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# Avian Acute Leukemia Virus OK10 Has an 8.2-Kilobase Genome and Modified Glycoprotein gp78

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We have analyzed the structure of OK10-BM virus, an avian acute leukemia virus produced by a bone marrow-derived cell line of macrophage origin, and compared it with that of OK10 AV, an associated virus originally present in the OK10 virus stock. The RNAs of OK10-BM virus and OK10 AV had the same mobility in agarose gels, corresponding to 8.0 to 8.5 kilobases, a size considerably larger than that of the transforming component (5 to 6 kb) of most other avian acute leukemia viruses. Fingerprint analysis showed a close relationship between OK10-BM virus and OK10 AV RNAs. The polypeptide compositions of OK10-BM and OK10 AV viruses were similar except for the envelope glycoproteins. In analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the large envelope glycoprotein of OK10-BM virus migrated at $M_r$ = 78,000 (gp78), whereas OK10 AV had the characteristic 85,000-dalton glycoprotein (gp85) of nondefective avian leukemia viruses. gp78 was weakly labeled with methionine, glycine, proline, or mannose, suggesting that purified OK10-BM virus had reduced amounts of the modified envelope glycoprotein. In cell-free rabbit reticulocyte lysates, OK10-BM virion RNA directed the synthesis of a 200,000-dalton polypeptide (p200), a 180,000-dalton polypeptide (pr180), and a 76,000-dalton polypeptide (pr76), whereas OK10 AV RNA gave rise only to pr180 and pr76, suggesting that p200 may represent an OK10-BM-encoded transforming protein. No biochemical evidence for the presence of an associated helper virus was found in the OK10-BM virus population produced by the macrophage cell line. However, when OK10-BM virus was serially passaged in chicken embryo fibroblasts, a virus having structural properties similar to those of OK10 AV (OK10 AV-specific oligonucleotides and gp85) appeared after three passages. Moreover, nonproducer clones of transformed cells could be readily obtained in OK10-BM virus-infected quail cell cultures. It is thus likely that the bone marrow-derived macrophage cell line produces a transforming virus defective in its *env* gene and low amounts of an associated helper virus, which upon transfer to fibroblasts is preferentially replicated.

Avian acute leukemia viruses cause rapid hematopoietic disorders and malignancies and various other types of neoplasms, such as renal and hepatic tumors, including carcinomas, in vivo. These viruses transform in vitro specific hematopoietic cells and in many cases also transform fibroblasts (18).

Three types of viral oncogenes of cellular origin, *erb* (erythroblast specific), *mac* (macrophage), and *myb* (myeloblast), are specifically involved in transformation of hematopoietic cells by the avian acute leukemia viruses (1, 38, 39). Oncogene-encoded transforming proteins found in target cells infected by these viruses are thought to be the mediators of transformation induced by the avian acute leukemia viruses (17-19, 22). The detailed mechanism by which oncogenes of the avian acute leukemia viruses and their oncogene-encoded transforming proteins act is, however, still undiscovered (2).

Avian acute leukemia viruses have been divided into three groups, i.e., the AEV (avian erythroblastosis virus), the AMV (avian myeloblastosis virus) and the MC29 (avian myelocytomatosis) groups of viruses, based on their oncogenic spectrum and the above-mentioned oncogenes and transforming proteins. The genetic structures of these viruses are similar in that they are defective in all of the structural genes. Their genomes usually consist of the 5′ end of the *gag* gene, a specific internal sequence, the oncogene, and part of the 3′-located *env* and c region (3). Because of defectiveness in the genes needed for virus replication and assembly, the

acute avian leukemia viruses require a replication-competent helper virus, usually a lymphoid leukosis virus, to provide these functions (3, 4, 7, 18, 26).

OK10 virus, first isolated from an embryonated egg of a leukemic hen (31), induces multiple tumors in the mesentery, kidneys, liver, and testes after a short latency period and transforms both macrophages and fibroblasts in culture (1, 23, 31). Biologically, OK10 virus has been classified as an avian acute leukemia virus. The biological properties and *mac* gene-specific nucleotide sequences in its genome have indicated that OK10 virus is a member of the avian myelocytomatosis group of viruses, which includes the MC29, CMII, and MH2 viruses (1, 5, 6, 13, 19, 39). The RNA genomes of the MC29 group of viruses have been shown to be about 5 to 6 kilobases (kb) in size (11–13, 24, 25) and to be deleted in all three structural genes, *gag*, *pol*, and *env* (3).

We have established a bone marrow-derived stable cell line, OK10-BM, which continuously produces infectious transforming OK10 virus. The OK10-BM cell line has characteristics of transformed macrophage-like cells (36). We describe here the characterization of the molecular properties of OK10 virus grown in its target cells, OK-BM cells. This virus has, unlike the other viruses of the MC29 group, a large 8.2-kb genome and a modified glycoprotein, gp78.

## MATERIALS AND METHODS

**Cell and viruses.** The macrophage-like bone marrow-derived cell line OK-BM, producing transforming avian acute leukemia virus (OK10-BM virus), and chicken fibroblasts, both derived from Brown Leghorn chickens (C/O or C/E cell type), were grown as described previously (36). Before the present experiments, OK10-BM cells had been cultured for 350 passages.

OK10 AV virus, subgroup A, an associated virus isolated from the original OK10 virus stock (31), Rous sarcoma virus (RSV) Prague A, MC29 (RAV-2) virus (kindly provided by Thomas Graf, German Cancer Center, Heidelberg, West Germany), and MH2 (RAV-3) virus (kindly provided by L. Payne, Houghton Poultry Research Station, Huntingdon, England) were grown as described previously (36).

**Cultivation and purification of radioactively labeled virus.** Exponentially growing OK-BM cells and virus-infected chicken embryo fibroblast cultures were labeled for RNA analyses with $^{32}P_i$ (300 to 500 $\mu$Ci/ml) or [$^3$H]uridine (50 $\mu$Ci/ml) for 6 h. For labeling of proteins, either [$^3$H]glycine, [$^3$H]proline, [$^{35}$S]methionine, D-[2-$^3$H]mannose, or D-[6-$^3$H]glucosamine hydrochloride was used at 50 to 100 $\mu$Ci/ml for 6 to 12 h in medium lacking the respective precursor. All radiochemicals were purchased from the Radiochemical Centre, Amersham, England. Viruses were purified from the labeled culture media by previously described procedures (34).

For surface labeling, the galactose oxidase procedure (15) and freshly purified unlabeled virus were used.

**Analysis of virion RNA.** RNA from purified $^{32}$P- or [$^3$H]uridine-labeled virions was released either with 2% sodium dodecyl sulfate (SDS) or by phenol extraction. The RNA was fractionated on a 15 to 30% (wt/wt) sucrose gradient made in TSE-SDS buffer (0.01 M Tris-hydrochloride [pH 7.5], 0.15 M NaCl, 0.001 M EDTA, 0.1% SDS) for 5.5 h at 25,000 rpm and 22°C in an SW27 rotor. The 60 to 70S RNA was recovered by precipitation with ethanol at −20°C in the presence of 0.3 M sodium acetate, 100 $\mu$g of unlabeled yeast RNA (Sigma Chemical Co., St. Louis, Mo.), and 2.5 volumes of ethanol. The RNA was then heat denatured in 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA–0.5% SDS in sealed glass capillaries at 78°C for 1 min. Polyadenylic acid-containing RNA was recovered by purification on oligodeoxythymidylic acid [oligo-(dT)]-cellulose (P-L Biochemicals, Inc., Milwaukee, Wis.), and the RNA was ethanol precipitated twice to remove SDS.

RNA released by phenol extraction was purified similarly, except the sucrose gradient centrifugation step was omitted. Purified RNA was stored under ethanol at −20°C.

The oligo(dT)-purified 35S RNAs were electrophoresed in cylindrical 1% agarose gels, and the gels were analyzed by slicing them into 2-mm segments and determining the radioactivity. [$^3$H]uridine-labeled HeLa cell 28 and 18S rRNA's and Semliki Forest virus 42S RNA were used as markers.

**RNA fingerprint analysis.** For two-dimensional fingerprint analysis, oligo(dT)-cellulose-selected 60 to 70S or heat-denatured $^{32}$P-labeled RNA (100,000 to 500,000 cpm), adjusted to 100 $\mu$g with unlabeled yeast RNA, was digested with RNase $T_1$ (Calbiochem, La Jolla, Calif.; 8 U in 8 $\mu$l of 10 mM Tris-hydrochloride [pH 7.5]–1 mM EDTA) for 30 min at 37°C. The resulting oligonucleotides were then fractionated by two-dimensional polyacrylamide gel electrophoresis as described previously (10, 35). Radioactivity in the oligonucleotides was determined by cutting out the spots and quantitating the radioactivity by Cherenkov counting.

**In vitro translation.** For in vitro translation, either oligo(dT)-selected 60 to 70S or 35S unlabeled virion RNA was used at 3 to 10 $\mu$g/ml in reticulocyte extracts by the method of Pelham and Jackson (32), using commercial reagents (Radiochemical Centre, Amersham, England) and [$^{35}$S]methionine as a precursor. The amount of radioactivity incorporated into newly synthesized protein was measured by precipitation with 10% trichloroacetic acid.

**Immunoprecipitation.** Anti-p27 and anti-gp85/37 rabbit sera (9, 27) and anti-reverse transcriptase sheep serum (30) were kindly provided by H. Diggelmann and E. Buetti, and K. Moelling, respectively. Normal rabbit and sheep sera were used as controls. Immunoprecipitation was carried out as described by Persson et al. (33). Samples to be analyzed were preabsorbed with corresponding normal sera. Protein A containing formaldehyde-fixed bacteria, prepared as described previously (28), were used as the solid-phase reagent.

**SDS-polyacrylamide gel electrophoresis.** Sam-

ples to be analyzed were dissolved in Laemmli sample buffer containing 4% SDS and 10% mercaptoethanol, electrophoresed in vertical 5 to 16% gradient slab gels by the method of Laemmli (29), and processed for fluorography by the method of Bonner and Laskey (8). Commercially obtained (Amersham) $^{14}C$-labeled proteins were used as molecular weight markers.

## RESULTS

**Size of OK10-BM virion RNA.** RNA released from purified OK10-BM virus had a sedimentation value of 60 to 70S typical for retroviruses (data not shown). For an analysis of the size of the subunit, $^{32}P$-labeled OK10-BM virus 60 to 70S RNA was heat denatured, purified by oligo(dT)-cellulose chromatography, and run in a 1% agarose gel together with various $^{3}H$-labeled marker RNAs (Fig. 1A through D). OK10-BM virus yielded only one distinct and reproducible species of RNA, which migrated faster than the 10-kb RSV Prague A RNA (Fig. 1A). OK10-BM virus RNA comigrated with the 8.5-kb helper RNA of MC29 (RAV-2) (Fig. 1B) and with the RNA of the associated virus, OK10 AV, originally found in the OK10 virus stock (Fig. 1C). OK10-BM virus RNA was finally coelectrophoresed together with Semliki Forest virus RNA (42S, about 13 kb) and HeLa cell 28 and 18S rRNA's (Fig. 1D). The slowly migrating material present in all four gels probably represented residual undenatured 60 to 70S RNA, since it was also present in the marker retrovirus RNA preparations. Based on the above results, we conclude that the RNA genome of OK10-BM virus has a size of about 8.0 to 8.5 kb. No evidence for the presence of a component smaller than this could be obtained.

**Oligonucleotide fingerprint analysis of OK10-BM and OK10 AV RNAs.** To further study the biochemical relationship between OK10-BM and OK10 AV viruses and to determine whether an associated virus could be detected in the bone marrow-derived cell line by a sensitive biochemical method, we analyzed by two-dimensional polyacrylamide gel electrophoresis the RNase $T_1$-resistant oligonucleotide fingerprints of their genomic RNAs (Fig. 2A and B). When the fingerprints were compared, it was evident that the two RNA species were closely related. At least 30 long oligonucleotides were common to both RNAs (Fig. 2, lower panels, open circles). There were, however, also distinct differences. At least three oligonucleotides present in OK10 AV RNA were absent from OK10-

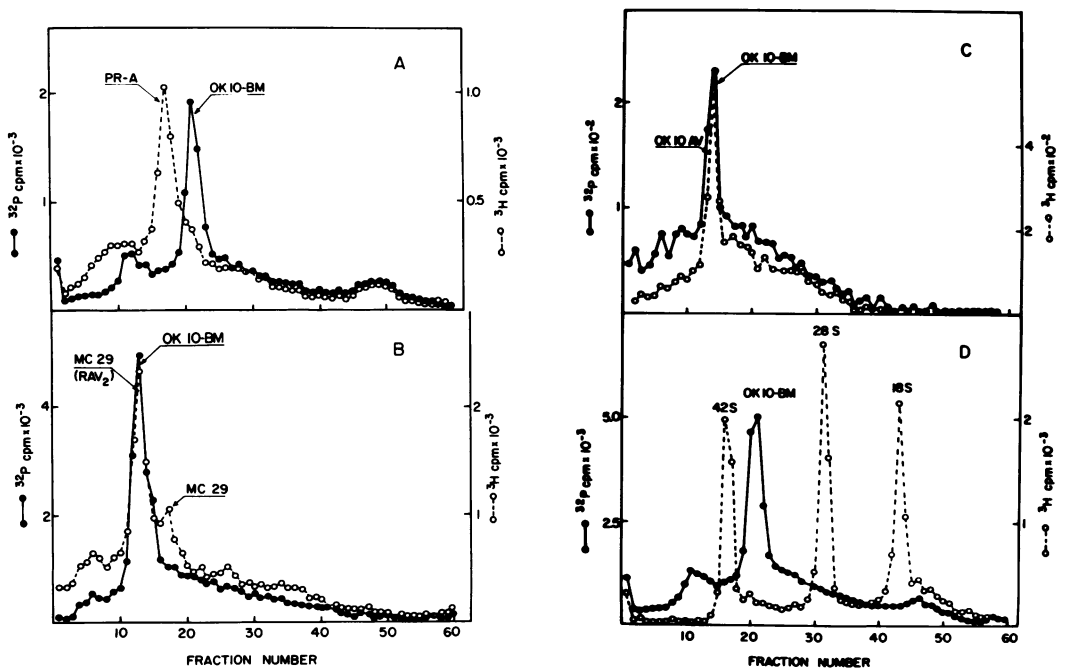


FIG. 1. *Electrophoretic size analysis of OK10-BM RNA in agarose gels. Heat-dissociated $^{32}P$-labeled OK10-BM 70S RNA (●) and [$^{3}H$]uridine-labeled RNAs (○) from various viruses were prepared and electrophoresed in 1% agarose gels as described in the text. (A) OK10-BM [$^{32}P$]RNA and Prague A [$^{3}H$]-RNA. (B) OK10-BM [$^{32}P$]RNA and MC29 [$^{3}H$]RNA. (C) OK10-BM [$^{32}P$]RNA and OK10 AV [$^{3}H$]RNA. (D) OK10-BM [$^{32}P$]RNA, HeLa cell 28 and 18S [$^{3}H$]rRNA's, and Semliki Forest virus 42S [$^{3}H$]RNA. For further explanations see text.*

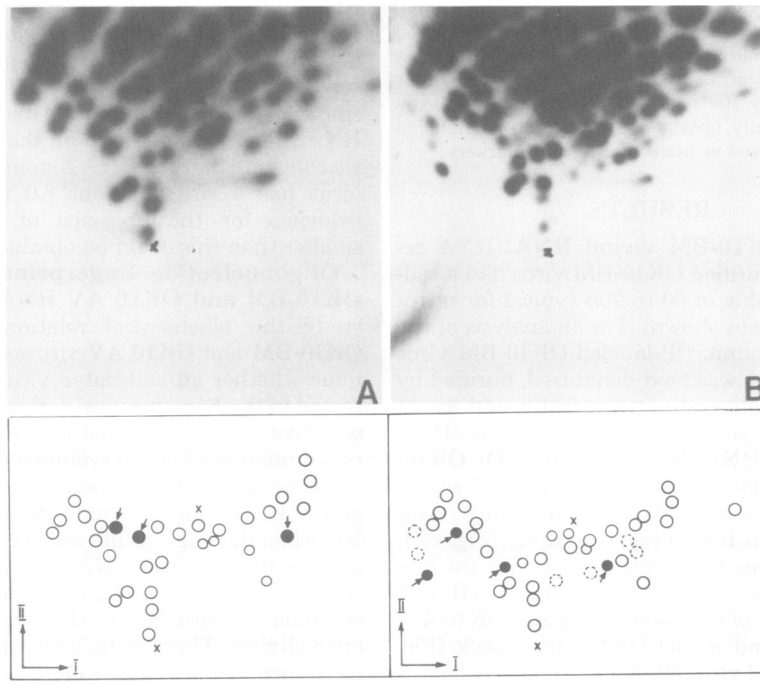


FIG. 2. *Two-dimensional polyacrylamide gel analysis of RNase $T_1$-resistant $^{32}P$-labeled oligonucleotides of OK10 AV (A) and OK10-BM (B) viral RNAs. Open circles indicate oligonucleotides common to both OK10-BM and OK10 AV viral RNAs. Filled circles indicated by arrows denote OK10 AV-specific (A) and OK-BM-specific (B) oligonucleotides, respectively. The faint (submolar) spots represented by broken circles in the OK10-BM RNA fingerprint have not been detected in fingerprints of OK10 AV RNA.*

BM virus RNA (Fig. 2, lower left panel, filled circles indicated by arrows). On the other hand, there were four strong spots present in the OK10-BM RNA fingerprint which were absent from the OK10 AV fingerprint (Fig. 2, lower right panel, filled circles indicated by arrows). In addition, there were a few fainter (submolar) spots in the OK10-BM RNA fingerprint which were absent from the OK10 AV fingerprint (Fig. 2, broken circles). These faint spots were present in both 60 to 70S and 30 to 35S RNAs regardless of whether the RNA was oligo(dT)-cellulose purified. No traces of the OK10 AV RNA-specific oligonucleotides were found in the OK10-BM RNA fingerprint.

**Structural proteins of OK10-BM and OK10 AV.** To compare the structural proteins of OK10-BM and OK10 AV viruses, the proteins were radiolabeled metabolically with amino acids and sugar precursors and externally by the galactose oxidase procedure. It was evident that there was one prominent difference between the two viruses. The major envelope glycoprotein gp85 was missing from OK10-BM virus and was replaced by a protein with an apparent molecular weight of about 78,000 (Fig. 3). This protein, gp78, could be labeled with [$^3$H]mannose (lane 5) and [$^3$H]glucosamine (lane 7) and externally by the galactose oxidase method (lane 9). It is, thus, like gp85, an externally located glycoprotein. gp78 of OK10-BM virus was less prominently labeled with amino acids, relative to, e.g., p27, than was gp85 (Fig. 3, lanes 1 through 4). gp78 and gp85 were both immunoprecipitated with anti-gp85/37 serum (data not shown).

No clear differences in the nonglycosylated proteins [p91($\beta$), p64($\alpha$), p27, p19, p15, and p12 [14]) of the two viruses were evident (Fig. 3). Identification of the different proteins was based on immunoprecipitation experiments with anti-p27, anti-reverse transcriptase, and anti-gp85/37 sera (data not shown).

**In vitro translation of virion RNA.** In vitro translation of rabbit reticulocyte extracts of the RNAs extracted from OK10-BM and OK10 AV virions gave rise in both cases to a large number of polypeptides of different sizes (Fig. 4). One distinct difference between the products of the two RNAs was, however, evident: unlike OK10 AV RNA (lane 2), OK10-BM virus RNA (lane 1) directed the synthesis of a polypeptide with an apparent molecular weight of about 200,000 (p200). Products which, based on their molecular weights, could be pr180$^{gag\text{-}pol}$ and pr76$^{gag}$ (14),

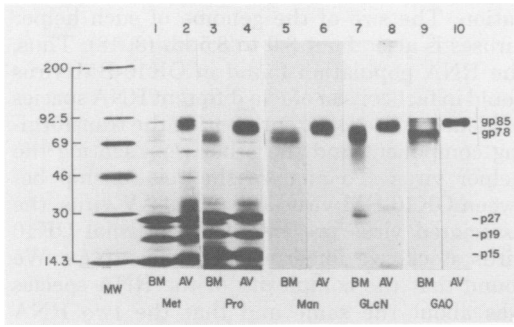


FIG. 3. *Structural proteins of OK10-BM and OK10 AV viruses. OK10-BM- and OK10 AV-infected cells were labeled with different radioactive precursors, the viruses were purified and disrupted, and the proteins were electrophoresed in 5 to 16% polyacrylamide gradient slab gels as described in the text. [$^{35}$S]methionine, lanes 1 and 2; [$^{3}$H]proline, lanes 3 and 4; D-[2-$^{3}$H]mannose, lanes 5 and 6; D-[6-$^{3}$H]-glucosamine, lanes 7 and 8; [$^{3}$H]galactose oxidase, surface labeled, lanes 9 and 10. The lane on the left indicates the migration of molecular weight marker proteins in thousands. BM, OK10-BM; AV, OK10 AV.*

as well as a number of smaller polypeptides, were found in the translates of both RNAs. The latter could represent pretermination products in the synthesis of pr76$^{gag}$, which are frequently seen in in vivo-labeled cell extracts (21).

pr200, pr180, pr76, and some smaller products could all be precipitated with anti-p27 serum (Fig. 4, lanes 4 and 5), but not with normal rabbit control serum (data not shown). These results suggest that p200, pr180, and pr76 are polyproteins containing p27-specific determinants. p200 and pr180 could, in addition, be immunoprecipitated with anti-reverse transcriptase serum (data not shown).

**Analysis of virion RNA and proteins after serial passages of OK10-BM virus in chicken embryo fibroblasts.** We found that the titer of focus-forming transforming virus increased up to 100-fold upon serial passaging of OK10-BM virus in chicken embryo fibroblasts. Typically, the titer of OK10-BM virus was about $10^3$ to $10^4$ focus-forming units (FFU) per ml. When such virus was used to infect chicken embryo fibroblasts at a multiplicity of infection of about 0.001 FFU per cell, the cells became fully transformed within two subcultures and by then released about $10^5$ to $10^6$ FFU/ml into the culture medium.

To study whether any changes in the genome of the passaged virus occurred, we fingerprinted the RNA of the virus from passages 3 to 6. As Fig. 5 shows, the fingerprint of the RNA from the virus released from the fibroblasts at passage 3 contained both the three OK10 AV-specific oligonucleotides (open arrows) and the four OK10-BM-specific oligonucleotides (solid arrows). Quantitation of the radioactivity in the spots indicated that OK10 AV RNA was present in a one- to twofold excess relative to that of OK10-BM virus. The relative amount of OK10 AV- and OK10-BM-specific spots remained roughly unaltered from passages 3 to 6. Faint submolar spots, similar in position to those seen in the OK10-BM fingerprint, were also detected in fingerprints of OK10-BM virus passaged in fibroblasts.

An analysis of the structural proteins (Fig. 6) indicated that after passaging of OK10-BM virus in fibroblasts gp78, characteristic of OK10-BM virus (lanes 1 and 5), was apparently fully replaced by gp85, characteristic for nontransforming OK10 AV virus grown in chicken fibroblasts (lanes 4 and 8); but the amount of gp85 relative to the other viral proteins seemed to increase with prolonged passaging of OK10-BM virus in chicken fibroblasts (lane 3). Nontransforming virus, as studied by using the interference assay from the culture media of the various passages (36), was first detected after five to six subcultures, but in some experiments it was not detected until after 15 passages of the bone marrow-derived virus in fibroblasts.

According to the interference assay, the generated nontransforming virus was of subgroup A, similar to that of OK10 AV virus. Thus, it appeared that the alteration in the glycoprotein and in the oligonucleotide fingerprint pattern

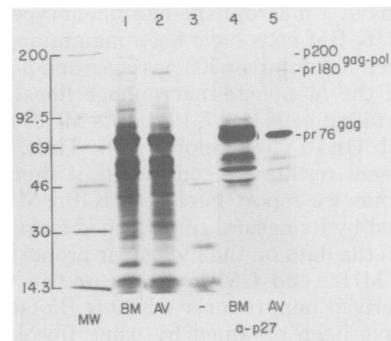


FIG. 4. *Analysis by polyacrylamide gel electrophoresis of in vitro translation products of OK10-BM and OK10 AV virion 70S RNAs. [$^{35}$S]methionine-labeled products in extracts programmed with OK10-BM RNA (lane 1) and OK10 AV RNA (lane 2) and without added RNA (lane 3). As shown in lane 4, the p200 specific for in vitro translates of OK10-BM RNA could be immunoprecipitated with anti-p27 rabbit serum in addition to pr180 and pr76, a characteristic seen also in in vitro translates of OK10 AV RNA (lane 5). MW, Molecular weight of the $^{14}$C-labeled marker proteins in thousands.*

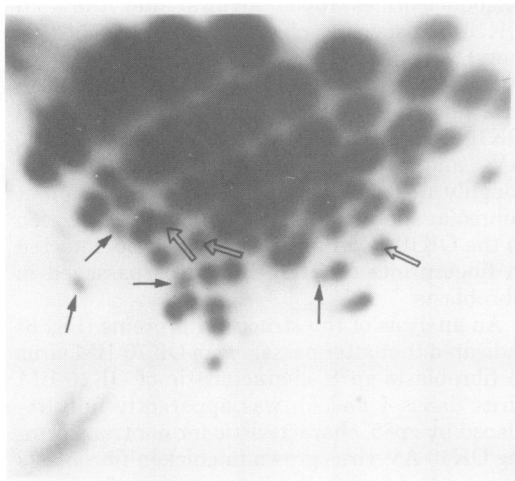

FIG. 5. *Fingerprint of OK10-BM virion RNA after three passages of the virus in chicken embryo fibroblasts. Analysis of RNase $T_1$-resistant $^{32}P$-labeled oligonucleotides in a two-dimensional polyacrylamide gel was as in Fig. 2. OK10-BM-specific oligonucleotides are indicated by solid arrows and OK10 AV-specific oligonucleotides are indicated by open arrows.*

preceded the appearance of nontransforming virus detectable by biological means.

## DISCUSSION

In this study we have characterized the structural properties of OK10 virus produced by transformed bone marrow-derived chicken cells, which have a macrophage-like phenotype (36). These OK-BM cells have been maintained as a stable cell line for over 500 passages and 5 years. Cells of the monocyte-macrophage lineage are natural target cells (1, 17, 19) of the MC29 group to which OK10 virus belongs (20). Thus, when the present results are considered, it should be noted that we report here on OK10-BM virus produced by its natural transformed target cell, whereas the data on the molecular properties of MC29, MH2, and CMII viruses or the OK10 virus-derived nonproducer OK10 Q B5 (see below) have been obtained by using fibroblastic cells (for references, see the introduction).

The genome of OK10-BM virus was found to consist of only one species of RNA, 8.0 to 8.5 kb in size. This is considerably larger than the transforming component of MC29, CMII, or MH2 virus, or any of the other avian acute leukemia viruses able to transform both fibroblasts and hematopoietic cells. These viruses all have truncated genomes of about 5 to 6 kb containing a completely deleted *pol* gene and partially deleted *gag* and *env* genes (12, 24). They therefore require a helper virus for replication. The size of the genome of such helper viruses is also about 8.0 to 8.5 kb (3, 18). Thus, the RNA population found in OK10-BM virus could in fact consist of two different RNA species of identical size, one representing the transforming component and the other representing the helper virus. To analyze the relationship between OK10-BM virus and OK10 AV virus, the associated virus present in the original OK10 virus stock, we fingerprinted their RNAs. We found that the complexity of the RNA species was about the same and that the two RNA species were closely related. OK10-BM virus yielded four specific long oligonucleotides that were not present in OK10 AV RNA. Reciprocally, OK10 AV RNA yielded three long oligonucleotides that were absent from OK10-BM RNA. Since OK10-BM RNA has been found to contain *mac*-related sequences (S. Saule, personal communication; B. Vennström and S. Pfeifer, unpublished data), the OK10-BM-specific oligonucleotides may be derived from this portion of the genome. Similarly, the three OK10 AV-specific oligonucleotides may be derived from the 5′ portion of the *env* gene, which according to hybridization and heteroduplex analyses has been deleted in OK10 Q B5 RNA (RNA of defective, noninfectious particles released by nonproducer quail fibroblasts) (8; Saule, per-

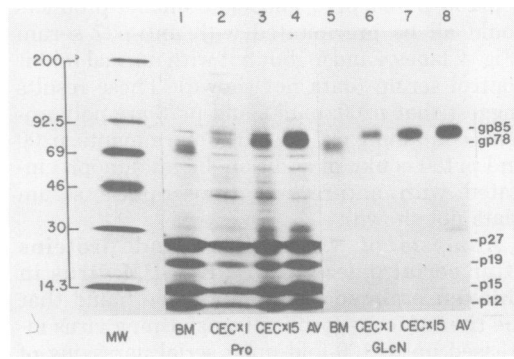


FIG. 6. *Analysis of structural proteins of OK10-BM virus after passage of the virus in chicken embryo fibroblasts. Virus-infected cells were labeled with [$^3$H]proline (lanes 1 through 4) or D-[6-$^3$H]glucosamine (lanes 5 through 8), and the viruses were purified, disrupted, and electrophoresed in 5 to 16% polyacrylamide gradient slab gels as described in the text. Lanes 1 and 5, OK10-BM virus produced by OK10-BM cells; lanes 2 and 6, OK10-BM virus subcultured once in chicken embryo fibroblasts (CEC); lanes 3 and 7, OK10-BM virus after 15 subcultures in CEC; lanes 4 and 8, OK10 AV virus (grown in CEC). MW, Molecular weight of the $^{14}$C-labeled marker proteins in thousands. Note the transition in OK10-BM virus of its characteristic gp78 to the gp85 characteristic of OK10 AV after only one subculture of OK10-BM virus in CEC.*

sonal communication). Physical mapping of these oligonucleotides must be carried out to verify this. No traces of the OK10 AV-specific oligonucleotides were seen in the OK10-BM fingerprints, suggesting that the original associated virus was not present in the OK10-BM virus population or was present in amounts too small to be detected by this method (ca. ≤1:10).

Polyacrylamide gel electrophoresis and immunoprecipitation experiments indicated that the OK10-BM virus particles contained apparently intact products of the *gag* and *pol* genes, whereas the major product of the *env* gene was conspicuously altered. Instead of the major envelope protein gp85 typical of nondefective avian leukemia/sarcoma viruses, we found a protein of $M_r$ = 78,000. This protein was glycosylated, externally located, and immunoprecipitated by anti-gp85/35; hence, it is defined as the major glycoprotein gp78 of OK10-BM virus. Compared with gp85 of the nontransforming virus, gp78 was very weakly labeled with methionine and poorly labeled also with proline, glycine, or mannose. This suggests that there may be a decreased amount of envelope proteins in the OK10-BM virus particle. Prolonged exposure of fluorographs indicated that the poorly labeled minor glycoprotein of nondefective avian leukemia/sarcoma viruses (gp37) was also altered in OK10-BM virus having an apparent molecular weight of about 30,000. Since both the large and the small envelope glycoproteins of OK10-BM virus had an increased migration rate in SDS-polyacrylamide gels, altered glycosylation could account for the change. To further characterize the nature of gp78, peptide mapping and analysis of the glycan moieties are necessary.

In vitro translation of OK10-BM viral RNA, combined with immunoprecipitation experiments, also indicated the capacity to code for apparently intact products of the *gag* and *pol* genes. In a manner similar to that of the RNA from nontransforming virus, OK10-BM virus RNA encoded a pr180 and a pr76, which could be immunoprecipitated with anti-p27. The former could also be precipitated with anti-reverse transcriptase. In addition, a p200 was found in the translates of OK10-BM viral RNA. Recently, Ramsay and Hayman (37) have reported on a p200 found in immunoprecipitates of the OK10-BM-transformed nonproducer quail fibroblasts, OK10 Q B5 (see above). This p200 contained *mac* gene-encoded methionine-labeled tryptic peptides and was suggested to be a possible transforming protein. The in vitro-made pr180 and p200 could both be translated from the genomic mRNA by a read-through mechanism. This would mean that suppression of termination at the C-terminus of pr180 is incomplete, resulting in roughly equimolar amounts of the two products. Alternatively, pr180 and p200 could be translated from different mRNA's. We have obtained preliminary results suggesting that at least p200 is made in the transformed bone marrow cells.

We have been unable to detect any associated virus in the culture medium of OK10-BM cells by employing several biological tests (36, 36a). However, several lines of evidence point to the presence of a helper virus. The strongest evidence is the ease by which nonproducer quail fibroblasts can be obtained (20; S. Pfeifer, unpublished data). Second, the serial passaging of the virus in chicken embryo fibroblasts indicated the reappearance of gp85, oligonucleotides which are typical of nontransforming viruses, and ultimately, after 5 to 15 passages, the recovery of interfering virus of subgroup A. In addition, Saule (personal communication) and Vennström and Pfeifer (unpublished data), using an *env* gene probe, have detected in OK10-BM cell extracts RNA sequences covering also the 5′ portion of the *env* gene, which according to heteroduplex analyses is deleted in OK10 Q B5 RNA, the RNA of the noninfectious defective particles released by the nonproducer quail fibroblasts (20). Thus, the OK10-BM cells may contain small amounts of an associated virus. It has been shown that the ratio of MH2 transforming to helper virus is greatly increased in macrophages, whereas in fibroblasts helper virus is produced in a 100-fold excess (24). Moreover, Gazzolo et al. (16) have reported that nontransforming viruses of subgroup A grow poorly in macrophages. Therefore, it is possible that the transforming OK10 virus is preferentially replicated in the macrophage-like OK10-BM cells, whereas in fibroblasts the helper has a selective advantage for replication.

## ACKNOWLEDGMENTS

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