JOURNAL OF VIROLOGY, Mar. 1979, p. 856-862
0022-538X/79/03-0856/07$02.00/0

Vol. 29, No. 3

Formation of Rous Associated Virus-60: Origin of the Polymerase Gene

R. C. SAWYER,†* C. W. RETTENMIER, AND H. HANAFUSA
The Rockefeller University, New York, New York 10021

Received for publication 4 August 1978

The DNA of normal chicken embryos contains sequences related to the avian leukosis-sarcoma viruses. RNA-dependent DNA polymerase of these viruses is encoded by a genetic element known as the *pol* gene. The nature of the endogenous virus *pol* gene in chicken cells was investigated by testing its ability to participate in genetic recombination. Rous-associated virus-60-type recombinant viruses isolated after infection of chicken cells with strains tsLA337PR-B or tsNY21SR-A, both of which produce a temperature-sensitive DNA polymerase, also possessed the temperature-sensitive lesion. These results are consistent with the hypothesis that the endogenous viral information used for the generation of Rous-associated virus-60 is deficient in at least part of the *pol* gene and that the defect includes that portion represented by the lesions in NY21 and LA337. The frequency of polymerase-negative BH-Rous sarcoma virus α formation was not affected by the levels of endogenous viral expression, which suggests that the α defect is not derived from the endogenous *pol* gene.

The *gag*, *pol*, and *env* genes of avian leukosis-sarcoma viruses (ALSV) direct the synthesis of viral internal structural proteins, RNA-dependent DNA polymerase, and envelope glycoprotein, respectively. Normal chicken cells contain as an integral part of their DNA genetic information related to ALSV (1, 2, 7, 36, 40). Several lines of chickens spontaneously release a virus, Rous-associated virus-0 (RAV-0), which possesses subgroup E glycoprotein specificity (6, 41). The majority of chicken cells do not spontaneously release virus particles, but they do produce varying amounts of viral RNA, group-specific (gs) antigens, and subgroup E envelope glycoprotein (chicken helper factor [chf]) (2, 3, 7, 10, 14, 19, 32, 39, 43, 44).

Infection of chicken cells with an ALSV results in the production not only of the progeny of the exogenous virus but also of a group of new subgroup E viruses called Rous-associated virus-60 (RAV-60) (15, 16). The titers of RAV-60 obtained from chicken cells reflect the amounts of endogenous viral RNA present in these cells. RAV-60 differs from RAV-0 in its more rapid growth in avian cells that are susceptible to subgroup E viruses (13, 27, 34). Nucleic acid hybridization studies comparing the RNAs of different RAV-60 isolates with those of the exogenous ALSV parent and endogenous viral sequences indicated that RAV-60 is a group of genetic recombinants (13, 22, 39). Analysis of electrophoretic markers in ALSV structural proteins suggested that crossing-over could take place between the exogenous and endogenous viral *gag* genes during the formation of RAV-60 (33).

In this paper we have examined the capacity of the endogenous ALSV-related *pol* gene to participate in genetic recombination. We have attempted to determine the origin of the *pol* gene of RAV-60 by infecting cells with parental avian sarcoma viruses containing a temperature-sensitive (ts) lesion in the *pol* gene and examining the polymerase of the recovered RAV-60. Two parental ts *pol* mutants were used for this purpose, tsLA337PR-B (26, 28) and tsNY21SR-A (Metroka and Hanafusa, manuscript in preparation), and in all cases the RAV-60 isolates obtained were found to contain the ts polymerase gene of the parental sarcoma virus.

We next tested the possibility that α-type BH-Rous sarcoma virus (BH-RSVα), which does not synthesize functional polymerase, might be formed by recombination of polymerase-positive BH-RSV(−) with a defective endogenous *pol* gene. However, unlike RAV-60 production, the frequency of BH-RSVα formation is independent of the levels of endogenous viral gene expression. The defect in BH-RSVα can apparently be localized to a portion of the *pol* gene because these variants are able to recombine with tsLA337, but not with tsNY21, to generate viruses having wild-type polymerase.

† Present address: Cancer Research Laboratories, Woodhaven, NY 11421.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo cells were prepared from fertile eggs (SPAFAS, Inc., Norwich, Conn.) and were assayed for gs antigen by complement fixation and for chf by the formation of BH-RSV(chf) (16, 17). Embryos were designated gs^-chf^- (negative for gs antigen and chf), gs^+chf^+ (positive for gs antigen and chf) or gs_Lh_E (low levels of gs antigen and extremely high levels of chf). Japanese quail embryo cells were prepared from fertile eggs (also from SPAFAS) in the same manner and were first used for virus propagation after the third subculture. The media, conditions for tissue culture, and propagation of ALSV have been previously described (17, 24).

LA337 subgroup A was derived by mutagenesis of the Prague strain of Rous sarcoma virus (PR-RSV) (26, 28). It contains a ts defect in the *pol* gene. The permissive and nonpermissive temperatures are 35 and 41°C, respectively. NY21 also contains a ts *pol* gene and was derived by mutagenesis of Schmidt-Ruppin RSV-A (SR-RSV-A). The permissive temperature is 37°C and the nonpermissive temperature is 41°C (Metroka and Hanafusa, manuscript in preparation). Stocks of the two mutants were grown on gs^-chf^- cells at the permissive temperature.

RAV-60 is formed after infection of chicken cells with an ALSV (15, 16). It was isolated by passaging serial 10-fold dilutions of culture fluids from ALSV-infected gs^+chf^+ or gs_Lh_E chicken cells on quail cells susceptible to subgroup E infection. The quail cells were subcultured periodically, and virus production was assayed by the appearance of virus-associated reverse transcriptase in the culture fluids. The progeny virus from the highest dilution producing a productive infection was again passaged on quail cells using serial 10-fold dilutions. Unless the original gs^+chf^+ or gs_Lh_E stock contained titers of a recombinant avian sarcoma virus of subgroup E in excess of the RAV-60 titer, the second passage on quail cells usually yielded stocks containing only RAV-60, as judged by the lack of transforming virus or virus infectious for C/E cells. For the isolation of RAV-60 from NY21, the original chick cell culture fluids were treated with antiserum prepared in chickens against SR-RSV-A prior to inoculation of the quail cells. In addition, the culture media contained anti-SR-RSV-A serum during the first passage in quail cells.

Polymerase assay and gel electrophoresis of viral proteins. Virus-associated RNA-dependent DNA polymerase activity was determined by pelleting the virus in 10 ml of culture fluid in a Beckman type 40 rotor. The reaction mixture in 0.1 ml contained 7.5 μg of $(rC)_n \cdot (dG)_{12-18}$ (7:3), 0.1% Nonidet P-40, 5.0 μmol of Tris-hydrochloride (pH 8.3), 2.0 μmol of dithiothreitol, 6.0 μmol of NaCl, 1.0 μmol of magnesium acetate, and 500 pmol of [^{3}H]dGTP (500 cpm/pmol). The virus pellet was first suspended in the reaction mixture without $(rC)_n \cdot (dG)_{12-18}$ and [^{3}H]dGTP. Samples were incubated at 35 and 41°C (LA337) or 37 and 45°C (NY21) for 30 min. The template primer and [^{3}H]-dGTP were then added, and the incubation was continued. At the end of 60 min, trichloracetic acid-precipitable radioactivity was determined.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of viral proteins labeled with L-[^{35}S]methionine in tissue culture was done as described elsewhere (33).

Cloning of BH-RSV(−). BH-RSV(−) grown in both gs^-chf^- and gs^+chf^+ cells was cloned in the absence of helper virus by soft-agar colony formation using UV-treated Sendai virus (17). A sample of 28×10^6 normal cells (gs^+chf^+, gs^-chf^-, or gs_Lh_E) was incubated with DEAE-dextran, UV-treated Sendai virus, and 1×10^6 to 5×10^6 focus-forming units of BH-RSV. The cells were divided into six plates and cultured in soft-agar medium. After 12 to 17 days (17 to 21 days for gs_Lh_E cells), individual colonies were picked and incubated in 0.1 ml of trypsin-EDTA solution for 10 to 15 min at room temperature (trypsin-EDTA: 0.05% trypsin; 0.02% EDTA; 0.025 M Tris-hydrochloride, pH 7.4; 0.14 M NaCl; 0.0005 M KCl; 0.3 mM Na_2HPO_4; and 6 mM glucose). The cells were plated with 0.4×10^6 normal chicken cells on 35-mm culture plates and overlaid with soft agar medium 24 h later. BH-RSVα(−) is defined as a virus particle that lacks reverse transcriptase activity (12). Transformed cultures that did not produce infectious sarcoma virus and did not contain polymerase activity in the culture fluids, but which did produce infectious sarcoma virus after superinfection with RAV-1, were classed as BH-RSVα(−)-transformed cultures. These BH-RSVα(−)-transformed cells produce physical particles, lacking polymerase, which can be assayed by [^{3}H]uridine labeling, and the level of the production of noninfectious particles generally correlated with the level of the titer of BH-RSV (RAV-1) obtained upon rescue with RAV-1.

Cultures of BH-RSVα (polymerase negative)-transformed chick cells were grown from individual soft-agar colonies using BHRSVα (RAV-1) as previously described (37).

Crosses between BH-RSVα and LA337 and NY21. Cultures of BH-RSVα-transformed chicken cells were superinfected with either LA337 or NY21 at 35 or 37°C, respectively, and maintained at these temperatures. Culture fluids were changed daily. Virus-associated RNA-dependent DNA polymerase activity was assayed on culture fluids harvested 4 days postinfection. Progeny virus in culture fluids harvested 5 days postinfection were then cloned by soft-agar colony formation. Soft-agar colonies of cells infected with LA337 or NY21 were also prepared as controls. Absorption and colony growth were done at 41°C, the nonpermissive temperature for LA337 and NY21. Polymerase activity from cultures derived from individual soft-agar colonies was assayed as described.

RESULTS

***pol* gene of RAV-60.** RAV-60 has been shown to be a recombinant virus containing genomic RNA sequences from both the endogenous provirus and exogenous infecting virus (13, 22, 38). With the exception of RAV-0-producing cells, normal uninfected chicken cells do not produce a virus-related reverse transcriptase (12, 23, 29, 46). We were interested in determining whether the endogenous *pol* gene could rescue known mutations in the *pol* genes of exogenous viruses during the formation of RAV-60.

Two such mutants, LA337 and NY21, were grown on gs^+chf^+ and gs_Lh_E chicken cells. RAV-60 was isolated from these stocks by repeated passage at end-point dilutions on Q/B Japanese quail cells. After RAV-60 isolates were determined to be free of parental sarcoma virus, they were tested for the temperature sensitivity of the *pol* gene. The data presented in Table 1 are expressed as the ratio of counts per minute incorporated at the nonpermissive temperature to that at the permissive temperature. The first column for each virus details the degree of temperature sensitivity of the different stocks of parental sarcoma virus from which each isolate of RAV-60 was derived. (RAV-60 titers are about 10^{-3} of that of sarcoma virus in culture fluids from gs^+chf^+ and gs_Lh_E chicken cells fully transformed by RSV and, thus, do not contribute measurably to these data.) In each case the parental LA337 and NY21 incorporate only 10 to 20% as much [^{3}H]dGMP at the nonpermissive temperature as at the permissive temperature using $(rC)_n \cdot (dG)_{12-18}$ as the template primer in the polymerase assay. Wild-type Prague RSV and SR-RSV incorporated slightly more radioactivity at the higher temperature. In some polymerase assays, control SR-RSV or RAV-2 incorporated slightly fewer counts per minute at 45 than at 37°C (45/37°C = 0.85 to 1.0).

RAV-60 isolated from wild-type Prague RSV-B and SR-RSV-A contained a wild-type polymerase gene. In addition, RAV-60 prepared from RAV-1, RAV-2, and BH-RSV(−) also contained a wild-type polymerase gene (data not shown). However, when a temperature-sensitive virus was used as the exogenous parent, all of the RAV-60s isolated produced a ts polymerase that was temperature sensitive to the same degree as the parental virus. We noted only one exception, RAV-60 [NY21], isolate 83a. This isolate, while still temperature sensitive, was significantly less so than the NY21 stock from which it was isolated (45/37°C of 0.58 versus 0.16). However, two other isolates of RAV-60 (83b and 83c) were purified from the same virus stock obtained as culture fluid of NY21-infected gs^+chf^+ cells and used for the isolation of 83a. Both 83b and 83c were as temperature sensitive as the NY21 of the original stock. With the exception of RAV-60 [NY21] 07 and 08, all isolates were derived from gs^+chf^+-type cells. The use of gs_Lh_E-type cells did not affect the recovery of ts RAV-60 as demonstrated by isolates 07 and 08 for RAV-60 [NY21].

Origin of RAV-60 *gag* gene. We have shown that electrophoretic variants of ALSV structural proteins can be used to determine the genetic origin of RAV-60 *gag* gene products (33). The most convenient marker for this purpose is the major structural protein p27. The p27 encoded by RAV-0 has a lower electrophoretic mobility in sodium dodecyl sulfate gels than the p27 of exogenous ALSV. One RAV-60 isolate was previously shown to have a p19 marker specific for the exogenous virus used in its production and a p27 that comigrated with that of RAV-0 (33). From this we concluded that the endogenous viral *gag* gene encodes a p27 closely related to that of RAV-0. We have examined the electrophoretic mobilities of the structural proteins of RAV-60s obtained in the present study. For this analysis, Prague RSV-B, SR-RSV-A, RAV-0, and the RAV-60s were labeled with [^{35}S]methionine, and the viral proteins were separated by

TABLE 1. *Thermal lability of RAV-60 polymerase derived from two ts polymerase mutants*

RAV-60 [LA337] isolate no.	Ratio of polymerase activity[a] 41/35°C		RAV-60 [NY21] isolate no.	Ratio of polymerase activity[a] 45/37°C	
	Parental LA337	RAV-60		Parental NY21	RAV-60
01	0.13	0.13	06	0.11	0.10
04	0.11	0.15	07	0.12	0.27
06	0.17	0.25	08	0.07	0.13
07	0.13	0.11	83 (a)[b]	0.09	0.58
14	0.12	0.08	83 (b)[b]	0.09	0.09
15	0.12	0.11	83 (c)[b]	0.09	0.15
WT-PR-RSV-B[c]	1.15	1.19	93	0.10	0.15
			94	0.12	0.20
			96	ND	0.07
			WT-SR-RSV-A	1.22	1.06

[a] Samples were preincubated at the indicated temperature in the absence of template-primer and deoxytriphosphate for 30 min. [^{3}H]dGTP and $(rC)_n \cdot (dG)_{12-18}$ were added, and incubations were continued at the respective temperature for 60 min. Trichloroacetic acid-precipitable radioactivity was then determined. ND, Not done.

[b] Three separate isolations of RAV-60 from the same original virus stock.

[c] WT, Wild-type; PR-RSV-B, Prague RSV subgroup B; SR-RSV-A, Schmidt-Ruppin RSV subgroup A.

electrophoresis in 12% polyacrylamide slab gels containing 0.1% sodium dodecyl sulfate. The p27 proteins of all RAV-60 isolates listed in Table 1 comigrated with the p27's of the exogenous parental viruses.

Formation of BH-RSVα. BH-RSV(−) spontaneously segregates a polymerase-minus mutant, BH-RSVα, that is unable to direct synthesis of any polymerase-related protein (11, 12, 35). It is possible that BH-RSVα is generated in a manner analogous to the formation of RAV-60, in which recombination occurs between an exogenous infecting virus and the endogenous sequences. During RAV-60 formation the polymerase gene is derived from the exogenous virus. The reciprocal recombinant, if it occurs, would presumably contain an endogenously supplied polymerase gene. The α form of BH-RSV(−) might represent this reciprocal recombinant. Since the amount of RAV-60 produced in chicken cells is proportional to the level of endogenous viral expression (15, 16, 45), we tested the hypothesis that BH-RSVα might be formed in a similar manner by measuring the frequency of α formation from stocks of BH-RSV(−) grown and subsequently cloned in chicken cells with varying levels of endogenous gene expression. If the hypothesis were true, the percentage of α clones should be highest in those cells with the highest levels of endogenous gene expression. The results are presented in Table 2. They show that, regardless of the cell type used for cloning the virus, 5 to 10% of the resulting clones were of the α type. Unlike RAV-60 production, BH-RSVα titers do not appear to be dependent on the level of expression of the endogenous viral genes. Therefore, the result seems to suggest that the formation of BH-RSVα probably does not represent recovery into virus of a defective endogenous polymerase gene linked to gs antigen and chf. Further, extensive efforts to isolate a *pol*⁻ mutant from SR-RSV have consistently been unsuccessful (unpublished data). For this reason we did not attempt to isolate a *pol*⁻ LA337 or NY21.

TABLE 2. *Effect of cell phenotype on the generation of polymerase-negative BH-RSVα*

Cell type used for growth of BH-RSV (*pol*⁺)	Cell type used for colony formation[a]	No. of colonies BH-RSVα/colonies tested[b]
gs^-chf^-	gs^-chf^-	4/35
gs^-chf^-	gs^+chf^+	3/30
gs^+chf^+	gs^+chf^+	4/60
gs^+chf^+	gs_Lh_E	5/54

[a] Virus was cloned by soft-agar colony formation.

[b] Individual colonies were tested for infectious virus (for those colonies grown on chf^+ or h_E cells) and polymerase activity in culture fluids. Colonies suspected of being BH-RSVα were further tested for positive rescue by avian leukosis virus RAV-1, and for lack of rescue by reticuloendotheliosis virus and pheasant virus (43).

Recombination between BH-RSVα and a ts mutant. Infectious pseudotypes of BH-RSVα are readily formed by superinfecting with a leukosis virus such as RAV-1. Upon subsequent cloning of such pseudotyped stocks, some clones will be polymerase-positive BH-RSV(−), of which at least a portion of the polymerase gene is presumably derived from the helper virus. To determine whether BH-RSVα contained a partial polymerase gene capable of genetic recombination, we analyzed crosses between it and two mutants, LA337 and NY21, containing different temperature-sensitive defects in the polymerase gene. The progeny of these crosses were screened for wild-type recombinants by cloning at the nonpermissive temperature (41°C). Cloning in this manner preferentially selects for wild-type virus if they exist in the stock. The results (Table 3) show that BH-RSVα readily recombines with LA337 to yield wild-type virus. By this selection technique more than 90% of the BH-RSVα × LA337 progeny that are able to initiate colony formation at 41°C contain either a wild-type or only partially temperature-sensitive polymerase. On the other hand, only 1 out of 11 BH-RSVα × NY21 progeny cloned in this manner was wild type for polymerase (data not shown). Both crosses utilized the same BH-RSVα stock. Thus BH-RSVα contains at least the part of the polymerase gene encompassing the lesion in LA337 but not NY21 and is capable of genetic recombination with LA337 but not NY21 to form wild-type polymerase.

DISCUSSION

The expression of the endogenous retrovirus-related *gag* and *env* genes of chicken cells in the form of gs antigens and chf is well documented (2, 3, 7, 10, 32, 39, 44). However, no functional product of the endogenous *pol* gene has been detected (12, 34, 39, 46). That the endogenous viral genes do contain at least some *pol* gene sequences can be inferred from the following lines of evidence. Hybridization data using a complementary DNA probe representing the ALSV *pol* gene revealed that some, but not all, ALSV *pol* gene sequences are represented in the polyadenylic acid-containing RNA of gs^+chf^+ chicken cells (43). Additional sequences apparently lacking in gs^+chf^+ cells are found in the RNA of gs_Lh_E-type cells. The gs^+chf^+ cells also contain a protein of molecular weight 120,000 which contains some of the tryptic peptides of

TABLE 3. *Recovery of a wild-type polymerase gene from a cross of BH-RSV(α) × LA337*

Type of *pol* gene[a]	LA337 no. of clones	BH-RSVα × LA337[b] no. of clones
Wild type (41/37°C ≥ 0.9)	1	10
Partially ts (41/37°C = 0.50 to 0.89)	6	3
ts (41/37°C < 0.50)	13	3

[a] An exogenous polymerase assay measured the incorporation of [^{3}H]dGMP using $(rC)_n \cdot (dG)_{12-18}$ as a template primer. Assignment of gene type was based on the ratio of counts per minute incorporated at 41°C to that at 35°C. Wild-type *pol* had a 41/35°C ratio of ≥ 0.90; 41/35°C of 0.50 to 0.89 = partially ts; 41/35°C of <0.50 = ts.

[b] Stocks of LA337 or the progeny of a mixed infection of BH-RSVα and LA337 were cloned by soft-agar colony formation at 41°C. The same stock of LA337 was used for cloning and the crosses with BH-RSVα.

reverse transcriptase (8). Finally, Panet et al. (31) demonstrated that uninfected cells contained small amounts of material antigenically related to reverse transcriptase. These results are consistent with the hypothesis that the endogenous viral genes contain a defective *pol* gene. The defectiveness of this gene is further supported by the observations that BH-RSVα grown in chicken cells does not spontaneously revert to the polymerase-positive β type, and RAV-60 cannot be obtained from BH-RSVα-infected cells (12).

As part of our attempts to determine the origin of the various genes of RAV-60, we examined the nature of the endogenous *pol* gene. We studied whether it could correct the ts defect in the *pol* gene of two different avian sarcoma viruses by testing for the recovery of wild-type RAV-60. In addition, we examined whether the polymerase-negative defect of BH-RSVα might be derived from the endogenous *pol* gene by comparing the frequency of α-type formation by polymerase-positive BH-RSV(−) in chicken cells with varying degrees of endogenous gene expression. That the BH-RSVα *pol* gene was different from the endogenous gene was further confirmed by demonstrating that the BH-RSVα could recombine with LA337 to yield wild-type polymerase.

In every RAV-60 isolate we tested, the polymerase was characteristic of the exogenous parent. Using two different ts *pol* mutants as exogenous parents, we were unable to recover RAV-60 that was not temperature sensitive for polymerase from either gs^+chf^+ or gs_Lh_E chicken cells. Although the total number of RAV-60 examined is not very large, if one considers that all of them contain the *env* gene which specified subgroup E glycoprotein and that *pol* and *env* are often genetically linked (5, 9, 28, 47), the failure to isolate a RAV-60 with wild-type reverse transcriptase is clearly significant. Although more RAV-60 isolates from gs_Lh_E cells must be studied before it can be concluded that the *pol* gene of these cells cannot recombine, the results from the two embryos tested are identical to those from gs^+chf^+ embryos. Furthermore, we demonstrated that wild-type ALSV yielded wild-type RAV-60. These results are consistent with those of Wang et al. (43), who have shown that the endogenous viral RNA of gs^+chf^+ (but not gs_Lh_E) cells contains a deletion in the *pol* sequences. Apparently the additional sequences in the larger endogenous viral RNA transcript found in gs_Lh_E-type cells is not sufficient to enable it to correct the ts defects of LA337 or NY21. One RAV-60 isolate (RAV-60 [NY21] 83a) was less temperature sensitive (the 45/37°C ratio for polymerase assay was 0.58, versus that for the original NY21, 0.16). Our experience with NY21 is that upon cloning we observe about 10% of clones that are considerably less temperature sensitive than the 45/37°C ratio of 0.1 to 0.2 usually obtained (data not shown). We believe that if RAV-60 was repeatedly cloned from any one stock of NY21 grown in gs^+chf^+ cells, 5 to 10% of the isolates would probably be only partially temperature sensitive.

The *gag* gene proteins are initially synthesized as a single precursor polypeptide in which p19 has been mapped at the amino-terminal end and p15 at the carboxy-terminal end (42). Eisenman et al. (8) failed to detect the Pr76 *gag* precursor protein in gs^+chf^+ cells, but did observe a 120,000-dalton polypeptide containing the tryptic peptides of p27, p19, and p12 but not p15. Thus the lack of p15 and viral reverse transcriptase in normal cells can be most easily explained by postulating a deletion in the gs^+chf^+ transcripts of some 1,900 nucleotides encompassing the 3′ (p15) region of the *gag* gene and extending substantially into the *pol* gene. In a previous study in which the appropriate gs protein markers were used, the p15 of RAV-60 was always supplied by the exogenous virus (33). By a similar analysis, every RAV-60 isolate in this study was found to have the p27 of the exogenous parental virus. We have observed very few clones of RAV-60 in which the entire *gag* gene was not supplied by the exogenous virus. The relative scarcity of RAV-60 containing any endogenous *gag* information is probably due to the fact that an additional recombination event is required to pick up a portion of the endogenous *gag* gene.

Analysis of the "c" region of three isolates of

RAV-60, derived from RAV-1, RAV-2, and BH-RSV(−), respectively, suggested that this region was of exogenous origin in all three isolates (W. Hayward, personal communication). Taken together, the data suggest that the majority of RAV-60 are formed by a double crossover mechanism with the crossovers occurring at or near the 5′ and 3′ ends of the *env* gene.

If the endogenous *pol* gene does not recombine with the ts mutants LA337 and NY21 to restore wild-type polymerase, it might still be recovered into virus as a defective polymerase gene. One candidate for such a recombinant was BH-RSVα, since this mutant does not contain any detectable polymerase protein. BH-RSVα is spontaneously generated by BH-RSV (12). We examined the frequency of BH-RSVα formation on chicken cells with different levels of endogenous virus gene expression. We reasoned that, just as the titers of RAV-60 are proportional to the level of endogenous gene expression, so too should the titers of BH-RSVα be proportional if the *pol* gene of BH-RSVα is derived from a defective endogenous *pol* gene. In fact, regardless of the cell type used to grow BH-RSV stocks or to clone them, 5 to 10% of the progeny were BH-RSVα. Thus, the level of endogenous viral gene expression does not seem to affect the frequency of α-type formation, leading us to conclude that BH-RSVα does not represent recovery of the endogenous polymerase gene into virus. It seems likely that this defect, thought to be a deletion, is generated in some other manner. If, however, recombination between exogenous and endogenous viral genetic information is unrelated to the extent of the expression of the endogenous gene (e.g., direct recombination at the DNA level), the above experiment would not resolve this question.

The ability of BH-RSVα to recombine with LA337 (but not NY21) further substantiates that the defect in BH-RSVα is different from that in the endogenous polymerase gene. In these studies cultures that produced virus-associated polymerase in the culture fluid but no sarcoma virus infectious for C/E chicken cells were presumed to have been initiated by infection with BH-RSVα that had recombined with LA337 to become BH-RSV(−). By these criteria, slightly less than half the cultures were derived from this type of recombinant.

We conclude from these data and those of Wang et al. (43) that normal chicken cells contain as part of the endogenous virus genes a defective virus-related reverse transcriptase gene. There is evidence, however, that normal chicken cells contain genetic information capable of recombining with LA337 to yield wild-type virus. LA337-transformed chicken cells yield a significantly higher percentage of wild-type virions after treatment of the cells with the DNA of normal chicken cells by DNA transfection techniques (4). In this study, DNA from Japanese quail cells was also effective. Since quail cells appear to contain little or no DNA sequences homologous to ALSV RNA (W. S. Hayward, personal communication), these results suggest that the LA337 *pol* gene may be capable of recombining with a non-ALSV cellular DNA polymerase gene in this system.

ACKNOWLEDGMENTS

We thank C. M. Metroka for supplying NY21. In addition, we thank Lynn Baldassari for excellent technical assistance and Randie Davidson for help in preparing the manuscript.

This work was supported by Public Health Service research grant CA-14935 from the National Cancer Institute and by grant VC-225 from the American Cancer Society. R.C.S. was a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

LITERATURE CITED

1. **Baluda, M. A.** 1972. Widespread presence, in chickens, of DNA complementary to the RNA genome of avian leukosis viruses. Proc. Natl. Acad. Sci. U.S.A. **69**:576–580.
2. **Chen, J. H., and H. Hanafusa.** 1974. Detection of a protein of avian leukoviruses in uninfected chick cells by radioimmunoassay. J. Virol. **13**:340–346.
3. **Chen, J. H., W. S. Hayward, and H. Hanafusa.** 1974. Avian tumor virus proteins and RNA in uninfected chicken embryo cells. J. Virol. **14**:1419–1429.
4. **Cooper, G.** 1978. Marker rescue of endogenous cellular genetic information related to the avian leukosis virus gene coding RNA-directed DNA polymerase. J. Virol. **25**:788–796.
5. **Cooper, G. M., and S. G. Castellot.** 1977. Assay of noninfectious fragments of DNA of avian leukosis virus-infected cells by marker rescue. J. Virol. **22**:300–307.
6. **Crittenden, L. B., J. V. Motta, and E. J. Smith.** 1977. Genetic control of RAV-0 production in chickens. Virology **76**:90–97.
7. **Dougherty, R. M., and H. S. DiStefano.** 1966. Lack of relationship between infection and avian leukosis virus and the presence of COFAL antigen in chick embryos. Virology **29**:586–595.
8. **Eisenman, R., R. Shaikh, and W. S. Mason.** 1978. Identification of an avian oncovirus polyprotein in uninfected chick cells. Cell **14**:89–104.
9. **Friis, R. R., W. S. Mason, V. C. Chen, and M. S. Halpern.** 1975. A replication defective mutant of Rous sarcoma virus which fails to make a functional reverse transcriptase. Virology **64**:49–62.
10. **Hanafusa, H., T. Aoki, S. Kawai, T. Miyamoto, and R. E. Wilsnack.** 1973. Presence of antigen common to avian tumor viral envelope antigen in normal chick embryo cells. Virology **56**:22–32.
11. **Hanafusa, H., and T. Hanafusa.** 1968. Further studies on RSV production from transformed cells. Virology **34**:630–636.
12. **Hanafusa, H., and T. Hanafusa.** 1971. Noninfectious RSV deficient in DNA polymerase. Virology **43**:313–316.
13. **Hanafusa, H., W. S. Hayward, J. H. Chen, and T. Hanafusa.** 1974. Control of expression of tumor virus genes in uninfected chicken cells. Cold Spring Harbor Symp. Quant. Biol. **39**:1139–1144.
14. **Hanafusa, H., T. Miyamoto, and T. Hanafusa.** 1970. A cell associated factor essential for formation of an infec-

tious form of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **68**:314-321.

15. **Hanafusa, T., H. Hanafusa, and T. Miyamoto.** 1970. Recovery of a new virus from apparently normal chick cells by infection with avian tumor viruses. Proc. Natl. Acad. Sci. U.S.A. **67**:1797-1803.
16. **Hanafusa, T., H. Hanafusa, T. Miyamoto, and E. Fleissner.** 1972. Existence and expression of tumor virus genes in chick embryo cells. Virology **47**:475-482.
17. **Hanafusa, T., T. Miyamoto, and H. Hanafusa.** 1970. A type of chick embryo cell that fails to support formation of infectious RSV. Virology **40**:55-64.
18. **Hayward, W. S.** 1977. Size and genetic content of viral RNAs in avian oncornavirus-infected cells. J. Virol. **24**: 47-63.
19. **Hayward, W. S., and H. Hanafusa.** 1973. Detection of avian tumor virus RNA in uninfected chicken embryo cells. J. Virol. **11**:157-167.
20. **Hayward, W. S., and H. Hanafusa.** 1975. Recombination between endogenous and exogenous RNA tumor virus genes as analyzed by nucleic acid hybridization. J. Virol. **15**:1367-1377.
21. **Hunter, E., and P. K. Vogt.** 1976. Temperature sensitive mutants of avian sarcoma viruses: genetic recombination with wild type sarcoma virus and physiological analysis of multiple mutants. Virology **69**:23-34.
22. **Kang, C. Y., and H. M. Temin.** 1972. Endogenous RNA-directed DNA polymerase activity in uninfected chicken embryos. Proc. Natl. Acad. Sci. U.S.A. **69**:1550-1554.
23. **Kang, C. Y., and H. M. Temin.** 1973. Lack of sequence homology among RNAs of avian leukosis-sarcoma viruses, reticuloendotheliosis viruses, and chicken endogenous RNA-directed DNA polymerase acitivity. J. Virol. **12**:1314-1324.
24. **Kawai, S., and H. Hanafusa.** 1972. Plaque assay for some strains of avian leukosis virus. Virology **48**:126-135.
25. **Kawai, S., and H. Hanafusa.** 1973. Isolation of defective mutant of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **70**:3493-3497.
26. **Linial, M., and W. S. Mason.** 1973. Characterization of two conditional early mutants of Rous sarcoma virus. Virology **53**:258-273.
27. **Linial, M., and P. E. Neiman.** 1976. Infection of chick cells by subgroup E viruses. Virology **73**:508-520.
28. **Mason, W. S., R. Friis, M. Linial, and P. K. Vogt.** 1974. Determination of the defective function in two mutants of Rous sarcoma virus. Virology **61**:559-574.
29. **Mizutani, S., and H. M. Temin.** 1973. Lack of serological relationship among DNA polymerases of avian leukosis-sarcoma viruses, reticuloendotheliosis viruses, and chicken cells. J. Virol. **12**:440-448.
30. **Neiman, P.** 1973. Measurement of endogenous leukosis virus nucleotide sequences in the DNA of normal avian embryos by RNA-DNA hybridization. Virology **53**:196-204.
31. **Panet, A., D. Baltimore, and T. Hanafusa.** 1975. Quantitation of avian RNA tumor virus reverse transcriptase by radioimmunoassay. J. Virol. **16**:146-152.
32. **Payne, L. N., and R. C. Chubb.** 1968. Studies on the nature and genetic control of an antigen in normal chick embryos which reacts in the COFAL test. J. Gen. Virol. **3**:379-391.
33. **Rettenmier, C., and H. Hanafusa.** 1977. Structural protein markers in the avian oncoviruses. J. Virol. **24**:850-864.
34. **Robinson, H. L.** 1976. Intracellular restriction on the growth of induced subgroup E avian type C viruses in chicken cells. J. Virol. **18**:856-866.
35. **Robinson, W. S., and H. L. Robinson.** 1971. DNA polymerase in defective Rous sarcoma virus. Virology **44**:456-465.
36. **Rosenthal, P. N., H. L. Robinson, W. S. Robinson, T. Hanafusa, and H. Hanafusa.** 1971. DNA in uninfected and virus-infected cells complementary to avian tumor virus RNA. Proc. Natl. Acad. Sci. U.S.A. **68**: 2336-2340.
37. **Sawyer, R., and H. Hanafusa.** 1977. Formation of reticuloendotheliosis virus pseudotypes of Rous sarcoma virus. J. Virol. **22**:634-639.
38. **Shoyab, M., and M. A. Baluda.** 1975. Homology between avian oncornavirus RNAs and DNA from several avian species. J. Virol. **16**:1492-1502.
39. **Smith, E. J., J. R. Stephenson, L. B. Crittenden, and S. A. Aaronson.** 1976. Avian leukosis-sarcoma virus gene expression. Noncoordinate control of group-specific antigens in virus-negative avian cells. Virology **70**: 493-501.
40. **Varmus, H. E., R. A. Weiss, R. R. Friis, W. Levinson, and J. M. Bishop.** 1972. Detection of avian tumor virus-specific nucleotide sequences in avian cell DNAs. Proc. Natl. Acad. Sci. U.S.A. **69**:20-24.
41. **Vogt, P. K., and R. R. Friis.** 1971. An avian leukosis virus related to RSV(0): properties and evidence for helper activity. Virology **43**:223-234.
42. **Vogt, V. M., R. Eisenman, and H. Diggelmann.** 1975. Generation of avian myeloblastosis virus structural proteins by proteolytic cleavage of a precursor polypeptide. J. Mol. Biol. **96**:471-493.
43. **Wang, S. Y., W. S. Hayward, and H. Hanafusa.** 1977. Genetic variation in the RNA transcripts of endogenous viral genes in uninfected chicken cells. J. Virol. **24**:64-73.
44. **Weiss, R. A.** 1969. The host range of Bryan strain Rous sarcoma virus synthesized in the absence of helper virus. J. Gen. Virol. **5**:511-528.
45. **Weiss, R. A., W. S. Mason, and P. K. Vogt.** 1973. Genetic recombination and heterozygotes derived from endogenous and exogenous avian RNA tumor viruses. Virology **52**:535-552.
46. **Weissbach, A., A. Bolden, R. Muller, H. Hanafusa, and T. Hanafusa.** 1972. Deoxyribonucleic acid polymerase activities in normal and leukovirus-infected chicken embryo cells. J. Virol. **10**:321-327.
47. **Wyke, J. A., J. G. Bell, and J. A. Beamand.** 1974. Genetic recombination among temperature sensitive mutants of Rous sarcoma virus. Cold Spring Harbor Symp. Quant. Biol. **39**:897.