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Envelope Assembly Mutant of Rous Sarcoma Virus

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The properties of a novel nonconditional envelope mutant of Rous sarcoma virus, rdPN3/2-SR-D, defective in the assembly of viral glycoproteins into mature virions, are described.

There have been four genes identified in the genome of Rous sarcoma virus (RSV): the *gag* gene, which codes for the structural proteins of the virus core (p27, p19, p15, and p12); the *pol* gene, which codes for the virion RNA-dependent polymerase; the *env* gene, which codes for the envelope glycoproteins (gp85 and gp37); and the *src* gene, which codes for the information for cellular transformation (for reviews, see 1 and 30). The analysis of virus mutants has been essential in identifying these genes, in determining their order within the genome, and in defining the sequence of events in the virus life cycle. However, there are still many aspects of RSV replication that remain elusive. We have been isolating additional mutants, particularly nonconditional mutants, in an attempt to learn more about virus replication mechanisms.

In a previous report (26), the genetic characterization of a novel nonconditional envelope mutant, rdPN3/2-SR-D (abbreviated PN3/2; also referred to as LR3/2 virus), was described. This mutant was isolated by using nonpermissive mammalian cells rather than permissive avian cells as hosts for establishing clonal RSV-transformed cell lines. RSV-transformed mammalian cells do not produce infectious virus, but virus is rescued after cell fusion with permissive chicken cells (29). However, if the mammalian cells are transformed by replication-defective RSV, they must be fused with chicken cells infected with helper virus or expressing the appropriate endogenous virus gene to recover infectious transforming RSV. The defect of PN3/2 virus was analyzed by fusing the mammalian cells harboring this virus with various uninfected and infected chicken cells and assaying for virus rescue. This mutant was found to be defective in the *env* gene but not in the *gag* or *pol* genes. From the rescue assays, it was concluded that the lesion results in a decrease rather than a complete block in the expression of functional host range determinants. When PN3/2 virus was rescued and cloned in permissive avian cells, all of the clones produced infectious transforming virus, but the titer was 500- to 1,000-fold lower than that produced by wild-type virus-infected clones. Since the only other nonconditional mutants in the envelope gene of RSV that have been characterized are deletion mutants (12, 15, 25) with complete absence of envelope function, it was important to further characterize this mutant.

Avian RNA tumor viruses are classified into subgroups on the basis of their host range, interference properties, and antigenicity (6, 14, 31). The virus produced by clones of PN3/2 virus-infected chicken cells exhibited the host range of parental Schmidt-Ruppin D (SRD) virus when titrated on avian cells with various genetic susceptibilities (26). Viral interference studies also indicate that PN3/2 virus is a subgroup D virus (Table 1). Cultures of chicken embryo cells (CEC) were infected with subgroup A (Rous-associated virus type 1 [RAV-1]), subgroup B (RAV-6), subgroup C (RAV-7), or subgroup D (RAV-50) leukosis viruses and challenged with various sarcoma viruses. Because subgroup D viruses are cytotoxic to CEC (5, 9, 33) the interference assay was modified to test for subgroup D interference (see Table 1). The data are reported as the ratio of the virus titer on RAV-infected cultures to that on uninfected CEC. PN3/2 virus plated efficiently on RAV-1- and RAV-7-infected CEC, but not on RAV-6- or RAV-50-infected CEC, a pattern similar to that exhibited by parental SRD virus. Challenge of RAV-1-, RAV-6-, RAV-7-, and RAV-50-infected CEC by PRA (Prague A RSV), PRB, PRC, and SRD viruses, respectively, confirmed that these cells were completely infected by leukosis virus and capable of interfering with virus of the appropriate subgroup. These results, in combination with the host range studies reported previously, demonstrate that the infectious virus produced by PN3/2 virus-infected chicken cells is of the same subgroup as the parental SRD virus from which this mutant was derived.

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TABLE 1. *Interference studies with PN3/2 virus*

Challenge virus	Plating efficiency on:[a]				
	Uninfected CEC	RAV-1-infected CEC (subgroup A)[b]	RAV-6-infected CEC (subgroup B)	RAV-7-infected CEC (subgroup C)	RAV-50-infected CEC (subgroup D)[c]
PRA	1.0	0.00005	0.58	0.78	0.19
PRB	1.0	0.76	0.00003	0.68	<0.0001
PRC	1.0	0.92	0.54	0.003	0.12
SRD	1.0	0.68	0.0002	0.74	<0.00003
PN3/2	1.0	0.63	<0.01	0.65	<0.008

[a] The relative efficiencies of infection of the various RAV-infected cultures by challenge virus were calculated by dividing the titer obtained on the RAV-infected cells by that obtained on uninfected CEC.

[b] RAV-infected cultures were prepared by inoculating 2×10^6 CEC with 1 ml of virus and passaging the cells twice before use. A total of 3×10^5 cells in 35-mm dishes were challenged with virus, the cultures were overlaid with focus agar, and foci were counted 7 to 10 days later.

[c] For challenge of RAV-50-infected cells, 10^6 cells in 60-mm culture dishes were inoculated with 0.001 to 0.1 focus-forming unit of challenge virus per cell. The cultures were trypsinized 18 h later and plated on indicator monolayers of 3×10^5 uninfected CEC in 35-mm dishes. Four hours after plating, the cultures were overlaid with focus agar. Foci were counted 7 to 10 days later.

When PN3/2 virus was passaged, it retained the property of production of low titers of infectious virus. Virus harvested from clonal cultures of PN3/2 virus-infected Reasheath C chicken cells was used to infect cultures of SPAFAS CEC. Virus production by these secondarily infected SPAFAS cells was compared with that by the original Reasheath C clones and with that by control cultures infected with SRD virus (Table 2). The average titer of virus produced per cell during a 24-h harvest period by PN3/2 virus-infected Reasheath C clones and the secondarily infected SPAFAS CEC ranged from 0.02 to 0.03 focus-forming unit. In contrast, SPAFAS and Reasheath C CEC infected with wild-type SRD virus produced 10.5 and 9.0 focus-forming units per cell, respectively.

Avian cells infected by the envelope deletion Bryan strain of RSV produce virus particles, but these virions are noninfectious due to an absence of the viral envelope glycoproteins (4, 8, 10, 13). PN3/2 virus-infected chicken cells also produced virions. The production of virus particles, quantitated by assaying virus polymerase activity in culture supernatants (22), was comparable to that of wild-type virus-infected CEC. In addition, the buoyant density of PN3/2 virions (1.16 g/cm^3), when analyzed by sucrose equilibrium density centrifugation, was similar to that of wild-type RSV (not shown).

To examine the polypeptide composition of PN3/2 virions, isotopically labeled virus was purified and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cultures were labeled with a ^{14}C-amino acid mixture for analysis of the total structural protein profile of virions or with [^3H]glucosamine for analysis of the viral glycoproteins. ^{14}C-amino acid-labeled mutant and wild-type virions contained the normal content of *gag* proteins. However, gp85,

TABLE 2. *Comparison of virus production by clones of PN3/2 virus-infected Reasheath C (RC) and secondarily infected SPAFAS chicken cells*

Clone designation	Virus production (FFU/cell per 24 h):[a]	
	Original clone[b]	Passaged virus[c]
3/2RC4	0.02	0.02
3/2RC5	0.03	0.03
3/2RC6	0.02	0.03
SRD[d]	9.0	10.5

[a] To determine the focus-forming units (FFU) of virus produced per cell during a 24-h harvest period, infected cultures at densities ranging from 1×10^6 to 4×10^6 cells per 60-mm dish were fed 5 ml of fresh medium and incubated at 37°C for 24 h, and the culture supernatants were collected. The total number of infected cells in each culture was determined by trypsinizing the cells and plating them as infectious centers on monolayers of uninfected CEC. Cell counts, using a hemocytometer, were also done, and the efficiency of focus formation per cell was near unity for all of the cultures. The values reported were obtained by dividing the total amount of virus produced by the number of infected cells in the culture.

[b] The clones of PN3/2 virus-infected Reasheath C CEC described previously (26) were used.

[c] Secondarily infected SPAFAS cultures were prepared by adding 1.0 ml of culture supernatant from the various 3/2RC clones to 35-mm dishes containing 2×10^5 SPAFAS CEC. Each 25-mm dish was passaged to a 60-mm dish 3 days after infection. The 60-mm dishes were passaged to two 60-mm dishes 3 days later, and 24-h harvests were collected 2 days after the final transfer. At the time that the virus was harvested, the cultures appeared to be confluently transformed.

[d] Cultures of Reasheath C and SPAFAS CEC infected by SRD virus were prepared by adding virus at a multiplicity of 5 focus-forming units per cell to 5×10^5 cells in 60-mm dishes. Supernatants were collected, the virus was titrated, and the number of infected cells in the culture was determined (see footnote *a*) when the cultures appeared to be confluently transformed.

which migrated in fractions 10 through 15 in the gels of wild-type virus, was absent in the gels of ^{14}C-amino acid-labeled mutant virions (Fig. 1). Purified [^{3}H]glucosamine-labeled wild-type virus exhibited two peaks corresponding to gp85 and gp37. In contrast, there were no distinct peaks in the gels of glucosamine-labeled mutant virus, and a higher level of background was present throughout the gel (Fig. 2).

The *env* gene product is a polypeptide of 57,000 to 64,000 daltons (4, 24, 27), which is glycosylated to form a 92,000- to 94,000-dalton polyprotein precursor (7, 18, 23). This precursor is incorporated into virions and is cleaved into

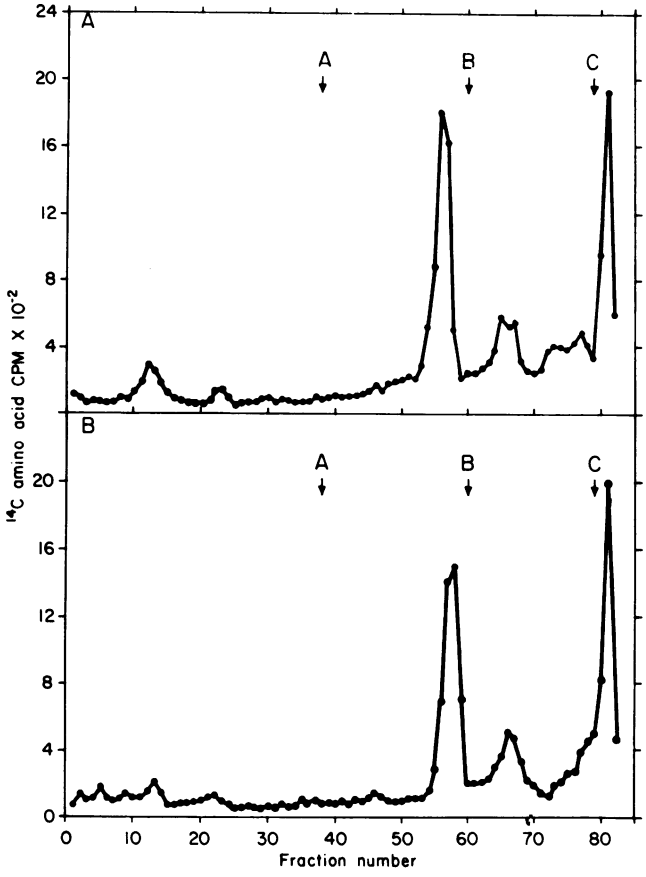

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ^{14}C-amino acid-labeled virus. Cultures in 100-mm dishes were incubated for 24 h in 5 ml of Earle balanced salts containing 5% Eagle amino acids, 5% calf serum, 1% dimethyl sulfoxide, and 20 μCi of ^{14}C-amino acid mixture (New England Nuclear Corp., Boston, Mass.). A 100-μg portion of avian myeloblastosis virus carrier virus was added to each labeled culture supernatant before virus purification. The culture fluid was clarified by centrifugation at 10,000 × g for 20 min in a Sorvall SS-34 rotor, and the virus from the clarified supernatants was concentrated by centrifugation at 40,000 rpm in an SW41 rotor for 60 min. The virus pellet was resuspended in 2 ml of NTE buffer (0.1 M NaCl, 0.01 M Tris, 0.001 M EDTA, pH 7.4) and layered onto a discontinuous gradient of 3 ml of 50% and 7 ml of 25% (wt/wt) sucrose in NTE. These gradients were centrifuged for 120 min at 38,000 rpm in an SW41 rotor. The visible virus band at the interface between 25 and 50% sucrose was collected, diluted 1:2 with NTE, and layered onto a 10-ml continuous gradient of 25 to 50% sucrose in NTE. After centrifugation for 16 h at 40,000 rpm in the SW41 rotor, the visible virus band was collected, diluted in NTE, and centrifuged at 40,000 rpm in the SW41 rotor. The virus pellet was resuspended in electrophoresis sample buffer. A total of 20,000 cpm of purified virus in 0.1 ml of sample buffer was analyzed on 12% sodium dodecyl sulfate-polyacrylamide gels (0.8 by 10 cm) as described by Laemmli (20). After electrophoresis, the gels were sliced into 1-mm fractions, the fractions were added to scintillation vials containing 3% Protosol in Econofluor (New England Nuclear Corp.), the vials were incubated for 18 h at 37°C, and the radioactivity in each vial was determined by scintillation counting. Migration was from left to right. (A) Purified SRD virus; (B) purified PN3/2 virus. Molecular weight markers were: A, 53,000; B, 22,500; C, 13,500.*

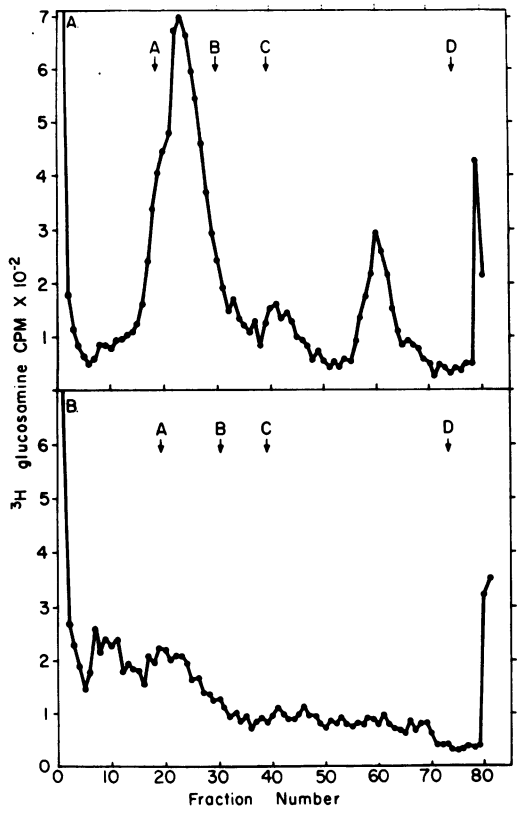

FIG. 2. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{3}H]glucosamine-labeled virus. Cultures were labeled with [^{3}H]glucosamine as described in footnote a, Table 3. A total of 15,000 cpm of purified virus in 0.1 ml of sample buffer was analyzed on 10% sodium dodecyl sulfate-polyacrylamide gels. The procedures for purifying virus are described in the legend to Fig. 1. (A) Purified SRD virus; (B) purified PN3/2 virus. Molecular weight markers: A, 94,000; B, 68,000; C, 53,000; D, 22,500.*

envelope glycoproteins gp85 and gp37 after virus budding (19). The PN3/2 virus lesion might be affecting the synthesis of the *env* gene product, its glycosylation, or the assembly of the glycosylated precursor into virions. To distinguish between these possibilities, intracellular envelope glycoprotein precursors were examined by immunoprecipitation analysis. Cultures were labeled with [^{3}H]glucosamine, and the glycoprotein-related material was precipitated with antiserum prepared against purified B77 virus gp85. This antiserum precipitated equivalent amounts of [^{3}H]glucosamine-labeled material from mutant and wild-type virus-infected CEC but did not react with extracts prepared from uninfected CEC. In addition, preimmune serum did not cross-react with any of these extracts (Table 3). Autoradiographs of these immunoprecipitates analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis are presented in Fig. 3. The material precipitated by gp85 antiserum from SRD (lane B) and PN3/2 (lane D) virus-infected CEC exhibited similar molecular weight profiles migrating as broad bands of approximately 60,000 to 94,000 daltons, with the majority of the material in the 60,000- to 65,000-dalton range. As a control, the virus produced by these cultures was purified and analyzed.

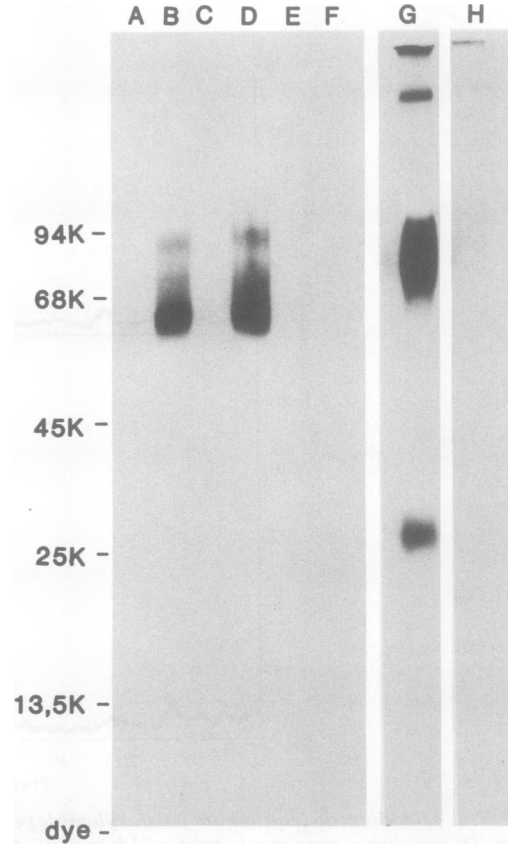

FIG. 3. *12% polyacrylamide gel electrophoresis of gp85 immunoprecipitates. The immunoprecipitates described in Table 3 were solubilized in sample buffer, and equivalent proportions were layered into 12% discontinuous sodium dodecyl sulfate-polyacrylamide slab gels (20, 28) as modified by Mason et al. (21), using the formula for determining the concentrations of acrylamide and bisacrylamide described by Blattler et al. (2). After electrophoresis, the gels were prepared for fluorography as described by Bonner and Laskey (3), and the dried gels were exposed to X-Omat RP-5 film. The samples were: (a) SRD, preimmune serum; (b) SRD, immune serum; (c) PN3/2, preimmune serum; (d) PN3/2, immune serum; (e) uninfected cells, preimmune serum; (f) uninfected cells, immune serum; (g) purified SRD virus; (h) purified PN3/2 virus.*

TABLE 3. *Immunoprecipitation analysis of [^{3}H]glucosamine-labeled cell extracts with gp85 antisera*[a]

Cells tested	Serum used	Input cpm	Total cpm in precipitate	% cpm in precipitate
Uninfected CEC	Preimmune[b]	1.0×10^6	9,338	0.9
	Immune[b]	1.0×10^6	11,006	1.1
SRD-infected CEC	Preimmune	1.8×10^6	7,227	0.4
	Immune	1.8×10^6	135,363	7.7
PN3/2-infected CEC	Preimmune	1.9×10^6	11,365	0.6
	Immune	1.9×10^6	164,722	8.9

[a] Cultures were labeled for 24 h with 50 μCi of [^{3}H]glucosamine (Amersham Corp.; specific activity, 20 Ci/mmol) in 5 ml of medium 199 containing 5% calf serum and 1% dimethyl sulfoxide. The cell monolayers were washed twice with ice-cold phosphate-buffered saline, and the labeled cells were lysed in 1 ml of ice-cold phosphate-buffered saline containing 0.02 sodium azide, 0.1% bovine serum albumin, 1% Nonidet P-40 (NP40), and 0.5% sodium deoxycholate. The lysates were clarified by centrifugation at 12,000 × *g* for 10 min. gp85 antiserum was added to the lysates, and the mixture was incubated on ice for 10 min. The immune complexes were collected by adding Formalin-fixed *Staphylococcus aureus*, Cowan I strain (10%, wt/vol), according to the method of Kessler (16, 17). A 5-μl amount of antiserum was used for precipitating the material from lysates of 1×10^6 to 2×10^6 cells, and 100 μl of bacteria was used to collect the antigen-antibody complexes. This quantity of antiserum was in excess of that needed to precipitate all of the gp85-specific material from 4×10^6 SRD-infected CEC. After addition of the bacteria, the extracts were incubated on ice for 15 min and then centrifuged for 30 s at 12,000 × *g* in an Eppendorf microfuge. The bacterial pellets were washed in: 20 mM Tris–1 M NaCl–0.1% NP40–0.3% sodium dodecyl sulfate (pH 7.5); 10 M Tris–0.1 M NaCl–1 mM EDTA–1% NP40 (pH 7.5); and 10 mM Tris–0.1% NP40 (pH 7.5). The final pellet was resuspended in 100 μl of electrophoresis sample buffer and boiled for 2 min, and the bacteria were pelleted at 12,000 × *g* for min. Total incorporation was determined by counting 5 μl of each sample.

[b] Rabbit preimmune serum and antiserum to purified gp85 from B77 virus were gifts of J. Li, Duke University Medical Center, Durham, N.C.

Wild-type virus exhibited the normal glycoprotein profile (lane G), but mutant virus (lane H) lacked detectable gp85 and gp37. A glycosylated protein related to gp85 of 70,000 daltons, identified as an incompletely glycosylated envelope precursor, has been found in RSV-infected chicken (11, 23) and quail (23) cells. In our gel system this precursor migrates as a band of 60,000 to 65,000 daltons. From these results, it appears that the failure of envelope proteins to be incorporated into PN3/2 virions results not from a deficiency in the intracellular envelope glycoprotein precursor but rather from a failure of the glycosylated precursor to be assembled into virions. If the PN3/2 mutation affects glycosylation, the differences are too subtle to be detected by the methods that we have used.

Chicken cells infected with avian RNA tumor viruses are resistant to superinfection by viruses of the same or a closely related subgroup (6, 31). This property is mediated by the virus glycoproteins. To determine whether the PN3/2 glycoprotein retained this property, mutant-infected chicken cells were challenged with various nontransforming helper viruses and the culture supernatants were collected in the interval from 24 to 48 h after superinfection (Table 4). Successful superinfection should increase the average titer of virus produced per cell above the control value. The average titer of virus produced per cell by the control culture (not superinfected) and cultures superinfected with B and D viruses were similar. Superinfection with RAV-1, however, increased the virus production per cell by 50-fold. Although PN3/2 virus glycoproteins are not incoporated into virions during assembly, they are still capable of mediating virus interference.

TABLE 4. *Superinfection studies of PN3/2 virus-infected CEC*[a]

Superinfecting virus	Subgroup of superinfecting virus	FFU/cell per 24 h
None		0.02
RAV-6	B	0.02
RAV-50	D	0.02
td-21[b]	D	0.04
RAV-1	A	1.0

[a] SPAFAS CEC confluently infected with PN3/2 virus were plated in 60-mm dishes at a density of 10^6 cells per dish. Twenty-four hours later, 1 ml of high-titer stock of superinfecting RAV was added. The supernatants from these cultures were collected in the interval from 24 to 48 h after superinfection. The titer of virus in these harvests and the number of infected cells in each culture were assayed as described in Table 2, footnote *a*. The data are reported as focus-forming units (FFU) of virus produced during the 24-h harvest period.

[b] td-21 is a transformation-defective virus derived from a mammal-tropic SRD isolate by P. Vogt. Stocks of td-21 were the gift of J. M. Bishop, University of California, San Francisco.

The results presented in this communication confirm our previous conclusions that PN3/2 virus is defective in the envelope gene. As pre-

dicted by our complementation analysis (26), the lesion is not an absolute mutation. PN3/2 virus-infected CEC produce virus particles, but these particles are largely noninfectious due to a deficiency of viral glycoproteins. The PN3/2 lesion does not prevent the synthesis or glycosylation of the intracellular envelope glycoprotein precursor. In addition, the mutant intracellular glycoprotein precursor retained the ability to interfere with superinfecting viruses of the appropriate subgroup. There are many possibilities that might explain the defect of assembly of glycoprotein precursors into PN3/2 virions. Additional studies with PN3/2 virus and other envelope assembly mutants may prove useful in understanding RNA tumor virus assembly and the mechanism of virus interference.

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LITERATURE CITED

1. **Bishop, J. M.** 1978. Retroviruses. Annu. Rev. Biochem. **47**:35–88.
2. **Blattler, D., F. Garner, K. Van Slyke, and A. Bradley.** 1972. Quantitative electrophoresis in polyacrylamide gels of 2–40%. J. Chromatogr. **64**:147–155.
3. **Bonner, W., and R. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
4. **Diggelmann, H.** 1979. Biosynthesis of an unglycosylated envelope glycoprotein of Rous sarcoma virus in the presence of tunicamycin. J. Virol. **30**:799–804.
5. **Dougherty, R. M., and R. Rasmussen.** 1964. Properties of a strain of Rous sarcoma virus that infects mammals. Natl. Cancer Inst. Monogr. **17**:337–350.
6. **Duff, R., and P. Vogt.** 1969. Characteristics of two new avian RNA tumor virus subgroups. Virology **39**:18–30.
7. **England, J., D. Bolognesi, B. Dietzschold, and M. Halpern.** 1977. Evidence that a precursor glycoprotein is cleaved to yield the major glycoprotein of avian tumor virus. J. Virol. **21**:810–814.
8. **Friis, R.** 1972. Abortive infection of Japanese quail cells with avian sarcoma viruses. Virology **50**:701–712.
9. **Graf, T.** 1972. A plaque assay for avian RNA tumor viruses. Virology **50**:567–578.
10. **Halpern, M., D. Bolognesi, and R. Friis.** 1976. Viral glycoprotein synthesis studies in an established line of Japanese quail embryo cells infected with the Bryan high-titer strain of Rous sarcoma virus. J. Virol. **18**: 504–510.
11. **Halpern, M. S., D. P. Bolognesi, and L. J. Lewandowski.** 1974. Isolation of the major glycoprotein and a putative precursor from cells transformed by avian sarcoma viruses. Proc. Natl. Acad. Sci. U.S.A. **71**:2342–2346.
12. **Hanafusa, H.** 1977. Cell transformation by RNA tumor viruses, p. 401–483. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology. Plenum Press, New York.
13. **Hanafusa, H., and T. Hanafusa.** 1968. Further studies on RSV production from transformed cells. Virology **34**:630–636.
14. **Ishizaki, R., and P. Vogt.** 1966. Immunological relationships among envelope antigens of avian tumor viruses. Virology **30**:375–387.
15. **Kawai, S., and H. Hanafusa.** 1973. Isolation of a defective mutant of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **70**:3493–3497.
16. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with staphylococcal protein A-antibody absorbent: parameters of the interaction of antibody-antigen complexes with protein A1. J. Immunol. **115**:1617–1624.
17. **Kessler, S. W.** 1976. Cell membrane antigen isolation with the staphylococcal protein A-antibody absorbent. J. Immunol. **117**:1482–1490.
18. **Klemenz, R., and H. Diggelmann.** 1978. The generation of the two envelope glycoproteins of Rous sarcoma virus from a common precursor polypeptide. Virology **85**:63–74.
19. **Klemenz, R., and H. Diggelmann.** 1979. Extracellular cleavage of the glycoprotein precursor of Rous sarcoma virus. J. Virol. **29**:285–292.
20. **Laemmli, U.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
21. **Mason, W., C. Yeater, J. Bosch, J. Wyke, and R. Friis.** 1979. Fourteen temperature-sensitive replication mutants of Rous sarcoma virus. Virology **99**:226–240.
22. **Mizutani, S., C. Kang, and H. Temin.** 1974. Endogenous RNA-directed DNA polymerase activity in virions of RNA tumor viruses and a fraction from normal chicken cells. Methods Enzymol. **29**:119–124.
23. **Moelling, K., and M. Hayami.** 1977. Analysis of precursors to the envelope glycoproteins of avian RNA tumor viruses in chicken and quail cells. J. Virol. **22**:598–607.
24. **Pawson, T., P. Mellon, P. H. Duesberg, and G. S. Martin.** 1980. *env* gene of Rous sarcoma virus: identification of the gene product by cell-free translation. J. Virol. **33**:993–1003.
25. **Scheele, C., and H. Hanafusa.** 1971. Proteins of helper dependent RSV. Virology **45**:401–410.
26. **Steimer, K., and D. Boettiger.** 1979. Cell fusion for genetic analysis of two nonconditional Rous sarcoma virus replication mutants. J. Virol. **32**:175–186.
27. **Stohrer, R., and E. Hunter.** 1979. Inhibition of Rous sarcoma virus replication by 2-deoxyglucose and tunicamycin: identification of an unglycosylated *env* gene product. J. Virol. **32**:412–419.
28. **Studier, W.** 1974. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.
29. **Svoboda, J., and R. Dormashkin.** 1969. Rescue of Rous sarcoma virus from virogenic mammalian cells associated with chicken cells and treated with Sendai virus. J. Gen. Virol. **4**:523–529.
30. **Vogt, P.** 1977. Genetics of RNA tumor viruses, p. 341–455. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology. Plenum Press, New York.
31. **Vogt, P., and R. Ishizaki.** 1966. Patterns of viral interference in the avian leukoses and sarcoma complex. Virology **30**:368–374.
32. **Weller, S., A. Joy, and H. Temin.** 1980. Correlation between cell killing and massive second-round superinfection by some subgroups of avian leukosis virus. J. Virol. **33**:494–506.