarcoma, leukemia and carcinoma viruses, p. 95-111. *In* S. Barlati and C. de Giuli-Morghen (ed.), Avian RNA tumor viruses. Piccin Ed., Padova, Italy.
15. **Eisenman, R., V. M. Vogt, and H. Diggelmann.** 1974. Synthesis of avian RNA tumor virus structural proteins. Cold Spring Harbor Symp. Quant. Biol. **39**:1067-1075.
16. **Graf, T., and H. Beug.** 1978. Avian leukemia viruses. Interaction with their target cells in vivo and in vitro. Biochim. Biophys. Acta **516**:269-299.
17. **Graf, T., B. Royer-Pokora, W. Meyer-Glauner, M. Claviez, E. Götz, and H. Beug.** 1977. *In vitro* transformation with avian myelocytomatosis virus strain CMII: characterization of the virus and its target cells. Virology **83**:96-109.
18. **Hanafusa, H.** 1977. Cell transformation by RNA tumor viruses, p. 401-408. *In* H. Fraenkel-Conrat and R. Wagner (ed.), Comprehensive virology, vol. 10. Plenum Press, New York.
19. **Hayman, M. J., K. Bister, P. K. Vogt, B. Royer-Pokora, and T. Graf.** 1978. Viral polyprotein synthesis in cells infected with avian sarcoma-leukemia viruses, pp. 214-226. *In* S. Barlati and C. de Giuli-Morghen (ed.), Avian RNA tumor viruses. Piccin Ed., Padova, Italy.
20. **Hu, S. S. F., C. Moscovici, and P. K. Vogt.** 1978. The defectiveness of Mill Hill 2, a carcinoma-inducing avian oncovirus. Virology **89**:162-178.
21. **Ishizaki, R., A. J. Langlois, J. Chabot, and J. W. Beard.** 1971. Component of strain MC29 avian leukosis virus with the property of defectiveness. J. Virol. **8**:821-827.
22. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617-1624.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
24. **Löliger, H.-C.** 1964. Experimentelle Untersuchungen zur Übertragung der Geflügelleukose. II. Serienübertragungen mit einem Myelosestamm. III. Vergleichende Untersuchungen zwischen zwei aus Spontanfällen isolierten Myelosestämmen und einem Lymphomatosestamm. Dtsch. Tieraerztl. Wochenschr. **71**:207-212.
25. **Löliger, H.-C.** 1964. Histogenetic correlations between the reticular tissue and the different types of avian leukosis and related neoplasms. Natl. Cancer Inst. Monogr. **17**:37-61.
26. **Mellon, P., A. Pawson, K. Bister, G. S. Martin, and P. H. Duesberg.** 1978. Specific RNA sequences and gene products of MC29 avian acute leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **75**:5874-5878.
27. **Oppermann, H., J. M. Bishop, H. E. Varmus, and L. Levintow.** 1977. A joint product of the genes *gag* and *pol* of avian sarcoma virus: a possible precursor of reverse transcriptase. Cell **12**:993-1005.
28. **Paterson, B. M., D. J. Marciani, and T. S. Papas.** 1977. Cell-free synthesis of the precursor polypeptide for avian myeloblastosis virus DNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **74**:4951-4954.
29. **Purchase, H. G., and B. R. Burmester.** 1972. Leukosis sarcoma group, p. 502-568. *In* M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder (ed.), Diseases of the poultry, 6th ed. The Iowa State University Press, Ames.
30. **Sheiness, D., L. Fanshier, and J. M. Bishop.** 1978. Identification of nucleotide sequences which may encode the oncogenic capacity of avian retrovirus MC29. J. Virol. **28**:600-610.

30a. **Vogt, P. K., K. Bister, S. S. F. Hu, and M. J. Hayman.** 1979. On the nature of defectiveness in avian oncoviruses MC29 and MH2, p. 83-93. *In* Y. Ikawa and T. Odaka (ed.), Oncogenic viruses and host cell genes. Academic Press Inc., New York.

31. **Wang, L.-H., P. Duesberg, K. Beemon, and P. K. Vogt.** 1975. Mapping RNase T_1-resistant oligonucleotides of avian tumor virus RNAs: sarcoma-specific oligonucleotides are near the poly(A) end and oligonucleotides common to sarcoma and transformation-defective viruses are at the poly(A) end. J. Virol. **16**:1051-1070.
32. **Wang, L.-H., P. Duesberg, S. Kawai, and H. Hanafusa.** 1976. Location of envelope-specific and sarcoma-specific oligonucleotides on RNA of Schmidt-Ruppin Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **73**: 447-451.
33. **Wang, L.-H., P. Duesberg, P. Mellon, and P. K. Vogt.** 1976. Distribution of the envelope-specific and sarcoma-specific nucleotide sequences from different parents in the RNAs of avian tumor virus recombinants. Proc. Natl. Acad. Sci. U.S.A. **73**:1073-1077.
34. **Wang, L.-H., P. H. Duesberg, T. Robins, H. Yokota, and P. K. Vogt.** 1977. The terminal oligonucleotides of avian tumor virus RNAs are genetically linked. Virology **82**:472-492.