Two Avian Sarcoma Virus Mutants with Defects in the DNA Polymerase-RNase H Complex

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Two mutants of avian sarcoma virus which exhibit different phenotypes have been analyzed for the properties of their RNA-dependent DNA polymerase and RNase H activities. LA 338 is a complex multiple mutant with at least one lesion each in transformation and replication functions. The purified RNA-dependent DNA polymerase-RNase H complex from the mutant is twofold more thermolabile than that from the wild-type parent. A peculiarity of this mutant is that the ability of the enzyme to respond to synthetic template-primers is lost more rapidly than is the response to native RNA as template. The mutant enzyme cannot be protected from inactivation by the addition of synthetic template-primers. LA 672 represents a different phenotype among reverse transcriptase mutants, showing a "late"-acting block in replication which affects only production of progeny by infected cells grown at the nonpermissive temperature. The purified DNA polymerase-RNase H complex of LA 672 is not thermolabile; rather, progeny grown at the nonpermissive temperature yield purified enzyme with a 20-fold-reduced specific activity in both DNA polymerase and RNase H. The content of reverse transcriptase protein in such noninfectious progeny, furthermore, did not appear to be significantly diminished since immunologically active enzyme could be demonstrated in a competition test for anti-reverse transcriptase antibody and since β and α subunits of reverse transcriptase could be identified after polyacrylamide gel electrophoresis of partially purified enzyme preparations. The amounts of β and α from the mutant were about twofold lower.

Isolation of avian sarcoma virus mutants with temperature-sensitive lesions in reverse transcriptase provided the first proof that this activity is encoded in the viral genome (7, 8, 16). The mutants on which this conclusion was based, LA 335 and LA 337, typify the classic phenotype which is now accepted for mutants defective in the *pol*, or polymerase, gene (1); these mutants show an early defect which prevents infection of cells at the nonpermissive temperature by blocking the synthesis of proviral DNA in the first hours after infection. If infection was performed for even 12 h at the nonpermissive temperature before a shiftdown to the permissive temperature, infection, host cell transformation, and progeny replication were all coordinately interdicted.

This paper will greatly extend previous information (4, 5, 11) about the nature of the lesion in LA 672, a late-acting mutant also defective in *pol*. Furthermore, evidence which helps to explain the very complicated phenotype of LA 338 (2, 17, 20) will be presented.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo cells were

prepared from fertile White Leghorn embryos obtained from Heisdorf and Nelson Farms, Redmond, Wash., and embryos negative for the expression of chick helper factor (6) were selected by the method of Friis et al. (5). Dulbecco-modified Eagle medium supplemented with 10% tryptose phosphate broth, 5% calf serum, and 1% dimethyl sulfoxide was employed for virus stock preparations. Focus tests were performed with an 0.8% agar overlay made with the same medium, essentially as described by Rubin (12). For virus stock preparations, chicken embryo cells were infected with viruses in the presence of 2 μ g of Polybrene (Aldrich Chemical Co.) per ml (14), and cultures were maintained with regular transfers at the permissive temperature (35°C) for 10 to 14 days until 30 to 40 dishes (90-mm diameter) each of wild-type (wt) and mutant virus-infected cells had been prepared. Harvests were then made either from cultures further maintained at 35°C or after a 2-day adaption period from cultures at the nonpermissive temperature (42°C). These latter cultures were harvested in a warm room at 40.5°C on a warm table adjusted to 42°C to avoid temperature shifts. Harvests were usually made at 24-h intervals, except for LA 672, which in some experiments was harvested as frequently as every 3 h. Pooled culture supernatants were frozen at -70°C until a volume of 3 to 5 liters was available, at which time virus was pelleted in a Beckman type 19 rotor (19,000 rpm for 2 h), followed by purification of virus

on linear 20 to 70% sucrose gradients. Biological controls were performed with all virus preparations to demonstrate that each stock exhibited the typical temperature-sensitive properties of the respective mutants and the correct subgroup.

The mutants were obtained from the Prague strain of Rous sarcoma virus, either subgroup A or subgroup C (Pr-A or Pr-C): LA 338, subgroup C, transformation and replication defective (20), and LA 672, subgroup A, replication defective and late reverse transcriptase defective (4). For control purposes, either the wt Pr-C or wt Pr-A was always included in each experiment.

Assays of heat lability of reverse transcriptase-RNase H. Heat inactivation studies were performed by incubating the virion-associated or purified DNA polymerase-RNase H complex in a preincubation mixture containing 0.05 M Tris-hydrochloride, pH 7.9, 8 mM MgCl₂, 80 mM KCl, and 10 mM dithiothreitol. Nonidet P-40 (0.02%) was added for the virion-associated enzyme assays. The mixture was incubated at the temperatures indicated in the figure legends, which had been chosen empirically as appropriate for the mutant under study. At various times, 0.05-ml samples were removed and were added to 0.05 ml of a reaction mixture which contained the same ions as did the preincubation mixture supplemented with either template-primer and [³H]TTP for a DNA polymerase reaction or substrate for an RNase H assay (9). This mixture was kept in melting ice for up to 40 min until all samples had been collected and then was incubated for 15 min at 38°C. Acid-precipitable radioactivity was measured. The rate of the enzyme reactions at 38°C was linear for at least 15 min. RNase H activity was assayed analogously, the substrate used being fd DNA-RNA hybrid as previously described (9).

The specific activity of the labeled deoxyribonucleoside triphosphate was 400 cpm of [³H]TTP per pmol in the case of synthetic template-primers and 5,000 cpm of [³H]TTP per pmol in the case of native RNA templates or endogenous reactions. The RNase H assay contained 20,000 cpm of RNA-DNA hybrid, with a specific radioactivity of 3,500 cpm of [³H]UMP per pmol.

Purification of reverse transcriptase-RNase H. The reverse transcriptase-RNase H complexes from the different wt viruses and their mutants were purified according to published procedures by DEAE-cellulose and subsequent phosphocellulose column chromatography (9). The DEAE column was washed with 0.1 M phosphate buffer, pH 7.2, and the enzyme was eluted stepwise with 0.3 M phosphate buffer to speed up the purification procedure (18). The phosphocellulose columns were eluted with linear salt gradients of 0 to 0.8 M KCl in phosphate buffer, pH 8.2. wt and mutant enzymes were extracted simultaneously as rapidly as possible and assayed immediately.

Inhibition of neutralization of AMV DNA polymerase with antipolymerase IgG by LA 672. The experiment for inhibition of neutralization of avian myeloblastosis virus (AMV) DNA polymerase with antipolymerase immunoglobulin G (IgG) by LA 672 was performed by the procedure of Nowinski et al. (10) as used for the analysis of Rous sarcoma virus- α . In a 50- μ l reaction mixture (0.01 M Tris-hydrochloride, pH 8.2, 0.15 M KCl), 1 μ g of the IgG fraction from

antisera to AMV DNA polymerase was mixed with LA 672 virus that had been previously disrupted with 0.2% Nonidet P-40 for 10 min at 4°C. After 60 min at 37°C, glycerol (final concentration, 20%), bovine serum albumin (50 μ g), and detergent-disrupted AMV virion DNA polymerase (1 U of activity) were added. This reaction mixture (70 μ l) was incubated at 37°C for 60 min. The residual enzyme activity was tested by the addition of synthetic template-primer, triphosphates, Mg²⁺, and dithiothreitol as required for the standard DNA polymerase assay (110- μ l final volume). Samples (50 μ l) were removed from reaction mixtures at 15 and 30 min at 39°C and processed for the determination of radioactivity.

Unit of enzyme activity. One unit of enzyme activity is defined as the amount of enzyme required to incorporate 1 pmol of TMP at 39°C in 1 min with the synthetic template-primer polyriboadenylic acid-oligodeoxythymidylic acid [poly(rA)-oligo(dT)] in a standard polymerase assay mixture.

Sera. Monospecific antiserum prepared in rats against purified AMV DNA polymerase was a generous gift of R. C. Nowinski. The IgG fraction was purified from the serum by Sephadex G-200 chromatography (19).

Buffers and solutions. TNE, pH 7.4, consisted of 0.05 M Tris-hydrochloride, pH 7.4, 0.1 M NaCl, and 0.005 M EDTA pH 7.2; sucrose and glycerol solutions were made in TNE, pH 7.4; phosphate buffers consisted of a mixture of KH₂PO₄ to give the indicated pH values. For enzyme extractions, the phosphate buffers (20 mM) contained 0.2% Nonidet P-40, 20% glycerol, and 1 mM dithiothreitol.

RESULTS

Virion-associated and purified DNA polymerase-RNase H complex of wt Pr-C and LA 338. The inactivation kinetics of the virion-associated enzymes from mutant virus preparations grown at permissive and nonpermissive temperatures were compared with those of the wt virus. The mutant virus preparations were consistently more thermolabile than the wt virus; the DNA polymerase activity exhibited by both mutants, using either endogenous template or the synthetic template-primer, showed half-lives reduced by a factor of three- to fivefold (Fig. 1a and b). The virion-associated RNase H activity was also temperature sensitive for both mutant preparations compared with the wt (Fig. 1c).

The enzymes were purified by column chromatography as described above. The column elution profiles are shown in Fig. 1d. The inactivation kinetics of the purified enzymes from mutant virus which had been grown at 35°C and from wt virus after reactions with various templates can be seen in Fig. 2a through c. For example, the half-life of purified mutant enzyme compared with wt enzyme was reduced by a factor of about 2 when native RNA plus oligo(dT) was used as template-primer. The pu-

FIG. 1. Thermal inactivation kinetics of the virion-associated reverse transcriptase activity from the mutant LA 338 grown at 35 and 42°C and its wt Pr-C. Mutant and wt viruses were concentrated 1,000 times from 3 liters of culture fluid each; 1.5 μ l of the mutant and 1 μ l of the wt viruses were preincubated at 47°C. At the indicated times, samples were removed from the preincubation mixtures and tested for residual enzyme activities with (a) the endogenous RNA plus oligo(dT) and (b) the synthetic template-primer poly(rA)·oligo(dT). (c) Twice the amount of mutant and wt virus was used for RNase H assay with 3 H-labeled RNA·DNA hybrid as substrate. (d) Purification of LA 338 grown at 35°C (●) and wt Pr-C (○) DNA polymerases. Elution profiles of poly(rA)·oligo(dT)-directed DNA synthesis from DEAE-cellulose (left) and phosphocellulose (right) columns are shown. Ten microliters of each fraction was used for determination of DNA polymerase activity. The molar concentration of the KCl gradient is indicated.

rified mutant enzyme also demonstrated a thermostable RNase H activity compared with the wt enzyme (Fig. 2d). Although the degree of thermostability appeared less striking with purified mutant preparations than with virion-associated enzyme preparations, half-lives of purified mutant DNA polymerase and RNase H activities were, in four separate experiments, found to be significantly reduced.

Preincubation of the purified DNA polymerase in the presence of synthetic template-primer

did not result in stabilization of activity (Fig. 2c). Furthermore, LA 338 enzyme exhibited a peculiar instability for activity on synthetic template-primer. A freshly prepared purified enzyme preparation showed a 10-fold reduction of activity for reaction with poly(rA)·oligo(dT) template-primer after storage for 24 h at 0°C (Fig. 2e). The residual activity showed an inactivation rate indistinguishable from that of the wt enzyme (Fig. 2e). In comparison, a twofold loss of activity for reactions with native RNA

FIG. 2. Thermal inactivation kinetics of the purified reverse transcriptase from the mutant LA 338 grown at 35°C and its wt Pr-C. Twenty microliters of the peak fraction of the enzyme activities eluted from the phosphocellulose column was used per assay. The enzymes were preincubated at 47°C for the times indicated and subsequently tested for residual activity in a standard enzyme assay at 37°C for 15 min. (a) 70S viral RNA plus oligo(dT) (0.1 µg) was used as template; (b) poly(rA)-oligo(dT); (c) poly(rA)-oligo(dT) present during preincubation; (d) ³H-labeled RNA-DNA substrate was added for determination of RNase H activity; (e) and (f) show thermal inactivation kinetics similar to (a) and (b), except that the enzymes were kept at 0°C for 24 h before the inactivation assay. The response of the mutant enzyme to synthetic template-primer was reduced by a factor of 10 (note differences in scale), whereas the response to native viral RNA plus oligo(dT) remained unchanged.

templates was observed during that time, and the residual activity was more thermolabile than that of the wt enzyme in an inactivation experiment (Fig. 2f). Addition of oligo(dT) primers did not result in further stimulation (data not shown). Such a loss of activity for synthetic template-primer was not observed with the wt enzyme; therefore, this behavior apparently represents a specific lability of the mutant enzyme. In some virus preparations, the virion-associated

DNA polymerase reaction showed almost no stimulation at all after addition of poly(rA)-oligo(dT) or oligo(dT) to the endogenous reaction. Hence, with this mutant, speed of preparation was essential for meaningful results.

Virion-associated and purified DNA polymerase-RNase H complex of wt Pr-A and LA 672. The virion-associated DNA polymerase activities of wt Pr-A grown at 41°C and of LA 672 grown at 35°C were compared. No difference in specific activities of DNA polymerase in an endogenous reaction, with added synthetic template-primer poly(rA)-oligo(dT), with or poly-ribocytidylic acid-oligodeoxyguanydic acid, or with native 70S AMV RNA could be observed between the wt virus and LA 672 mutant virus (data not shown). The RNase H activities were likewise very similar (data not shown). Furthermore, no increased thermolability of the DNA polymerase-RNase H activities could be detected in LA 672 grown at 35°C in comparison with wt Pr-A (data not shown).

LA 672 grown at the nonpermissive temperature (41°C) showed, however, at most 5% of the polymerase activity detectable with LA 672 grown at the permissive temperature (35°C) or wt Pr-A grown at 41°C (data not shown). The RNase H activities were slightly reduced, i.e., only three- to fivefold.

The question arose whether LA 672 virus grown at the nonpermissive temperature might be lacking the DNA polymerase-RNase H complex or whether the enzyme complex was present in an enzymatically inactive form. This question has been answered in the following two ways: (i) by identifying an immunologically active enzyme complex in LA 672 grown at 41°C according to its ability to compete with a functional reverse transcriptase for antipolymerase antibodies [a similar approach has been used by Nowinski et al. (10) for analysis of Rous sarcoma virus-α (0)]; (ii) by purifying the enzyme from the mutant virus grown at 41°C and comparing it on sodium dodecyl sulfate-polyacrylamide gels with the enzyme contained in an equivalent amount of LA 672 grown at 35°C.

LA 672 was allowed to compete with AMV virion DNA polymerase for antipolymerase IgG (Fig. 3). Small amounts of LA 672 which did not give rise to polymerase activity (reaction I in Fig. 3) were allowed to adsorb antipolymerase IgG. The amount of antipolymerase IgG was adjusted such that it was able to inhibit 80% of a standard AMV DNA polymerase activity (reaction II in Fig. 3). The uninhibited AMV DNA polymerase activity can maximally reach the activity shown in reaction III. If LA 672 DNA polymerase adsorbs all antiserum, then subse-

FIG. 3. Competition of LA 672 grown at 35°C (a) and 41°C (b) with AMV DNA polymerase for antipolymerase serum. Detergent-disrupted LA 672 virions were incubated with the IgG fraction of antipolymerase serum (reaction IV) or normal serum (reactions I and II) for 60 min at 37°C. Reaction II did not receive virus, but received antipolymerase IgG only. Detergent-disrupted AMV was then added to reactions I, II, and IV and allowed to compete with LA 672 for the immunoglobulins for 60 min at 37°C. The residual enzyme activity was then tested with the synthetic template-primer poly(rA)-oligo(dT). Samples were removed from the reaction mixtures after 15 and 30 min at 39°C and processed for determination of radioactivity. Reaction I (LA 672 plus normal IgG) represents a control which shows that LA 672 does not contribute any activity to the final polymerase assay. Reaction II (antipolymerase IgG plus AMV) establishes the amount of neutralization of AMV by antipolymerase IgG (about 8%). Reaction III (LA 672 plus normal IgG plus AMV) gives the optimum value of enzyme activity which can be reached if all of the neutralizing antisera were adsorbed by LA 672, leaving the AMV polymerase uninhibited. Reaction IV (LA 672 plus antipolymerase IgG plus AMV) shows the result of the competition of LA 672 and AMV for the antipolymerase IgG. If LA 672 adsorbs all antiserum, the AMV DNA polymerase remains unblocked and reaction IV will be identical to reaction III. If LA 672 does not contain a DNA polymerase which can adsorb the antipolymerase serum, all antiserum will be available for inhibition of the AMV DNA polymerase and reaction IV will be similar to reaction II.

quently added AMV DNA polymerase will remain unblocked and this reaction (IV) will then maximally reach the activity of reaction III. If LA 672 does not contain an immunologically active DNA polymerase which can absorb the antipolymerase IgG, all antipolymerase IgG will be available for inhibition of the AMV DNA polymerase, and reaction IV will be similar to reaction II. LA 672 virus grown at 35°C was able to absorb antipolymerase IgG and thus reduce the inhibition of the AMV DNA polymerase (reaction IV is similar to reaction III); LA 672

grown at 41°C was likewise able to absorb equivalent amounts of IgG. This method is sensitive enough to detect enzyme molecules at a level at which no activity with synthetic template-primers can be detected anymore. However, it does not allow a precise quantitation of the amount of enzyme present in LA 672 virions.

The DNA polymerase-RNase H complex was, therefore, isolated from LA 672 virions grown at 35 and 41°C to determine the amount of detectable enzyme protein. The residual enzyme activity present in LA 672 grown at 41°C was sufficient to locate the enzyme during elution from a DEAE-cellulose and a phosphocellulose column. The amount of DNA polymerase activity of LA 672 (41°C) shown in the Fig. 4 insert was about 5% of that of LA 672 (35°C) or wt Pr-A (41°C). The amount of detectable RNase H was also reduced, but not as much. The amount of viral protein of the starting material was identical as tested by optical density. Neither the residual DNA polymerase activity nor the RNase H of the purified LA 672 enzyme exhibited a higher thermal lability than did the wt Pr-A enzymes. The residual DNA polymerase and RNase H activities of the LA 672 enzymes were both inhibitable to about 90% by 5 µg of antipolymerase IgG, indicating that they were both of viral origin (data not shown). The pooled enzyme activity peaks of LA 672 at 41 and 35°C were analyzed on sodium dodecyl sulfate-polyacrylamide gels (Fig. 4). Despite several contaminating bands in the enzyme preparations, α and β could be distinguished, and their amounts were twofold reduced at 41°C if compared with the amount of the residual contaminating protein bands. Analysis of the polypeptide composition of core preparations from mutant and wt virus similarly showed a twofold decrease in the amounts of β and α in the mutant preparation (data not shown).

DISCUSSION

Reverse transcriptase is essential for the replication of retroviruses and is responsible for their name. Mutants in the viral *pol* gene (1), which codes for reverse transcriptase, have been available for several years (7, 8, 16). The prototype *pol* mutant carries in the virion a thermolabile enzyme which imposes an "early" block on infection by preventing synthesis of the DNA provirus at the nonpermissive temperature; examples of this kind of mutant are LA 335 and LA 337 (7).

With LA 672, a different phenotype among *pol*-defective mutants is encountered. Although it was early recognized that LA 672 particles

FIG. 4. Absorbancy pattern of partially purified enzyme protein of LA 672 grown at 41 and 35°C after sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 5% polyacrylamide gels. The gels were subsequently stained with Coomassie brilliant blue and scanned for the absorbancy pattern at 600 nm. Migration was from left to right. Pr76^{nas} migrating between β and α was present in this preparation and might serve as an internal protein standard. (Insert) Twelve liters of wt Pr-A and 8 liters of LA 672 culture fluid were concentrated 1,000-fold. After purification, the virus was disrupted and purified by DEAE-cellulose and phosphocellulose column chromatography. The elution profiles of poly(rA)·oligo(dT)-directed DNA synthesis (○) and RNase H activities (Δ) from the phosphocellulose columns were obtained by using 5 μ l of wt and 20 μ l of mutant enzyme per assay. The residual enzyme activities of LA 672 are very low (note the scale). The total enzyme activity peaks were pooled and concentrated for gel electrophoretic analysis.

produced at the nonpermissive temperature, in addition to being noninfectious biologically, exhibited a 20-fold reduction in DNA polymerase activity (5), direct proof that even purified reverse transcriptase from such particles was deficient in DNA polymerase and RNase H activ-

ities has been presented for the first time in this communication. LA 672 is not an early mutant, and infections performed at the nonpermissive temperatures are not blocked. Neither are the virions or purified polymerase thermolabile. However, progeny production from infected cells

is thermolabile, and within 3 h after a temperature shift to 42°C, cultures yield only noninfectious particles. Hence, the phenotype of this mutant is a "late" one, reflecting a defect which acts at a stage later than integration of provirus into the host DNA.

In studies with several mutants, Panet et al. (11), using virion-associated enzyme preparations, obtained evidence based on a radioimmunoassay which indicated that LA 672 particles matured at the nonpermissive temperature had a fivefold-reduced complement of enzyme molecules relative to wt PrA or mutant virus grown at the permissive temperature. In the present study, in which in addition to a radioimmuno-competition test partially purified reverse transcriptase preparations were examined on polyacrylamide gels, evidence for a twofold reduction in reverse transcriptase incorporation was obtained. Apparently normal reverse transcriptase, consisting of both β and α species, could be demonstrated in particles of LA 672 obtained after growth at the nonpermissive temperature.

LA 338 has posed a puzzle for many years. Wyke and Linial (20) observed, in addition to a conversational T-class late transformation lesion and a late replication defect blocking production of infectious progeny at 42°C, an early-acting defect in a function which was believed to be responsible for "initiation of transformation." At present, no genetic element is known which is responsible for an early event essential for transformation and which is not also essential for replication. Nonetheless, four mutants exhibiting this phenotype have been reported: LA 30, LA 338, LA 343 (20), and MI 100 (3). Recently, Tato et al. (13) have demonstrated that for one of these mutants, LA 30, the actual cause of the apparent lesion in the initiation of transformation was a leaky defect in the product of the viral *env* gene, envelope glycoproteins. This defect had the effect of causing infections performed at the nonpermissive temperature to be inefficient. The finding of Tato et al. (13) have made the existence of a special function for the initiation of transformation extremely unlikely; therefore, it is appropriate to look at others of the four mutants with this phenotype to see whether a similar early, leaky replication lesion can be detected.

Analyses of LA 338 infection and replication at 42°C have shown that the early block does interdict coordinately both transformation and replication and, furthermore, that this defect can be complemented by making phenotypically mixed virus stocks between LA 338 and the avian leukosis virus Rous-associated virus type 5, subgroup A (Friis, unpublished observations).

Furthermore, the complementation of the early defect of LA 338 which occurs in such phenotypically mixed virus stocks does not result from incorporation of the leukosis virus envelope glycoprotein gp85 into the mutant, since focus tests on selectively resistant cells indicate that the complementation still occurs on C/AE phenotype cells. In summary, biological evidence indicates that the early defect in LA 338 replication does not affect exclusively the initiation of transformation, but also affects the initiation of replication. Furthermore, in agreement with Blair et al. (2), who confirmed that LA 338 had no *env* lesion, it seems that complementation does not occur because of exchange of glycoproteins; i.e., the lesion is not in *env*.

Although Verma et al. (17) could not demonstrate thermolability in the purified RNA-dependent DNA polymerase or RNase H, they reported that LA 338 must have a very early defect since cells infected at the nonpermissive temperature failed to make DNA provirus according to DNA hybridization experiments. Our own evidence presented above indicates that LA 338 has a defect in reverse transcriptase, since a subtle thermolability could be reproducibly demonstrated with purified reverse transcriptase for both DNA polymerase and RNase H reactions. The half-lives of these activities of the mutant enzyme have been reduced by a factor of about 2 relative to the wild type. Furthermore, a curious specific lability of the mutant enzyme which renders the enzyme unable to respond to synthetic template-primer without affecting the reaction on native viral RNA has also been observed repeatedly. Addition of oligo(dT) primers did not further stimulate RNA transcription. This peculiarity cannot be explained now, except to speculate that the mutant enzyme may have a particularly labile initiation capability.

We conclude that LA 338, in addition to a T-class transformation lesion, carries at least one other replication lesion acting early to prevent the function of reverse transcriptase in infections performed at the nonpermissive temperature. Probably, this latter lesion also acts late and is thus responsible for the failure of cells infected with LA 338 at the permissive temperature to continue the production of infectious virus after a subsequent shift to the nonpermissive temperature. Such a lesion could account for the labile initiation capability mentioned above.

Table 1 summarizes the findings of the present study and those of previous reports of many laboratories, including one investigation of the Rauscher leukemia virus reverse transcriptase mutant *ts29*. It can be observed that three phe-

TABLE 1. Summary of findings of the present study and those of previous reports of other laboratories

Mutant designation	Parent virus	Biological phenotype	Biochemical characterization ^a			
			DNA polymerase	RNase H	Stabilization through template	Binding to primer tRNA
LA 335	Pr-C	Early block in provirus synthesis	tl (16)	tl (17)	No (16)	tl (11)
LA 336	B77		tl (17; this study)	tl (17; this study)	Yes (17; this study)	tl (11)
LA 337	Pr-C		tl (16)	tl (17)	No (16)	ND
<i>ts29</i>	Rauscher murine leukemia virus	Early block in provirus synthesis	tl (15)	tl (Moelling, unpublished observation)	Yes (15; Moelling, unpublished observation)	ND
LA 338	Pr-C	Early block in provirus synthesis and late block in production of functional enzyme	tl (this study)	tl (this study)	No (this study)	Not tl (11)
LA 343	Pr-C		tl (11)	ND	ND	Not tl (11)
LA 672	Pr-A	Late block in production of functional enzyme	Not tl (this study)	Not tl (this study)	No (this study)	Not tl (this study)

^a Biochemical characterization is intended to show the properties in vitro of purified or virion-associated enzyme. Hence, "thermolabile" (tl) indicates an enzyme which in preincubations is more rapidly inactivated than the enzyme from the appropriate virus. Numbers within parentheses indicate references. ND, Not determined.

notypic categories exist for reverse transcriptase mutants. Probably, in addition to these early-acting, early- and late-acting, and late-acting categories, other features will emerge, such as mutants uniquely defective for primer binding, template binding, initiation, or elongation functions.

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