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Specific RNA sequences and gene products of MC29 avian acute leukemia virus

(defective RNA tumor virus/oligonucleotide mapping/cell-free translation/transforming genes)

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ABSTRACT **The 28S RNA of the defective avian acute leukemia virus MC29 contains two sets of sequences: 60% are hybridized by DNA complementary to other avian tumor virus RNAs (group-specific cDNA) and 40% are hybridized only by MC29-specific cDNA. Specific and group-specific sequences of viral RNA, defined in terms of their large RNase T_1-resistant oligonucleotides, were located on a map of all large T_1 oligonucleotides of viral RNA. Oligonucleotides representing MC29-specific sequences of viral RNA mapped between 0.4 and 0.7 unit from the 3′-poly(A) end. Oligonucleotides of group-specific sequences mapped between 0 and 0.4 and between 0.7 and 1 map unit. Cell-free translation of viral RNA yielded three proteins with approximate molecular weights of 120,000, 56,000, and 37,000, termed $P120^{mc}$, $P56^{mc}$, and $P37^{mc}$. $P120^{mc}$ contained both MC29-specific peptides and serological determinants and peptides of the conserved, internal group-specific antigens of avian tumor viruses. $P120^{mc}$ is translated only from full-length 28S RNA. Furthermore, MC29 RNA contains sequences related to the group-specific antigen gene (*gag*), near the 5′ end, which are followed by MC29-specific sequences. We conclude that this protein is translated from the 5′ 60% of the RNA, and that it includes a segment translated from the specific sequences. It is suggested that the transforming (*onc*) gene of MC29 may consists of the specific and some group-specific RNA sequences and that $P120^{mc}$, which is also found in transformed cells, may be the *onc* gene product.**

MC29 is an avian RNA tumor virus that causes acute leukemia and carcinoma and also transforms fibroblasts in culture (1–4). The transforming or *onc* (5) gene of MC29 has not been defined genetically or biochemically. The viral genome is a 28S RNA (5700 nucleotides) that is identified as being MC29-specific by its absence from pure helper virus and because the sequence of 28S RNA is conserved when propagated with different helper viruses (6–8). It contains two sets of sequences: 60% are hybridized by DNA complementary to other avian tumor virus RNAs (group-specific cDNA) and 40% are hybridized only by MC29-specific cDNA (6–8). It has been suggested that this virus contains a specific *onc* gene, because it does not contain sequences related to *src*, the *onc* gene of Rous sarcoma virus (RSV) (6–9).

The purpose of this study is to biochemically define the *onc* gene of MC29 and to compare it to the *src* gene of RSV. The *src* gene has been unambiguously defined by analyses of *src* deletion mutants and *src* recombinants as a contiguous sequence of about 1500 nucleotides that segregates with sarcomagenicity (10, 11, 12). The *src* gene was mapped near the 3′ end of RSV RNA (11, 13) and appears to be translated into a protein of 60,000 daltons (14, 15). However, due to the defectiveness of MC29 in all three replicative genes (3, 4, 16) termed *gag* (for internal group-specific antigens), *pol* (for DNA polymerase) and *env* [for envelope glycoprotein (5)], *onc* deletion mutants and recombinants of MC29 would lack biologically detectable genetic markers in replicative genes. Moreover, the specific sequences of MC29 are not expected to recombine readily with other avian tumor viruses lacking them, because analysis of tumor virus recombination has demonstrated that efficient recombination only occurs between closely related, allelic sequences (17). Therefore, the approach that was used to define *src* of RSV cannot yet be used to define the *onc* gene of MC29 or to define the *onc* genes of other defective transforming viruses.

However, if the specific sequences of MC29 are contiguous and if they code for a specific protein, they are candidates for a MC29 *onc* gene analogous to the *src* gene of RSV. Therefore we identified and located the MC29-specific sequences on the viral 28S RNA and then investigated the proteins encoded by these sequences. Preliminary work has been described recently (8).

RESULTS

Mapping MC29-Specific and Group-Specific Sequences of MC29 RNA. Due to its defectiveness, MC29 virus can only be propagated in the presence of a helper virus. The helper virus used here was ring-neck pheasant virus (RPV) (4). The 50–70S RNA extracted from a mixture of MC29 and its helper contains two distinct monomer RNA species, a 34S helper virus RNA and a 28S MC29 RNA (6). The 28S MC29 RNA has been electrophoretically isolated and its RNase T_1-resistant oligonucleotides have been analyzed by fingerprinting (Fig. 1*A*). The compositions of the RNase A-resistant fragments of each oligonucleotide have been reported (6–8) and were extended and revised here as described in the legend of Fig. 1. To locate MC29-specific and group-specific sequences on MC29 RNA, the following strategy was used: RNA segments were defined in terms of their RNase T_1-resistant oligonucleotides. The location of a given oligonucleotide on the RNA (oligonucleotide map) was then deduced from the size of the smallest poly(A)--tagged RNA fragment from which the oligonucleotide could be obtained (Fig. 2 and ref. 13).

MC29-specific RNA segments were recovered from RNA·DNA hybrids formed between viral RNA and MC29-specific cDNA. MC29-specific cDNA was prepared by hybridizing MC29(RPV) cDNA to an excess of unlabeled RNA of RPV and of Prague RSV of subgroup B (PR-B) under conditions of moderate stringency (36) (Fig. 1). In this way all but the MC29-specific sequences of this cDNA were converted to heteroduplexes, leaving only MC29-specific cDNA single-

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Abbreviations: RSV, Rous sarcoma virus; RPV, ring-neck pheasant virus; PR-B, Prague RSV of subgroup B.

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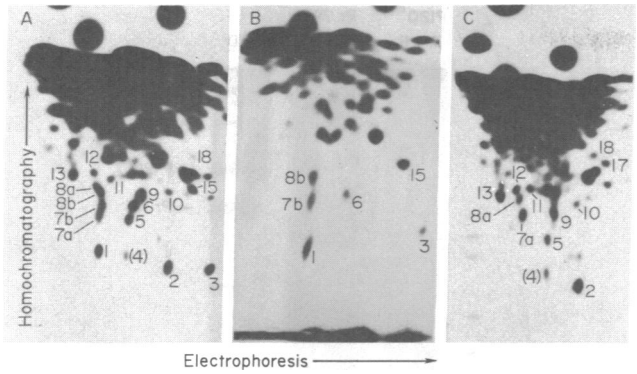

FIG. 1. RNase T_1-resistant oligonucleotides of whole 28S MC29 RNA (*A*), of MC29 RNA sequences that are not sequence-related to other avian tumor virus RNAs (MC29-specific) (*B*), and of MC29 RNA sequences that are sequence-related to other avian tumor virus RNAs (group-specific) (*C*). (*A*) 28S MC29 [^{32}P]RNA was prepared electrophoretically from 50–70S MC29(RPV) RNA (6–8). After partial fragmentation and removal of poly(A)-tagged fragments to derive an oligonucleotide map (see Fig. 2), the RNA was digested with RNase T_1 and subjected to 2-dimensional electrophoresis/homochromatography as described (6, 13). Oligonucleotides were numbered as previously and their RNase A-resistant fragments were confirmed (6–8). The composition of no. 10 was revised to 3 U, 2 C, 2 (AU), (AG), (AAU) and that of no. 15 to 4 U, 4 C, G, (AC), 2 (AU), (AAU). The double spots nos. 7 and 8 were resolved by fingerprinting poly(A)-tagged RNA fragments and in fingerprints such as *A* into distinct oligonucleotides denoted alphabetically (Figs. 1 and 2). Their compositions were 7a: 2 U, 6 C, G, 2 (AC), (AU), (AAC); 7b: 3 U, 8 C, (AC), (AAG); 8a: 2 U, 2 C, G, 2 (AC), (AAAC); 8b: 2 U, 4 C, (AC), (AG), (AAU), (AAAC). Oligonucleotide no. 4 is in parentheses because it is thought to derive from contaminating RPV RNA (6) rather than from MC29 RNA. (*B*) To prepare MC29-specific sequences from MC29 RNA, 50–70S MC29(RPV) [^{32}P]RNA was hybridized to MC29-specific cDNA. MC29-specific cDNA was made by incubating 2 μg of MC29(RPV) cDNA (6–8) with 15 μg of RPV RNA and 12 μg of RNA from Prague subgroup B RSV (PR-B) for 12 hr at 40°C in 10 μl of 50% (wt/vol) formamide containing 0.45 M NaCl, 0.045 M sodium citrate, and 0.01 M sodium phosphate at pH 7.0. Subsequently, 1.5 μg of MC29(RPV) [^{32}P]RNA (5×10^6 cpm/μg) was added in 20 μl of the above formamide buffer and incubation was continued for 1 hr. After digestion for 30 min at 40°C in 200 μl of 0.3 M NaCl/0.03 M sodium citrate containing 5 μg of RNase A and 50 units of RNase T_1 per ml, the resistant hybrid was isolated from the void volume of a Bio-Gel P-100 column (12×0.6 cm) equilibrated in 0.1 M NaCl/0.01 M Tris·HCl, pH 7.4/1 mM EDTA/0.2% sodium dodecyl sulfate. The hybrid was extracted three times with phenol in the presence of 30 μg of carrier yeast tRNA, then ethanol-precipitated, heat-dissociated in buffer of low ionic strength, digested with RNase T_1, and subjected to fingerprint analysis as above. The oligonucleotides from MC29-specific RNA segments so identified are underlined in the oligonucleotide map shown in Fig. 2. (*C*) To prepare avian tumor virus group-specific sequences of MC29 RNA, 0.25 μg of electrophoretically prepared 28S MC29 [^{32}P]RNA (as in *A*) (2×10^6 cpm/μg) was hybridized with 1 μg of PR-B and 1 μg of RPV cDNA for 12 hr in 25 μl of 70% formamide/0.15 M NaCl/0.03 M sodium citrate/0.02% sodium dodecyl sulfate/0.01 M sodium phosphate, pH 7.0. The reaction product was heated to 50°C for 1 min in 0.15 M NaCl/0.015 sodium citrate, pH 7.0, and treated with RNase T_1 (but not with RNase A) and otherwise as described for *B*.

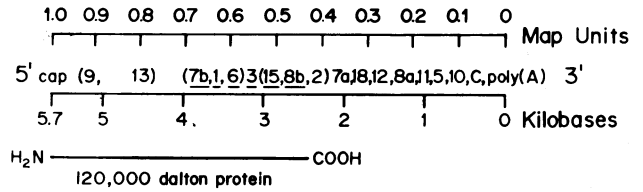

FIG. 2. Oligonucleotide map of 28S MC29 RNA. The 28S MC29 [^{32}P]RNA (about 6×10^6 cpm) was prepared electrophoretically from 50–70S MC29(RPV) RNA (6). The RNA was degraded by incubating three equal aliquots for 3, 6, and 9 min, respectively, in 0.05 M Na_2CO_3 at pH 11 and 50°C. Fragments were combined and poly(A)-tagged species was selected on oligo(dT)-cellulose and fractionated into different size classes as described (13). The T_1 oligonucleotides of six size classes of RNA fragments differing by approximately 1000 nucleotides from each other were fingerprinted (not shown). Oligonucleotides of fragments were identified by their chromatographic properties and by their RNase A-resistant fragments (Fig. 1). The resulting order of oligonucleotides is plotted on two scales, one denoting the approximate distance of an oligonucleotide from the 3′ poly(A) coordinate in kilobases, the other denoting it in relative map units. When the relative order of oligonucleotides is uncertain, they are shown in parentheses. Oligonucleotides from strain-specific sequences of MC29 RNA (Fig. 1*B*) are underlined and those from group-specific sequences (Fig. 1*C*) are not underlined. The bottom line represents the MC29-specific protein $P120^{mc}$. It is aligned colinearly with the RNA segment from which it was probably translated, on the basis of data described in the text.

stranded. This cDNA was then hybridized in a second step with 50–70S MC29(RPV) [^{32}P]RNA under essentially the conditions described above. However, incubation was for a shorter time, to minimize displacement of unlabeled RNA from heteroduplexes present in our preparation of MC29-specific cDNA by related or identical sequences of MC29 [^{32}P]RNA. After digestion of unhybridized MC29(RPV) [^{32}P]RNA with RNases A and T_1, hybridized RNA was recovered and digested with RNase T_1. The resulting MC29-specific, RNase T_1-resistant oligonucleotides were detected by fingerprint analysis and their RNase A-resistant fragments were determined (Fig. 1*B*).

The remaining oligonucleotides of MC (34) 29 RNA were expected to be derived from RNA segments that are sequence related to the RNAs of other members of the avian tumor virus group. These group-specific RNA sequences were isolated as follows: 28S MC29 [^{32}P]RNA, prepared electrophoretically from 50–70S MC29(RPV) RNA (7), was hybridized to an excess of PR-B and RPV cDNA as above (Fig. 1*C*). After the hybridization reaction, unhybridized RNA was digested with RNase T_1 alone. RNase A was not used in order to preserve small MC29-specific oligonucleotide segments that are part of larger, group-specific polynucleotide segments of MC29 RNA hybridized with PR-B and RPV cDNAs. Group-specific sequences of avian tumor virus RNAs defined by hybridization are known to differ if compared by T_1 oligonucleotide fingerprints (11, 13, 18). This is because fingerprinting detects specific oligonucleotides even in RNA sequences that differ by only a few percent of their nucleotides and that are closely related if compared by RNA·cDNA hybridization. Because neither RPV nor PR-B is an immediate predecessor of MC29, it was to be expected that the hybrids formed between MC29 RNA and PR-B and RPV cDNAs would contain small mismatches. Mismatches involving oligonucleotide segments with fewer than two Gs would register as complete hybrids in our conditions. The T_1 oligonucleotides of the resulting hybrid are shown in Fig. 1*C*. They represent RNA sequences of MC29 RNA that are closely related to, but not identical with, sequences of PR-B and RPV RNA. It can be seen in Fig. 1 (and Fig. 2) that the T_1 oligonucleotides of MC29 RNA fall into two nonoverlapping sets, those representing MC29-specific and those representing group-specific sequences of MC29 RNA.

An oligonucleotide map of 28S MC29 RNA is shown in Fig. 2. It can be seen that the specific oligonucleotides (underlined in Fig. 2) map together between 0.4 and 0.7 units. Oligonucleotides of group-specific sequences (not underlined) are found at the 5′ end and in the 3′ half of viral RNA. The 3′ half contained oligonucleotide no. 11, a conserved oligonucleotide probably belonging to the *env* gene (11–13, 18, 19) and the highly conserved C oligonucleotide, which maps near the 3′ end of avian tumor virus RNAs (13). The group-specific sequences of the 5′ end include one oligonucleotide, no. 9, previously identified as a conserved element of the *gag* gene of other avian tumor viruses (18, 19). It is conceivable that this oligonucleotide

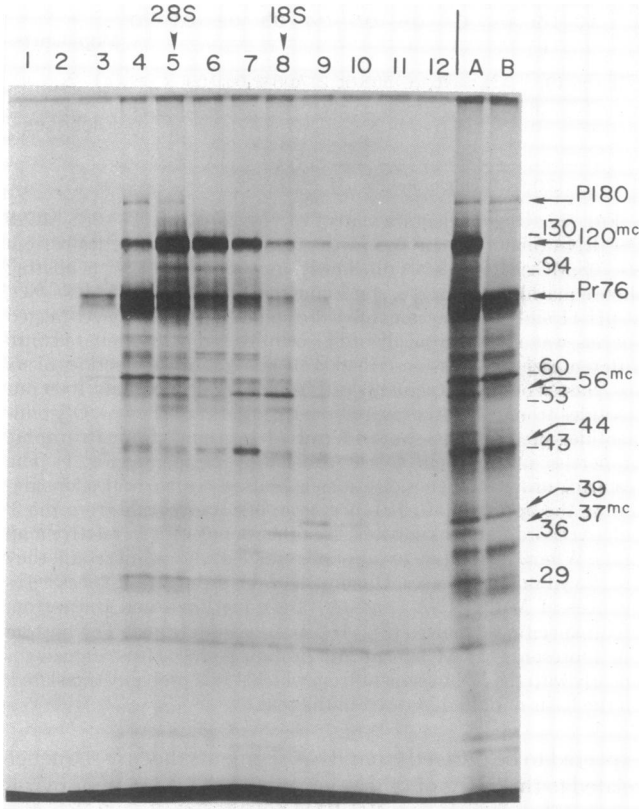

FIG. 3. Electrophoretic analysis of *in vitro* translation products of viral RNAs. RNAs were translated in the messenger-dependent rabbit reticulocyte lysate. [^{35}S]Methionine was present at 400 μCi/ml (600–1200 Ci/mmol) (1 Ci = 3.7 × 10^{10} becquerels). Products were analyzed by electrophoresis in the presence of sodium dodecyl sulfate on a 12.5% polyacrylamide slab gel in which the ratio of acrylamide to bisacrylamide was 120:1. The gel was autoradiographed after fixing and drying (20). Tracks A and B show the products of total heat-denatured, poly(A)-selected (13), 50–70S virion RNAs from MC29(RPV) and RPV (track A), and from RPV alone (track B). Tracks 1–12 represent the products of the MC29(RPV) RNA used in track A after fractionation on a density gradient. A total of 25 μg of poly(A)-selected MC29(RPV) RNA was centrifuged on a 12-ml 15–30% (vol/vol) glycerol gradient containing 0.1 M NaCl, 0.01 M Tris·HCl at pH 7.5, 1 mM EDTA, and 0.1% sodium dodecyl sulfate for 5 hr at 40,000 rpm in a Beckman SW 41 rotor at 20°C. RNA in μg per fraction was 1, 0.3; 2, 0.09; 3, 1.8; 4, 3.0; 5, 3.2; 6, 2.9; 7, 2.9; 8, 2.7; 9, 2.5; 10, 2.2; 11, 1.6; 12, 1.2. The RNA of each fraction was precipitated with ethanol in the presence of 20 μg of yeast RNA, and 1/40th of each fraction was added to the cell-free system. Each track shows the products of the fraction of the gradient corresponding with the track number shown on the gel. The positions of 18S and 28S RNA markers from a parallel gradient are shown. The migration of molecular weight markers, which were located by Coomassie blue staining, are indicated by horizontal bars, by which the molecular weight × 10^{-3} of each is given. Cell-free products of interest are indicated by arrows.

might be derived from a fragment of 34S RPV RNA present in our pool of 28S RNA. Because this *gag* oligonucleotide maps near the 5′ end of viral RNA, a RPV RNA fragment carrying no. 9 would have to lack a segment at its 3′ end in order to electrophorese with 28S RNA. Therefore a pool of poly(A)-selected (13) 28S MC29 RNA (recovery 50%) was fingerprinted. The result that oligonucleotide no. 9 was present (not shown) indicated that it was an integral part of 28S MC29 RNA.

***In Vitro* Translation of MC29 RNA.** To identify the products of the MC29 RNA genome, the RNA was translated in a cell-free system. By using this technique it has been possible to identify the *gag*, *pol*, *src*, and possibly also the *env* gene products of RSV (15, 20–22). This technique also allows us to define that region of the genome from which proteins are made by

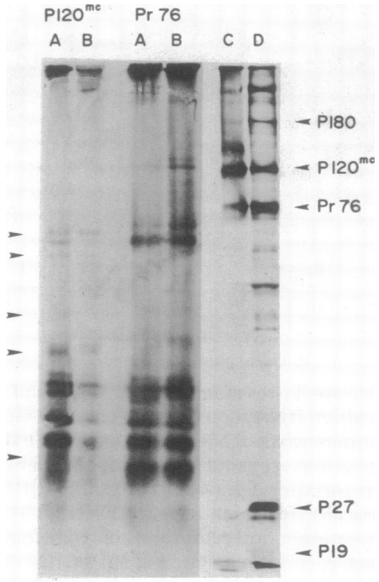

FIG. 4. Peptide analyses of viral proteins P120^{mc} and Pr76 and electrophoretic comparison between P120^{mc} made *in vitro* and its presumed counterpart from infected cells. P120^{mc} synthesized from MC29 RNA and Pr76 synthesized from transformation-defective PR-B RSV RNA were purified by electrophoresis on a 10% polyacrylamide slab gel, located by autoradiography, excised from the gel, and applied in the gel slice to slots in a new 15% polyacrylamide gel. They were then digested *in situ* with 500 ng (slots A) or 50 ng (slots B) of *Staphylococcus aureus* V8 protease, and the digestion products were analyzed by polyacrylamide gel electrophoresis as described (27). The arrows mark P120^{mc}-specific peptides. Track C shows the cell-free products of MC29(RPV) and RPV RNA (as in Fig. 3, track A), and track D shows the proteins precipitated with antiserum prepared against disrupted avian myeloblastosis virus (which contains mainly antibody to *gag* protein) from MC29(RPV)-infected cells. Immunoprecipitations were carried out as described (14). The viral proteins precipitated by antiserum to avian myeloblastosis virus were not precipitated by control serum, and P120^{mc} was not synthesized in RPV-infected cells (not shown).

analyzing the sizes of the RNAs that code for the various products. This latter definition is based on the observation that translation initiates near the 5′ end of eukaryotic mRNAs (23–25) and on the fact that all viral RNAs tested have a common 3′ end because they have been poly(A)-selected (13).

MC29(RPV) 50–70S RNAs were heat-denatured, poly(A)-selected, and translated in the messenger-dependent rabbit reticulocyte lysate. Products were analyzed by polyacrylamide gel electrophoresis. To identify the products specified by the MC29 RNA, the products of total poly(A)-selected MC29(RPV) RNAs (Fig. 3, track A) were compared with those of RPV RNA alone (Fig. 3, track B). A number of products were common to the two tracks, and these were assumed to be products of the RPV RNA. These include a 180,000 molecular weight polypeptide (P180), which is believed to be the *gag-pol* gene product (22), and Pr76, the primary product of the *gag* gene (20, 21, 26). A number of smaller products, most of which are immunoprecipitated by antiserum to the *gag* gene protein, P27, were synthesized only from full-length (30–40S) RNA, suggesting that they are premature termination products of the *gag* gene. Other smaller products common to MC29(RPV) and RPV are believed to represent products of the *env* and *C* regions of viral RNA (unpublished).

In addition to these products there were three polypeptides that are specific to MC29 RNA. They have molecular weights of 120,000, 56,000, and 37,000 on this gel system, and will be denoted as P120^{mc}, P56^{mc}, and P37^{mc} (Fig. 3). P120^{mc} was a major product of the MC29(RPV) RNA mixture, and was synthesized with the same order of efficiency as Pr76 (because

they have similar numbers of methionine residues; see below). A protein of similar size was recently found in MC29-infected cells and was shown to contain serological determinants of the viral *gag* gene proteins (4). To test the relationship of the two proteins, P120^{mc} was compared electrophoretically to its presumed counterpart precipitated from MC29-infected cells with antiserum prepared against disrupted avian myeloblastosis virus, which includes antibody to *gag* proteins. It can be seen that the two proteins were electrophoretically identical (Fig. 4 *C* and *D*). In addition, P120^{mc} synthesized *in vitro* was specifically immunoprecipitated by antiserum to P27 of avian myeloblastosis virus, the major *gag* gene protein, indicating that it contains determinants of *gag* proteins (not shown). P120^{mc} was not recognized by antisera against products of the *pol* and *env* genes (not shown). The same is true for the *in vivo* counterpart (4).

The partial proteolysis technique of Cleveland *et al.* (27) has been used to compare P120^{mc} biochemically with the primary *gag* gene protein, Pr76, synthesized *in vitro* from RPV or transformation-defective (td) PR-B RSV RNAs. Polypeptide bands were prepared electrophoretically and digested *in situ* with *Staphylococcus aureus* V8 protease (27). Fig. 4 shows that of 12 digestion products produced when P120^{mc} is digested, 7 are also found with Pr76, indicating that P120^{mc} must contain sequences encoded by the *gag* gene. These are derived from the *gag* proteins P19 and P27 (not shown). P27 in particular is rich in methionine, accounting for the more intense exposure of these bands. The presence of peptides in Pr76 that are not found in P120^{mc} is consistent with the suggestion (4) that part of the *gag* gene is missing from that region of the MC29 genome coding for P120^{mc}. The observation that five P120^{mc} digestion products (marked by arrows in Fig. 4) were produced that are unique to P120^{mc} suggests that it contains polypeptide sequences not coded for by the *gag* gene. We conclude that the P120^{mc} translated *in vitro* from viral RNA and that found in MC29-infected cells are probably the same and that P120^{mc} contains both *gag*-related and MC29-specific peptides.

In an attempt to define which regions of the MC29 or RPV RNAs code for which polypeptides, we investigated the sizes of the RNAs coding for the various products produced when MC29(RPV) RNAs are translated in the reticulocyte lysate. Total poly(A)-selected, heat-denatured MC29(RPV) RNAs were separated on the basis of size by density gradient sedimentation. The RNA from each gradient fraction was then translated in the reticulocyte lysate, and the products were analyzed by polyacrylamide gel electrophoresis as shown in Fig. 3. The positions of 18S and 28S marker RNAs are shown. Both Pr76 and P180 were clearly synthesized from 34S RPV RNA (Fig. 3, fraction 4). A 120,000 molecular weight polypeptide was synthesized from 28S RNA (fraction 5). Because, on a molar basis, 28S RNA was by far the most efficient template for the synthesis of P120^{mc}, we conclude that this protein was translated from full-length 28S MC29 RNA. Translation of P120^{mc} from smaller RNA species is thought to reflect trailing of 28S MC29 RNA in the gradient and perhaps the presence of degraded RNA. Minor RNA species are readily detected by this technique because *in vitro* translation is a very sensitive assay for template-active RNA species. A minor polypeptide with a molecular weight of 53,000 was also synthesized from 28S MC29 RNA (Fig. 3) and is thought to be a premature termination product of P120^{mc}, because it was immunoprecipitated with antiserum to P27. A 56,000 molecular weight MC29-specified polypeptide was synthesized with approximately equal efficiency from fractions 7 and 8 (18–24S RNA), and a 37,000 molecular weight polypeptide from 15–18S RNA. This latter polypeptide (P37^{mc}) is immunoprecipitated by anti-gp85 serum, which suggests that it may be the product of a defective *env* gene (not shown). The nature of the 56,000 molecular weight polypeptide specified by 18–24S MC29 RNA is as yet unclear.

DISCUSSION

Correlating MC29 RNA Sequences and Translation Products. The finding that specific sequences of MC29 map together on viral RNA suggests that they code for a specific gene product, rather than being scattered strain-specific elements of MC29 RNA. Assuming colinearity of viral RNA and proteins, our data indicate that this product is the P120^{mc} protein. We propose that P120^{mc} is translated from the 5′ 60% of the viral RNA (as depicted in Fig. 2), which includes *gag* sequences and specific sequences of MC29 RNA, for the following reasons: (*i*) About 3600 nucleotides are required to code for P120^{mc}. An MC29 oligonucleotide, no. 9, which was previously identified as a highly conserved element of the *gag* gene of other avian tumor viruses, maps near the 5′ end of MC29 RNA and is followed within a 3600-nucleotide distance by MC29-specific oligonucleotides. (*ii*) The P120^{mc} contains unique as well as *gag* protein-related peptides, as would be expected for a protein translated from the 5′ 60% of MC29 RNA. The unique peptides probably represent non-*gag* peptides, although they may derive from strain-specific elements of the defective *gag* gene of MC29. The presence of non-gag peptides in P120^{mc} is also suggested by its size, which exceeds that of Pr76, the primary *gag* gene product, by approximately 44,000 daltons. (*iii*) The observation that only full-length MC29 RNA can be translated into P120^{mc} also argues for a 5′ map location of this protein, because eukaryotic mRNAs use effectively but one initiation site near the 5′ terminus (23–25). (*iv*) P120^{mc} contains determinants and peptides of the *gag* proteins P27 and P19, which are known to map at the NH_2-terminal end of *gag* (26) and thus are coded for by sequences that usually map at the 5′ end of avian tumor virus RNAs (18, 19).

Because the virus does not express replication genes, we can only speculate on the function of the group-specific sequences of MC29 RNA. Some of these sequences must play direct roles in virus replication, providing specific sites for packaging of viral RNA by helper virus proteins, for reverse transcription of viral RNA, and for dimer linkage of 28S RNA monomers. The *gag*-related, group-specific sequences of MC29 are translated into P120^{mc} and may as such be involved in transformation (see below). Further analyses of P56^{mc} and P37^{mc} are necessary to determine whether their sequences overlap with P120^{mc} or with each other; it is possible that they correspond to distinct segments of viral RNA, because MC29 RNA may code for approximately 200,000 daltons of protein.

What Is the *onc* Gene of MC29? There are two different hypotheses as to which sequences on the MC29 RNA genome represent the *onc* gene. One suggests that the MC29-specific sequences constitute the *onc* gene, while the alternative suggests that the defective replicative genes present in MC29 RNA function as transforming genes.

We favor the idea that specific RNA sequences apparently unrelated to the replicative genes might be specific *onc* genes. This is proven for RSV (10–13) and has also been postulated for an avian leukemia virus, MH2 (7, 8), as well as for defective murine sarcoma viruses (28–30) and the Friend murine leukemia virus (7, 31). These studies have also suggested that, within a given RNA tumor virus family, specific sequences and probably transforming genes may differ, whereas replicative genes are relatively conserved. The finding that MC29-specific and group-specific sequences are translated into a specific protein P120^{mc} and that the same protein is also found in transformed cells (4) [and not in large quantity in the virion (unpublished)] suggests that this protein may be involved in cell transformation. A possibly analogous nonstructural protein of 120,000 daltons that contains *gag* gene-related and specific peptides has been found in cells transformed by the defective avian MH2 (35) and Abelson murine leukemia viruses (32). Our

findings that MC29 contains a specific RNA segment and produces a specific nonstructural protein product are consistent with the existence of a specific *onc* gene in MC29. This gene would differ from that of avian sarcoma viruses in its sequence (2, 6, 9, 10), location (12, 13), and mode of expression (14). This also corresponds to the phenotypic differences observed between RSV- and MC29-transformed fibroblasts (33).

Further work is necessary to determine whether the 56,000 and 37,000 molecular weight proteins synthesized *in vitro* are also synthesized and functional, and perhaps also required for transformation, in MC29-infected cells. While a definite answer to the question of whether the specific sequences of MC29 and the $P120^{mc}$ are involved in transformation can only be given if genetic variants of MC29 become available, our data allow us to conclude that the *onc* genes and gene products in two different prototypes of the avian tumor virus family—i.e., MC29 and RSV—are different. Thus, it is possible that the mechanisms of transformation are also different.

Note Added in Proof. A preliminary comparison of the peptides of $P120^{mc}$ generated by proteolytic cleavage with *Staphylococcus aureus* V8 protease indicates that $P120^{mc}$ synthesized *in vitro* and in infected cells (see Fig. 4) share their methionine-containing peptides. This provides further evidence that they are similar or identical.

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