Synthesis and Processing of Viral Glycoproteins In Two Nonconditional Mutants of Rous Sarcoma Virus

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We have studied the pattern of glycoprotein synthesis in two nonconditional mutants of Rous sarcoma virus. One mutant, SE33, produces no viral particles but synthesizes Pr92em, which is cleaved intracellularly to mature glycoproteins. The second mutant, SE521, encodes a gPr92em which is not cleaved to gp85 or gp37 and therefore produces virions with the phenotype of Bryan RSV(−) or NY8. Neither of these mutants have detectable genomic deletions. The study of these mutants has led to the following conclusions. (i) In the absence of particle production or p15 synthesis, gPr92em can be cleaved to the mature glycoprotein which is found on the cell surface. (ii) Noncleaved gPr92em is not packaged into virions but is found on the cell surface. (iii) gPr92em alone can account for subgroup specific viral interference. (iv) gPr92em is probably transported to the cell surface before additional glycosylation or cleavage to mature virion glycoprotein. The nonprocessed precursor of SE521 appears to be glycosylated normally, and thus far we have been unable to determine the basis for the defect in this mutant.

The env gene of Rous sarcoma virus (RSV) encodes the envelope glycoproteins gp85 and gp37, which are found on the surface of virions and are required for infection. Several nonconditional mutants have been previously described which lack both gp85 and gp37 glycoproteins: Bryan high-titer RSV(−) [BH RSV(−)] and Schmidt-Ruppin NY8 (SR-NY8) (13, 34). In both cases, the virions are noninfectious, but if they are artificially introduced into cells by inactivated Sendai virus-mediated fusion, they can replicate normally, indicating that the glycoproteins are only required for absorption or penetration or both. The virus produced is again glycoprotein negative and noninfectious (13). Both RSV(−) and NY8 contain large deletions in their genomes in the region of the env gene (5).

The two viral glycoproteins gp85 and gp37 are found on the surface of virions as dimers molecules linked by disulfide bonds and form knobbed spikes (1, 17). Biosynthesis of the two glycoproteins occurs through a single glycosylated precursor molecule of 90,000 to 95,000 daltons (90 to 95K) (called here gPr92em) (3, 7, 10, 15, 21, 29). Although gPr92em contains all the tryptic peptides of gp85 and gp37, the two glycoproteins do not share any tryptic peptides. gp85 is located at the N-terminus of Pr92em, and gp37 is located at the C-terminus of the precursor. (15, 29). If glycosylation is inhibited by either 2-deoxyglucose or tunicamycin, a nonglycosylated protein of 57 to 62K (called here P57em) can be precipitated with antiviral or antitumor antibody (4, 33). Further evidence showing that the 57 to 62K protein is the primary env gene product comes from the work of Pawson et al. (24), who demonstrated that a 64K protein could be translated in vitro from 28S viral RNA. This 64K protein is totally absent in the translation products of the env mutant NY8. P57em does not appear to be cleaved into subunits and is not incorporated into virions.

There are conflicting data on the site of cleavage of gPr92em into mature glycoprotein. Hayman (10) has reported, using cell fractionation techniques, that gPr92em is cleaved in a cellular membrane fraction (endoplasmic reticulum) and that the gp85 and gp37 cleavage products are then transported to the plasma membrane for viral assembly. On the other hand, Klemenz and Diggelmann found that newly synthesized virions contained uncleaved precursor which was cleaved to mature viral glycoprotein in the newly budded virions (14). The enzyme responsible for cleavage of glycoprotein precursor to mature glycoproteins has not been identified. It has been shown that one of the virion structural proteins, p15, is a protease involved in cleavage of Pr76em to the virion structural proteins (37). The relationship of p15 to Pr92em cleavage has not been demonstrated in vivo, although Moelling et al. (22) have shown that p15 does not cleave gPr92em in vitro.
We have analyzed glycoprotein synthesis in two nonconditional mutants of Prague strain RSV, SE521 and SE33. SE521 encodes a gPr92<sup>env</sup> which is not cleaved to gp85/gp37, whereas SE33 cells do not make virions but cleave gPr92<sup>env</sup> to gp85 and gp37. Results with these mutants relate to questions concerning the site and mechanism of glycoprotein processing.

**MATERIALS AND METHODS**

Cells and viruses. Cultures of chicken fibroblasts (CEF) were prepared from fertile eggs obtained from H and N, Inc., Redmond, Wash., and are of the C/E chf(−) phenotype. Embryonated quail eggs were obtained from Life Sciences Inc., St. Petersburg, Fla., and used to prepare quail fibroblasts (QEF). Wild-type Prague-E (PR-E) 52+ has been described previously (19), and the cloned derivative PR-E 52+ E15-5 has been used in all these studies as a wild-type control. SE521 is a derivative of PR-E 52+ which was obtained by picking a single focus and recloning under conditions which prevented reinfection. It is maintained as a line of transformed quail cells. SE33 is a derivative of PR-E S82h (a ts pol PR-E obtained from a cross between LA335-PR-C and Rouss-associated virus-0 [RAV-0]) which contains the recombinant forms of p19, p19a, and β (27). It was recloned from an agar colony produced by cells from an S82h focus producing low-titer virus. It is also maintained as a line of transformed quail cells. Both SE521 and SE33 have been maintained in culture for over 2 years. RSV-Q clone 3 [a cloned derivative of en(-) BH RSV(−)] was obtained from W. Mason and was originally isolated by R. Pries. All cells were grown in Ham’s F10 supplemented with 5% calf serum. All procedures were described (20, 25).

Antisera and chemicals. Anti-RSV-B<sub>a</sub> (α-RSV-B<sub>b</sub>) was prepared by injecting rabbits with detergent-disrupted proteins from gradient-purified RSV PR-B. Anti-gp85 and anti-p27 were prepared in rabbits injected with RAV-0 virion proteins purified by gel filtration in 6 M guanidine-hydrochloride. Anti-p-19 was a gift from V. Vogt, and antipolymerase was from H. Oppermann. Tunicamycin was a gift of R. Hamill, Lilly Research Laboratories, Indianapolis, Ind.

Labeling of cells and viruses and analysis of proteins on polyacrylamide gels. Amino acid labeling was performed with [3H]leucine in leucine-free medium or [35S]methionine in methionine-free medium containing 2.5% calf serum. [3H]glucosamine labeling was performed in glucose-free medium containing 10 mM fructose and 2.5% calf serum. Labeling details are given in individual figure legends. Viral proteins were analyzed by immunoprecipitation procedures and sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (27). All gels containing [3H]-labeled proteins were fluorographed (2). Viral proteins were analyzed after clarification of viral supernatants at low speed and pelleting of virus through a 20% sucrose cushion.

RESULTS

Characterization of SE521 and SE33 virions. Both mutants were isolated as clones of transformed quail cells which produced no infectious virus (<2 focus-forming units [FFU] per ml, assayed on QEF). The parental virus of both clones was subgroup E PR-RSV (recombinants between PR-RSV-C and RAV-0 [20, 27]). Preliminary screening revealed that supernatants from SE33 cells yielded no polymerase-containing particles as measured in an exogenous RNA-dependent DNA polymerase reaction with RNA: DNA as primer template, whereas SE521 supernatants contained roughly comparable amounts of polymerase activity compared with wild-type PR-E 52+ (data not shown).

To determine whether any viral proteins could be detected in SE33 cell supernatants, a labeling
experiment was performed with 100-fold more [3H]leucine to label the mutant than to label the wild type (1 mCi to label SE33 versus 10 μCi to label PR-E 52+). Supernatants were clarified, pelleted through a 20% sucrose cushion, and immunoprecipitated with α-RSV-B3 serum (data not shown). We were unable to detect any specific viral proteins in the SE33 supernatant. Therefore, we conclude that SE33 cells synthesize less than 1% of the amount of viral particles produced by wild-type-infected quail cells. Immunoprecipitation experiments were performed to determine which viral proteins were synthesized in SE521-released virions. The products of the viral gag and pol genes were analyzed by labeling virions with [35S]methionine and precipitating with α-RSV serum or α-pol serum. Again, we could not detect any virion proteins from SE33 supernatants; SE521 pol and gag proteins were indistinguishable from those of PR-E 52+ (data not shown). We next examined the glycoproteins of SE521 and PR-E 52+ virions by labeling cells with [3H]glucosamine and precipitating clarified viral supernatants with α-gp85 antiserum and analyzing the proteins on polyacrylamide gels (Fig. 1). Lane B shows wild-type virion gp85 and gp37. Lane C shows that SE521 virions contain no gp85 or gp37. There is a small amount of glucosamine-containing material at about 48,000 daltons, which also appears in wild-type virions and may represent nonspecific cellular material. We therefore conclude from the labeling of released virions that SE33 is a particle-negative mutant, whereas SE521 produces particles that lack envelope proteins.

Experiments were performed to see whether the genomes of SE521 and SE33 could be rescued by phenotypic mixing with avian leukosis virus (RAV-61). Table 1 shows that RAV-61 can rescue both mutants, albeit not as efficiently as the rescue of BH RSV (−), the envelope glycoprotein-deficient, naturally occurring variant of RSV. This indicates that both SE33 and SE521 contain functional src gene products and that the mutations leading to the phenotypes are not cellular, since RAV-61 proteins must be normally processed in both cell clones.

Characterization of intracellular proteins synthesized by SE33. Although no released virions could be detected in SE33 cultures, we were interested in determining whether any gag-, pol-, or env-related gene products were synthesized intracellularly. To examine the nonglycosylated intracellular proteins, SE33 cells were labeled for 6 h with [35S]methionine and immunoprecipitated with different specific viral antisera (Fig. 2). Anti-RSV antiserum (lane A) precipitates primarily a 30,000-dalton protein (size determined from molecular weight markers). This protein is also precipitated with α-p19 serum (lane B) but not α-p27 serum (lane C). The α-p27 serum precipitated a faint small protein; however, this band is irreproductibly precipitated and is not precipitated with antiserum to total virion proteins which contain α-p27 activity (lane A). No polymerase-related bands were noted with α-pol serum (lane D), and a very faint high-molecular-weight band was noted after precipitation with the α-gp85 serum (lane E). Therefore, the major nonglycosylated virus-related intracellular protein in SE33 cells is a 30K gag-related protein which contains p19, but not p27, antigenic determinants.

![Fig. 1. Glycoproteins in SE521 and PR-E 52+ virions. Confluent 1.5 ml plates were labeled for 18 h with 50 μCi of [3H]glucosamine in glucose-free medium. Viral supernatants were clarified and pelleted through 1.5 ml of 20% sucrose in standard buffer in an SW50.1 rotor at 40,000 rpm for 60 min. Pellets were resuspended in electrophoresis sample buffer and electrophoresed on a 12.5% gel. (A) [3H]leucine PR-C virus gag marker; (B) [3H]glucosamine PR-E 52+ virions; (C) [3H]glucosamine SE321 virions.](http://jvi.asm.org/)

**Table 1. Rescue of mutants by RAV-61**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Virus titer (FFU/ml)*</th>
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<tbody>
<tr>
<td></td>
<td>−RAV-61</td>
</tr>
<tr>
<td>BH RSV(−)</td>
<td>&lt;3</td>
</tr>
<tr>
<td>SE521</td>
<td>&lt;3</td>
</tr>
<tr>
<td>SE33</td>
<td>&lt;3</td>
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* Dishes (60 mm) seeded with 6 x 10⁵ mutant cells were infected with RAV-61 in the presence of 2 μg of polynye per ml or left uninfected. The medium was changed daily to GM+D and harvested after 5 days. Viral supernatants were assayed on C/E CEF and QEF for focus formation.

FFU, Focus-forming units.
The precursor is cleaved to mature gp85, indicating the rate of cleavage relative to precursor in the mutant. The ratio of gPr92env to gp85 was 1.35 in PR-E 52+ and 0.33 in SE33 cells. This result is not consistent with a model of glycoprotein cleavage occurring in newly budded virions rather than intracellularly.

To examine any intracellular glycoproteins encoded by SE33, cells were labeled for 16 h with [3H]glucosamine, and proteins were immunoprecipitated with α-gp85 serum (Fig. 3). Lane B shows the glycoproteins in wild-type PR-E 52+ cells, gPr92env and gp85. The same two proteins are visible in SE33 cells (lane D).

A densitometer scanning of the gel revealed that SE33 cells contained about 19% as much glucosamine-labeled gPr92env + gp85 as the wild-type control (both from confluent plates plated at the same initial density and containing approximately the same number of cells). This result indicates that in the case of SE33, glycoprotein precursor is synthesized intracellularly and cleaved to mature gp85 in the absence of virion synthesis. The glycoproteins in SE33 are functional, since Sendai-mediated fusion of SE33 cells with envelope-defective BH RSV (−) cells yields infectious virus with subgroup E glycoprotein specificity (data not shown).

Characterization of intracellular proteins synthesized by SE521. SE521 produces particles containing normal gag and pol proteins but no glycoproteins (Fig. 1). To ascertain whether any intracellular glycoprotein is synthesized, cells were labeled with [3H]glucosamine, and proteins were precipitated with α-gp85. Figure 3, lane C, shows that a protein similar to wild-type gPr92env (lane B) is synthesized. The total amount of glucosamine-labeled protein (by densitometer scanning) relative to wild-type (gPr92env + gp85) is 63%. This protein is not cleaved to mature gp85 intracellularly in 6 h, nor does gp85 appear in virions. A summary of the viral proteins synthesized by SE33 and SE521 is presented in Table 2.

**Glycoprotein synthesis and processing in mutant cells.** Densitometer tracings of the relative amounts of gPr92env and gp85 in SE33 and wild-type cells after a 16-h label (Fig. 3) suggested that there was more cleavage product relative to precursor in the mutant. The ratio of gPr92env to gp85 was 1.35 in PR-E 52+ and 0.33 in SE33 cells. This result is not consistent with a model of glycoprotein cleavage occurring in newly budded virions rather than intracellularly.

Also, Fig. 3 suggested that the gPr92env is not processed after 16 h in SE521 cells. To study the kinetics of glycoprotein synthesis and processing in SE33 cells and to determine whether any turnover of SE521 gPr92env into unstable products might occur, a series of continuous labelings with [3H]glucosamine was done (Fig. 4). In this experiment, the gp85 protein band is much more diffuse than that of the band in Fig. 3, making quantitation difficult. However, it can be seen that under the labeling conditions used, in wild-type PR-E 52+ cells, gPr92env is first seen after 40 min of labeling, and gp85 appears after 60 min.

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min. In contrast, in SE521 cells, whereas gPr92<sup>nu</sup> is detected at 40 min, no other protein products can be detected at any time. In SE33 cells, very little glycoprotein is detected until 60 min of labeling, and even at that time gp85 is present in a greater amount than is the precursor protein. Although the total amount of glycoprotein is reduced, it appears that processing to mature glycoprotein gp85 occurs more rapidly in the mutant, which does not synthesize detectable numbers of virions.

To determine where in the cell the glycoproteins of SE521 and SE33 are localized, immunofluorescence on viable cells with anti-gp85 serum was performed (Fig. 5). The serum used was specific for viral glycoproteins and does not cross-react with either viral structural proteins or uninfected quail proteins, since both uninfected QEF and RSV(−) QEF were completely fluorescence negative. PR-E 52+ wild-type cells show clear surface fluorescence, as do SE521 and SE33 cells. The fluorescence on all three cell types had the same granular appearance; the variation in surface fluorescence in the photographs is caused by focal plane differences. This result indicates that inactive SE521 gPr92<sup>nu</sup> not destined for inclusion in viral particles, as well as SE33 glycoprotein (gPr92<sup>nu</sup> or mature gp85 or both) which is not assembled into virions, is localized at the cell surface.

Since SE521 produces a noncleaved glycoprotein precursor molecule at the cell surface, it was of interest to determine whether the gPr92<sup>nu</sup> alone could be responsible for viral interference. Interference is a well-documented phenomenon in which preinfection of a cell with a leukemia virus of a specific subgroup prevents superinfection of the cells by viruses of the same, but not different, subgroup (36). Since SE521 was derived from a virus of subgroup E, if the gPr92<sup>nu</sup> can induce viral interference, SE521 cells should allow replication by subgroup A but not E. The results of this experiment are shown in Table 3. It can be seen that SE521 cells prevent replication of two subgroup E viruses (RAV-60 and PR-E 52+, its parental virus) but not two subgroup A viruses. Interference is complete with PR-E 52+ and less so with RAV-60, which derives its glycoproteins from endogenous c<sup>hf</sup> (11) rather than from RAV-0, as do PR-E 52+ and SE521. This result shows that uncleaved glycoprotein precursor can account for subgroup-specific interference.

**Charaterization of the mutation in SE521.** To ascertain whether the failure of the SE521 glycoprotein precursor to be cleaved is caused by a deletion in the genome of the virus, we characterized the integrated proviral DNA sequences with restriction enzyme digestion and Southern gel transfer (30). The results are shown in Fig. 6. Lane C is the EcoRI digestion pattern of wild-type PR-E 52+ parental proviral DNA. Three major virus-specific bands at 2.5 × 10<sup>6</sup>, 2.0 × 10<sup>6</sup>, and 1.5 × 10<sup>6</sup> daltons are seen. In addition, three larger bands are seen which represent endogenous quail sequences (data not shown) probably related to endogenous src (31). Lane B is BH RSV(−) proviral DNA; this mutant con-
**TABLE 3. Interference pattern of SE521 cells**

<table>
<thead>
<tr>
<th>Infecting virus</th>
<th>Virus titer (FFU/ml) grown on:</th>
<th>SE521</th>
<th>QEF</th>
</tr>
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<tbody>
<tr>
<td>RSV PR-A</td>
<td>$2.4 \times 10^4$</td>
<td>$3.2 \times 10^4$</td>
<td></td>
</tr>
<tr>
<td>RSV PR-E 52+</td>
<td>$&lt;5$</td>
<td>$5.0 \times 10^3$</td>
<td></td>
</tr>
<tr>
<td>RAV-1 (subgroup A)</td>
<td>$7 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAV-60 (subgroup E)</td>
<td>$1.4 \times 10^3$</td>
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</table>

* Dishes (60 mm) of SE521 or secondary-passage QEF ($1.5 \times 10^6$/plate) were infected with 0.1 ml of virus in the presence of 2 µg of polybrene per ml. Medium was changed daily to GM+D, and supernatants were harvested after 6 days. Viral supernatants were assayed on QEF for focus formation.

* FFU, Focus-forming units.

**FIG. 5. Immunofluorescence of viable cells.** Immunofluorescence with α-gp85 antiserum was performed as described in the text. The bar on the PR-E 52+ panel represents 20 µm.

Virus contains an approximately $1.3 \times 10^6$-dalton deletion in the env gene. However, no deletion can be detected in SE521 (lane D). We estimate that deletions of about 50 base pairs could have been detected by this method. This indicates that SE521 does not contain a large deletion in the env gene or in any other structural gene which might encode a glycoprotein-specific protease. Since SE521 virions contain p15 (data not shown) and cleave Pr76 to mature internal structural proteins, we can eliminate this protein as the cause of the SE521 phenotype. Furthermore, since other viruses can replicate normally in SE521 cells (Tables 1 and 3), a host enzyme is not responsible for the phenotype either. A sur-
LINIAL and gPr92env contained the no although gels.

Transfer from transformed quail cells infected by SE521, SE33, PR-E 52+, or RSV(-) as described by Hughes et al. (12). A 15-μg amount of each DNA was digested with EcoRI in the buffer described by Bethesda Research Labs. Digestion was monitored with bacteriophage lambda DNA. Electrophoresis was in 0.8% agarose gels. Transfer onto nitrocellulose filters and hybridization was by the method of Shank et al. (28). The cDNA probe used was 32P-cDNA synthesized complementary to the entire RNA of RSV-SR-D, obtained from M. Groudine. (A) SE33+; (B) RSV(-); (C) PR-E 52+; (D) SE521.

A surprising result also depicted in Fig. 6 is that we could detect no genomic deletion of greater than 50 base pairs in the SE33 provirus (lane A), despite the fact that the mutant is lacking functional gag and pol gene products. Further analysis will be necessary to determine the nature of the defect in this mutant.

Since no large deletion could be detected in the genome of SE521, we attempted to determine whether subtle changes in the gPr92env could be responsible for the lack of maturation of this molecule into functional glycoproteins. One possibility was that the gPr92env of SE521 is aberrantly glycosylated and therefore is missing a recognition signal for cleavage. We compared the [3H]glucosamine-labeled precursors of SE521 and PR-E 52+ in two-dimensional polyacrylamide gels which separate molecules according to charge and size (Fig. 7). PR-E 52+ gp85 could be resolved into at least 16 differently charged subspecies. gPr92env resolved into two major and two minor species. (The faintest, most acidic spot is visible on the original fluorograph). Although no gp85 was noted in SE521, its gPr92env contained the same two major and two minor charged species as the wild type. Therefore, it appears to be glycosylated similarly to the wild type.

Although no deletion was noted in the SE521 provirus, it was possible that a small deletion existed in the env protein. To analyze the size of the nonglycosylated env gene product, cells were labeled with [3H]leucine in the presence of tunicamycin, which prevents glycosylation of avian oncovirus glycoproteins (4, 33). Proteins were then precipitated with α-gp85 serum and analyzed on polyacrylamide gels (Fig. 8). It can be again noted that in the absence of tunicamycin, gPr92env of similar mobilities are synthesized by the wild-type and SE521-infected cells. The mobility of the wild-type gPr92env might be slightly slower than that of the mutant. In the presence of tunicamycin, a 57,000-dalton protein is synthesized which is the nonglycosylated env gene product (4, 33). More of this molecule is present in the wild-type cells than in SE521 cells. Since identical amounts of gPr92env are synthesized, this indicates that the nonglycosylated P57env might be less stable in the mutant. This is consistent with the existence of two smaller proteins in greater amounts in the SE521 cells, which could be degradation products of the P57env. The
The general scheme for viral glycoprotein biosynthesis and maturation is as follows. The env gene encodes a nonglycosylated protein of about 57,000 to 62,000 daltons (4, 33), which is glycosylated to a 92,000-dalton precursor protein (3, 7, 10, 15, 21, 29). The 28S mRNA which encodes the env gene product is found predominantly on membrane-bound polysomes (18). Additional glycosylation occurs, and cleavage by an unknown protease leads to the structural glycoproteins gp85 and gp37, which are found in extracellular virions. The temporal relationship of glycosylation and cleavage has not been determined. Some workers claim that cleavage of glycoprotein precursor occurs at the cell mem-

FIG. 8. Glycoprotein precursor synthesized in the presence of tunicamycin in SE521 and PR-E 52+ cells. Confluent 60-mm plates of PR-E 52+ or SE521 were treated for 4 h with or without 1 µg of tunicamycin per ml (T). Cells were then washed three times with Tris ± 1 µg of tunicamycin per ml and then labeled with 150 µCi of [3H]leucine for 4 h ± tunicamycin. Cell lysates were immunoprecipitated with α-gp85 antiserum, and precipitated proteins were electrophoresed on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. Molecular weight markers are shown in the leftmost lane. WT indicates wild-type PR-E 52+.

FIG. 9. Precipitation of PR-E 52+ and SE521 cellular glycoproteins with α-env and α-whole virus antibodies. Confluent 60-mm plates were labeled with 100 µCi of [3H]glucosamine or 200 µCi of [3H]leucine for 6 h. Plates were lysed, and lysates were divided into two equal parts. One part was precipitated with α-gp85 and the other with α-RSV-Bd (antibody made to detergent-disrupted RSV PR-B virions). As usual, immunoprecipitation was done in the presence of 0.3% sodium dodecyl sulfate. Proteins were electrophoresed in a 12.5% gel. (A) [35S]methionine-labeled PR-E 52+ whole virus markers; (B) [3H]-labeled leu SE521 α-gp85; (C) [3H]-labeled leu PR-E 52+ α-gp85; (D) [3H]-labeled leu SE521 α-RSV-Bd; (E) [3H]-labeled leu PR-E 52+ α-RSV-Bd; (F) [3H]-labeled glc-NH2 SE521 α-gp85; (G) [3H]-labeled glc-NH2 PR-E 52+ α-gp85.

The 70K protein seen with tunicamycin might represent the partially glycosylated intermediate observed by Halpern et al. (8). The results of this experiment indicate no sizeable deletion in the env structural gene product. Thus, slight difference in mobilities of the glycoprotein precursor (seen more clearly in Fig. 9) is probably due to difference in glycosylation. It is unlikely that a single base change is responsible for the SE521 mutation, since in over 2 years of continuous culture of SE521 cells we have never obtained a wild-type revertant virus.

To ascertain whether there is a small size change in the SE521 gpPr92wu, we labeled the precursors with [3H]leucine as well as [3H]glucosamine and precipitated virus-related glycoproteins with two sera, α-gp85 and α-RSV-Bd (made to RSV PR-B detergent-disrupted virions) (Fig. 9). It is especially apparent in the [3H]leucine-labeled proteins that a small size difference exists between the precursor of SE521 (lanes B and D) and that of the wild type (lanes C and E). The gpPr92wu of the mutant migrates slightly faster than the wild type. The α-RSV-Bd does not seem to precipitate glycoproteins from [3H]leucine-labeled wild-type (lane E) as efficiently as from the mutant (lane D); however, the significance of this is unclear, since [3H]glucosamine-labeled glycoprotein is as efficiently precipitated (data not shown). The α-RSV-Bd serum also precipitates a major protein of about 60,000 daltons, which might represent one of the gag-related cleavage intermediates since the cells were labeled for 6 h.
brane and mature gp85 and gp37 are assembled into virions (10, 17), whereas others find that gPr92env is packaged and cleaved rapidly in newly released virions (14). We have analyzed the biosynthesis of virion proteins in cells producing two nonconditional mutants of RSV, SE521 and SE33. SE33 cells synthesize apparently normal glycoprotein in the absence of particle production, whereas SE521 cells produce viral proteins lacking glycoprotein but synthesize a form of gPr92env intracellularly. These two mutants have proven useful in resolving some of the details of viral glycoprotein synthesis.

We have found in SE33 infected cells that gp85 is synthesized in the absence of viral particle production. We are unable to conclude whether gp37 is present as well, since we cannot detect intracellular gp37 with our labeling conditions and antisera, and SE33 produces no particles. However, we infer that gp37 is synthesized since SE33 can complement BH RSV(−) after fusion to produce env+ infectious virions. One question has been whether the gag gene protein p15 might be involved in glycoprotein cleavage in vivo. It is known that p15 cleaves the gag gene precursor to the internal structural proteins (37). Since SE33 does not synthesize p15 but does cleave gPr92env gp85, this hypothesis can be ruled out. A similar conclusion has been reached by Moelling et al. (22), who found that p15 was unable to cleave gPr92env in vitro. In fact, cleavage appears to occur more rapidly in SE33 cells than in wild-type cells (Fig. 4). Furthermore, the efficient cleavage of gPr92env in the absence of viral particle synthesis is more consistent with a model of intracellular glycoprotein cleavage than cleavage in newly budded virus.

The fact that SE521 cells produce particles and intracellular precursor which is not cleaved has allowed us to study some of the properties of the precursor molecule. We have shown as have others (6, 15) that gp85 is more highly glycosylated and positively charged (Fig. 7) than the precursor proteins. The noncleaved Pr92env of SE521 exhibits the same four differentially charged species as wild-type gPr92env (two major and two minor). Despite the fact that SE521 gPr92env is underglycosylated relative to gp85, it is still found at the cell surface (Fig. 5). This tends to eliminate additional glycosylation as a signal for transport of gp85 to the membrane. Furthermore, we have never seen a more highly glycosylated form of gPr92env in SE521 cells. If anything, this molecule contains slightly less carbohydrate, because it migrates slightly faster on sodium dodecyl sulfate-polyacrylamide gels (Fig. 8). This is consistent with the notion that cleavage to mature gp85 precedes additional glycosylation. The caveat in this idea is that since the SE521 precursor is mutant, it may be lacking a site necessary for additional glycosylation as well as a cleavage signal. Despite the localization of at least some SE521 gPr92env at the cell surface, budding virions do not incorporate this molecule, suggesting that the additional processing is necessary for assembly.

Leukosis and sarcoma viruses isolated from chickens have been classified into five subgroups, A to E. One of the criteria for this classification has been the phenomenon of late interference, that is, the ability of a virus of one subgroup to prevent subsequent infection by another virus of the same, but not different, subgroup (36). This is believed to be caused by blockage of cellular receptor sites by the resident virus (32). Our results (Table 3) show that the glycoprotein precursor alone has the ability to cause subgroup-specific interference and therefore can block viral receptor sites. We have not tried to see whether the SE521 glycoprotein precursor can enhance infection by subgroups B and C, as reported earlier for replication competent subgroup E viruses (9).

The actual cause of the phenotype that we observed in SE33 has not as yet been determined. Our data (Fig. 6) show that there is no large deletion (≥50 base pairs) in the proviral DNA, yet the virus does not encode functional gag proteins or any polymerase-related protein that we can detect. The env and src genes code for functional gene products. A nonconditional RSV mutant with a similar phenotype but different genotype has been isolated by W. S. Mason. The mutant PH14 synthesizes no particles, but synthesizes a 30K gag-related protein as well as gPr92env which is processed to gp85. However, PH14 has a genomic deletion of about 0.06 × 10⁶ daltons in the gag gene (T. Hsu, J. Taylor, and W. S. Mason, personal communication). It is possible that SE33 contains a small deletion in gag leading to a frame shift and termination of translation. Alternatively the mutation might be at the level of transcriptional control. Experiments to isolate and characterize these viral mRNA’s in SE33 cells are currently under way.

Delineation of the nature of the mutation in SE521 has proven problematic. The proviral genome contains no deletion (Fig. 6), and the nonglycosylated env gene protein is the same size as that of the wild type (Fig. 8). There may be a small difference in size of the gPr92env compared to parental PR-E 52+; however, this would likely be due to different carbohydrate content. On the other hand, the charged species of the mutant and wild-type precursor are iden-
tical (Fig. 7). Since the mutant does not revert to the wild type, more than a single base change must be responsible. In addition, the mutation is neither in a cellular enzyme, since superinfecting viral glycoproteins are processed normally, nor in p15, which cleaves the gag genes normally. It is possible that a small deletion, insertion, or cluster of base changes has resulted in alteration of the cleavage site recognized by a specific protease. If the protease responsible for glycoprotein cleavage is an enzyme with trypsin-like activity, comparison of the trypsin-generated polypeptides of mutant and wild-type nonglycosylated P57s might reveal a difference. It has proven difficult to obtain sufficient amounts of radiolabeled P57s to do trypic mapping, especially in the case of the mutant protein which appears to be less stable (Fig. 8).

SE251 should be a useful tool to study localization of and transport of the glycoprotein precursor in the cell in the absence of processing. In the same way, SE33 could be used to study mature glycoprotein in the absence of particle production.

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virus DNA: termini of linear DNA bear 300 nucleotides 
present once or twice in two species of circular DNA.


