Electron Microscopic Characterization of the Defectiveness of a Temperature-Sensitive Mutant of Moloney Murine Leukemia Virus Restricted in Assembly

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The effect of temperature shiftdown on the assembly of ts3 virions was investigated by both scanning (SEM) and transmission (TEM) electron microscopy. Ts3 is a spontaneous temperature-sensitive mutant of Moloney murine leukemia virus (Mo-MuLV) which previous studies indicated to be defective in assembly or release of the virions. In the present study, both SEM and TEM revealed the following: (i) there were more cell-associated virions in ts3-infected cells grown at the nonpermissive temperature (39°C) than either in cells grown at the permissive temperature (34°C) or in wild-type MuLV-infected cells grown at 39°C; (ii) there were more normal single particles than multiploids (virions with two or more pieces of genomic RNA) in ts3-infected cells grown at the nonpermissive temperature; (iii) there were more multiploids in ts3-infected cells grown at the nonpermissive temperature than either in cells grown at the permissive temperature or in wild-type MuLV-infected cells grown at the nonpermissive temperature; (iv) upon temperature shift from 39 to 34°C, about 90% of the cell-associated virions dissociated from the cell surface. TEM studies also indicated that upon temperature shiftdown, virion assembly rapidly occurred. The above observations suggest that faulty assembly, which results in the production of multiploids, may not be the reason why ts3 virions accumulate on the cell surface at the nonpermissive temperature. The relatively higher proportion of multiploids found in ts3-infected cells grown at 39°C compared with those grown at 34°C may be due to the higher density of budding virions at the cell surface at the nonpermissive temperature, which increases the possibility of two or more particles assembling close to one another. The accumulation of ts3 virions in all stages of assembly at the nonpermissive temperature, together with the fact that rapid assembly and release of ts3 virions occurred on temperature shiftdown, indicates that virion assembly is restricted after it has been initiated. The probable role of altered glycoprotein(s) in restricting virion assembly is discussed.

One of the major events in the replication of RNA tumor viruses is the assembly of the virions at the cell membrane. As yet the mechanism involved is poorly understood. It has been postulated (1, 4) that assembly of the virions is regulated by protein-protein interactions. One approach toward understanding the processes involved in the assembly of RNA tumor viruses is by defining the temperature-sensitive (ts) defects of viral mutants blocked in assembly. Recently, such mutants from both avian and mammalian RNA tumor viruses have been isolated and partially characterized (2, 3, 6-9, 11). Ts3 is a spontaneous ts mutant of Moloney murine leukemia virus (Mo-MuLV) isolated by Wong et al. (9). Previous studies (7, 8) have

indicated that the ts defect of ts3 may be due to a block either late in the assembly process or in the release of the virions. The present investigation on the assembly of ts3 virions further defines the ts defect of ts3.

MATERIALS AND METHODS

Cells. Detailed procedures for the propagation of TB cells, a fibroblastic cell line established from mixed cultures of bone marrow and thymus CFW/D mouse cells, have been previously described (7).

Virus. TB cells were infected in suspension at a multiplicity of infection of 5 with wild-type Mo-MuLV or ts3, a ts mutant of MuLV. Infected cells (3×10^6) were plated on each 60-mm plastic petri dish and incubated at the permissive (34°C) or nonpermissive (39°C) temperature for 48 h. Ts3-infected

cells were also incubated at the nonpermissive temperature for 48 h and then shifted to the permissive temperature for 30 or 60 min.

Transmission electron microscopy (TEM). Viral pellets and wild-type MuLV- or ts3-infected TB cells scraped off with a rubber policeman were fixed in 2% glutaraldehyde in sodium cacodylate buffer or phosphate-buffered saline and then postfixed in 2% osmic acid overnight or in a freshly prepared mixture of 0.12 g of potassium ferrocyanide in 6 ml of 1% osmic acid. After dehydration through graded ethyl alcohol, the cells were embedded in Spurr's plastic. Sections were mounted directly on 300-mesh grids and stained in uranyl acetate at 70°C in a water bath and in Reynolds lead citrate. Specimens were viewed in a Siemens 102 electron microscope at 80 kV. For quantitation of virions in thin sections, cell sections were chosen at random, and the type of virion and the stage in assembly of the virions were scored by viewing at a magnification of $\times 80,000$.

SEM. The details for preparing infected cells for scanning electron microscopy (SEM) and quantitation of the cell-associated virions have been described (8).

RESULTS

Relative proportion of normal C-type particles to virion aggregates in ts3- or MuLV-infected cells as determined by SEM. We reasoned that if most of the ts3 particles produced at the nonpermissive temperature (39°C) are "polymorphic" as reported by Wong and McCarter (7), in contrast to the production of normal C-type particles at the permissive temperature (34°C), faulty assembly of the virion at 39°C may be indicated. Ts3-infected cells were therefore grown at 34 or 39°C for 48 h or 39°C for 48 h and then shifted to 34°C for 15 or 60 min; MuLV-infected cells were grown at 39°C for 48 h and prepared for SEM as described in Materials and Methods.

Figure 1A shows a representative portion of the cell surface of a ts3-infected cell at the nonpermissive temperature 48 h postinfection. Most of the particles present were normal C-type spherical forms. Scattered among them were duplexes (black arrow) and short microvillus-like structures (white arrow), which may consist of two or more virions aligned in tandem (Fig. 1B). Triplexes (Fig. 1C and D), quadruplexes (Fig. 1E), and larger aggregates (Fig. 1F and G) were also observed in ts3-infected cells grown at the nonpermissive temperature or grown at the nonpermissive temperature for 48 h and then shifted to the permissive temperature for 15 min.

In ts3-infected cells grown at 34°C and in MuLV-infected cells grown at 39°C, the number of particles observed on the cell surface was greatly reduced as compared with ts3-infected cells grown at the nonpermissive temperature. Duplexes and occasionally triplexes were also

observed in the former cultures; however, larger virion aggregates were not found.

The relative proportions of normal C-type particles to aggregates in the different cultures examined are shown in Table 1. The figures presented in Table 1 may be only rough estimates, because only virions in the late stages of assembly could be identified with certainty. The total number of aggregates may also be underestimated, since rod-shaped virions may be mistaken for microvilli. Atypically shaped or unusually large spherical particles were not included in the counts recorded in Table 1. Since the same types of errors were encountered in all the samples examined, the figures presented in Table 1 should be an indication of real differences among the samples studied.

The results presented in Table 1 may be summarized as follows: (i) there were more virions on the cell surface of ts3-infected cells grown at 39°C than on ts3-infected cells grown at 34°C or on MuLV-infected cells grown at 39°C; (ii) more than 90% of the ts3 particles that had accumulated on the cell surface at 39°C disappeared from the cell surface within 1 h after temperature shiftdown; (iii) there were more normal single particles than aggregates in the ts3-infected cells grown at 39°C; (iv) there were more aggregates in the ts3-infected cells grown at 39°C than in either ts3-infected cells grown at 34°C or MuLV-infected cells grown at 39°C.

The above findings suggest that formation of morphologically aberrant particles may not be the reason why ts3 virions fail to complete assembly and release from the cell surface at the nonpermissive temperature. The relatively higher proportion of aggregates found in the ts3-infected cells grown at 39°C could be due to the higher density of budding virions at the cell surface, which increases the probability of two or more particles assembling close to one another. Furthermore, the accumulation of virions at the nonpermissive temperature may be attributed either to a block in some process(es) in virion assembly or to a ts defect in the release of the virion per se. This latter possibility would be indicated if most of the particles were completely assembled, but failed to dissociate from the cell surface at the nonpermissive temperature.

TEM studies. To investigate the above possibilities, TEM studies were conducted on ts3-infected cells to determine the stage in assembly at which the assembly of ts3 is blocked at the nonpermissive temperature, and whether further assembly of these ts3 virions can take place on temperature shiftdown.

Ts3-infected TB cells grown at 34 or 39°C for 48 h, and ts3-infected TB cells grown at 39°C for

FIG. 1. (A) SEM of a portion of a *ts3*-infected TB cell surface grown at the nonpermissive temperature showing normal spherical virions as well as duplexes (black arrow) and short microvilli that may be short rod-shaped virions (white arrow). Scale = 500 nm. (B-G) Scale = 250 nm. (B) Short rod-shaped virions; (C and D) triplexes; (E) quadruplexes; (F and G) large virion aggregates.

48 h and then shifted to 34°C for 30 or 60 min, were prepared for TEM as described in Materials and Methods.

Ts3 virions. Thin-section electron microscopy of ts3-infected cells grown at the nonpermissive temperature revealed the presence of both normal C-type particles and multiploids (particles with more than one ribonucleoprotein component) in all stages of assembly. The structure and assembly of both types of particles have been reported recently (12).

Effect of temperature shiftdown on the assembly of ts3 virions. A composite view of ts3-

TABLE 1. Relative proportions of normal C-type particles to aggregates^a in ts3- or MuLV-infected cultures determined by SEM

Type of culture	Total cell surface area examined (μm^2)	Total no. of particles/100- μm^2 area	% of aggregates to total no. of particles
ts3, 39°C (48 h)	705	250	18
ts3, 39 → 34°C (15 min)	326	228	18
ts3, 39 → 34°C (60 min)	285	11	3
ts3, 34°C (48 h)	367	38	8
MuLV, 39°C (48 h)	825	29	8

^a Duplexes, triplexes, etc.

infected cells grown at the nonpermissive temperature for 48 h is shown in Fig. 2. TEM indicates that although both normal particles and multiploids in different stages in assembly were present in ts3-infected cells grown at the nonpermissive temperature, less than 20% of these particles were completely assembled (Fig. 2d) and on the verge of dissociating from the cell surface. This finding is consistent with the infrequent observation of extracellular immature C-type particles and the virtual absence of mature C-type particles in the intercellular spaces of ts3-infected cells grown at the nonpermissive temperature (Fig. 3A). The above observations suggest that at the nonpermissive temperature, virion assembly is initiated but probably restricted. It was further reasoned that if ts3 is temperature sensitive in some factor (function) restricting assembly, on temperature shiftdown, one should be able to detect rapid virion assembly and release. This would be reflected in a change in the composition of virions in the different stages of assembly. In an attempt to verify the above hypothesis, the relative proportions of virions in the various stages of assembly were compared in the following cultures: ts3 grown at 39°C for 48 h, ts3 grown at 39°C for 48 h and then shifted to 34°C for 30 or 60 min, and ts3 grown at 34°C for 48 h. The normal budding C-type particles were arbi-

FIG. 2. Composite electron micrograph of a ts3-infected TB cell grown at the nonpermissive temperature for 48 h. Scale = 100 nm. (a) Normal virion at an early stage in assembly with an arc-shaped ribonucleoprotein (RNP). (b) Normal virion at an intermediate stage in assembly with a semicircular RNP. (c) Spherical duplex at a late stage in assembly showing a break in the RNP (arrow) and a larger size and broader linkage to the cell compared with (d). (d) Normal virion at a very late stage in assembly showing complete assembly of the RNP, narrow linkage to the cell, and resealing of the cellular membrane. (e) Normal virion at a very late stage in assembly sectioned through the core shell. (f) Multiploid virion at an early stage in assembly showing breaks in the RNP and broad linkage to the cell. (g) Multiploid during the final stages in assembly showing similar characteristics as pointed out for the normal virion shown in (d).

FIG. 3. (A) Uncommon view showing an accumulation of budding virions (a), complete assembled normal virions (b), and multiplouids (c) in the intercellular space of *ts3*-infected cells grown at the nonpermissive temperature. Scale = 200 nm. Not all the completely assembled virions are necessarily extracellular, since the section could have been cut through the transverse axis of some of the virions. (B) Accumulation of mature particles in the intercellular space of *ts3*-infected cells grown at the permissive temperature showing variation in the shape and size of the cores of the virions. Scale = 200 nm. Virion with a regular pentagonal core (single black arrow); virion with an elongated core (double black arrow); spherical virion with two cores (white arrow).

trarily separated into four groups according to their stage in assembly: early (Fig. 2a), intermediate (Fig. 2b), late (Fig. 4b), and very late (Fig. 2d and 4c).

The results of this investigation are summarized in Table 2. In the ts3-infected cells grown at the permissive temperature, almost 90% of the particles observed were non-cell associated, and more than 90% of these were mature particles with electron-dense nucleoid (Fig. 3B). Only 2% of the cell-associated virions were multiploids. The normal C-type cell-associated particles appeared to be evenly distributed among the four stages of assembly.

Some marked differences were observed between ts3 virions from cells grown at the permissive temperature as compared with those grown at the nonpermissive temperature. There were 22 cell-associated virions per cell section in ts3-infected cells grown at 39°C as compared with 0.5 virion per cell section in ts3-infected cells grown at 34°C. In contrast to the virions found in cells grown at the permissive temperature, 96% of the virions observed in the nonpermissive culture were cell associated. The few extracellular virions observed were immature (Fig. 3A). Mature virions may have been present in such low numbers that they were not detected. In the culture grown at the nonpermissive temperature, 29% of the total virions observed were multiploids, whereas only 2% of the virions in the culture grown at the permissive temperature were multiploids. Fifty percent of the normal C-type virions observed in the culture grown at the nonpermissive temperature were early-budding virions, whereas less than 20% of them were in a very late stage in assembly.

A composite view of ts3-infected cells grown at 39°C for 48 h and then shifted to 34°C for 30 min is shown in Fig. 4. Several marked changes were observed. The number of cell-associated virions decreased to about 2 virions per cell section, whereas the ratio of multiploids to normal particles remained the same. The number of extracellular particles increased from about 4% to almost 50% of the total number of particles observed, and more than 80% of these were immature particles. Among the cell-associated normal C-type virions, over 30% were late-budding particles, and almost 60% were in the final stages of assembly.

As compared with a ts3-infected culture shifted to the permissive temperature for 30 min, a longer shift-down period (60 min) further reduced the number of cell-associated virions to about 0.5 virion per cell section. There was a further increase in the proportion of non-cell-associated virions to cell-associated virions and

a reduction in the proportion of multiploids to total virions. Among the normal C-type particles, there appeared to be a slight increase in the proportion of early-budding virions.

The above findings may be summarized as follows: multiploids were present in ts3-infected cells grown at both the permissive and nonpermissive temperature. Consistent with our SEM findings, the proportion of multiploids to normal virions was higher in ts3-infected cells grown at the nonpermissive than at the permissive temperature. In spite of the fact that 50% of the normal particles in the very early stages in assembly could not be identified in the SEM studies, a higher proportion of multiploids was observed in the TEM studies. This suggests that the duplexes, triplexes, etc., observed by SEM were only a portion of the total multiploids present. The proportion of multiploids calculated from the TEM studies did not exceed 30% of the total virions observed in ts3-infected cells grown at the nonpermissive temperature. Virion assembly rapidly occurred on temperature shift-down with both normal particles and multiploids dissociating from the cell surface.

DISCUSSION

Our SEM and TEM studies confirmed the findings of Wong and MacLeod (8) that there were substantially more virions on the cell surface of ts3-infected TB cells grown at the nonpermissive temperature (39°C) than of cells grown at the permissive temperature (34°C) or of MuLV-infected TB cells grown at 39°C. Our SEM studies agree with their observations that more than 90% of the virions that had accumulated at the cell surface at 39°C dissociated from the cell surface during the first 60 min after temperature shift-down. However, our TEM studies indicate that when the accumulation of virions on the cell surface is maximal, the total cell surface area that can be occupied by virus particles is greater than the 10% estimated by Wong and MacLeod (8), since neither virions in the very early stages of assembly (about 50% of the total number of normal cell-associated C-type particles) nor many of the multiploids (such as the rod-shaped virions, the abnormally large spherical forms, or the atypically shaped virions) were identified by SEM.

Our present findings suggest that the production of atypically shaped particles (multiploids) at the nonpermissive temperature may not be a direct result of the ts defect of ts3. Less than 30% of the virions observed by TEM from ts3-infected cells grown at the nonpermissive temperature were multiploids. Moreover, multiploids, primarily duplexes, were also consist-

FIG. 4. *Ts3*-infected TB cells grown at the nonpermissive temperature for 48 h and then shifted to the permissive temperature for 30 min showing extracellular immature normal virions (d) and multiploids (e-g) and normal virions (b and c) and multiploids (a) at a late or very late stage in assembly. Scale = 100 nm.

TABLE 2. *Relative proportions of normal virions to multiploids and virions in various stages of assembly in different ts3-infected cultures determined by TEM*

Type of culture	Total no. of particles observed	No. of cell-associated virions/cell section	% Membrane-associated particles of total particles	% Multiploids of total particles	% Free immature particles of non-membrane-associated particles	% of different development stages of membrane-associated normal particles ^a			
						Early	Intermediate	Late	Very late
ts3, 34°C	548	0.5	11	2	8	18	34	28	20
ts3, 39°C	622	22	96	29	100 ^b	50	13	18	19
ts3, 39 → 34°C (30 min)	559	2	53	28	82	3	7	31	59
ts3, 39 → 34°C (60 min)	644	0.5	36	16	90	16	9	44	31

^a See legend to Fig. 2 and 4.^b Mature particles were probably present in such low numbers that they were not detectable.

ently observed, although in lower numbers, in ts3-infected cells grown at 34°C, in MuLV-infected cells grown at 39°C (12), and in a Moloney murine sarcoma virus (Mo-MuSV) cell line (unpublished data). The higher proportion of multiploids to total particles found in ts3-infected cells grown at the nonpermissive temperature may be due to the greater chance of two or more particles assembling in juxtaposition because of the accumulation of budding viruses at the cell surface. The above suggestion is consistent with the observed decrease in the proportion of multiploids to total particles in interferon-treated cells infected with ts3 (unpublished data). It was shown in another study (10) that the total number of ts3 virions decreased by two- to threefold in interferon-treated cells as compared with the controls. The production of atypically shaped virions in Friend leukemia virus-infected cells after dimethyl sulfoxide treatment has also been reported by Sato et al. (5). In these dimethyl sulfoxide-treated cells, there was a significant increase in the number of budding virions as compared with the controls.

The ts defect of ts3 appears to be due to some restriction in virion assembly. At the nonpermissive temperature, both normal and multiploid virions in all stages of assembly were observed. Only about 20% of the normal particles observed were at a very late stage in assembly (Fig. 2d). Four percent of the total particles observed were extracellular, none of which were mature virions. However, after a 30-min shift to permissive conditions, about 90% of the normal C-type virions observed were late-budding virions (Fig. 4b), with almost 60% of these at a very late stage in assembly (Fig. 4d). This rapid increase in the proportion of budding virions in the final stages of assembly on temperature shiftdown, in conjunction with a simultaneous decrease in the number of cell-associated

virions and an increase in the number of extracellular immature virions, suggests that rapid assembly and release of the virions occurred during the 30 min after shift to the permissive temperature. On temperature shiftdown, rapid assembly and release of the multiploid virions also seem to have occurred. In the 39°C-infected culture, most of the multiploids observed were broadly linked to the cell at their bases (Fig. 2c). On temperature shiftdown, most of them showed complete assembly of viral components, resealing of the cellular membrane, and shrinking of the cytoplasmic linkage to the cell (Fig. 2g and 4a). The large number of incompletely assembled ts3 virions in all stages of assembly at the nonpermissive temperature suggests that restriction in assembly only occurs after it has been initiated and that the ts lesion is a step common to the whole assembly process.

We have as yet no conclusive evidence as to how virion assembly is restricted at the nonpermissive temperature. Previous findings (7) indicate that ts3 virions partially assembled on the cell membrane of cultures held at 39°C before the addition of actinomycin D or cycloheximide are able to complete assembly and dissociate from the cell membrane on temperature shiftdown to 34°C in the presence of the inhibitors. This suggests that the ts defect lies beyond events associated with the synthesis of viral DNA, DNA-dependent RNA, and protein. The ts defect has also been demonstrated (7) to be reversible, because even in an established infection a shift from 34 to 39°C turns off virus production almost immediately. In the same study, the ability of ts3 to rescue MuSV from an MuSV-infected nonproducer cell line was also examined. Whereas ts3 was found to rescue MuSV at 34°C, a shift to 39°C immediately blocked release of both viruses. A subsequent shift from 39 to 34°C again resulted in the acti-

nomycin D- and cycloheximide-insensitive release of both ts3 and MuSV during the first hour after shiftdown. Recently, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (J. A. Ptak and P. K. Y. Wong, manuscript in preparation) indicated that the mobility of the glycoprotein fraction of ts3 virions grown at the permissive temperature is different from that of wild-type MuLV. In addition, the mobility of viral proteins precipitated by anti-MuLV gp71 from ts3-infected cells grown at the nonpermissive temperature was also different from that precipitated from wild-type MuLV-infected cells. Furthermore, the proteins precipitated from ts3-infected cells grown at the nonpermissive temperature showed an increase in the amount of the 85,000-molecular-weight glycoproteins and a concomitant decrease in gp69/71.

All the above findings indicate that the defect of ts3 may reside in either the processing or conformation of the viral envelope components(s). It has been suggested that the assembly of enveloped viruses is regulated by protein-protein interactions (4). The conversion of a membrane area into a budding patch may be brought about by insertion of glycoprotein molecules into the lipid bilayer (1). It was further suggested that the shape and rigidity of the viral envelope is maintained by a viral structural component laid down in numerical correspondence to the envelope glycoprotein(s). It is conceivable that other structural proteins, including the ribonucleoprotein, may be similarly assembled in their correct order. We suggest that if the glycoprotein(s) of ts3 is unable to assume its correct conformation at the nonpermissive temperature, then the assembly of the rest of the viral components may be hampered by either an inefficiency or inaccuracy in the protein-protein interactions. However, once permissive conditions are restored, the defective conformation of the glycoprotein is corrected and normal assembly can be resumed.

Among the late ts mutants of Rauscher leukemia virus isolated by Stephenson and Aaronson (6), ts24 exhibits characteristics similar to those of ts3. Recent microscopic studies (11) suggest that ts24 is defective either in the late stages of assembly of the particles or in the release of the virus particles from the cell surface. Ts24 resembles ts3 in that there are more cell-associated virions at the nonpermissive than at the permissive temperature and that virions are rapidly released on temperature shiftdown. However, unlike ts3, there appear to be far fewer cell-associated virions in ts24-infected cells grown for 6 days at the nonpermissive temperature (4 to 5 virions/cell section) as compared with ts3-infected cells grown for 48 h at 39°C (22 virions/cell section). Furthermore,

all the budding ts24 virions observed in infected cells grown at the nonpermissive temperature were almost completely assembled, normal virions. By contrast, both normal particles and multiploids in all stages of assembly were observed in ts3-infected cells grown at the nonpermissive temperature.

It is possible that although ts3 and ts24 have certain characteristics in common, they are in fact temperature sensitive in different functions. Alternatively, they may be defective in the same function to varying degrees. An approach in resolving this possibility may be achieved by means of complementation and recombination tests between these mutants.

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LITERATURE CITED

1. Blough, H. A., and J. M. Tiffany. 1975. Theoretical aspects of structure and assembly of viral envelopes. *Curr. Top. Microbiol. Immunol.* 70:1-30.
2. Friis, R. R., H. Ogura, H. Gelderblom, and M. S. Halpern. 1976. The defective maturation of viral progeny with a temperature-sensitive mutant of avian sarcoma virus. *Virology* 73:259-272.
3. Hunter, E., M. J. Hayman, R. W. Rongey, and P. K. Vogt. 1976. An avian sarcoma virus mutant that is temperature sensitive for virion assembly. *Virology* 69:35-49.
4. Klenk, H.-D. 1974. Viral envelopes and their relationship to cellular membranes. *Curr. Top. Microbiol. Immunol.* 68:29-58.
5. Sato, T., C. Friend, and E. de Harven. 1971. Ultrastructural changes in Friend erythroleukemia cells treated with dimethyl sulfoxide. *Cancer Res.* 31:1402-1417.
6. Stephenson, J. R., and S. A. Aaronson. 1973. Characterization of temperature-sensitive mutants of murine leukemia virus. *Virology* 54:53-59.
7. Wong, P. K. Y., and J. A. McCarter. 1974. Studies of two temperature sensitive mutants of Moloney murine leukemia virus. *Virology* 58:396-408.
8. Wong, P. K. Y., and R. MacLeod. 1975. Studies on the budding process of a temperature-sensitive mutant of murine leukemia virus with a scanning electron microscope. *J. Virol.* 16:434-442.
9. Wong, P. K. Y., L. J. Russ, and J. A. McCarter. 1973. Rapid, selective procedure for isolation of spontaneous temperature-sensitive mutants of Moloney murine leukemia virus. *Virology* 51:424-431.
10. Wong, P. K. Y., P. H. Yuen, R. MacLeod, E. H. Chang, M. W. Myers, and R. M. Friedman. 1977. The effect of interferon on *de novo* infection of Moloney leukemia virus. *Cell* 10:245-252.
11. Yeger, H., V. I. Kalnins, and J. R. Stephenson. 1976. Electron microscopy of mammalian Type-C RNA viruses: use of conditional lethal mutants in studies of virion maturation and assembly. *Virology* 74:459-469.
12. Yuen, P. H., and P. K. Y. Wong. 1977. A morphologic study on the ultrastructure and assembly of murine leukemia virus using a temperature-sensitive mutant restricted in assembly. *Virology* 80:260-274.