JOURNAL OF VIROLOGY, Dec 1981, p. 971-976
0022-538X/81/120971-06$02.00/0

Vol. 40, No. 3

env Gene Products of AKR Dual-Tropic Viruses: Examination of Peptide Maps and Cell Surface Expression

NANCY G. FAMULARI* AND KAREN JELALIAN ENGLISH
Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received 24 March 1981/Accepted 1 July 1981

The *env* gene products of nine AKR dual-tropic murine leukemia viruses were compared by peptide mapping and were assayed for expression on the cell surface of infected fibroblasts. Seven virus isolates expressed the *env* gene polyprotein on the cell surface. The *env* gene products of six of these seven viruses had identical peptide maps. The analysis of structure and expression of *env* gene products carried out in this study characterizes a subset of dual-tropic murine leukemia viruses shown by others to be thymotropic.

Recent experiments have shown that injection of dual-tropic murine leukemia virus (MuLV) of AKR origin into young AKR mice can initiate the process of leukemogenesis in these animals (3, 13, 16). Neither the endogenous ecotropic or xenotropic MuLV of the AKR strain has been shown to have this capacity (3, 8, 15). These findings have been interpreted to suggest that the appearance of dual-tropic MuLV in AKR mice initiates the spontaneous development of leukemia in this strain. However, the specific mechanisms by which dual-tropic MuLV is involved in leukemogenesis in the AKR strain are unknown at present. An approach to dissecting the involvement of dual-tropic MuLV in leukemogenesis is to examine gene products of this class of MuLV to determine whether structural or functional characteristics of viral proteins correlate with the biological activity of an individual isolate. Of particular interest in this regard are the *env* gene products of dual-tropic MuLV since the *env* gene appears to be the site of the recombinational event which gives rise to these viruses (2, 4, 7, 12, 15, 17). We have examined the *env* gene products of a series of nine dual-tropic viruses isolated from the AKR strain of mice by the physical parameters of sizing with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and peptide mapping and by examination of cell surface expression of *env* gene products in infected fibroblasts. The isolates studied fall into three categories in terms of in vivo activity (16; see Table 1). The first (A^+L^+ viruses) includes isolates which induce the amplification (A) of MuLV-related antigens on thymocytes (a late preleukemic event in the development of spontaneous AKR leukemias [9]), followed by onset of leukemia (L) within 100 days. Viruses in the second category (A^+L^- viruses) induce MuLV antigen amplification but do not accelerate leukemia development. Both A^+L^+ viruses and A^+L^- viruses have been shown to be thymotropic (3, 16). Viruses in the third category (A^-L^- viruses) neither induce antigen amplification nor accelerate leukemogenesis and appear to lack the phenotype of thymotropism (16). An analysis of the structure of the protein products of the recombinant *env* gene of this series of viruses affords a means of determining whether a particular type of gene product is found in conjunction with a particular in vivo phenotype.

The primary *env* gene products (PrENV proteins) of these three classes of recombinants were compared with one another and with those of an AKR ecotropic virus (69E5) and xenotropic virus (69X9) by migration in SDS-PAGE. Virus-infected cells (mink lung cells in the case of the dual-tropic viruses and the xenotropic virus, and SC-1 cells in the case of the ecotropic virus) were pulse-labeled for 15 min with ^{14}C-amino acid mixture, and PrENV protein was isolated from cell extracts by immunoprecipitation with goat antiserum to Rauscher MuLV (R-MuLV) gp70 and SDS-PAGE analysis as previously described (5) (Fig. 1). With the exception of MCF 13 virus, all the recombinants, regardless of in vivo activity, encoded a PrENV protein with an apparent molecular weight in SDS-PAGE of approximately 76,000. The PrENV protein of MCF 13 virus migrated with a molecular weight of approximately 71,000. We had previously reported that the PrENV protein of the recombinant virus, MCF 247, could be distinguished from that of the AKR ecotropic MuLV by migration in SDS-PAGE; the ecotropic virus PrENV protein migrates with a molecular weight of approximately 80,000 (5). This finding is extended here to indicate that a common feature of AKR dual-tropic viruses is a small

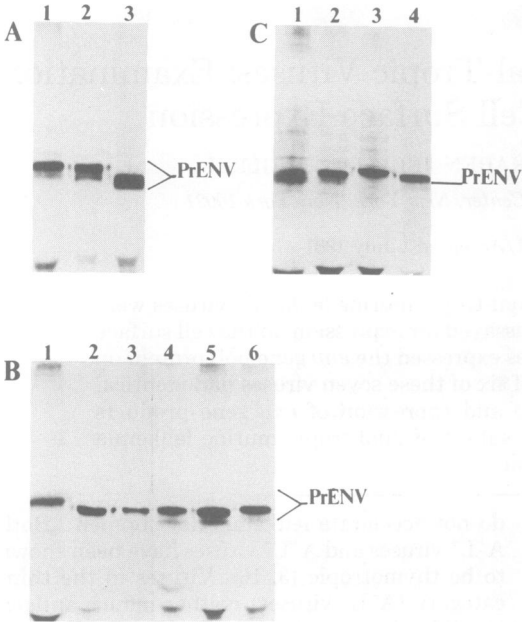

FIG. 1. *SDS-PAGE analysis of the PrENV protein of AKR dual-tropic viruses. Cytoplasmic extracts were prepared from virus-infected cells radiolabeled with ^{14}C-amino acids in a 15-min pulse. Extracts were reacted with goat anti-R-MuLV gp70 serum, and the resulting precipitates were subjected to electrophoresis in 7.5% acrylamide slab gels as previously described (5). (A) Mink lung cells infected with: Track 1, MCF 247 virus; track 2, MCF 69L1 virus; track 3, MCF 13 virus. (B) Track 1, 69E5 virus-infected SC-1 cells; tracks 2 through 5, mink lung cells infected with (track 2) SC30, (track 3) SC37, (track 4) MCF 28-7, (track 5) MCF 30-2, and (track 6) MCF 247 virus. (C) Mink lung cells infected with: Track 1, 69X9 virus; track 2, 69-4 virus, track 3, 26-4 virus; track 4, MCF 247 virus.*

PrENV protein relative to that of ecotropic virus. This size difference is of potential use for detecting the expression of both ecotropic and dual-tropic MuLV in the same cell. In contrast, the xenotropic virus PrENV protein has a migration very similar to that of the recombinant viruses (5; Fig. 1C, track 1). A minor protein species migrating slightly faster than the dual-tropic virus PrENV protein is present variably in immunoprecipitates of extracts of cells infected by cloned dual-tropic viruses (Fig. 1A, tracks 1 and 2; Fig. 1B, tracks 4 to 6; Fig. 1C, track 2). This protein species appears to be a form of the PrENV protein, due to its recognition by anti-p15(E) serum (data not shown). Its relationship to the major PrENV species is under investigation.

Pulse-labeled PrENV proteins isolated by SDS-PAGE were analyzed further by mapping partial digest fragments produced by cleavage with *Staphylococcus aureus* V8 protease (1). This method has been used previously to identify the expression of recombinant *env* gene products in AKR leukemia cells and readily distinguishes MCF 247 virus *env* gene products from those of ecotropic and xenotropic MuLV (6). The PrENV maps of two of the A^+L^+ recombinants, MCF 247 and MCF 69L1, are indistinguishable; the map of MCF 13 virus PrENV is distinct (Fig. 2A), as expected from the size difference observed between the PrENV proteins of MCF 13 and of MCF 247 and MCF 69L1 viruses. In addition, partial digest maps of gp70 of MCF 247 and MCF 69L1 were identical to one another and distinguishable from that of MCF 13 virus (data not shown). This result is consistent with the fact that the gp70 of MCF 13 is also smaller than those of MCF 247 and MCF 69L1 viruses (see Fig. 3A) and confirms the report of Elder et al. (4) that tryptic peptide maps of gp70 of MCF 13 and MCF 247 viruses are distinguishable. The PrENV maps of A^+L^- recombinants SC30, SC37, 28-7, and 30-2 were compared with those of the A^+L^+ recombinant MCF 247 and the ecotropic virus 69E5. By this method of analysis the PrENV proteins of the

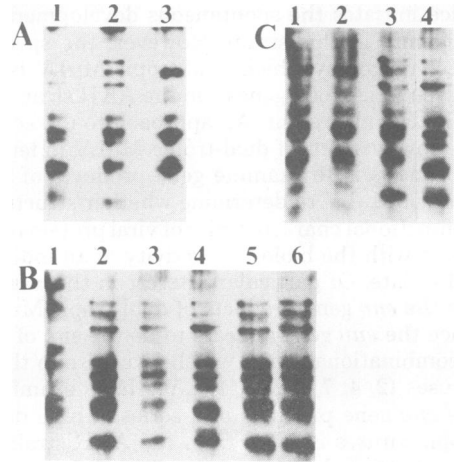

FIG. 2. *Comparison of the PrENV proteins of AKR dual-tropic viruses by partial protease digestion mapping. PrENV proteins, isolated by electrophoresis of immunoprecipitates, were subjected to partial digestion with S. aureus V8 protease according to Cleveland et al. (1), and protein fragments were analyzed in 15% acrylamide slab gels. Details of conditions used have been previously reported (5). Shown are the PrENV proteins of the following viruses: (A) track 1, MCF 247; track 2, MCF 69L1; track 3, MCF 13. (B) track 1, 69E5; track 2, SC30; track 3, SC37; track 4, MCF 28-7; track 5, MCF 30-2; track 6, MCF 247. (C) track 1, 69X9; track 2, 69-4; track 3, 26-4; track 4, MCF 247.*

four A^+L^- viruses were indistinguishable from one another and from that of MCF 247 virus. Two of the A^+L^- recombinants, SC37 and SC30, exhibit a preferential tropism for mouse cells relative to mink cells (16). The PrENV proteins of these two isolates were compared with that of 69E5 virus by migration in SDS-PAGE (Fig. 1B, tracks 1 to 3) and by peptide mapping (Fig. 2B, tracks 1 to 3). However, these recombinants bear no resemblance to the ecotropic virus by these criteria.

The third class of recombinants (A^-L^- viruses) appear to bear closer resemblance to xenotropic virus than do the dual-tropic recombinants of the A^+L^+ or A^+L^- classes. Although the *env* gene products of these isolates carry both ecotropic and xenotropic virus type-specific antigenic markers, a finding which indicates that they are recombinants, a number of ecotropic virus type-specific markers commonly carried by dual-tropic virus are not expressed by the *env* gene products of these viruses (14, 15). Furthermore, these recombinants have a 2-log higher titer on mink lung cells than on mouse SC-1 cells (11, 16). No preleukemic antigen amplification, progeny virus replication, or leukemia acceleration occurs after intrathymic injection of AKR mice with this class of recombinant virus, a finding which indicates that this class of recombinant is unable to infect thymocytes (16). Partial protease digest maps of PrENV proteins of two of these isolates, 69-4 and 26-4, were compared with those of the xenotropic virus 69X9 and MCF 247 (Fig. 2C). Maps of 69X9 and 69-4 virus PrENV proteins (Fig. 2C, tracks 1 and 2) appear to be identical, whereas the map of the PrENV protein of 26-4 (Fig. 2C, track 3) shares features in common with that of MCF 247 virus (Fig. 2C, track 4) and those of 69X9 and 69-4. Peptide maps of gp70 of these four viruses were also examined. The data obtained from these maps are consistent with those generated by comparisons of PrENV (data not shown).

The third aspect of the analysis of this series of dual-tropic viruses was the examination of cell surface expression of *env* gene products. We have previously described the cell surface expression of the PrENV protein of the A^+L^+ virus MCF 247 on infected mouse SC-1 cells and mink lung cells (5, 10). This phenotype of *env* gene expression was not observed for ecotropic and xenotropic MuLV in fibroblasts. In addition, cell surface localization of the PrENV protein has been shown to be characteristic of dual-tropic virus expression in AKR leukemia cells (6). It is not known whether this aspect of *env* gene expression plays a role in dual-tropic virus-induced leukemogenesis. Analysis of the AKR recombinant viruses described above for this phenotype afforded us a means of determining whether the cell surface expression of PrENV protein correlated with the in vivo activity of these isolates.

Suspensions of virus-infected mink lung fibroblasts were subjected to lactoperoxidase-catalyzed radioiodination, and extracts of labeled cells were immunoprecipitated with goat anti-R-MuLV gp70 serum or rabbit anti-R-MuLV p15(E) serum as previously described (5). Precipitates were subjected to SDS-PAGE analysis to determine whether a protein containing both gp70 and p15(E) antigenic determinants was present on the cell surface (Fig. 3). In the case of A^+L^+ and the A^+L^- recombinants (Fig. 3A and B), anti-gp70 serum recognized two protein species on the surface of infected cells, a major band, which is gp70, and a minor, slow-migrating protein species. This minor protein, recognized by both anti-gp70 and anti-p15(E) serum, is the PrENV protein. The identification of the PrENV protein has been confirmed by partial protease digest mapping (data not shown). Neither 69-4 nor 26-4, viruses of the A^-L^- category, was shown to induce the cell surface expression of PrENV protein in infected fibroblasts (Fig. 3C, tracks 3 to 6). Anti-gp70 serum recognized only gp70 in extracts of surface-iodinated cells infected by these viruses; anti-p15(E) serum did not recognize any MuLV-specific proteins in these extracts. The analysis of 69X9 and MCF 247 virus-infected fibroblasts for cell surface expression of PrENV protein is included for comparison (Fig. 3C, tracks 1, 2, 7, and 8). 69X9 virus has previously been shown to be negative for the phenotype of cell surface expression of the PrENV protein (5).

As a result of characterizing the nine dual-tropic viruses outlined in Table 1, we have determined that six of the isolates encode very similar *env* gene products. Since these isolates include both A^+L^+ (MCF 247 and MCF 69L1) and A^+L^- (SC37, SC30, MCF 30-2, and MCF 28-7) viruses, no correlation was detected between the parameters of *env* gene expression measured in this analysis and the ability of an isolate to accelerate leukemogenesis in AKR mice. These findings indicate that the simple expression of products of a dual-tropic virus *env* gene in a thymocyte does not put that cell at risk for transformation within the time limits set by AKR acceleration tests carried out in young adult mice (16). It remains to be determined whether particular aspects of expression of these gene products in thymocytes may be involved either with priming a cell population for transformation or with transformation directly.

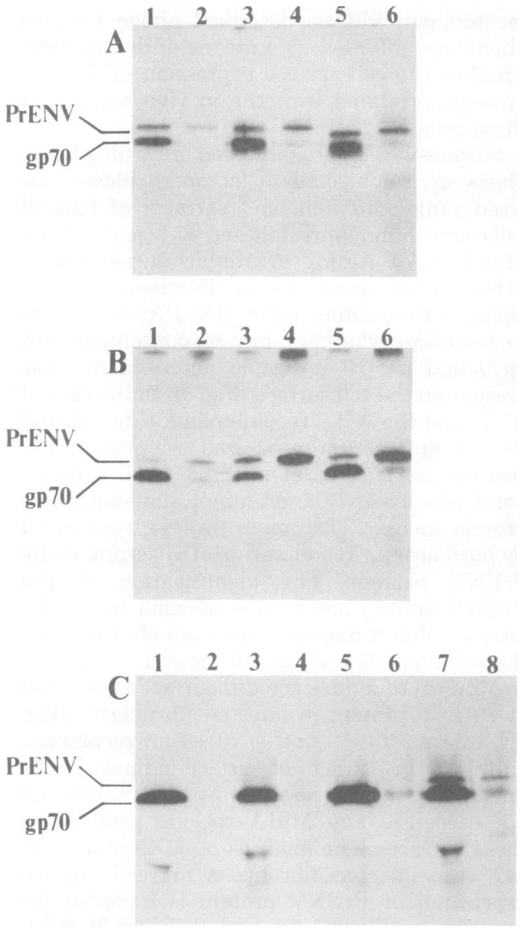

FIG. 3. *Analysis of virus-infected fibroblasts for cell surface expression of the PrENV protein. Cytoplasmic extracts were prepared from virus-infected mink lung cells which had been subjected to lactoperoxidase-catalyzed radioiodination in suspension. Extracts were reacted with either goat anti-R-MuLV gp70 serum or rabbit anti-R-MuLV p15(E) serum, and the resulting precipitates were analyzed in step gradient acrylamide slab gels (7.5, 10, 12.5% acrylamide) as previously described (5). Infecting viruses and reactive sera were as follows. (A) Track 1, MCF 247 virus, anti-gp70 serum; track 2, MCF 247 virus, anti-p15(E) serum; track 3, MCF 69L1 virus, anti-gp70 serum; track 4, MCF 69L1 virus, anti-p15(E) serum; track 5, MCF 13 virus, anti-gp70 serum; track 6, MCF 13 virus, anti-p15(E) serum. (B) Track 1, SC30 virus, anti-gp70 serum; track 2, SC30 virus, anti-p15(E) serum; track 3, MCF 28-7 virus, anti-gp70 serum; track 4, MCF 28-7 virus, anti-p15(E) serum; track 5, MCF 30-2 virus, anti-gp70 serum; track 6, MCF 30-2 virus, anti-p15(E) serum. (C) Track 1, 69X9 virus, anti-gp70 serum; track 2, 69X9 virus, anti-p15(E) serum; track 3, 69-4 virus, anti-gp70 serum; track 4, 69-4 virus, anti-p15(E) serum; track 5, 26-4 virus, anti-gp70 serum; track 6, 26-4 virus, anti-p15(E) serum; track 7, MCF 247 virus, anti-gp70 serum; track 8, MCF 247 virus, anti-p15(E) serum.*

TABLE 1. *In vivo properties of AKR dual-tropic MuLV*

Virus[a]		Activity in AKR mice[b]	
Phenotype[c]	Designation	Amplification of MuLV antigens	Leukemia acceleration
A^+L^+	MCF 247	+	+
	MCF 69L1	+	+
	MCF 13	+	+
A^+L^-	SC30	+	–
	SC37	+	–
	MCF 28-7	+	–
	MCF 30-2	+	–
A^-L^-	69-4	–	–
	26-4	–	–

[a] The viruses MCF 247 and MCF 13 were isolated by Hartley and co-workers (7). The viruses MCF 69L1, SC30, SC37, MCF 28-7, MCF 30-2, 69-4, and 26-4 were isolated by O'Donnell and co-workers (16).

[b] Data of O'Donnell et al. (16). AKR mice (25 to 60 days old) were injected intrathymically with virus. Thymocytes were obtained from these animals by thymic biopsy 32 to 38 days postinjection and were tested for expression of MuLV cell surface antigens by cytotoxicity assay using (W/Fu × BN)F_1 rat anti-W/Fu leukemia (C58NT)D serum. Biopsied animals were observed for development of leukemia and scored as accelerated if disease developed before 180 days of age.

[c] Virus phenotype was based on ability to induce antigen amplification (A) and to accelerate leukemia development (L) in AKR mice.

In contrast, a correlation was observed between structure and expression of *env* gene products and the ability of a dual-tropic MuLV isolate to infect thymocytes and to induce amplification of MuLV antigens in vivo. Six of seven recombinants which display these two phenotypes were shown to encode *env* gene products which were indistinguishable by Cleveland mapping. All seven isolates which are thymotropic and induce antigen amplification expressed the PrENV protein on the surface of infected fibroblasts. These two findings suggest that a selection is exerted on the type of recombinant *env* gene which will confer thymotropism. Evidence is emerging to indicate that antigen amplification is the result of direct expression in thymocytes of the *env* gene products of the input virus (O'Donnell and Nowinski, submitted for publication; Famulari, unpublished data), but whether the ability to induce antigen amplification in vivo is dependent on characteristics of virus in addition to thymotropism is not known. It should be borne in mind that "amplified" dual-tropic virus *env* gene products are expressed at unusually high levels on preleukemic

thymocytes relative to *env* gene products of ecotropic MuLV (9, 20). That the similarities observed between A^+ isolates are not due to selection resulting from in vitro isolation procedures is supported by partial protease digest analysis of the major PrENV species expressed in cultured and primary AKR leukemias. The predominant PrENV species expressed in 19 out of 20 leukemias studied was shown to be closely related if not identical to that encoded by the six indistinguishable A^+ isolates cited above. The expression of the PrENV protein of the A^+ isolate, MCF 13, was detected in 1 of 20 leukemias (6; unpublished data).

The recombinants 69-4, 26-4, and MCF 13 do not fall into the group of indistinguishable A^+ isolates defined by peptide mapping. Both 69-4 and 26-4 appear to contain more xenotropic virus-related information in their *env* gene than do the other AKR dual-tropic viruses, as judged by peptide maps of PrENV and gp70 (Fig. 3C). Consistent with the structural similarity of PrENV and gp70 of 69-4 and 26-4 to that of xenotropic MuLV is the finding that these two recombinants have preferential tropism for mink lung cells relative to SC-1 cells (11, 16) and are lacking all but one of the ecotropic virus type-specific antigens found on gp70 of the six indistinguishable, A^+ recombinants (14, 15). In addition, the oligonucleotide map of the genome of 26-4 virus indicates that considerably more xenotropic virus-related information has been substituted in the *env* gene of 26-4 than is the case for the other AKR dual-tropic viruses examined (N. Hopkins, personal communication). Oligonucleotide mapping of 69-4 virus has not been carried out. In this regard, it is interesting that 69-4 and 26-4 do not encode $G_{(AKSL2)}$ antigen, a type-specific antigen of AKR dual-tropic virus gp70 (15, 19). This antigen is found on normal bone marrow of all high-leukemia-incidence mouse strains and appears to be a marker for the xenotropic virus-related parent involved in the generation of most AKR recombinant viruses (16, 19). That gp70 of 69-4 and 26-4 lacks this antigen, but contains substantial xenotropic virus-related information, may indicate that these recombinants have a different xenotropic virus-related parent from the $G_{(AKSL2)}$-positive recombinants.

The *env* gene products of MCF 13 are also structurally distinguishable from those of the other A^+ recombinants studied here. gp70 of this isolate lacks three of five ecotropic virus type-specific antigens carried by gp70 of the other A^+ recombinants (14, 15), but unlike the A^- recombinants 69-4 and 26-4, MCF 13 has equal titers of infectivity for mink lung cells and SC-1 cells (16), is thymotropic (3, 16), and has an oligonucleotide fingerprint that is extremely similar to that of MCF 247 virus (17). MCF 13 and the other A^+ recombinants all induce the expression of the PrENV protein on the surface of virus-infected fibroblasts, a phenotype not shared with the xenotropic-like recombinants 26-4 and 69-4 (see Fig. 3) or with AKR ecotropic or xenotropic MuLV (5). In addition, MCF 13 encodes the dual-tropic virus-specific antigen $G_{(AKSL2)}$ (15). These data suggest relatedness between the xenotropic virus-related parent of MCF 13 and that of the other A^+ recombinants despite the structural differences in their *env* gene products. A comparison of endonuclease H-treated, i.e., unglycosylated (10, 18, 21), PrENV protein of MCF 13 with that of several other recombinants (MCF 247, MCF 69L1, and SC37) has been carried out. It appears that the low molecular weight of the MCF 13 PrENV protein (Fig. 1A) is due to a smaller polypeptide backbone rather than differential glycosylation relative to the other recombinants (unpublished data). These findings suggest the possibility that deletion during recombination is responsible for the differences which exist between the *env* gene products of MCF 13 and the other A^+ recombinants isolated from AKR mice.

We thank P. O'Donnell, E. Fleissner, P. Etkind, N. Hopkins, E. Stockert, and E. Tress for many helpful discussions and critical readings of the manuscript. We are indebted to P. O'Donnell and N. Hopkins for permission to quote their unpublished data.

This work was supported by Public Health Service grant CA-27950 awarded by the National Cancer Institute.

LITERATURE CITED

1. **Cleveland, D., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102-1106.
2. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1979. Cell-surface antigens associated with recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **149**:702-712.
3. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1980. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **151**:542-552.
4. **Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Hartley, and W. P. Rowe.** 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (*env*) gene recombinants. Proc. Natl. Acad. Sci. U.S.A. **74**:4676-4680.
5. **Famulari, N. G., and K. Jelalian.** 1979. Cell surface expression of the *env* gene polyprotein of dual-tropic MCF murine leukemia virus. J. Virol. **30**:720-728.
6. **Famulari, N. G., J.-S. Tung, P. V. O'Donnell, and E. Fleissner.** 1980. Murine leukemia virus *env* gene expression in preleukemic thymocytes and leukemia cells of AKR strain mice. Cold Spring Harbor Symp. Quant. Biol. **44**:1281-1287.
7. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. U.S.A. **74**:789-792.
8. **Hays, E. F., and D. L. Vredevoe.** 1977. A discrepancy in

XC and oncogenicity assays for murine leukemia virus in AKR mice. Cancer Res. **37**:726–730.

9. **Kawashima, K., H. Ikeda, E. Stockert, T. Takahashi, and L. J. Old.** 1976. Age-related changes in cell surface antigens of preleukemic AKR thymocytes. J. Exp. Med. **144**:193–208.
10. **Kemp, M. C., N. G. Famulari, and R. W. Compans.** 1981. Glycopeptides of murine leukemia viruses. III. Glycosylation of *env* precursor glycoproteins. J. Virol. **39**:463–470.
11. **Kemp, M. C., N. G. Famulari, P. V. O'Donnell, and R. W. Compans.** 1980. Glycopeptides of murine leukemia viruses. II. Comparison of xenotropic and dual-tropic viruses. J. Virol. **34**:154–161.
12. **Lung, M. L., C. Hering, J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1979. Analysis of the genomes of mink cell focus-inducing murine type-C viruses: a progress report. Cold Spring Harbor Symp. Quant. Biol. **44**: 1269–1274.
13. **Nowinski, R. C., and E. F. Hays.** 1978. Oncogenicity of AKR endogenous leukemia viruses. J. Virol. **27**:13–18.
14. **O'Donnell, P. V., and R. C. Nowinski.** 1980. Serological analysis of antigenic determinants on the *env* gene products of AKR dual-tropic (MCF) murine leukemia viruses. Virology **107**:81–88.
15. **O'Donnell, P. V., E. Stockert, Y. Obata, A. B. DeLeo, and L. J. Old.** 1980. Murine leukemia virus-related cell surface antigens as serological markers of AKR ecotropic, xenotropic, and dual-tropic viruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1255–1264.
16. **O'Donnell, P. V., E. Stockert, Y. Obata, and L. J. Old.** 1981. Leukemogenic properties of AKR dual-tropic (MCF) viruses: amplification of murine leukemic virus-related antigens on thymocytes and acceleration of leukemia development in AKR mice. Virology **112**:548–563.
17. **Rommelaere, J., D. V. Faller, and N. Hopkins.** 1978. Characterization and mapping of RNase T_1-resistant oligonucleotides derived from the genomes of AKV and MCF murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **75**:495–499.
18. **Rosner, M., L. S. Grinna, and P. W. Robbins.** 1980. Differences in glycosylation patterns of closely related murine leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **77**:67–71.
19. **Stockert, E., A. B. DeLeo, P. V. O'Donnell, Y. Obata, and L. J. Old.** 1979. $G_{(AKSL2)}$: a new cell surface antigen of the mouse related to the dual-tropic MCF class of MuLV detected by naturally occurring antibody. J. Exp. Med. **149**:200–215.
20. **Tung, J.-S., and E. Fleissner.** 1980. Amplified *env* and *gag* products on AKR cells. Origin from different murine leukemia virus genomes. J. Exp. Med. **151**:975–979.
21. **Witte, O. N., and D. F. Wirth.** 1979. Structure of murine leukemia virus envelope glycoprotein precursor. J. Virol. **29**:735–743.

U.S. POSTAL SERVICE

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

(Required by 39 U.S.C. 3685)

1. TITLE OF PUBLICATION	A. PUBLICATION NO.	2. DATE OF FILING
Journal of Virology		5 October 1981
3. FREQUENCY OF ISSUE	A. NO. OF ISSUES PUBLISHED ANNUALLY	B. ANNUAL SUBSCRIPTION PRICE
Monthly	12	$130 nonmem, $30 mem

4. COMPLETE MAILING ADDRESS OF KNOWN OFFICE OF PUBLICATION (Street, City, County, State and ZIP Code) (Not printers)

1913 I Street, N.W., Washington, DC 20006

5. COMPLETE MAILING ADDRESS OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS (Not printers)

1913 I Street, N.W., Washington, DC 20006

6. FULL NAMES AND COMPLETE MAILING ADDRESS OF PUBLISHER, EDITOR, AND MANAGING EDITOR (This item MUST NOT be blank)

PUBLISHER (Name and Complete Mailing Address)

American Society for Microbiology, 1913 I Street, N.W., Washington, DC 20006

EDITOR (Name and Complete Mailing Address)

Director of Publications
Walter G. Peter III, 1913 I Street, N.W., Washington, DC 20006

MANAGING EDITOR (Name and Complete Mailing Address)

Walter G. Peter III, same as above

7. OWNER (If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.) (Item must be completed.)

FULL NAME	COMPLETE MAILING ADDRESS
American Society for Microbiology	1913 I Street, N.W., Washington, DC 20006

8. KNOWN BONDHOLDERS, MORTGAGEES, AND OTHER SECURITY HOLDERS OWNING OR HOLDING 1 PERCENT OR MORE OF TOTAL AMOUNT OF BONDS, MORTGAGES OR OTHER SECURITIES (If there are none, so state)

FULL NAME	COMPLETE MAILING ADDRESS
None	

9. FOR COMPLETION BY NONPROFIT ORGANIZATIONS AUTHORIZED TO MAIL AT SPECIAL RATES (Section 411.3, DMM only)
The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes (Check one)

☐ (1) HAS NOT CHANGED DURING PRECEDING 12 MONTHS ☐ (2) HAS CHANGED DURING PRECEDING 12 MONTHS (If changed, publisher must submit explanation of change with this statement.)

10. EXTENT AND NATURE OF CIRCULATION	AVERAGE NO. COPIES EACH ISSUE DURING PRECEDING 12 MONTHS	ACTUAL NO. COPIES OF SINGLE ISSUE PUBLISHED NEAREST TO FILING DATE
A. TOTAL NO. COPIES (Net Press Run)	7,357	6,408
B. PAID CIRCULATION 1. SALES THROUGH DEALERS AND CARRIERS, STREET VENDORS AND COUNTER SALES		
2. MAIL SUBSCRIPTION (Member)	3,193	3,341
(Nonmember)	1,551	1,758
C. TOTAL PAID CIRCULATION (Sum of 10B1 and 10B2)	4,744	5,099
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	100	100
E. TOTAL DISTRIBUTION (Sum of C and D)	4,844	5,199
F. COPIES NOT DISTRIBUTED 1. OFFICE USE, LEFT OVER, UNACCOUNTED, SPOILED AFTER PRINTING	2,513	1,209
2. RETURN FROM NEWS AGENTS	---	---
G. TOTAL (Sum of E, F1 and 2 - should equal net press run shown in A)	7,357	6,408

11. I certify that the statements made by me above are correct and complete. SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER: Walter G. Peter III, Managing Editor

PS Form 3526 June 1980 (Page 1) (See instruction on reverse)