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Complementation Studies with Rous Sarcoma Virus *gag* and *gag-pol* Polyprotein Mutants

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Avian retroviruses (with the notable exception of spleen necrosis virus) express their protease (PR) both in their *gag* and their *gag-pol* polyprotein precursors, in contrast to other retroviruses, notably, the mammalian retroviruses, in which PR is encoded in the *gag-pol* polyprotein or in a separate reading frame as a *gag-pro* product. The consequence is that the avian PR is expressed in stoichiometric rather than catalytic amounts. To investigate the significance of the particular genome organization of the avian retrovirus prototype Rous sarcoma virus, we developed an assay that measures complementation between the *gag* and the *gag-pol* polyproteins by expressing them from two different plasmids in transfected cells. By using this assay, we showed that the protease PR from the *gag-pol* polyprotein is capable of autocatalytic self-cleavage and -activation when coexpressed with a protease-deficient *gag* protein and that the PR domain has a role in viral particle assembly. Furthermore, this complementation assay can be used to investigate the role of the *gag* domain in the *gag-pol* polyprotein by determing whether it can rescue a defect in the *gag* polyprotein. We report here the results of such an experiment, which studied a mutation in the N terminus of the *gag* gene.

The retroviral genome encodes at least three genes: 5′ *gag-pol-env* 3′. The *env* gene codes for the envelope protein of the virion. Expression of the *gag* gene yields a polyprotein precursor ($Pr76^{gag}$ for Rous sarcoma virus [RSV]) which migrates to the plasma membrane of the host cell, where it is cleaved during virus assembly and budding to yield the five mature *gag* proteins. The *pol* gene is always expressed as a *gag-pol* fusion polyprotein precursor ($Pr180^{gag\text{-}pol}$ for RSV) by translational suppression of the *gag* gene termination codon at a frequency of about 5%. The *gag-pol* precursor incorporated into the viral particle during budding (at a ratio of 1:19 with respect to the *gag* precursor) is then processed to yield the enzyme reverse transcriptase, which is entirely coded by the *pol* gene (for reviews, see references 2, 9, and 15). Thus, the *gag* gene is expressed both as a separate entity and as a fusion product with the *pol* gene. This raises the question of the role, if any, of the *gag* portion of the *gag-pol* fusion protein. It has been shown that the NH-terminal portion of *gag* is sufficient to allow release of fusion proteins from cells but that sequences extending as far as the capsid (CA) protein are required for formation of viruslike particles (6), suggesting that *gag* is necessary to transport *pol* to the membrane. Analysis of virus mutants expressing the *gag-pol* fusion protein in the absence of the *gag* precursor has been performed with Moloney murine leukemia virus, spleen necrosis virus, human immunodeficiency virus type 1, and RSV (1, 3, 4, 12, 14, 17). In all cases, it was found that the *gag-pol* fusion protein failed to induce formation of viral particles (unless rescued by coexpression of the *gag*-encoded protein). However, the stability of the *gag-pol*-encoded polyproteins within the cell varied, being processed in spleen necrosis virus and human immunodeficiency virus type 1 but stable in Moloney murine leukemia virus and RSV.

The structure and expression of the genome of RSV are unique among retroviruses in that the protease PR is encoded in both the *gag* and the *gag-pol* polyprotein precursors, with the consequence that this enzyme is expressed in stoichiometric rather than catalytic amounts. This raises the question of whether the PR of RSV *gag-pol* polyprotein is active (as is the case for the PRs of other retroviruses, which only express PR as part of *gag-pol*). To investigate the significance of the particular genome organization and expression of RSV, we developed an assay that measures complementation between the *gag* and *gag-pol* polyproteins by expressing them in chicken embryo fibroblasts (Gs^- and Chf^-; Spafas, Norwich, Conn.) from two different plasmids derived from pAPrC (a nonpermuted copy of provirus RSV Prague C [10]). By using this assay, we showed that protease PR from the *gag-pol* polyprotein can be activated autocatalytically when complemented by a protease-deficient *gag* protein. Furthermore, this complementation assay can be used to determine whether any defect in the *gag* gene can be rescued by the *gag* portion of the *gag-pol* gene, and we report the results of such an experiment that used a mutation in the N terminus of the *gag* gene.

Expression of the *gag-pol* fusion protein without *gag* protein does not induce virion formation or proteolytic processing. A mutant expressing a *gag-pol* fusion protein but no *gag* protein was constructed (Fig. 1) by deleting the first two nucleotides of the *gag* gene termination codon, thus overcoming the translation arrest and constitutively expressing the *pol* gene in the same reading frame as *gag* (5). In this mutant, U180, the *gag-pol* fusion protein differs from that of the wild type by deletion of the first amino acid (isoleucine) of the junction peptide. The U180 mutant proviral DNA was transfected into cultures of chicken embryo fibroblasts, and the viral proteins, either incorporated into particles released into the culture media or synthesized in the transfected cells, were analyzed by immunoblotting using anti-CA serum as already described (10). As shown in Fig. 2A and 3A (lane U180), the mutant U180 synthesized a stable fusion protein in the transfected cells but, in the absence of the *gag* protein

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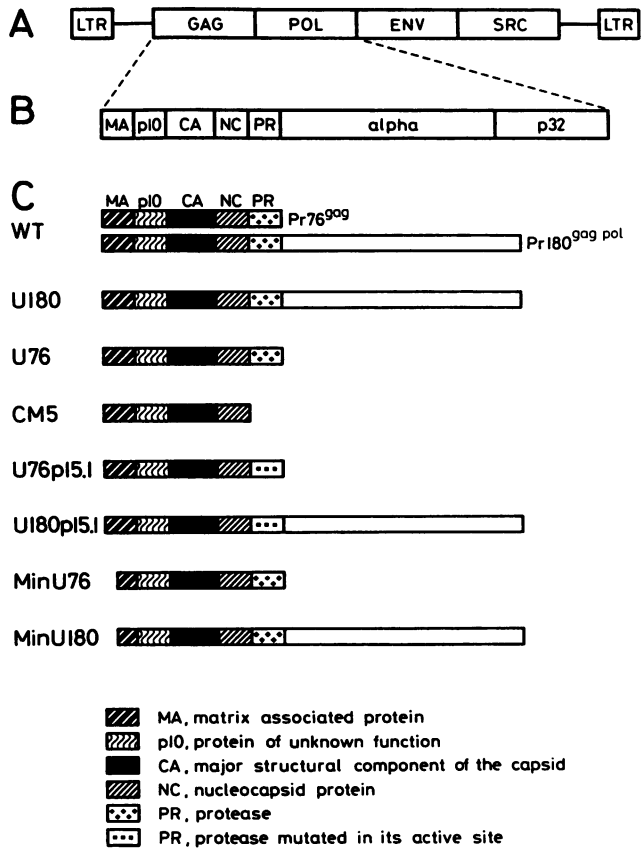

FIG. 1. Structures of *gag* and *gag-pol* polyprotein mutants. (A) Complete genome of RSV PrC as a linear provirus. The regions encoding the *gag*, *pol*, *env*, and *src* proteins are shown in boxes. LTR, long terminal repeat. (B) Enlargement of the *gag* and *pol* genes. MA, matrix-associated protein; p10, protein of unknown function; CA, capsid protein; NC, nucleocapsid protein; PR, protease; alpha, 58-kDa component of reverse transcriptase; p32, integrase. (C) Schematic representation of the mutants (symbols for the coding regions are shown at the bottom). The wild type synthesizes both *gag* precursor $Pr76^{gag}$ and *gag-pol* precursor $Pr180^{gag\text{-}pol}$. Mutant U180 was constructed by deleting the first two nucleotides of the stop codon of the *gag* gene by site-directed mutagenesis (8, 16) using the oligodeoxynucleotide 5′ GGCCCTCCCTAAATT TGTCAAGCGG 3′; it differs from the wild-type (WT) *gag-pol* polyprotein by the absence of the first amino acid (isoleucine) of the *gag-pol* junction peptide. Mutant U76 has already been described: it synthesizes only the $Pr76^{gag}$ polyprotein and produces noninfectious viral particles (11). Double mutants MinU180 and MinU76 were obtained by changing the initiation codon of the *gag* (or *gag-pol*) polyprotein from AUG to TTC by site-directed mutagenesis (8, 16) with the oligodeoxynucleotide 5′ TATGACGGCTTC GAAGCTTGATCCACC 3′. CM5, a mutant lacking the PR domain, has already been described (11). Double mutants U180p15-1 and U76p15-1 were constructed by exchanging the 2,393-bp *Eco*RI restriction fragment containing the protease active site mutation p15-1 (a change from Asp to Arg at the active center of PR [11]) with the same restriction fragment of the DNA proviral vector carrying either the U76 or the U180 mutation outside of this restriction fragment. All of the mutations were confirmed by DNA sequencing (19).

$Pr76^{gag}$, there was no viral particle formation. In addition, no CA antigen was detected in the culture medium, showing that the *gag-pol* fusion protein itself was not released or not stable enough to be immunoprecipitated (Fig. 3B). Similar results have been obtained with a mutant expressing only the first 85 amino acids of the *pol* gene fused to the *gag* gene (1). Our results thus indicate that the phenotype of the shorter fusion mutant is not due to the absence of the remainder of the *pol* gene. These data are compatible with those published after our work was completed, showing no particle formation when *gag-pol* is expressed alone (3, 14).

The *gag-pol* fusion mutant can be rescued in *trans* by coexpression of the *gag* polyprotein. We have already described a mutant U76 expressing only the *gag* polyprotein precursor (11). This mutant, upon transfection, yields fully processed viral particles as efficiently as the wild type. To rescue *gag-pol* fusion mutant U180, we cotransfected the two plasmids (U180 and U76) at different U180-U76 ratios from 1:10 to 4:1 and analyzed the intracellular and viral proteins and production of viral particles by immunoblotting and the presence of the *pol* gene product in the particles by the reverse transcriptase assay (10). The results presented in Fig. 3C and D show that at all input ratios of U180 to U76 up to 1:1, viral particles were formed with an efficiency similar to that of the wild-type virus or transfection with mutant U76 alone (some transfection efficiency-related variation was observed). At a U180-U76 input ratio of 4:1, efficiency of virus particle formation was markedly impaired (<5% of the wild-type level). Results of the reverse transcriptase assay of these coexpression experiments (Table 1) showed that while all particles contained this enzymatic activity, its level increased when the input ratio of U180 to U176 increased. At high U180-U76 input ratios, particles that contain more reverse transcriptase (and therefore more *gag-pol* polyprotein) than the wild-type virions were formed. It was only at a U180-U76 input ratio of 1:4 that the reverse transcriptase activity of the particles reached the level found in the wild type and, therefore, the stoichiometry of *gag* and *gag-pol* incorporated into particles was probably the same as in wild-type particles. Examination of the intracellular proteins (Fig. 2B and C) shows that at increasing concentrations of input U180 DNA, the amount of *gag-pol* precursor synthesized increased concordantly. However, the efficiency of U180 transcription-translation appeared to be lower than that of U76, although there was variation between different transfection experiments and different plasmid preparations (compare Fig. 2A, B, and C). At U180-U76 ratios which gave particles with reverse transcriptase activities close to that of wild-type particles, the relative proportions of *gag-pol* and *gag* precursors in the cell lysate were similar. However, the efficiency of particle formation with *gag* and *gag-pol* present at wild-type levels was also dependent on the requirement that a single cell express both plasmids. The results of these complementation experiments demonstrate that (i) the *gag-pol* fusion polyprotein can be packaged into particles when the *gag* polyprotein is provided in *trans* (in agreement with other reports [3, 14]), which implies an interaction between the two polyproteins during the process of particle assembly, and (ii) particles which contain more *gag-pol* fusion polyprotein than the wild type can be formed. The inability of *gag-pol* to be released from cells and to form virus particles when expressed alone can be explained in two ways. (i) The presence of *gag* is necessary to transport *gag-pol* to the cell membrane, or (ii) the *gag-pol* molecule is sterically inflexible and cannot condense into a capsid structure unless "spaced" by the presence of *gag* molecules. Similar results, showing that *gag-pol* can be released from a cell and form particles only when rescued by *gag* expression, were published after our studies were completed (3, 14).

The protease from the *gag-pol* polyprotein can be activated

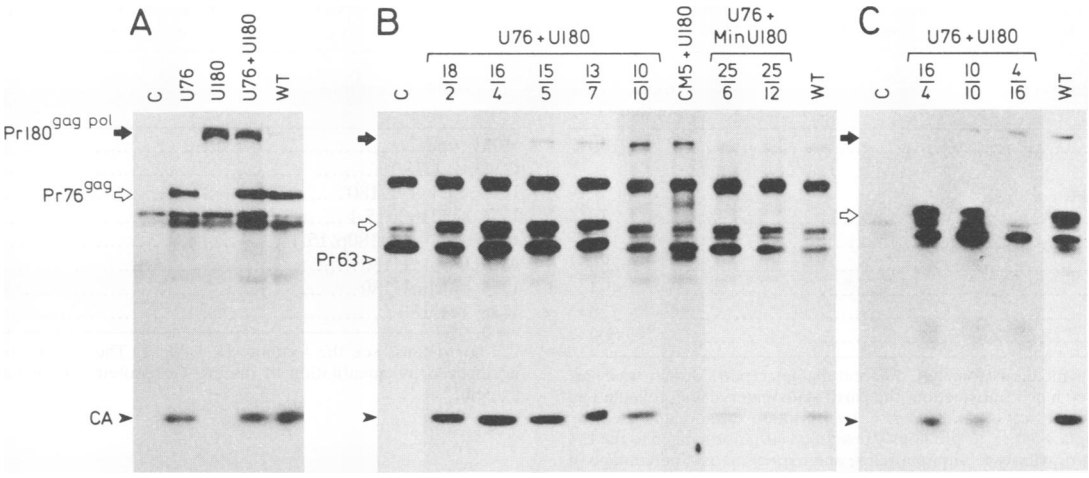

FIG. 2. Analysis of intracellular viral proteins from cells transfected with the viral mutants DNAs. (A) Proteins of cell lysates from two petri dishes were immunoprecipitated with a polyclonal antibody against CA, followed by protein A-Sepharose adsorption. The eluted proteins were resolved by polyacrylamide gel electrophoresis followed by immunoblotting with anti-CA serum and ^{125}I-labelled protein A, as previously described (10). Single transfections were performed with 15 μg of plasmid DNA per plate. For cotransfection (U76 plus U180), the plasmid DNA input was 30 μg of U76 and 10 μg of U180. (B and C) Analysis was performed as described for panel A. Cotransfections were performed either with the amounts of plasmid DNA indicated above the lanes (per plate) or with 25 μg of plasmid-encoding *gag* sequences plus 10 μg of plasmid-encoding *gag-pol* sequences. C, control; WT, wild type.

autocatalytically by coexpression with a *gag* polyprotein. To investigate the potential of the protease encoded in the *gag-pol* polyprotein of RSV, we used our complementation assay to mimic a mammalian retroviral system: we cotransfected mutant U180 with mutant CM5. In this mutant, which has already been described (11), codon 1 of the protease PR is replaced by a stop codon. It therefore synthesizes only a truncated *gag* precursor without PR (analogous to that of mammalian retroviruses) and no *gag-pol* protein (Fig. 1) but forms nonprocessed viral particles at a lower efficiency than the wild type (Fig. 3B in reference 11). Cotransfection of CM5 and U180 resulted in virus particle production less efficient than that of the wild type (Fig. 4A): they contained a protein of about 63 kDa (the translation product of CM5), a small amount of Pr180$^{gag\text{-}pol}$ (made visible by overexposure of the autoradiograph), and a significant amount of mature CA (the amount of processing was variable between transfections, but CA was always demonstrable). Furthermore, the particles produced had a relative level of reverse transcriptase activity comparable to that of the wild-type virus, albeit slightly reduced (Table 2). That the amount of CA in the progeny particles is greater than unprocessed Pr63 suggests either that CA is derived from *gag* (and *gag-pol*) or that the ratio of *gag-pol* to *gag* is much higher than in wild-type particles and that CA is derived only from *gag-pol*. However, the relative reverse transcriptase activity is similar to (even slightly lower than) that of the wild-type virus, suggesting that the amount of *gag-pol* incorporated is not significantly higher (assuming partial activation of reverse transcriptase). Increasing the proportion of U180 to CM5 had an effect similar to that produced by complementation of U180 by U76: particles were formed with a similar efficiency

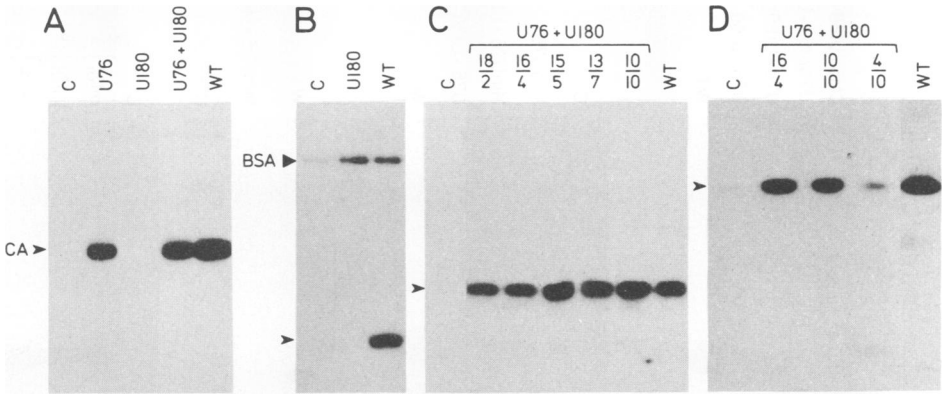

FIG. 3. Analysis of *gag* proteins of viral particles produced by cells after transfection. (A) Culture medium was harvested 48 to 60 h posttransfection, the particulate material was collected by high-speed centrifugation, and *gag* protein content was analyzed by immunoblotting using anti-CA antibody as previously described (10). Transfections were carried out as described in the legend to Fig. 2A. (B) The medium at 48 to 60 h posttransfection was immunoprecipitated, and the eluted proteins were electrophoresed as described in the legend to Fig. 2A. (C and D) Analysis of viral proteins was performed as described for panel A. The numbers above the lanes are ratios of U76 to U180 plasmid DNAs in micrograms. C, control; WT, wild type; BSA, bovine serum albumin.

TABLE 1. Reverse transcriptase activities of particles produced by complementation of U76 and U180[a]

Complementation	Reverse transcriptase activity (%)
Wild type	100%
U76 +U180 at:	
18/2	38
16/4	128
15/5	286
13/7	494
10/10	728
4/16	>1,000

[a] The culture medium from two 100-mm-diameter petri dishes was harvested at 48 to 60 h posttransfection, the particulate material was collected by high-speed centrifugation, and reverse transcriptase was quantitated by exogenous template assay, as previously described (10), normalized to the CA protein content of wild-type viral particles, and expressed as a percentage of the wild-type particles after subtraction of the negative control.

TABLE 2. Reverse transcriptase activities of viral particles in complementation experiments[a]

Complementation	Reverse transcriptase activity (%)
Wild type	100
CM5 + U180	60
U76p15.1 + U180	30
U76 + U180p15.1	150
U76p15.1 + U180p.15.1	0
MinU76 + U180	>500
U76 + MinU180	140
U76 + U180	150

[a] For details, see the footnote to Table 1. The number of particles was normalized by quantitation of mature CA protein and/or unprocessed *gag* protein.

at a U180-CM5 input ratio of 1:1, but at 4:1 particle formation was greatly reduced (data not shown). However, as with U180-U76 cotransfection, the relative reverse transcriptase activity of the particles increased and was detectable even at the highest input DNA ratio. These data support the idea that the particles, formed at a U180-CM5 input ratio of 2:5, shown in Fig. 4A did not incorporate more *gag-pol* than *gag* during virus assembly and that CA was derived, at least partially, from *gag*.

Analysis of the intracellular viral proteins revealed the presence of $Pr180^{gag\text{-}pol}$ and $Pr63^{gag}$ at levels similar to those of the wild-type virus (Fig. 2B, Pr180 and Pr76), suggesting that the lower efficiency of virus particle release is due to the absence of the PR domain in *gag*. A similar reduction in virus particle assembly has been reported for a protease-deficient *gag* mutant (14). These results show (i) that RSV PR can activate itself from the *gag-pol* polyprotein (as is the case for mammalian retroviruses), since we have shown that an unprocessed *gag-pol* polyprotein does not exhibit reverse transcriptase activity (11); (ii) that the PR encoded in the RSV *gag-pol* polyprotein can probably act in *trans* to process the PR-deleted *gag* polyprotein; (iii) that rescue of the U180 phenotype by coexpression of a *gag* polyprotein can occur in the absence of the PR domain of the latter protein, although the PR domain has a role in efficient rescue and virus assembly; and (iv) that the PR from RSV does not require a free carboxyl end for autocatalytic processing, as has been suggested recently (1). Why the PR of RSV is present also in the *gag* polyprotein is not understood. One of the reasons might be quantitative, since it has been shown that RSV PR intrinsically has a lower specific activity than nonavian retroviral proteases (7); thus, more enzyme may be needed. This would be compatible with the partial processing observed in our complementation experiments. It should be noted that input ratios of U180 to CM5 lower than those used in the experiments presented in Fig. 4A reduced processing and resulted in less mature CA (data not shown). Another explanation for the presence of PR in the *gag* polyprotein is the role of the PR, directly or indirectly, in RSV RNA packaging (11). Finally, it appears possible that PR plays a structural role in the assembly of the RSV virion, possibly through interaction of PR domains.

Another approach to investigate the role of the PR of the

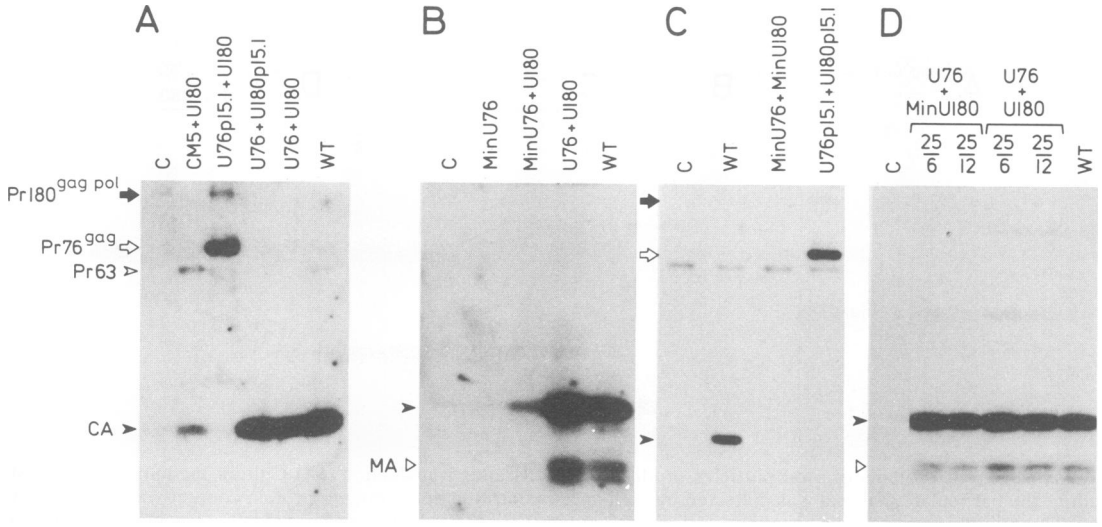

FIG. 4. Analysis of *gag* proteins of viral particles released by cells after transfection or cotransfection. The analysis was preformed as described in the legend to Fig. 3A, except that for B and D an anti-MA antibody was used in addition to an anti-CA antibody. Transfections were performed as described in the legend to Fig. 2B and C. C, control; WT, wild type.

gag-pol polyprotein is to use active-site mutants in complementation experiments. Two double mutants were constructed: a U76 mutant in which the active-center aspartic acid of PR has been mutated to arginine, destroying its proteolytic activity (U76p15.1 [Fig. 1 and reference 11]) and a U180 mutant with the same additional mutation in its PR (U180p15.1 [Fig. 1]). Transfection of cells by mutant U76p15.1 alone yielded viral particles as efficiently as the wild type with an unprocessed *gag* precursor protein, showing that the PR was fully inactivated (data not shown). Cotransfection of cells with mutants U76p15.1 and U180 produced viral particles as efficiently as the wild type, but by comparison with the coexpression of U180 and CM5, there were much more unprocessed *gag* and *gag-pol* polyproteins and much less mature CA (made visible by overexposure of the autoradiograph) (Fig. 4B and Table 2). Two features of retroviruses may offer an explanation: the facts that the active PR is a dimer and that many more molecules of *gag* than *gag-pol* are incorporated into virus particles. Assuming that only PR dimers homogeneous for a functional active site are active, the number of such active molecules will be small by comparison with the wild type or CM5-U180 complementation (in which there are fewer copies of PR but all are active). The results of the converse complementation experiment with mutants U76 and U180p15.1 support this hypothesis (Fig. 4A and Table 2). In this case, the active protease was in excess and the PR of the *gag* polyprotein was able to cleave the protease-deficient *gag-pol* polyprotein completely, resulting in reverse transcriptase activity similar to that observed in the complementation between U76 and U180. It was observed in another study (3) that some mutations in the PR active site of U180 affected the ability of PR to activate the RT, probably through an effect on the precursor structure: our mutation of aspartic acid to asparagine appeared to have no such effect. The control experiment in which U76 p15.1 and U180 p15.1 were coexpressed yielded viral particles containing unprocessed polyproteins (Fig. 4C). Thus, it appears that the PR present in *gag-pol* may be dispensable for processing of *gag* and *gag-pol* polyproteins although it is intrinsically capable of autocatalytic self-cleavage and precursor processing.

The data presented here are, at least in part, contradictory to two studies published while our studies were being completed and submitted (3, 14). The data from the analysis of point mutations of the active site of the PR of *gag* are similar to our data. In one report (3), no CA but some RT activity was detected in the released particles, and in the other no CA or RT was detected (14). However, in our study of active-site point mutants very low levels of CA and RT were detected. The analysis of *gag* PR deletion mutants by these groups yielded results essentially identical to the point mutation studies. Thus, these two groups concluded that the embedded PR is inactive. In contrast, our data from complementation between CM5 and U180 showed significant levels of polyprotein processing and, thus, that the embedded PR can be active. An explanation for the difference between our data and the study of Craven et al. (3) may be the nature of the protease deletion construct. With our construct CM5 (in which a stop codon replaces the first codon of PR), production of virus particles was significantly reduced when the construct was expressed alone or with U180 (reference 9 and Fig. 4A), suggesting a role for the PR domain of *gag* in particle assembly. This reduction was also observed by Stewart and Vogt (14) with an identical mutation. However, in mutant 3h of Craven et al. (3) the first seven amino acids of PR are present and this mutant is fully efficient in particle formation, suggesting that this part of PR is the domain involved in efficient assembly. If these amino acids are able to interact with the PR domain of *gag-pol*, the effect on PR activity may be similar to that observed with the active-site mutants in which an inactive dimer is formed.

In the study of Stewart and Vogt, they established a cell line expressing *gag-pol* constitutively and then introduced a plasmid encoding a protease-deficient *gag*. The reason for the discrepancy with out data may result from the levels of *gag* and *gag-pol* expression, since we have shown that at high ratios of *gag-pol* to *gag* expression particle formation is rendered much less efficient. However, it should be stated that their immunoblots of cell lysates do not suggest a large difference in expression.

During the construction of mutant U180, a one-amino-acid deletion occurred in the linker peptide. It is possible that this mutation has an effect on the activity of the PR of *gag-pol*. An earlier study showed that sequences at the C terminus of the PR of *gag* do affect activity (1). However, to explain our data, this mutation would have to result in increased susceptibility to processing of the precursor at this junction, either by PR itself or by a cellular protease (that *gag-pol* is not apparently processed intracellularly excludes the latter possibility).

Deletion of the N terminus of the *gag* polyprotein can be rescued by complementation with the *gag-pol* polyprotein. The complementation assay that we developed can also be used to study the role of the *gag* portion of the *gag-pol* polyprotein by assaying for the rescue of any *gag* gene mutants by wild-type *gag-pol* polyprotein provided in *trans*. It is thought that the MA protein located at the N terminus of the *gag* gene is involved in the interaction of the *gag* polyprotein with the plasma membrane (13). We therefore constructed double mutant MinU76 (Fig. 1), which carries a deletion of the N terminus of the MA protein. This was achieved by changing the normal AUG initiator of the *gag* gene to TTC so that translation should begin at a downstream AUG, probably the second AUG in the MA sequence, resulting in synthesis of a *gag* polyprotein shorter by 28 amino acids. Transfected alone, MinU76 synthesized a truncated *gag* polyprotein of about 72 kDa, as expected, at about half of the efficiency of Pr76 synthesized by the wild type (3a) but did not produce any particles (Fig. 4B). A similar mutant which displays the same phenotype has already been described (18), which is compatible with the hypothesis that the N terminus of the MA protein is needed for interaction of the *gag* polyprotein with the plasma membrane (since U76 produces particles as efficiently as the wild type). Mutant U180 was therefore used to complement mutant MinU76 (Fig. 4B). Relatively few particles were produced by complementing MinU76 by U180, compared with the cotransfection of U180 and U76 (<5% at the equivalent plasmid DNA input ratio; this is not overspill from the adjacent lane, as equivalent results were obtained when the proteins of the viral mutants were electrophoresed separately). The particles had reverse transcriptase activity about fivefold higher than that of the particles produced by coexpression of U180 and U76 when the activity was normalized to their CA protein content (Table 2). Thus, it appears that the N terminus of the *gag-pol* polyprotein is able to direct the *gag* polyprotein lacking the first 28 N-terminal amino acids to the plasma membrane, allowing formation of viral particles, although this rescue is inefficient and the proportion of *gag-pol* to *gag* is higher than in wild-type particles. The converse experiment was carried out by coexpressing another double mutant, MinU180 (analogous to MinU76 [Fig.

1]), with single mutant U76 (Fig. 3D and Table 2). In this case, the phenotype of the particles produced was similar to that obtained by coexpression of U180 and U76 at the levels of viral protein synthesis (Fig. 2B), particle formation (Fig. 3D), and reverse transcriptase activity (Table 2). Control experiments in which MinU76 and Min U180 were coexpressed did not yield any viral particles (Fig. 3C). Thus, the N terminus of the *gag-pol* polyprotein is dispensable for rescue of *gag-pol* fusion mutant U180 by the *gag* polyprotein. The different efficiencies of synthesis and incorporation of *gag* and *gag-pol* and the role of the N terminus of *gag* as a membrane "anchor" and in the transport of the precursor proteins to the plasma membrane could provide an explanation for the differences between these two complementations. It may be hypothesized that each *gag-pol* molecule interacts only with a small number of *gag* molecules during virus assembly (rather than all 19 in excess). Therefore, in the complementation between U180 and MinU76, the *gag-pol* molecules interact with, and transport to the membrane, fewer *gag* molecules than would reach the plasma membrane in the wild type. This results in the phenotype observed, i.e., fewer particles and an increased proportion of *gag-pol* in the virions. In the complementation between MinU180 and U76, according to this model, there will be no limitation or hindrance of the interaction between *gag* and *gag-pol* or the efficiency with which *gag-pol* is transported to the membrane, resulting in the phenotype observed. Rescue of a deletion mutant of the N terminus of *gag* by wild-type *gag* has been reported (18).

The observation that a mutant such as U180, expressing only the *gag-pol* polyprotein but producing no particles, can be rescued by coexpression with a mutant such as U76, expressing only the *gag* polyprotein, implies that there is an interaction between the two precursors and that some region(s) of the *gag* polyprotein is involved in this rescue. By using the complementation assay described here, we can exclude from this function the first 28 amino acids of the MA protein of the *gag-pol* polyprotein but the PR domain may have a role, since CM5 can only partially rescue mutant U180. The data are in agreement with those of Stewart and Vogt (14), but it should be noted that deletion mutant 3h of Craven et al. (3), which has the first seven amino acids of PR, is fully competent in particle formation. Studies of complementation between *gag* mutants should, therefore, prove useful in analyzing the role of this gene in the retrovirus life cycle.

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