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Viral Glycoprotein Synthesis Studies in an Established Line of Japanese Quail Embryo Cells Infected with the Bryan High-Titer Strain of Rous Sarcoma Virus

MICHAEL S. HALPERN,* DANI P. BOLOGNESI, AND ROBERT R. FRIIS

The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, and Institut für Virologie, Fachbereich Human Medizin, Justus Leibig Universität, 63 Giessen, West Germany*

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Although a glycoprotein with an approximate molecular weight of 43,000 is associated with purified virions of the Bryan high-titer strain of Rous sarcoma virus propagated on R(−)Q cells, these virions lack gp85, the major glycoprotein of the avian tumor virus envelope. As measured by immune precipitation with a specific antiserum, gp85 does not accumulate to detectable levels in R(−)Q cells.

The Bryan high-titer strain of Rous sarcoma virus (BH-RSV) is a defective virus that possesses the capacity to transform avian cells in culture, but not to direct the synthesis of infectious progeny virus (9, 10). Infectious pseudotypes of BH-RSV can nevertheless be produced by propagation of BH-RSV on chicken embryo cells that are positive for the chick helper factor (chf) (11, 17). The progeny virus, designated BH-RSV(chf), has the same envelope properties as the subgroup E endogenous leukosis virus that can be rescued from chf(+) chicken embryo cells. Because the BH-RSV that has been propagated on chf(−) cells lacks gp85, the major glycoprotein of avian tumor viruses, whereas BH-RSV(chf) does not (14), and because the expression of the subgroup E-specific form of the viral glycoprotein is detectable in uninfected chf(+) but not chf(−) chicken embryo cells (5, 8), it would follow that the synthesis of this glycoprotein in BH-RSV-infected chf(+) cells is specified by one or more genes of the host cell rather than of the superinfecting virus.

Whereas the major envelope glycoprotein of the infectious pseudotype BH-RSV(chf) is apparently not coded for by the genome of BH-RSV, it has not been established whether this genome codes for a structurally related glycoprotein that cannot be assembled into the viral envelope, perhaps as a result of some defect. Consistent with the possibility that no such component is synthesized, however, is the observation that the 35S subunits of the RNA of BH-RSV are smaller than those of nondefective avian sarcoma viruses (2, 15). The smaller size of the RNA may in turn reflect a deletion of some or all of the genetic information coding for the viral glycoprotein. An obvious corollary to the deletion hypothesis would postulate the absence of structures related to gp85 in cells lacking chf that are infected with BH-RSV.

Experiments described in this report were undertaken to determine whether the synthesis of gp85 proceeds in a helper-virus free line of BH-RSV-infected quail embryo cells, designated R(−)Q (4). For this purpose, an antiserum elicited to the purified gp85 of the subgroup C strain of Prague RSV, but reactive with the gp85s of viruses from subgroups A through E (5, 13), was used as a probe for the detection and quantitation of viral glycoprotein synthesis.

MATERIALS AND METHODS

Cells and viruses. The development of Japanese quail cell lines infected with BH-RSV has been previously described (4, 12). These lines were obtained from colonies arising from single infected cells in soft-agar suspension culture. Two lines are in regular culture and have obtained passage levels of 280 and 86, respectively: (i) R(−)Q clone 3, which produces noninfectious BH-RSV [hereafter designated BH-RSV(−)Q], and (ii) R(+)Q clone 4, which produces infectious BH-RSV(RAV-60), subgroup E [hereafter designated BH-RSV(+)Q]. R(+)Q clone 4 is presumed to produce infectious virus as a consequence of an initial superinfection with the helper virus present in the original infecting stock. As a standard subgroup E virus, BH-RSV(RAV-0) (16), which had been propagated on quail embryo cells, was employed as a marker in electrophoresis experiments.

Radioactive labeling. To obtain isotopically labeled virus for subsequent structural analyses, R(−)Q and R(+)Q cells, which had been transferred 2 days before and seeded at 4×10^6 cells per 100-mm plastic culture dish, were labeled for 12 h in the following medium: Eagle minimal essential medium, 90% deficient in amino acids and lacking glucose, but supplemented with 10 mM fructose as en-

ergy source, 500 μCi of [^{3}H]glucosamine per ml (D-[6-^{3}H]glucosamine hydrochloride; >10,000 mCi/mmol; Amersham Buchler, GmbH, Braunschweig, West Germany), and 50 μCi of [^{14}C]protein hydrolysate per ml (Amersham Buchler, GmbH) as well as 5% heat-inactivated calf serum.

For purposes of labeling cells to be lysed for immunoprecipitation, media either deficient for glucose but supplemented with fructose and [^{3}H]glucosamine at 200 μCi/ml or deficient in amino acids and supplemented with 200 μCi of [^{3}H]protein hydrolysate per ml (Amersham Buchler, GmbH), were employed. After a labeling interval of 12 h, plates were washed three times with phosphate-buffered saline and frozen at −70 C until the lysates were prepared.

Purification of virus. (i) Supernatant fluids were clarified by centrifugation in a Sorvall centrifuge at 6,000 rpm for 15 min. The virus was then pelleted by centrifugation for 60 min at 35,000 rpm in a Spinco SW50.1 rotor. The resultant pellet was gently suspended in standard buffer (3) with a Bransonic 12 sonifier and layered on a 5-ml linear preformed gradient of 24 to 48% sucrose (wt/wt). The virus was then sedimented to equilibrium for 16 h at 40,000 rpm in a Spinco SW50.1 rotor. Virus was pelleted out of sucrose solution by centrifugation for 90 min at 25,000 rpm in a Spinco SW27 rotor. The procedures of pelleting and sedimentation on sucrose gradients were repeated two more times except that the penultimate sedimentation on sucrose gradient was for 2.5 h at 40,000 rpm in a Spinco SW50.1 rotor.

Analysis of the structural polypeptides of purified virions. The procedures followed for the recovery, by phenol extraction, of the polypeptides of purified radioactively labeled virions were as described in Duesberg et al. (3). Viral polypeptides were analyzed by electrophoresis on sodium dodecyl sulfate (SDS) gels (5% acrylamide concentration) (3); the methods of slicing of gels, as well as determination of radioactivity in individual slices, were as described previously (7).

Isolation of gp85 from cell lysates. Cellular lysates were prepared as described (6) except that 1% (wt/vol) deoxycholate as well as 1% (vol/vol) Nonidet P-40 were included in the lysing buffer which also contained 0.15 M NaCl and 0.01 M Tris-hydrochloride, pH 8.1. Nuclei and large membranous fragments were removed by centrifugation for 10 min at 12,000 rpm in a Sorvall centrifuge. For the detection of cell-associated gp85, immune precipitation with rabbit anti-gp85 serum was effected by the indirect method as described previously (6). In certain experiments, before precipitation with anti-gp85 serum, a preprecipitate was formed in cell lysates by the addition of 20 μg of human serum albumin together with an equivalence concentration of goat anti-albumin serum (purchased from Antibodies Incorporated, Davis, Calif.). This step was performed to remove any labeled proteins that might adhere nonspecifically to an antigen-antibody lattice.

RESULTS

Analysis of viral structural polypeptides. Particles of BH-RSV(−)Q are noninfectious (4). By contrast, R(+)Q cells yield titers of the infectious pseudotype, BH-RSV(+)Q, which are of the order of 10^6 focus-forming units/ml when supernatant fluids are assayed on avian cells susceptible to infection with subgroup E virus (unpublished observations). It has been previously reported that BH-RSV propagated on chf(−) chicken embryo cells is noninfectious (11) and, in addition, lacks the envelope glycoprotein gp85 that is associated both with infectious pseudotypes of BH-RSV and with nondefective avian sarcoma viruses (14). To test if a comparable structural difference obtained for BH-RSV(−)Q and BH-RSV(+)Q, analyses of the polypeptides of each virus were carried out.

For this purpose, R(−)Q and R(+)Q cells were labeled with a mixture of [^{3}H]glucosamine and [^{14}C]amino acids. The virus released into the media of the labeled cultures was then purified by a succession of equilibrium and velocity sedimentations on sucrose gradients. The double label was used to provide a means to directly compare the ratios of glycoprotein to total protein in the two virus preparations. The profiles of the final equilibrium sedimentations are shown in Fig. 1. As judged by the relative amounts of [^{14}C]amino acid label associated with purified virions, R(−)Q cells produced only about 40% of the amount of virus that was produced by R(+)Q cells cultured under similar conditions. The greater than twofold increase in yield of physical particles from the R(+)Q cells is presumed to reflect the replication in these cells of RAV-60, which must then be present in excess of BH-RSV(RAV-60) in supernatant fluids of R(+)Q cells.

The gradient patterns shown in Fig. 1 also indicated that a significant level of glucosamine label was associated with both virus preparations although, as normalized to the amounts of amino acid label, approximately 30% more glucosamine label was associated with BH-RSV(+)Q. After their recovery by phenol extraction, the viral polypeptides were then resolved on SDS polyacrylamide gels. To compare relative levels of glucosamine-labeled proteins to amino acid-labeled proteins in the two virus preparations, samples for electrophoresis from each gradient were adjusted to contain equal amounts of [^{14}C]amino acid radioactivity. After electrophoresis of the polypeptides of BH-RSV(+)Q, two major peaks of glucosamine label corresponding to gp85 and gp37 were detected (Fig. 2a).

With BH-RSV(−)Q, a small peak of amino acid label (fractions 20 to 25, Fig. 2b) is detected at a position on the gel comparable to the position of the gp85 of the BH-RSV(+)Q; this peak contains approximately 20% of the amino

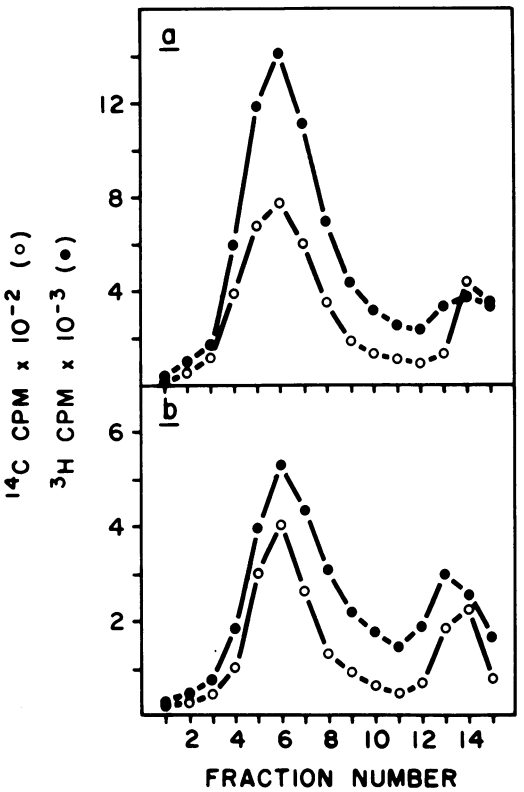

FIG. 1. *Equilibrium sedimentations on sucrose density gradients of (a) BH-RSV(+)Q labeled with [^{3}H]glucosamine and [^{14}C]amino acids. (b) BH-RSV(−)Q labeled with [^{3}H]glucosamine and [^{14}C]amino acids. The peaks at fractions 6 correspond to a density of 1.16 g/cm^{3}.*

acid label associated with the gp85 of BH-RSV(+)Q (Fig. 2a). It is not known at present whether this peak represents glycoprotein, since the background of glucosamine label in this region of the gel precludes identifying with certainty any glucosamine moiety covalently bound to the polypeptide. Nevertheless, these structural analyses do indicate that, in comparison with an infectious pseudotype, BH-RSV(−)Q possesses little, if any, glycosylated gp85.

The only clearly resolved peak of glucosamine label detected in the electropherogram of the polypeptides of BH-RSV(−)Q (fractions 36 to 45, Fig. 2b) had an electrophoretic mobility slightly less than the gp37 of BH-RSV(+)Q. Use of the viral glycoproteins (resolved in Fig. 2a) as molecular weight markers served to assign an approximate molecular weight of 43,000 to this component, and so we have designated it gp43. The origin of the low level of the glucosamine label trailing the gp43 peak is not known. As label is also detected between the two glycoprotein peaks in the electropherogram of the glucosamine-labeled BH-RSV(+)Q, the radioactivity may represent cellular glycoprotein which co-purifies with virus.

Expression of viral structural protein by R(−)Q and R(+)Q cells. Experiments were next undertaken to ascertain whether R(−)Q cells express a moiety antigenically related to gp85. To test for the presence of cross-reacting material, immune precipitation was effected with an anti-gp85 serum using a lysate of cells that had been labeled with [^{3}H]amino acids.

No peak of label was detected co-migrating with the [^{14}C]amino acid-labeled gp85 of BH-RSV(RAV-0) virus that was co-electrophoresed to serve as positional marker in the gel (Fig. 3b). The peaks designated a_1, a_2, and a_3 correspond to the nonspecifically precipitating proteins described previously (6); these peaks are invariably detected when immune precipitates (either specific or nonspecific) are formed in lysates of infected or noninfected cells that had been labeled with [^{3}H]amino acids. By contrast, a small but quantitatively significant peak of label, representing approximately 0.05% of the total trichloroacetic acid-precipitable counts/

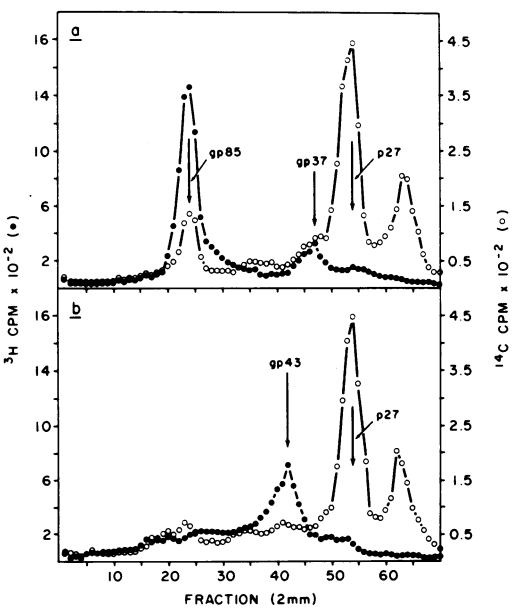

FIG. 2. *Electrophoreses of (a) BH-RSV(+)Q recovered from fractions 5 to 7 of the gradient in Fig. 1a. (b) BH-RSV(−)Q recovered from fractions 5 to 7 of the gradient in Fig. 1b. The peaks migrating ahead of gp37 correspond to the viral internal polypeptides; the positions of the major internal polypeptide p27 are indicated.*

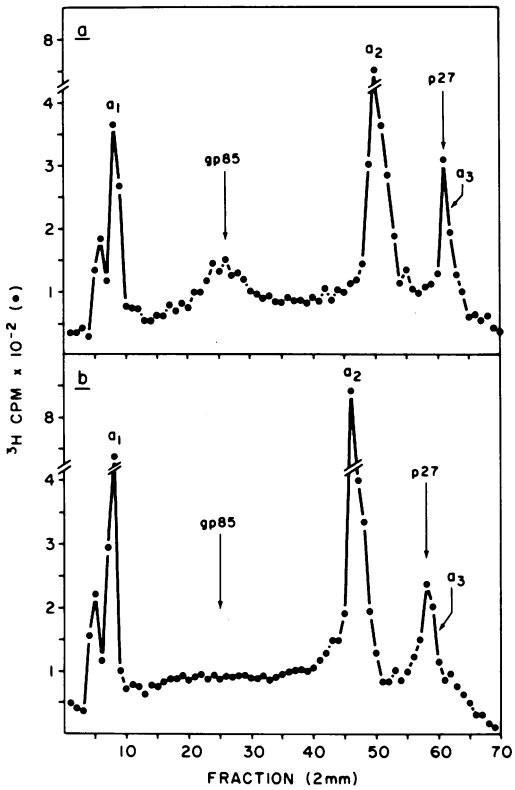

FIG. 3. *Electrophoreses of anti-gp85 immune precipitates prepared with: (a) 10^6 trichloroacetic acid-precipitable counts/min of a lysate of [^{3}H]amino acid-labeled R(+)Q cells. (b) 10^6 trichloroacetic acid-precipitable counts/min of a lysate of [^{3}H]amino acid-labeled R(−)Q cells. Prior to specific precipitation, preprecipitates of albumin-antialbumin were formed in each lysate. [^{14}C]amino acid-labeled BH-RSV(RAV-0) was co-electrophoresed on each gel and the positions of viral gp85 and p27 are indicated.*

min in the volume of lysate used for immune precipitation, was detected co-migrating with viral gp85 after electrophoresis of the anti-gp85 immune precipitate prepared from the lysate of R(+)Q cells (Fig. 3a). This peak was not detected when normal rabbit sera were substituted for the anti-gp85 serum (data not shown), although, as expected, equivalent levels of peaks a_1, a_2, and a_3 were detected. The specificity of the immune precipitation and the co-electrophoresis with viral gp85 therefore serve to identify this peak as gp85, whose synthesis must have been induced by superinfecting with RAV-60 helper virus.

Despite the nondetectability of amino acid-labeled gp85 in lysates of R(−)Q cells, nonglycosylated virion internal polypeptides could be recovered from these lysates by immune precipitation with an antiviral serum prepared against disrupted B_{77} avian sarcoma virus (unpublished observations). The major viral internal polypeptide, p27, was approximately 50% reduced in lysates of R(−)Q cells compared to its level in lysates of R(+)Q cells. The isolation of cell-associated viral polypeptides indicates that lysates of R(−)Q cells are not in some general way refractory to the recovery by the technique of immune precipitation of viral structural protein; this in turn strengthens the argument that the absence of labeled gp85 in immune precipitates reflects a lack of accumulation of this glycoprotein.

As is evident in Fig. 3, a relatively high background of label is detected in the region of the gel corresponding to the position of gp85 when immune precipitates prepared from lysates of amino acid-labeled cells were analyzed. This label presumably represents cellular protein nonspecifically bound to the antigen-antibody lattice. In the electropherograms presented here, formation of a preprecipitate of albumin-antialbumin prior to the formation of the anti-gp85 immune precipitate served to reduce the background radioactivity relative to the background detected in anti-gp85 immune precipitates prepared by omitting the preprecipitation step. Nevertheless, it is apparent that the remaining background would be sufficient to obscure an amount of amino acid-labeled gp85 in R(−)Q cells that is small relative to the level in R(+)Q cells. This consideration is important in the context of viral protein synthesis by R(−)Q cells since, as already noted, the level of virus production by these cells is reduced relative to the level of production by the superinfected cells.

As a more sensitive test for gp85 synthesis in R(−)Q cells, immune precipitates were prepared from lysates of cells labeled with [^{3}H]glucosamine. A peak of glucosamine-labeled gp85, representing approximately 0.45% of the total trichloroacetic acid-precipitable counts/min subject to immune precipitation, could be specifically precipitated from the lysate of the R(+)Q cells (Fig. 4a, comparison with the normal rabbit sera control shown in Fig. 4c). No comparable moiety was resolved when the anti-gp85 immune precipitate prepared from a lysate of R(−)Q cells was analyzed (Fig. 4b) although, as was the case in Fig. 4c, a minor peak was detectable co-migrating with the gp85 marker. A minor peak was also resolved, however, when the immune precipitate prepared with the lysate of R(−)Q cells and normal rabbit sera was analyzed (Fig. 4d), suggesting that the peak detected in Fig. 4b, 4c, and 4d represented a glycoprotein that nonspecifically binds to an antigen-antibody lattice.

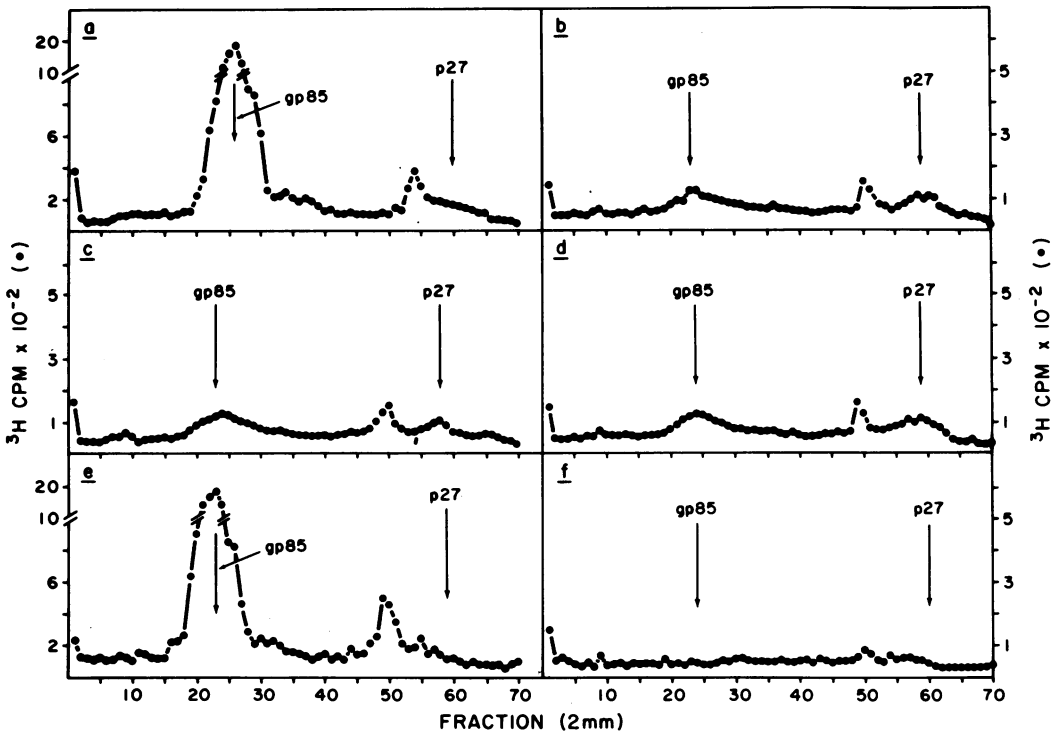

FIG. 4. *Electrophoreses of (a) an anti-gp85 immune precipitate prepared with 10^6 trichloroacetic acid-precipitable counts/min of a lysate of [^{3}H]glucosamine-labeled R(+)Q cells. (b) An anti-gp85 immune precipitate prepared with 10^6 trichloroacetic acid-precipitable counts/min of a lysate of [^{3}H]glucosamine-labeled R(−)Q cells. (c) The same as a, except normal rabbit sera were utilized. (d) The same as b, except normal rabbit sera were utilized. (e) The same as a, except a preprecipitate of albumin-antialbumin was formed prior to the anti-gp85 immune precipitate. (f) The same as b, except a preprecipitate of albumin-antialbumin was formed prior to the anti-gp85 immune precipitate. [^{14}C]amino acid-labeled BH-RSV(RAV-0) was co-electrophoresed on each gel, and the positions of viral gp85 and p27 are indicated.*

Additional evidence for the lack of cross-reactivity of this glycoprotein and gp85 followed from the observation that formation of a preprecipitate of albumin-antialbumin served to clear from the lysate of glucosamine-labeled R(−)Q cells any detectable label that adhered to an anti-gp85 precipitate and co-migrated with viral gp85 (Fig. 4f). Formation of the preprecipitate in the lysate of the glucosamine-labeled R(+)Q cells, however, did not serve to reduce significantly the level of labeled gp85 in the anti-gp85 immune precipitate (Fig. 4e).

DISCUSSION

Recent electron microscopy observations have indicated that BH-RSV(−)Q lacks at least the greater part of the spikelike surface projections that are associated with nondefective avian tumor virus (1, 12). As would be predicted from this result, resolution on SDS polyacrylamide gel of the polypeptides of BH-RSV(−)Q showed a striking reduction in the level of gp85 in comparison with the level of gp85 associated with an infectious pseudotype. We assume that the noninfectivity of BH-RSV(−)Q is a consequence of this lack of gp85.

Whether the lack of gp85 on BH-RSV(−)Q is absolute is as yet unknown; a polypeptide, migrating with the approximate mobility of viral gp85 but reduced in amount relative to the polypeptidyl moiety of the gp85 of BH-RSV(+)Q, was detected in the electropherogram of the polypeptides of BH-RSV(−)Q. Little, if any, glucosamine label is associated with this component, however, so that if it is structurally related to the gp85 of infectious avian tumor virus then it is probably a non- or incompletely glycosylated form of the latter. The observation that the component does not migrate with the greater mobility expected for an incompletely glycosylated form of gp85 but rather co-migrates with the gp85 of the infectious BH-RSV(+)Q virus raises the possibility, however, that the polypeptide is a nonvirus-related quail

cell protein that either binds to virions or co-purifies unbound.

Also co-purifying with virions of BH-RSV(−)Q is a glycoprotein which, on the basis of its electrophoretic mobility on SDS gel, we have designated gp43. The fact that gp43 was not detected in the electropherogram of the polypeptides of BH-RSV(+)Q may indicate that either the gp85 or gp37 that are coded for by the genome of RAV-60 had a higher affinity than gp43 for the same site on a virion substructure. Alternatively, gp43 may be present in virions of BH-RSV(RAV-60) though not in virions of RAV-60. Since, in supernatant fluids of R(+)Q cells, the latter is in excess of the former, the peak of gp37 may obscure the peak of gp43 on an SDS gel electropherogram of purified BH-RSV(+)Q.

Glycoprotein gp43 may represent (i) a non-virus-related quail cell glycoprotein that contaminates preparations of purified BH-RSV(−)Q, or (ii) a fragment of gp85 coded for by the remnant of a partially deleted segment of viral RNA that had once specified the synthesis of gp85. Experiments in progress to analyze the antigenic composition of isolated gp43 may serve to distinguish these possibilities, although the observation that gp43 was not specifically precipitated with anti-gp85 serum from intracellular lysates of R(−)Q cells perhaps itself argues for no determinants common to both gp43 and gp85.

To ascertain whether the lack of gp85 associated with virions of BH-RSV(−)Q derives from an assembly defect or a biosynthetic lesion, immune precipitation with an anti-gp85 serum was used as a probe for the synthesis of gp85 in R(−)Q cells. The results of these experiments indicated that isotopically labeled gp85 did not accumulate to detectable levels in R(−)Q cells even though identical methods of analysis sufficed for the detection of labeled gp85 in R(−)Q cells superinfected with RAV-60. These findings suggest that gp85 is not synthesized by R(−)Q cells, but a number of qualifications must attach to this interpretation of our results.

One is the possibility that gp85 is synthesized at a level below detection. Quantitation is particularly difficult with immune precipitates prepared from lysates of amino acid-labeled cells since a relatively high level of background label is invariably associated with these precipitates. Experiments with amino acid labeling, nevertheless, suggested that synthesis of gp85 in R(−)Q cells does not occur. This interpretation was strengthened by the results obtained with glucosamine labeling wherein the sensitivity would have been sufficient to resolve a peak of glucosamine-labeled gp85 containing greater than 5 to 10% of the radioactivity associated with the gp85 in the lysate of the superinfected cells. Thus, if synthesis of glycosylated gp85 does proceed in R(−)Q cells, the level of synthesis must be of the order of 10-fold less than the level that obtains in the RAV-60 superinfected R(−)Q cells.

A second possibility that cannot presently be excluded is that rapid catabolism of newly synthesized gp85 (but not of the newly synthesized virion internal polypeptides) proceeds in R(−)Q cells and prevents accumulation. Since gp85 accumulation does occur in the R(+)Q cells, this possibility seems unlikely, although one could suppose that BH-RSV codes for a defective form of gp85 that is subject to rapid degradation by a cellular protease. A similar line of reasoning must also allow for the possibility that BH-RSV codes for a form of gp85 that does not possess antigenic determinants recognized by the anti-gp85 serum. Antibodies in this serum react with both the type-specific determinants on the gp85 of subgroup C virus as well as the group-specific determinants on the gp85 of virus of all subgroups (5, 13). Any interaction between the anti-gp85 serum and a putative gp85 in R(−)Q cells would be expected to involve the group-specific determinants. The nature and number of these determinants on the gp85 of nondefective virus are as yet unknown, however, and, if they are not numerous, a relatively minor alteration in amino acid sequence or carbohydrate content associated with the gp85 of BH-RSV(−)Q could result in its nonrecognition by the anti-gp85 serum.

It should be emphasized that the possibilities of a greatly reduced level of cell-associated gp85, or the specification of an atypical form (one that is nonantigenic or highly susceptible to proteolysis), still imply an anomalous pattern of synthesis. The qualifications discussed here assume special significance only in terms of the predicted consequence of a model which, based on the size of viral RNA, postulates that BH-RSV represents a deletion mutant lacking the genetic information coding for gp85 (2, 15). Clearly any one of these qualifications is incompatible with such a model. At present, therefore, while we feel that the data presented here strengthen the argument for the deletion hypothesis, the point is still unresolved and will likely remain so until methods for the translation in vitro of the full complement of viral RNA become available.

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