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Biochemistry

Host restriction of Friend leukemia virus: Synthesis and integration of the provirus

(*Fv-1* locus)

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ABSTRACT **Host restriction of exogenous infection by murine leukemia viruses is controlled *in vitro* predominantly by the murine *Fv-1* locus. The mechanism of this host restriction was investigated by comparing the early events in the replication of N-tropic versus B-tropic Friend leukemia virus in NIH 3T3 cells. These cells, which are *Fv-1*^{nn} in type, are permissive for the N-tropic strain, but nonpermissive for the B-tropic strain, which replicates permissively in Balb/c cells. We have studied the synthesis, intracellular location, and molecular form of virus-specific DNA early in replication by means of molecular hybridization with a virus-specific DNA probe. Our results suggest that in the permissive infection viral DNA rapidly becomes integrated with cellular DNA. However, in the nonpermissive infection, although almost equal amounts of both positive and negative strand viral DNA are synthesized, integration of the provirus does not occur.**

The host response of the mouse to murine leukemia viruses is multigenic, but one gene, the *Fv-1* locus, predominates (1). *In vivo*, the gene determines the susceptibility or resistance of the mouse to the induction of Friend disease by the Friend virus complex (2). Both *in vivo* and *in vitro*, this gene controls the ability of the lymphatic leukemia virus of the Friend virus complex (LLV-F) as well as other murine leukemia viruses to replicate (3, 4).

The *Fv-1* locus exists in two allelic forms, *n* and *b*. NIH Swiss mice and cells derived from them represent the prototype of the homozygous N-type (*Fv-1*^{nn}), and Balb/c mice and cells derived from them represent that of the homozygous B-type (*Fv-1*^{bb}) (3, 4).

Exogenous infection by LLV-F and other murine leukemia viruses, which may be divided into N-tropic and B-tropic types according to their efficiency of replication on NIH or Balb/c cells, respectively, is affected by this locus.

Host restriction of most murine leukemia viruses is dependent upon the multiplicity of infection (MOI) (5, 6). A virus grown in its permissive host needs only a single infectious particle to establish an infectious center. However, in the nonpermissive cell, two or more infectious particles (as determined by titration in the permissive cell type) are necessary to establish a successful infection. Virus strains have been isolated, however, which demonstrate host restriction over a wide range of MOI (7).

As part of an investigation of the mechanism of the *Fv-1*-dependent host restriction, we and others have demonstrated that this genetic effect is mediated intracellularly, after adsorption, penetration, and uncoating of the virus (8–10). We have, furthermore, shown that late events in the replication cycle, i.e., the synthesis of viral proteins and viral messenger RNA, are markedly suppressed and probably absent in the nonpermissive cell (11, 12). Similar results concerning viral mRNA synthesis have recently been reported by Jolicoeur and Baltimore (13).

In this work, we will present data on early events leading to the synthesis of LLV-F proviral DNA and its integration into the host cell genome. Our results indicate that, after infection with a single infectious particle, proviral DNA appears to be synthesized equally in permissive and nonpermissive cells. However, only in the permissive case does integration of the provirus with cellular DNA occur.

MATERIALS AND METHODS

Cells. NIH 3T3 (N3T3) and Balb/c 3T3 (B3T3) continuous cell lines were obtained from Dr. George Todaro. They were maintained in Eagle's minimal medium (F-11, Gibco) supplemented with 10% fetal calf serum (North American Biologicals), Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 15 mM (Gibco), and penicillin–streptomycin (50 units/ml, 50 μg/ml).

Viruses. Friend leukemia viruses, both N-tropic and B-tropic, were obtained from Dr. Frank Lilly as spleen extracts of infected mice. Each virus strain was passaged *in vitro* in the permissive cell line. Media from infected cell cultures were tested for plaque-forming titer by the X-C assay (14), and frozen in aliquots of culture medium at −70° to provide a stock of tissue culture passaged virus, usually 5×10^6 to 5×10^7 plaque-forming units/ml. Repeated testing of such stock virus failed to reveal any residual spleen focus-forming agent (R. Steeves, personal communication). This stock virus exhibited two-hit kinetics on the nonpermissive cell (T. G. Krontiris and R. Soeiro, unpublished observations).

Labeling of Viral RNA with ^{32}P. Labeling of LLV-F viral RNA with [^{32}P]orthophosphate has been previously described (15). The specific activity of the radiolabeled viral RNA usually obtained was 1×10^7 cpm/μg of viral RNA.

Preparation of Friend Virus Single-Stranded Complementary DNA. The endogenous RNA-directed DNA polymerase (reverse transcriptase) reaction (16) was used to make single-stranded cDNA in the presence of actinomycin D (17). Reactions were carried out in 2.0 ml containing 0.01 M Tris·HCl at pH 7.8, 0.06 M KCl, 2 mM $MnCl_2$, 2 mM dithiothreitol, 0.03% Triton X-100, 50 μg/ml of actinomycin D (Sigma), 1 mM dCTP, dGTP, dATP (P-L Biochemicals), [^{3}H]dTTP at 0.02 mM (specific activity 47 Ci/mmol, Amersham Searle), and 0.6–0.8 mg/ml of N-tropic LLV-F viral protein. The reaction was incubated at 38° for 12 hr and was terminated by the addition of Sarkosyl (2%) and NaOH (0.3 M). The mixture was boiled at 100° for 10 min, was reneutralized with HCl, and was deproteinized with phenol saturated with chloroform–isoamyl alcohol (24:1). The single-stranded cDNA product of the reverse transcriptase reaction was 99% susceptible to nuclease S_1 (18), which indicated a completely single-stranded nature. N-tropic

Abbreviations: LLV-F, lymphatic leukemia virus of the Friend virus complex; MOI, multiplicity of infection; M_r, molecular weight; C_0t, initial concentration of DNA (moles of nucleotide/liter) × time (seconds).

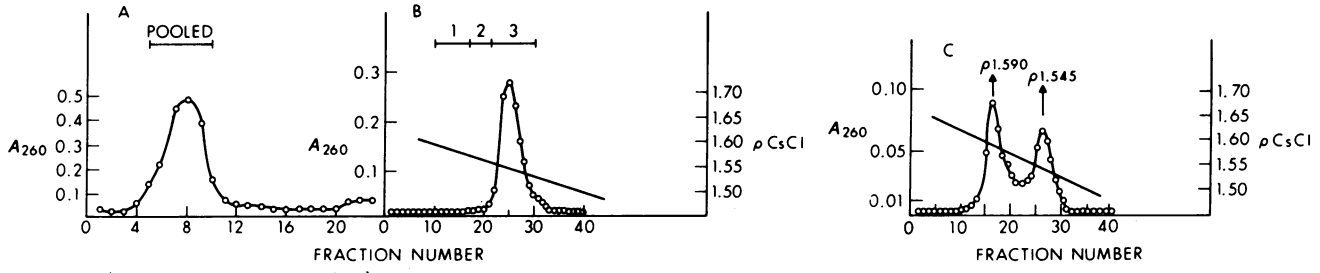

FIG. 1. Fractionation of cellular DNA on alkaline sucrose and ethidium bromide–cesium chloride density gradients. (A) Cells (1 to 5 × 10^7 per gradient) in TE buffer (0.01 M Tris·HCl at pH 8.5, 0.01 M EDTA) were mixed with 2.0 ml of lysing solution, 0.5 M NaOH, 0.1 M EDTA, 0.5% Sarkosyl, 1.0% Nonidet P40 over a preformed 15–30% alkaline sucrose gradient buffered with 0.3 M NaOH, 0.01 M EDTA, 0.5 M NaCl, 0.1% Sarkosyl and placed at 4° for 12 hr. Gradients were then subjected to centrifugation for 7 hr, at 22,000 rpm, 4° in an SW 27 rotor. Fractions were collected dropwise from below and the appropriate fractions were pooled for further treatment. Form I simian virus 40 DNA (53 S) sediments at fractions 13–14 under these conditions. (B) Pooled DNA was precipitated, deproteinized as previously described (19), reprecipitated, and resuspended in TE buffer. A volume of 0.4 ml of an ethidium bromide solution (3 mg/ml in TE buffer) was added to the resuspended DNA and the volume was adjusted to 3.4 ml. CsCl (3.2 g) was added to give a density of 1.58 g/cm^3. The final ethidium concentration was 300 μg/ml. The gradients were then centrifuged to equilibrium in an SW 50.1 rotor at 33,000 rpm for 55 hr. Fractions were collected dropwise from below and treated as previously described (19). ρ is density, in g/cm^3. (C) Mitochondrial DNA was prepared according to the method of Nass (22) and treated as the DNA in (B).

LLV-F cDNA serves to detect either N- or B-tropic viral DNA or RNA since extensive cross hybridization has been shown (M. M. Sveda and R. Soeiro, unpublished observations).

Ethidium Bromide–CsCl and Alkaline Sucrose Gradients. Ethidium bromide–CsCl and alkaline sucrose gradient centrifugation were carried out using previously published conditions (19).

Hybridizations. RNA·DNA hybridization was carried out as previously described (15). DNA·DNA reassociation was performed in 7–10 μl reaction mixtures at 65° containing 0.3 M NaCl, 0.01 M Tris·HCl at pH 7.5, and 0.1% sodium dodecyl sulfate in 50 μl sealed capillary tubes. Unlabeled cellular DNA was added at a concentration of 8.5 mg/ml, and labeled cDNA was added to a final concentration of 0.001 μg/ml. At the end of each incubation, hybridization reactions were diluted with 0.5 ml of S_1 buffer (0.002 mM $ZnCl_2$, 0.001 M NaCl, 0.03 M sodium acetate at pH 4.5) and digested with a predetermined amount of nuclease S_1 (Miles) at 38° for 45 min. Salmon sperm DNA was added and the samples were precipitated with trichloroacetic acid, filtered through Millipore glass fiber filters, and assayed for radioactivity in Omnifluor–toluene (New England Nuclear). Results of hybridization experiments were corrected to 0.18 M NaCl (20).

RESULTS

Integration of Viral DNA. Our initial studies attempted to define whether, under conditions of nonpermissive infection, evidence could be obtained for integration of the provirus into the host cell genomic DNA. To maximize the ratio of singly to doubly infected cells, we chose an MOI of 0.1. Under these conditions, we would anticipate that in the permissive case a maximum of 10% of the cells would be successfully infected. However, in the nonpermissive case the Poisson distribution equation would predict a ratio of nine singly infected cells to one multiply infected cell due to the multiplicity effect noted above. A maximum of 1% of infectious centers could be produced in nonpermissive cultures at this MOI.

We chose as our definition of an integrated viral genome the appearance of viral DNA physically linked to high-molecular-weight cellular DNA after separation by two physical properties, molecular weight and density. To accomplish this, N3T3 cells were infected either permissively (N-tropic LLV-F) or nonpermissively (B-tropic LLV-F) at an MOI of 0.1. Thirty-six hours postinfection, the cells were mixed with lysing solution (see *Materials and Methods* and *legend* to Fig. 1) to effect release of cellular DNA in a gentle manner. The released DNA was then subjected to centrifugation in alkaline sucrose gradients to separate high- and low-molecular-weight DNA. Fig. 1A shows a typical alkaline sucrose gradient of cellular DNA isolated by this method. The main band, which represents cellular DNA, has a sedimentation value >53 S as determined by cosedimentation with form I simian virus 40 DNA (fractions 13–14, not shown). Material denoted large DNA was pooled (see Fig. 1A) from the main band. Small DNA represented all DNA of lower molecular weight.

No mechanical trapping of low-molecular-weight single-stranded DNA could be demonstrated by reconstruction experiments using labeled viral cDNA (data not shown). To determine whether any trapping of other molecular forms of viral DNA occurred in the main band, and to eliminate "supercoiled" forms of proviral DNA that would cosediment with the large DNA (form I murine leukemia virus DNA is 63 S; ref. 21), large DNA was analyzed by resedimentation to equilibrium density in an ethidium bromide–CsCl density gradient. Fig. 1B represents the results of such an analysis of high-molecular-weight DNA. This gradient (Fig. 1B) would separate, simultaneously, any contamination of species of "supercoiled" or double-stranded "circular" forms of DNA from high-molecular-weight "linear" DNA. Low-molecular-weight linear DNA would have been eliminated by the initial alkaline sucrose gradient. We chose mitochondrial DNA, which is known to exist in several molecular forms, as the density marker for supercoiled DNA (ρ = 1.59 g/cm^3) and linear DNA (ρ = 1.545 g/cm^3) (19, 22). In Fig. 1C such a mitochondrial preparation is shown. We pooled three fractions (see Fig. 1B, 1, 2, 3) of material from the ethidium bromide–CsCl gradient according to the determined densities of "supercoiled" (ρ = 1.58–1.60 g/cm^3), "circular" (ρ = 1.575–1.585 g/cm^3), and "linear" (ρ = 1.53–1.57 g/cm^3) DNA to analyze by DNA·DNA reassociation with single-stranded LLV-F cDNA (see *Materials and Methods*).

Fig. 2 represents an analysis of the presence of virus-specific DNA in the main band (pool 3) of cellular DNA isolated from permissively versus nonpermissively infected cells. The association of such DNA over a range of C_0t [initial concentration of DNA (moles of nucleotide/liter) × time (seconds)] values is shown relative to self-annealing of host cell DNA to determine the C_0t value of unique sequence cell DNA ($C_0t \simeq 1.2 \times 10^3$

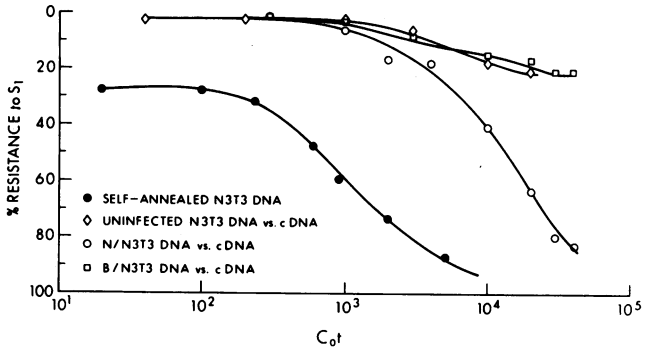

FIG. 2. Detection of integrated virus-specific DNA sequences. High-molecular-weight DNA was extracted from uninfected, and permissively and nonpermissively infected cells, 36 hr postinfection, as described in Fig. 1. DNA, pooled between CsCl densities 1.53–1.57 g/cm^3 (Fig. 1B-3) and sheared to uniform size (passed 20 times through a 26 gauge needle), was reassociated with cDNA (1009 cpm) as described in *Materials and Methods*. Uninfected cellular DNA was labeled *in vivo* with [^{3}H]thymidine, isolated as described in Fig. 1A, sheared to uniform size as above, and reassociated as described in *Materials and Methods* over a wide range of C_0t values. Only the values representing reannealing of unique sequences are shown.

mole·sec/liter, ref. 23). Viral DNA isolated from permissively infected cells protected 85% of our cDNA probe, and gave an estimated copy number of 0.1 genome equivalents per cell, a figure in keeping with the MOI of 0.1 used in these experiments. Annealing of similarly isolated uninfected cell DNA resulted in protection of only 20% of our cDNA, and that only at very high C_0t values. DNA from nonpermissively infected cells revealed no protection of our cDNA probe above background levels.

It should be noted at this point that the experiments shown were carried out only in NIH cells. The Balb/c cells are known to contain endogenous oncornavirus (24), which probably explains the high background levels of annealing of DNA from uninfected Balb/c cells with LLV-F cDNA (M. M. Sveda and R. Soeiro, unpublished work).

The conclusion from this experiment was that little to no new viral DNA becomes integrated with host cell DNA in *Fv-1* restricting cells by 36 hr postinfection.

Analysis of Total versus Integrated Viral DNA. To examine in greater detail the synthesis of proviral DNA, we studied a period of 10 hr postinfection. Other workers had determined that at this time both strands of viral DNA were present equally in the infected cell (21). N3T3 cells infected permissively or nonpermissively at MOI 0.1 were fractionated into nuclei and cytoplasm (15). Nuclei were lysed as above, and fractions containing large and small DNA were pooled from the alkaline sucrose gradient as in Fig. 1. Table 1 demonstrates the results of hybridization of pooled DNA fractions with cDNA, in this case to a single end point of C_0t 1×10^4 mol·sec/liter. Large DNA [molecular weight (M_r) $> 6 \times 10^6$ daltons] from the permissive infection protected 72% of cDNA, whereas that from the nonpermissive infection protected only slightly above the background level of the uninfected cell. These data confirmed our earlier results that little to no proviral DNA was integrated with host cell DNA. Of interest, however, was a comparison of smaller DNA isolated from the infected cells. In this case virtually equal amounts of cDNA were protected by DNA isolated from the permissively or the nonpermissively infected cells. The ratio of protection of cDNA by total nuclear DNA in the permissive versus the nonpermissive infection is 2.2, whereas that of the "integrated" form is 12.7. This suggests that total synthesis

Table 1. Hybridization of nuclear and cytoplasmic cellular DNA with viral cDNA

Cells	DNA	cpm hybridized	cpm hybridized less uninfected
Permissive	Large	727 (72%)	560 (56%)
	Small	310 (31%)	298 (30%)
	Cytoplasmic	ND	
Nonpermissive	Large	211 (21%)	44 (4%)
	Small	357 (35%)	345 (34%)
	Cytoplasmic	ND	
Uninfected	Large	167 (17%)	
	Small	12 (1%)	
	Cytoplasmic	ND	

Ratio
Permissive/nonpermissive: Large 12.7 (560/44)
Permissive/nonpermissive: Total 2.2 (858/389) (large + small)

DNA isolated from nuclei of cells infected (MOI 0.1) for 10 hr was fractionated into large and small DNA as in Fig. 1A. DNA from each pool was collected and annealed with viral cDNA (1009 cpm) to a final C_0t of 10^4 mole·sec/liter (large nuclear) or for a total of 65 hr (small nuclear and total cytoplasmic). S_1-resistant cDNA was analyzed as in *Materials and Methods*. ND = not detectable. Parallel infections of N3T3 cells with either N-tropic or B-tropic virus were assayed for progeny virus after 5 days to demonstrate restriction of virus growth. Log_{10} titers as assayed by the X-C procedure in the permissive cell: N-tropic LLV-F/N3T3 = 6.67; B-tropic LLV-F/N3T3 = 3.45.

of positive strand viral DNA was approximately equal, whereas integration of viral DNA in the nonpermissive case apparently did not occur. The little radioactivity above background noted for the nonpermissive high molecular weight DNA could represent cells multiply infected, and therefore successful infectious centers. Moreover, parallel annealing of total cytoplasmic DNA from either permissively or nonpermissively infected cells with the cDNA probe for periods of up to 65–74 hr (C_0t value not known) failed to demonstrate any positive strand viral DNA. This suggests that at 10 hr postinfection, under our conditions of infection, little to no viral DNA is cytoplasmically located. It also suggests that the effect of the *Fv-1* locus does not prevent the nuclear association of either infecting uncoated virus or, if proviral synthesis is cytoplasmic, the nuclear migration of its product DNA.

Analysis of "Supercoiled" and "Circular" Forms of Viral DNA. A similar 10 hr infection at MOI 0.1 was further analyzed to ascertain the presence in the nonpermissive cell of putative intermediates in the integration of viral DNA (21, 25). Large DNA obtained from purified cell nuclei and pooled (see Fig. 1A) after sedimentation through alkaline sucrose gradients was subjected to equilibrium density centrifugation as described in Fig. 1B. Fractions pooled from the linear, circular, and supercoiled regions were hybridized to viral cDNA. Table 2 gives the data obtained. In this case, again, the amount of presumably integrated viral DNA (fraction 3; ρ = 1.53–1.57 g/cm^3) was greater in the permissive case than in the nonpermissive case. The enrichment for viral DNA in this fraction in the permissive case (6.8-fold) was not as great as noted in the earlier experiment at 36 hr postinfection.

However, when density regions corresponding to "supercoils" and "circles" were analyzed in the permissive case, low but significant levels of protection of the cDNA probe were found. In the nonpermissive case, the levels of viral DNA in these regions were too close to background levels to be reliable. How-

Table 2. Hybridization of high-molecular-weight cellular DNA with viral cDNA

Cells	ρ of DNA, g/cm³	cpm hybridized		cpm hybridized less uninfected
Permissive	1.53–1.57	623		467
	1.575–1.585	82		66
	1.589–1.605	96		78
			Total	611
Nonpermissive	1.53–1.57	225		69
	1.575–1.585	47		31
	1.589–1.605	32		14
			Total	114
Uninfected	1.53–1.57	156		
	1.575–1.585	16		
	1.589–1.605	18		

Ratio

Permissive/nonpermissive: ρ = 1.53–1.57 6.8 (467/69)

Permissive/nonpermissive: Total 5.4 (611/114)

Cellular DNA isolated from uninfected and permissively and nonpermissively infected N3T3 cells (10 hr postinfection) was treated as described in Fig. 1. Regions of the ethidium bromide–CsCl gradients corresponding to "supercoiled" (ρ = 1.589–1.605 g/cm³, fraction 1), "circular" (ρ = 1.575–1.585 g/cm³, fraction 2), and "linear" DNA (ρ = 1.53–1.57 g/cm³, fraction 3) were treated as previously described (19). DNA taken from the "linear" region was annealed with cDNA (1009 cpm) to a $C_0t \geq 10^4$ mol·sec/liter, while DNA from the other regions was annealed with cDNA for 65–72 hr (see *Materials and Methods*).

ever, what is clear is that there does not appear to be an accumulation of "supercoiled" DNA in the nonpermissive infection as determined by this analysis. (Single-strand closed circles might exist in the low M_r fraction of the alkaline sucrose gradient.) In our earlier study (Table 1) of total nuclear DNA, almost equivalent amounts of total viral DNA were found in the nonpermissive infection. If circular and/or supercoiled forms of proviral DNA are necessary precursors to integration, then these data suggest that the effect of the *Fv-1* gene could occur during events leading to the circularization and/or supercoil formation of this DNA prior to integration. Alternatively, the proviral DNA could circularize, and/or form supercoils, and be subsequently cleaved by a restriction endonuclease.

Analysis of Negative and Positive Strand Viral DNA. We attempted to analyze further what viral DNA products were synthesized in the nonpermissive infection. If the predominant species were low molecular weight, were both strands made? In this instance we took advantage of the convention of separation of high-molecular-weight DNA (therefore presumably integrated) from low M_r DNA as obtained by the Hirt procedure (21, 26–28). Again, a 10 hr infection at MOI 0.1 was carried out, but in this case total cellular DNA was fractionated into Hirt pellet and Hirt supernatant.

Table 3 presents the results of annealing of such fractions, in this case not only to viral cDNA, but also to ^{32}P-labeled viral RNA. In this manner the presence of both viral DNA strands could be determined. The high M_r DNA (Hirt pellet) from the permissive infection, annealed to a C_0t of 10^4 mol·sec/liter, protected at least 50% of both positive and negative strand probes. In confirmation of our earlier studies (Table 1, Fig. 2), this fraction from the nonpermissive infection annealed to the same C_0t failed to protect any significant fraction of either probe above the background of uninfected cellular DNA.

In the low M_r (Hirt supernatant) fraction, however, there was protection of both positive and negative strand probes, and the level of protection was virtually identical for the permissive and nonpermissive infections.

Our conclusion, therefore, was that in the nonpermissive infection, amounts of low M_r viral DNA of both strands approximated that found in the permissive case. However, integration of viral DNA with cellular DNA did not occur in nonpermissively infected cells.

DISCUSSION

It has been shown elsewhere that the effect of the *Fv-1* genetic locus on the replication of murine leukemia viruses occurs intracellularly after adsorption and uncoating of the virus (8–10). It has further been shown that late events in the replication cycle, the synthesis of virus-specific proteins and messenger RNA, does not occur in the nonpermissively infected cell (11–13).

Our current studies have focused on the early events surrounding viral DNA synthesis and integration as influenced by

Table 3. Analysis of Hirt fractions for positive and negative strand viral DNA

		Hirt supernatant	Hirt pellet	Pellet less uninfected
Permissive (N/N3T3)	^{32}P	255 (28%)	664 (73%)	464 (51%)
	^{3}H	155 (29%)	396 (74%)	284 (53%)
Nonpermissive (B/N3T3)	^{32}P	200 (22%)	228 (25%)	28 (3%)
	^{3}H	128 (24%)	134 (25%)	22 (4%)
Uninfected N3T3	^{32}P	14	200 (22%)	
	^{3}H	21	112 (21%)	

Ratio

Permissive/nonpermissive: positive strand (^{3}H) 13

negative strand (^{32}P) 16

^{32}P-Labeled viral RNA was prepared as described in *Materials and Methods* to a specific activity of 9×10^6 cpm/μg of viral RNA. 910 cpm of ^{32}P were added to each hybridization reaction to assay for negative strand viral DNA. [^{3}H]cDNA was prepared as described in *Materials and Methods* to a specific activity of 1×10^6 cpm/μg of cDNA. 535 cpm of ^{3}H were added to each hybridization reaction to assay for positive strand viral DNA. Reaction mixtures were 100 μl and the reactions were carried out to a $C_0t \geq 10^4$ mol·sec/liter for the Hirt pellet and for 65–72 hr for the Hirt supernatant. ^{32}P-Labeled material was digested with 50 μg/ml of RNase A in 500 μl of buffer, 0.3 M NaCl, 0.02 M Tris·HCl at pH 7.4 at 38° for 60 min. ^{3}H was assayed using S_1 nuclease as described in *Materials and Methods*.

the *Fv-1* locus. Our findings show that a comparison of total virus-specific DNA synthesis under permissive versus nonpermissive conditions of infection reveals close to equivalent amounts of total virus-specific DNA have been formed. Moreover, we have shown that in the nonpermissive cell viral DNA of both strand polarities is synthesized. Additionally, these data reveal that little to no accumulation of putative intermediates in the integration of the provirus occurs in the nonpermissive infection. Most noticeably, there appears to be no integration of virus-specific DNA under nonpermissive conditions, whether measured grossly, as high-molecular-weight DNA isolated by the Hirt procedure, or more rigorously, by separation first of high-molecular-weight DNA and subsequently of DNA known from its density to be linear.

The conclusion is that the *Fv-1* gene effect is mediated at the level of integration or, possibly, at the level of circularization of viral DNA prior to integration. Since both viral DNA strands are made in the nonpermissive cell, and in amounts similar to that found under permissive conditions, it would appear that no gross dysfunction of reverse transcriptase action is effected. One possible reservation to this conclusion would be that all noninfectious particles participate in the synthesis of viral DNA, and that the true effect of the *Fv-1* locus is on the single infecting particle to prevent any viral DNA synthesis. Such an effect would presuppose that synthesis by the noninfectious particles would be unaffected by the *Fv-1* gene.

It has been proposed on the basis of the experiments of Bassin *et al.* (29) and of Rein *et al.* (30) that the *Fv-1* effector molecule interacts with a protein structure of the virus. If this protein is the reverse transcriptase molecule, then it is interfering with a yet-to-be-defined effect of the enzyme on circularization or integration of the proviral DNA. Alternatively, another virus-specific protein, unknown or as yet unidentified, which may serve such a function, may be involved.

Our earlier experiments used another methodology, that of following the fate of input viral genome RNA, to approach the question of reverse transcriptase activation. We found, unexpectedly, that virus-specific input RNA appeared integrated with the host genome (15). The "integration" of viral RNA appeared to conform to the known biology of oncornavirus infection (31). This occurred equally under both permissive and nonpermissive conditions of replication. Some questions regarding the conclusions drawn from those earlier experiments must now be held, since the conclusions are in conflict with the results of our more recent work using standard methodology.

Note Added in Proof. Conclusions similar to those presented here regarding the *Fv-1* gene effect have recently been obtained by Jolicoeur and Baltimore (32).

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