JOURNAL OF VIROLOGY, Feb. 1978, p. 553-561
0022-538X/78/0025-0553$02.00/0

Vol. 25, No. 2
Printed in U.S.A.

Impaired Processing of Precursor Polypeptides of Temperature-Sensitive Mutants of Rauscher Murine Leukemia Virus

W. J. M. VAN DE VEN,* D. VAN ZAANE,† C. ONNEKINK, AND H. P. J. BLOEMERS
Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

Received for publication 24 August 1977

The synthesis and processing of virus-specific precursor polypeptides in NIH/3T3 cells infected at the permissive temperature (31°C) with temperature-sensitive (*ts*) mutants of Rauscher murine leukemia virus was studied in pulse-chase experiments at the permissive and nonpermissive (39°C) temperatures. The newly synthesized virus-specific polypeptides were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis after immunoprecipitation with polyvalent and monospecific antisera against Rauscher murine leukemia virus proteins. In cells infected with *ts* mutants defective in early replication steps (the early mutants *ts*17 and *ts*29), and *ts* mutants defective in postintegration steps (the late mutants *ts*25 and *ts*26), the processing of the primary *gag* gene product was impaired at the nonpermissive temperature. *gag*-pr75 of all four mutants was converted into *gag*-pr65; however, *gag*-pr65 accumulated at the nonpermissive temperature, and the main internal virion polypeptide p30 was not formed. Therefore, the proteolytic cleavage is blocked beyond *gag*-pr65. Concomitantly, the formation of the *env* gene-related polypeptide p12(E) of all four mutants was blocked at the restrictive temperature. In contrast, cells infected with the late mutant *ts*28, which produced noninfectious virions at 39°C, showed a normal turnover of the *gag* and *env* precursor polypeptides.

In studies on the molecular biology of type C RNA tumor viruses, the isolated conditional lethal mutants of avian (6, 9, 22, 26) and mammalian (13, 15, 17, 25) RNA tumor viruses provide useful markers for the analysis of the viral genome. Temperature-sensitive (*ts*) mutants of murine leukemia virus (MuLV) have been derived from the Kirsten (17), Rauscher (15), and Moloney (25) strains. The mutants of the Rauscher strain (R-MuLV) have been divided into three different classes based upon the stage at which virus replication is impaired at the restrictive temperature (15). Through the courtesy of J. R. Stephenson, we have obtained representatives of the *ts* mutants of class I (at 39°C, defective in replication functions before the synthesis of viral group-specific antigens), class II (at 39°C, defective in functions between the synthesis of viral group-specific antigens and production of virions), and class III (noninfectious virions produced at 39°C).

The virus-specific structural polypeptides of the RNA tumor viruses are formed through proteolytic cleavage of precursor polypeptides (for a review see 14). Thus, in the case of R-MuLV, the primary *gag* gene product, *gag*-pr75, is converted into *gag*-pr65, which is split into certain intermediary products (with molecular weights between 40,000 and 50,000) and, subsequently, into the polypeptides of the viral core, p15, p12, p30, and p10 (2). Likewise, gp69/71 and p15(E), the virus-specific polypeptides located in the viral envelope as well as in the plasma membrane of infected cells, are derived from the primary *env* gene product *env*-pr82. Finally, p12(E), found in the viral envelope, is presumably derived from p15(E) (12, 24; D. van Zaane, thesis, University of Nijmegen, 1977). Using gel filtration columns that did not resolve the two *gag* precursor polypeptides *gag*-pr75 and *gag*-pr65, Stephenson et al. (18) showed that two *ts* mutants of R-MuLV, *ts*25 and *ts*26, possessed temperature-sensitive defects in the proteolytic cleavage of the *gag* gene-related precursor polypeptides. These studies were undertaken to localize the defects more precisely and to investigate whether the other thermosensitive replication mutants of R-MuLV also possess thermosensitive defects in gene expression. Therefore, the gene expressions of two early (*ts*17 and *ts*29) and one late (*ts*28) *ts* mutants

† Present address: Department of Virology, Central Veterinary Institute, Lelystad, The Netherlands.

were studied, and the results were compared with those obtained with the *ts* mutants *ts*25 and *ts*26 and with a clonal stock of wild-type (WT) R-MuLV.

MATERIALS AND METHODS

Materials. L-[^{35}S]methionine (specific activity, 380 Ci/mmol) and a ^{14}C-labeled amino acid mixture (specific activity, 54 mCi/matom) were obtained from the Radiochemical Centre, Amersham, England. Anti-gp69/71 and anti-p15 sera were the generous gift of M. Strand and J. T. August. Characteristics of these antisera were described by Strand and August (20). Anti-R-MuLV serum, anti-p30 serum, anti-p15(E),p12(E) serum, and an antiserum raised against bovine serum albumin (anti-BSA serum) were prepared and described by van Zaane et al. (24).

Cells and viruses. NIH/3T3 cells (21) were cultured as monolayers in Dulbecco's modification of Eagle medium supplemented with 10% fetal calf serum (Grand Island Biological Co. Bio-Cult Ltd.).

The viruses used included a clonal isolate of WT R-MuLV (WT-248) and five R-MuLV *ts* mutants, *ts*17, *ts*25, *ts*26, *ts*28, and *ts*29 (15). NIH/3T3 cells were infected with virus as described by Stephenson and Aaronson (15). Infection with *ts* R-MuLV was performed at 31°C, and that with WT R-MuLV was performed at 37°C.

JLS-V9 cells, derived from bone marrow cells of BALB/c mice (4) infected with and producing R-MuLV (V9-R-MuLV), were cultured as monolayers in Eagle minimal essential medium supplemented with 10% calf serum. V9-R-MuLV labeled with L-[^{35}S]methionine was isolated as described by Duesberg and Robinson (5) and was used as a reference.

Labeling of cells and preparation of lysates. Subconfluent monolayers of cells growing in plastic tissue culture flasks (Falcon Plastics, 25 cm^2) were starved for 10 min in labeling medium (Hanks basic salt solution supplemented with 10% dialyzed calf serum and amino acids except for the radioactive ones). Then, the medium was replaced by labeling medium with a radioactive amino acid(s), and the cells were incubated for 30 min. After being pulse-labeled, the cells were washed once, and 4 ml of normal culture medium was added; thereafter, the cells either were lysed immediately or radioactivity was chased for various times. In some experiments the temperature was shifted during the chase period. When indicated, the temperature shift from 39 to 31°C was performed 2 h after the beginning of the chase period; the temperature shift from 31 to 39°C was performed immediately after pulse-labeling of the cells.

After pulses as well as after chases, the cells were lysed by the addition to the cultures (i.e., cells and medium) of 1 ml of a buffer containing 50 mM sodium phosphate (pH 7.2), 4.5% sodium chloride, 5.0% Triton X-100, 2.5% deoxycholate, and 0.5% sodium dodecyl sulfate (fivefold-concentrated immunoprecipitation buffer [24]). After clearing the lysates by centrifugation at 220,000 × *g* for 10 min in a Ti50 rotor of a Spinco L-50 centrifuge, the lysates were stored at −80°C.

Immunoprecipitation and polyacrylamide gel electrophoresis. Detection of R-MuLV-specific polypeptides in cell lysates was performed with polyvalent and monospecific antisera against R-MuLV proteins according to the immunoprecipitation procedure described by van Zaane et al. (24). Immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Laemmli (10) on 7 to 18% polyacrylamide gradient slab gels. Radioactivity was visualized by scintillation autoradiography (3).

RESULTS

Synthesis and identification of virus-specific polypeptides in NIH/3T3 cells infected with WT R-MuLV (WT-248). The synthesis of virus-specific polypeptides in NIH/3T3 cells infected with WT R-MuLV was examined in pulse-chase experiments at 37°C (Fig. 1A–G). Cells were pulse-labeled for 30 min with a ^{14}C-labeled amino acid mixture, and, thereafter, radioactivity was chased for 0 and 20 h. The viral structural polypeptides were identified through immunoprecipitation analysis with well-defined (see Materials and Methods) monospecific antisera and a polyvalent antiserum against R-MuLV polypeptides. Radioimmunoprecipitation analysis of pulse-labeled cells with anti-R-MuLV serum revealed that in addition to the virus-specific precursor polypeptides *gag*-pr75, *gag*-pr65, and *env*-pr82 (cf. references 1, 24), small amounts of two high-molecular-weight polypeptides (indicated with ●) were precipitated (Fig. 1A). Upon analysis with monospecific antisera it appeared that both high-molecular-weight polypeptides carried antigenic determinants of the *gag*-related structural polypeptides p30 and p15, but not of the *env*-related structural polypeptide gp69/71 (not shown). These high-molecular-weight polypeptides are therefore probably virus-specific polypeptides, which in addition to the antigenic determinants of the *gag*-precursor contain other virus-specific antigenic determinants (possibly of reverse transcriptase [8]). They might represent virus-specific precursor polypeptides whose synthesis is due to an occasional readthrough at the *gag* gene termination site on a viral mRNA (14).

Moreover, after pulses as well as after chases, some additional high-molecular-weight polypeptides (>90,000) were precipitated with the antisera used, especially with the anti-R-MuLV serum (Fig. 1A–O) and the anti-p15(E),p12(E) serum (Fig. 1D, indicated with *). Probably, many of these polypeptides are not virus specific and represent cross-reacting host cell polypeptides. However, some of them might be degradation products of the high-molecular-weight precursor polypeptides synthesized according to a readthrough mechanism. We will not discuss the significance of these products here, because

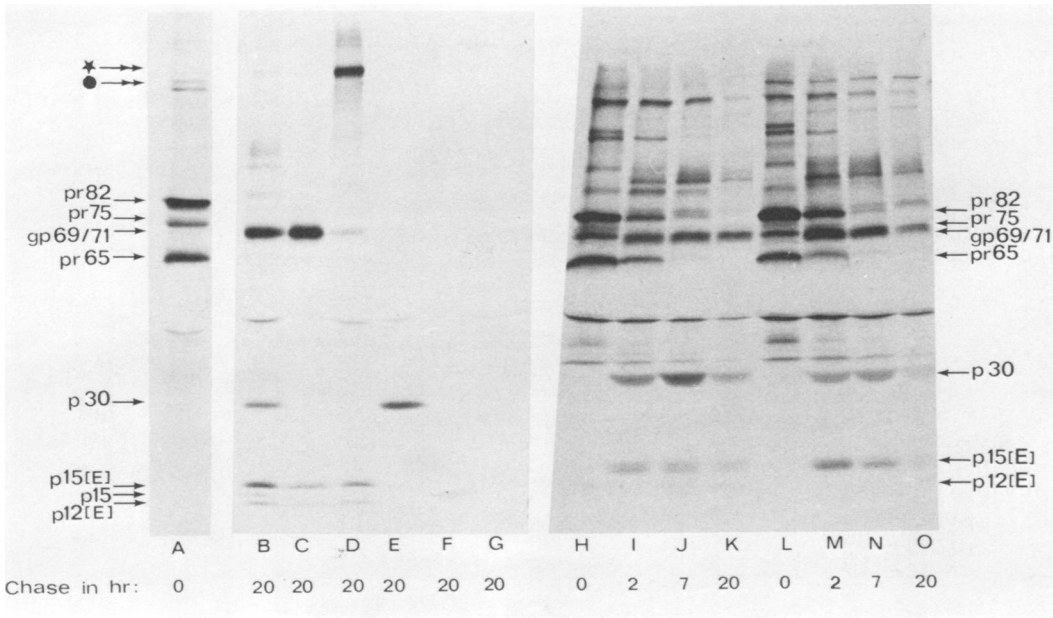

FIG. 1. *Normal processing of gag and env products of WT R-MuLV (WT-248). Subconfluent monolayers of NIH/3T3 cells infected with WT R-MuLV were pulse-labeled for 30 min with 100 μCi of a ^{14}C-amino acid mixture (A–G) or with 75 μCi of L-[^{35}S]methionine (H–O), and radioactivity was chased for 0, 2, 7, and 20 h as indicated. The pulse-chase experiments were performed at 31°C (H–K), 37°C (A–G), or 39°C (L–O). After lysis of the cells as described in the text, virus-specific polypeptides were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and scintillation autoradiography after immunoprecipitation with: anti-R-MuLV serum (A, B, H–O); anti-gp69/71 serum (C); anti-p15(E),p12(E) serum (D); anti-p30 serum (E); anti-p15 serum (F); or anti-BSA serum (G). Bands marked with ● and * are discussed in the text.*

their synthesis does not seem to be affected in any of the mutants we have investigated. After a chase period of 20 h, radioimmunoprecipitation analysis of labeled cultures (i.e., cells and medium) with anti-R-MuLV serum showed that the *gag* and *env* precursor polypeptides had disappeared and that the viral structural polypeptides gp69/71, p30, p15(E), and p12(E) were formed (Fig. 1B). These viral structural polypeptides were identified with monospecific antisera (Fig. 1C–F). In a control experiment none of the polypeptides could be precipitated with anti-BSA serum (Fig. 1G). It should be noted that the *gag* products p12 and p10 are not recognized by the anti-R-MuLV serum used.

As a control for the analysis of the *ts* mutants, Fig. 1(H–O) shows the results of a pulse-chase experiment at 31°C (Fig. 1H–K) and 39°C (Fig. 1L–O) with NIH/3T3 cells infected with WT R-MuLV (WT-248). Cells were pulse-labeled with L-[^{35}S]methionine for 30 min, and, thereafter, radioactivity was chased for 0, 2, 7, and 20 h. Radioimmunoprecipitation analysis was performed with anti-R-MuLV serum. It appeared that at both temperatures the precursor polypeptide *env*-pr82, as well as *gag*-pr75 and *gag*-pr65, was cleaved into virus-specific structural polypeptides (Fig. 1H–O). It should be noted that p15 does not contain methionine (7) and consequently is not detected when cells are labeled with L-[^{35}S]methionine.

Localization of the blockage in the cleavage of the *gag* gene-related precursor polypeptides of *ts*25 and *ts*26. The cleavage of the *gag* gene-related precursor polypeptides of *ts*25 and *ts*26 was shown to be blocked at the nonpermissive temperature (2, 19). To localize the blockage in the cleavage scheme, the synthesis and processing of virus-specific precursor polypeptides was examined in NIH/3T3 cells infected at 31°C with *ts*25 and *ts*26. Pulse-chase experiments were performed at 31 and 39°C, and the virus-specific polypeptides were immunoprecipitated with anti-R-MuLV serum. The results obtained with *ts*25 are presented in Fig. 2. At 31°C no differences in the synthesis and processing of the precursor polypeptides of *ts*25 and WT-248 could be observed (cf. Fig. 2A–D and Fig. 1H–K). In accordance with the work of Stephenson et al. (19), Fig. 2(E–H) shows that the processing of the *gag* gene precursor polypeptides was impaired at the nonpermissive

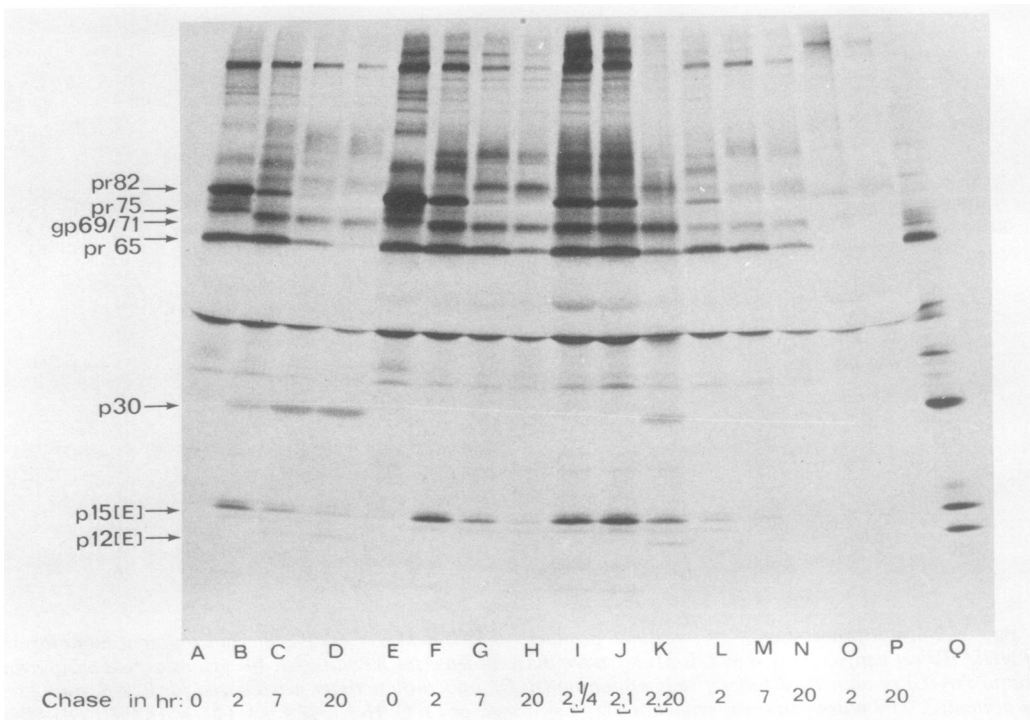

FIG. 2. *Thermosensitive processing of gag products of ts25. Subconfluent monolayers of NIH/3T3 cells infected at 31°C with ts25 were pulse-labeled for 30 min with 75 μCi of L-[35S]methionine, and radioactivity was chased for various times as indicated (A–P). Pulse-chase experiments were performed at 31°C (A–D, O–P) or 39°C (E–H). In some cases the temperature was shifted for a chase at 31°C (I–K) or 39°C (L–N), as described in the text. Thus, "chase in h 2¼" indicates a 2-h chase at 39°C followed by a 0.25-h chase at 31°C, etc. Virus-specific polypeptides were analyzed as described in the legend to Fig. 1. Immunoprecipitation with: anti-R-MuLV serum (A–N); anti-BSA serum (O–P); reference V9-R-MuLV labeled with L-[35S]methionine (Q).*

temperature; *gag*-pr65 of *ts*25 is accumulated and no p30 is formed. On the other hand, like WT *gag*-pr75, the *gag*-pr75 of *ts*25 is converted into *gag*-pr65. When the temperature was shifted to 31°C 2 h after pulse-labeling the cells at 39°C (Fig. 2I–K), the proteolytic cleavage was resumed and p30 was produced. When, in another experiment, the temperature was shifted from 31°C (during the pulse) to 39°C (during the chase), the proteolytic cleavage was blocked (Fig. 2L–N).

The results of parallel experiments with NIH/3T3 cells infected with *ts*26 (not shown) were similar to those observed with *ts*25; again, at 39°C *gag*-pr65 was accumulated, but the mutation was slightly leaky: cells infected with *ts*26 produced small amounts of p30 at the restrictive temperature (Fig. 3G–H).

A more detailed analysis of the blockage in the proteolytic cleavage of the *gag* precursor polypeptides of *ts*25 and *ts*26 was obtained after radioimmunoprecipitation analysis with monospecific antisera (Fig. 3). Cells infected with *ts*25 or *ts*26 were labeled for 30 min with a ^{14}C-amino acid mixture at 31 or 39°C, and radioactivity was chased for 20 h. Figure 3 shows that at the nonpermissive temperature *gag*-pr65 was accumulated in cells infected with *ts*25 (Fig. 3M–O) or *ts*26 (Fig. 3G–I); furthermore, the production of the *gag*-related structural polypeptides p30 and p15 was inhibited. In addition to the impaired processing of the *gag* precursor polypeptides of *ts*25 and *ts*26, a blockage in the production of an *env* gene-related structural polypeptide was also observed at the restrictive temperature. Radioimmunoprecipitation analysis with anti-R-MuLV serum (Fig. 2H and N; Fig. 3G and M), anti-gp69/71 serum (Fig. 3K and Q), and anti-p15(E),p12(E) serum (Fig. 3J and P) revealed that production of p12(E) was inhibited. Production of the structural envelope polypeptides gp69/71 and p15(E), however, seemed to be normal (Fig. 2F–H and Fig. 3G, J, K, M, P, and Q). It should be noted that some-

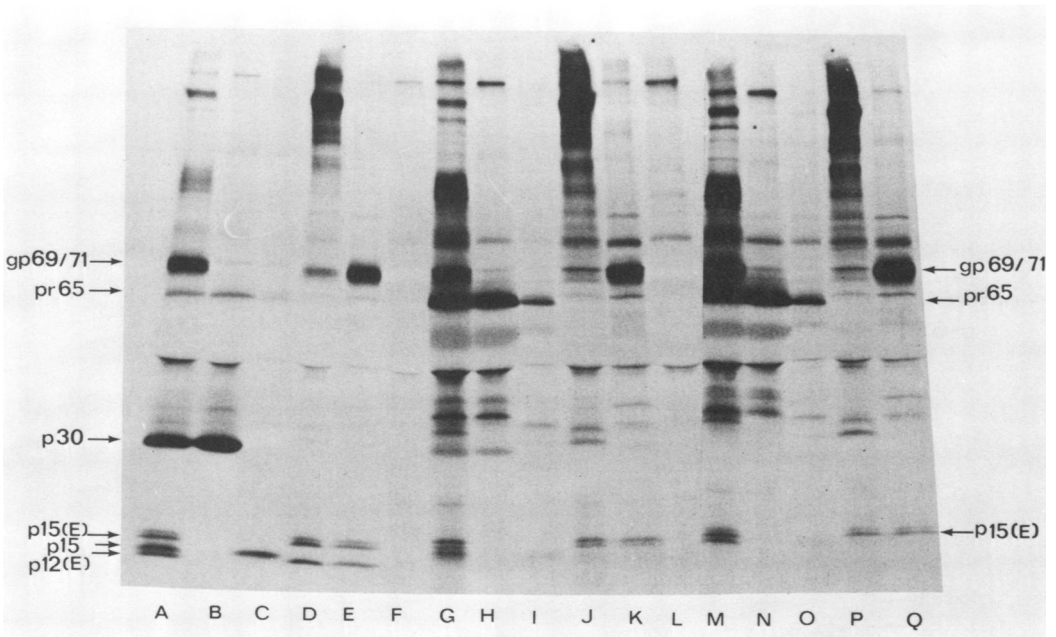

FIG. 3. *Leaky thermosensitive processing of gag products of ts26; thermosensitive production of the env product p12(E) in addition to the thermosensitive processing of gag products of ts25 and ts26. NIH/3T3 cells infected at 31°C with ts26 (A–L) or ts25 (M–Q) were pulse-labeled for 30 min with 100 μCi of a ^{14}C-amino acid mixture, and radioactivity was chased for 20 h. Pulse-chase experiments were performed at 31°C (A–F) or 39°C (G–Q). Virus-specific polypeptides were analyzed as described in the legend to Fig. 1. Immunoprecipitation with: anti-R-MuLV serum (A, G, M); anti-p30 serum (B, H, N); anti-p15 serum (C, I, O); anti-p15(E),p12(E) serum (D, J, P); anti-gp69/71 serum (E, K, Q); anti-BSA serum (F, L).*

times p15(E) seemed to be composed of two components (Fig. 1, 2, and 3). Both polypeptides were immunoprecipitated with anti-R-MuLV serum (Fig. 1, 2, and 3A, G, and M), anti-gp69/71 serum (Fig. 3E, K, and Q), and anti-p15(E),p12(E) serum (Fig. 3D, J, and P). [Incidentally, the precipitation of p15(E) and p12(E) with anti-gp69/71 serum is presumably due to the presence of a complex between gp69/71, p15(E), and p12(E) as discussed by van Zaane et al. (24) and Leamnson et al. (11).]

Synthesis of virus-specific polypeptides in NIH/3T3 cells infected with *ts*17, *ts*28, and *ts*29. Two early temperature-sensitive mutants of R-MuLV, *ts*17 and *ts*29, were shown to be defective in one or more functions before the synthesis of group-specific antigens (15). To examine the possibility that these mutants also had a defect in the proteolytic cleavage of their precursor polypeptides, the synthesis of virus-specific polypeptides was studied at 31 and 39°C, after infection at the permissive temperature. Cells were labeled with L-[^{35}S]methionine, and radioimmunoprecipitation was performed with anti-R-MuLV serum. The results obtained with *ts*17 and *ts*29 are presented in Fig. 4 and 5, respectively. In both cases, *gag*-pr75 was processed to *gag*-pr65 at the nonpermissive temperature. However, proteolytic cleavage was blocked beyond *gag*-pr65, since no p30 was formed. When the temperature was shifted from 39 to 31°C, cleavage was resumed, as could be concluded from the production of p30. Furthermore, the inhibition of the production of p12(E) at the restrictive temperature, as found in cultures infected with *ts*25 and *ts*26, was also observed. On the other hand, the late mutant *ts*28, similarly analyzed in parallel experiments, was not defective for the processing of its precursor polypeptides. The relevant pulse-chase experiments at 31 and 39°C are shown in Fig. 6.

DISCUSSION

Some temperature-sensitive mutants of R-MuLV are partially characterized (15, 16, 18, 19, 23, 27). Among them are the mutants *ts*17, *ts*25, *ts*26, *ts*28, and *ts*29, which were used in this study.

At 39°C, some of these mutants are defective in either early replication steps (class I represented by *ts*17 and *ts*29) or postintegration steps (class II represented by *ts*25 and *ts*26, and class

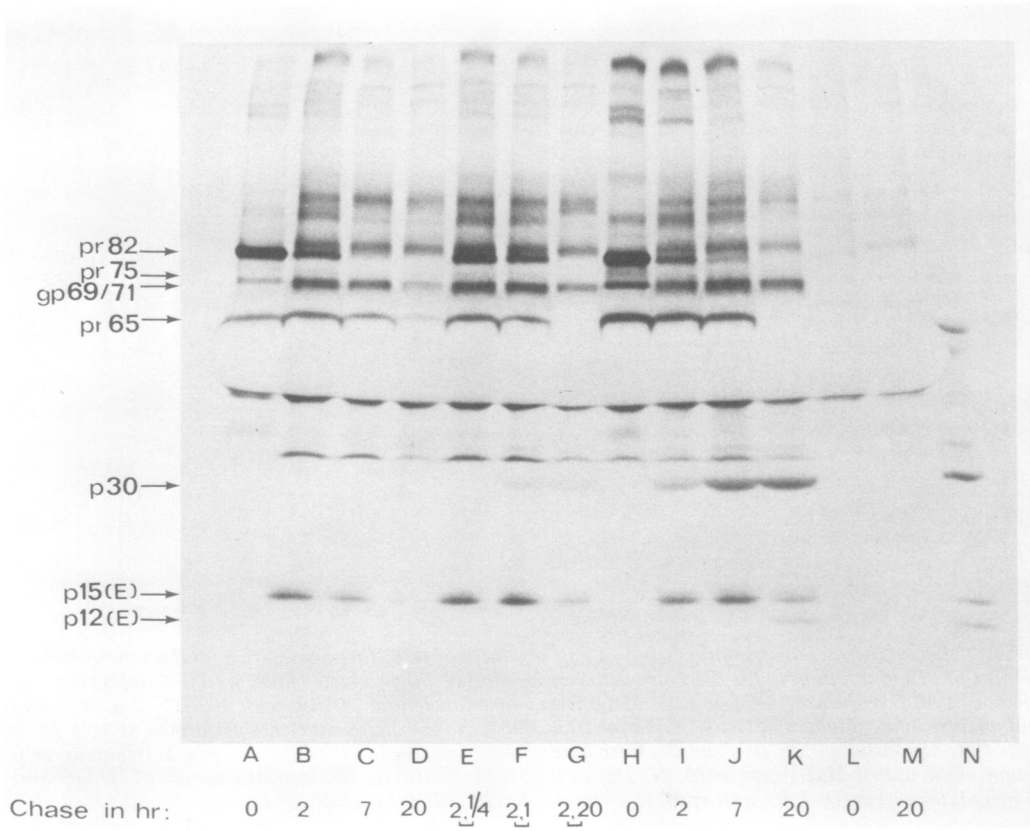

FIG. 4. *Thermosensitive processing of gag products of ts17 and concomitant failure to produce the env product p12(E). NIH/3T3 cells infected at 31°C with ts17 were pulse-labeled with 75 μCi of L-[35S]methionine for 30 min, and radioactivity was chased for various times as indicated (A–M). Pulse-chase experiments were performed at 39°C (A–D) or 31°C (H–M). The temperature was shifted from 39 to 31°C (E–G), as described in the text (see also legend to Fig. 2). Virus-specific polypeptides were analyzed as described in the legend to Fig. 1. Immunoprecipitation with: anti-R-MuLV serum (A–K); anti-BSA serum (L–M); reference V9-R-MuLV labeled with L-[35S]methionine (N).*

III represented by *ts*28) (15). *ts*25 and *ts*26 are postintegration defective mutants whose defect is known to involve the cleavage of the *gag* gene-related precursor polypeptides (2, 19). Mutant *ts*29 possesses a thermolabile reverse transcriptase (23) and *ts*28 has an unidentified *ts* defect at a late step in its replication (15, 27).

In this study cells were infected with these mutants at the permissive temperature (31°C), and subsequently the synthesis and processing of virus-specific precursor polypeptides were examined at the permissive and nonpermissive temperatures. At 31°C no differences in the processing of the *gag* and *env* gene-related precursor polypeptides were observed in cells infected with either WT R-MuLV or one of the *ts* mutants (Fig. 1, 2, 4, 5, and 6). However, at 39°C, a blockage in the processing of a *gag* gene-related precursor polypeptide of the mutants *ts*17, *ts*25, *ts*26, and *ts*29 was observed (Fig. 2, 3, 4, and 5). When the temperature was shifted down to 31°C after pulse-labeling the cells at 39°C, processing of the *gag* precursor polypeptides to the internal structural virion polypeptides p30 (Fig. 2, 3, 4, and 5) and p15 (Fig. 3) was observed. The impaired processing of the *gag* precursor polypeptides at the restrictive temperature was localized beyond *gag*-pr65, since *gag*-pr75 disappeared but *gag*-pr65 accumulated and p30 was not produced (Fig. 2, 3, 4, and 5). In all analyses, impaired production of p12 and p10 escaped detection, since these virus-specific polypeptides were not recognized by the anti-R-MuLV serum. However, presumably none of the *gag* gene-related structural polypeptides is produced at 39°C since all four subgene

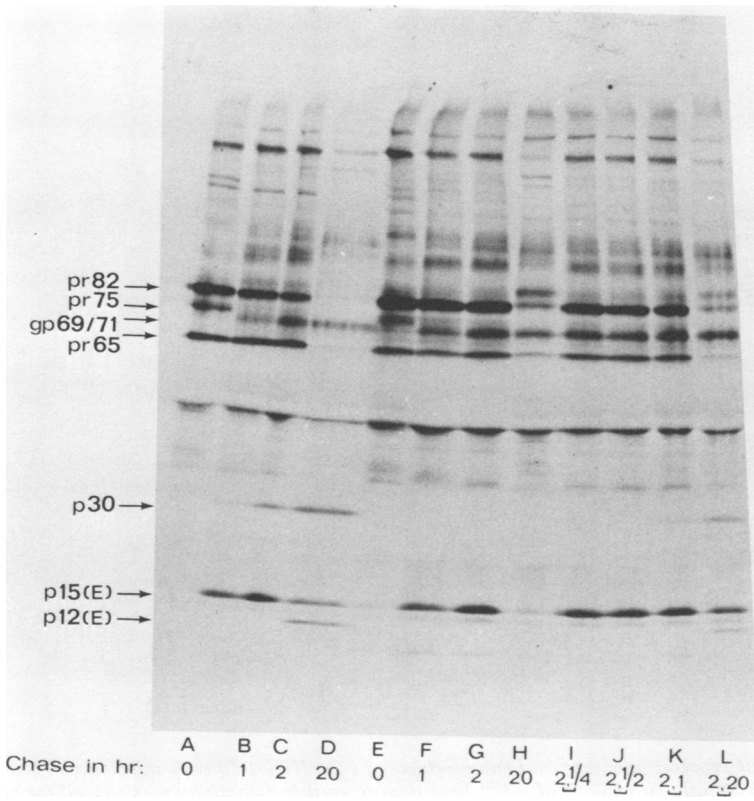

FIG. 5. *Thermosensitive processing of gag products of ts29 and concomitant failure to produce the env gene-related product p12(E). NIH/3T3 cells infected at 31°C with ts29 were pulse-labeled for 30 min with 75 μCi of L-[^{35}S]methionine, and radioactivity was chased for various times as indicated (A–L). Pulse-chase experiments were performed at 31°C (A–D) or 39°C (E–H). The temperature was shifted from 39 to 31°C (I–L), as described in the text (see also legend to Fig. 2). Virus-specific polypeptides were analyzed as described in the legend to Fig. 1. Immunoprecipitation with: anti-R-MuLV serum (A–L).*

products, p15, p12, p30, and p10, are present in the smaller *gag* precursor polypeptide *gag*-pr65 (van Zaane, thesis). This conclusion does not exclude the possibility that eventually *gag*-pr65 is degraded and may give rise to an incomplete set of *gag* subgene products (cf. Barbacid et al. [2]). The impaired processing of the *gag* precursor polypeptide of *ts*25 and *ts*26 is consistent with the observations of Stephenson et al. (19). The presence of the same defect in the mutants *ts*17 and *ts*29 is quite unexpected, since both were characterized as early mutants (class I). In the case of *ts*29 the early replication defect is associated with a thermolabile reverse transcriptase (23). Further evidence for a second replication defect in *ts*29 is obtained from an electron microscopic study by Yeger et al. (27).

Upon a shift to the nonpermissive temperature after infection at the permissive temperature, these authors found an accumulation of immature virions at the cell surface. The immature virions were in an early stage of budding, suggesting a replication defect at an early stage of virion assembly. Whereas cultures infected with *ts*17, *ts*25, *ts*26, and *ts*29 revealed an impaired processing of the *gag* precursor polypeptides at the nonpermissive temperature, production of the *env* gene-related polypeptide p12(E) was also inhibited (Fig. 2, 3, 4, and 5). It is not yet clear whether or not there is a relation between these defects. For instance, the defect could be primarily in virion assemblage, and, as a consequence, the cleavage of different precursor polypeptides could be blocked. As discussed above, two of the mutants of this phenotype (*ts*17 and *ts*29) possess an additional early defect on the basis of which they were listed as class I mutants. Again, it is not known whether they are double (or triple) mutants or single pleiotropic mutants.

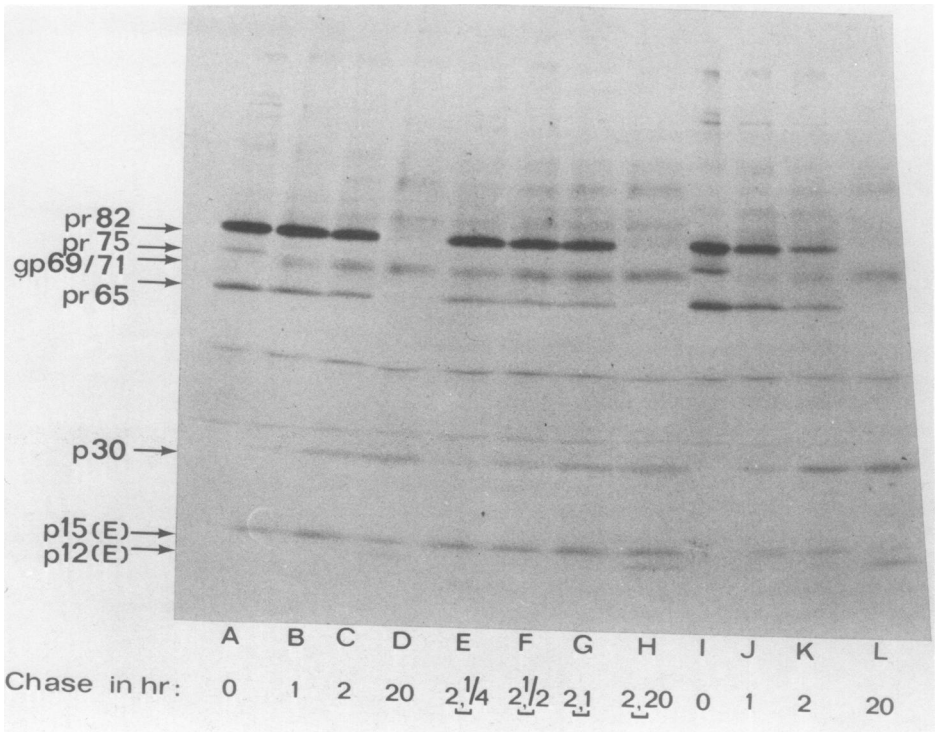

FIG. 6. *Normal processing of gag and env products of ts28. NIH/3T3 cells infected at 31°C with ts28 were pulse-labeled for 30 min with 75 μCi of L-[^{35}S]methionine, and radioactivity was chased for various times as indicated (A-L). Pulse-chase experiments were performed at 39°C (A-D) or 31°C (I-L). The temperature was shifted from 39 to 31°C (E-H), as described in the text. Virus-specific polypeptides were analyzed as described in the legend to Fig. 1 (see also legend to Fig. 2). Immunoprecipitation with: anti-R-MuLV serum (A-L).*

Finally, the structural polypeptides of *ts*28 are produced normally at the restrictive temperature (Fig. 6). However, the distorted morphology of the virions formed at 39°C (27) suggests a defect in a late stage of the assembly.

ACKNOWLEDGMENTS

We thank J. R. Stephenson and S. A. Aaronson for the clonal stock of wild-type R-MuLV and the *ts* R-MuLV mutants, and we thank M. Strand and J. T. August for the anti-gp69/71 and anti-p15 sera.

These investigations were supported by the Foundation for Medical Research FUNGO, which is subsidized by the Netherlands Organization for Advancement of Pure Research (Z.W.O.).

LITERATURE CITED

1. **Arcement, L. J., W. L. Karshin, R. B. Naso, G. Jamjoom, and R. B. Arlinghaus.** 1976. Biosynthesis of Rauscher leukemia viral proteins: presence of p30 and envelope p15 sequences in precursor polypeptides. Virology **69**:763-774.
2. **Barbacid, M., J. R. Stephenson, and S. A. Aaronson.** 1976. *Gag*-gene of mammalian type-C RNA tumour viruses. Nature (London) **262**:554-559.
3. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83-88.
4. **Chopra, H. C., and G. P. Shibley.** 1967. Infection of an established mouse cell line with cell-free Rauscher leukemia virus. I. Cytological study with the electron microscope. J. Natl. Cancer Inst. **39**:241-256.
5. **Duesberg, P. H., and W. S. Robinson.** 1966. Nucleic acids and proteins isolated from the Rauscher mouse leukemia virus (MLV). Proc. Natl. Acad. Sci. U.S.A. **55**:219-227.
6. **Friis, R. R., K. Toyoshima, and P. K. Vogt.** 1971. Conditional lethal mutants of avian sarcoma viruses. I. Physiology of ts75 and ts149. Virology **43**:375-389.
7. **Ikeda, H., W. Hardy, Jr., E. Tress, and E. Fleissner.** 1975. Chromatographic separation and antigenic analysis of proteins of the oncorna viruses. V. Identification of a new murine viral protein, p15(E). J. Virol. **16**:53-61.
8. **Jamjoom, G. A., R. B. Naso, and R. B. Arlinghaus.** 1977. Further characterization of intracellular precursor polyproteins of Rauscher leukemia virus. Virology **78**:11-34.
9. **Kawai, S., and H. Hanafusa.** 1972. Plaque assay for some strains of avian leukosis virus. Virology **48**:126-135.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
11. **Leamnson, R. H., M. H. M. Shandler, and M. S. Halpern.** 1977. A structural protein complex in Moloney leukemia virus. Virology **76**:437-439.
12. **Naso, R. B., L. J. Arcement, W. L. Karshin, G. A. Jamjoom, and R. B. Arlinghaus.** 1976. A fucose-deficient precursor to Rauscher leukemia virus gp69/71. Proc. Natl. Acad. Sci. U.S.A. **73**:2326-2330.
13. **Scolnick, E. M., J. R. Stephenson, and S. A. Aaron-**

son. 1972. Isolation of temperature-sensitive mutants of murine sarcoma virus. J. Virol. **10**:653–657.

14. **Shapiro, S. Z., and J. T. August.** 1976. Proteolytic cleavage events in oncornavirus protein synthesis. Biochim. Biophys. Acta **458**:375–396.
15. **Stephenson, J. R., and S. A. Aaronson.** 1973. Characterization of temperature-sensitive mutants of murine leukemia virus. Virology **54**:53–59.
16. **Stephenson, J. R., and S. A. Aaronson.** 1974. Temperature-sensitive mutants of murine leukemia virus: III. Mutants defective in helper functions for sarcoma virus fixation. Virology **58**:294–297.
17. **Stephenson, J. R., R. K. Reynolds, and S. A. Aaronson.** 1972. Isolation of temperature-sensitive mutants of murine leukemia virus. Virology **48**:749–756.
18. **Stephenson, J. R., S. R. Tronick, and S. A. Aaronson.** 1974. Temperature-sensitive mutants of murine leukemia virus. IV. Further physiological characterization and evidence for genetic recombination. J. Virol. **14**:918–923.
19. **Stephenson, J. R., S. R. Tronick, and S. A. Aaronson.** 1975. Murine leukemia virus mutants with temperature-sensitive defects in precursor polypeptide cleavage. Cell **6**:543–548.
20. **Strand, M., and J. T. August.** 1976. Structural proteins of ribonucleic acid tumor viruses. J. Biol. Chem. **251**:559–564.
21. **Todaro, G., and H. Green.** 1963. Quantitative studies on the growth of mouse embryo cells in culture and their development into established lines. J. Cell. Biol. **17**:299–313.
22. **Toyoshima, K., and P. K. Vogt.** 1969. Temperature-sensitive mutants of an avian sarcoma virus. Virology **39**:930–931.
23. **Tronick, S. R., J. R. Stephenson, I. M. Verma, and S. A. Aaronson.** 1975. Thermolabile reverse transcriptase of a mammalian leukemia virus mutant temperature-sensitive in its replication and sarcoma virus helper functions. J. Virol. **16**:1476–1482.
24. **van Zaane, D., M. J. A. Dekker-Michielsen, and H. P. J. Bloemers.** 1976. Virus-specific precursor polypeptides in cells infected with Rauscher murine leukemia virus: synthesis, identification and processing. Virology **75**:113–129.
25. **Wong, P. K. Y., and J. A. McCarter.** 1973. Genetic studies of temperature-sensitive mutants of Moloney murine leukemia virus. Virology **53**:319–326.
26. **Wyke, J. A.** 1973. The selective isolation of temperature-sensitive mutants of Rous sarcoma virus. Virology **52**:587–590.
27. **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.