SEARCH FOR VIRUS-SPECIFIC SEQUENCES IN DNA OF CELLS PERSISTENTLY INFECTED BY RABIES VIRUS

O. G. Andzhaparidze, Yu. S. Boriskin,
N. N. Bogomolova, I. D. Drynov,
M. S. Bektemirova, and A. V. Shishkov

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During hybridization of highly radioactive transcript ^{3}H-DNA or rabies virus ^{3}H-RNA with cellular DNA no virus-specific sequences were found in the DNA of BHK 21/13S and HEp-2 cells persistently infected with rabies virus. In experiments on transfection of virus-sensitive cells with DNA preparations from persistently infected cultures, negative results also were obtained.

KEY WORDS: rabies virus; persistent infection of cells; DNA–DNA and RNA–DNA hybridization; transfection.

The idea of integration of the virus genome with the cell genome as the mechanism of prolonged coexistence of virus and cell, which has undoubtedly been confirmed for oncogenic DNA- and RNA-containing viruses, has more recently been spread also to persistent infections caused by infectious viruses [7, 10]. Previously the writers obtained two cultures persistently infected with rabies virus [3] and constantly producing infectious virus and virus antigen.

The object of the present investigation was to verify the possibility of integrating the virus genome with the cell genome by hybridization and transfection methods, which required the prior obtaining of a concentrated, purified preparation of rabies virus for isolation of virus RNA and for obtaining highly radioactive virus RNA and its DNA transcript in vitro.

EXPERIMENTAL METHOD

The method of culture of the persistently infected cultures, of titrating rabies virus (RV) in mice, and of immunofluorescence were described previously [3]. The RV was propagated on monolayer primary cultures of quail fibroblasts and concentrated by ultrafiltration at 4°C by means of "Amicon" concentrator through a cartridge with membranes of HiP-100 type. The RP was sedimented at 20,000 rpm in the course of 2 h at 4°C in the 6 × 250 rotor of an MSE-65 centrifuge, and the residue was suspended in 0.5-0.8 ml NTE buffer, pH 7.8 [9], and purified in 5-30% linear sucrose gradient in the SW-40 rotor of a Beckman L5-65 centrifuge at 34,000 rpm (1 h). RV was sedimented from gradient fractions containing the maximum infectivity, diluted with NTE buffer, at 110,000g for 2 h. RNA was isolated with a mixture of phenol and chloroform [8]. The DNA transcript of RV RNA was obtained as described in [2], using 50 units Escherichia coli DNA-polymerase (from Sigma), deoxyadenosine triphosphate-^{3}H (specific activity 7.2 Ci/mmole, from "Izotop"), deoxyguanosine triphosphate-^{3}H (specific activity 10.3 Ci/mmole, from Amersham), and deoxycytidine triphosphate-^{3}H (specific activity 25 Ci/mmole, from Amersham). The specific activity of the synthesized complementary DNA (cDNA), calculated on the basis of the specific activity of ^{3}H-precursors, was 6.7 and 10 cpm/μg. The synthesized product was analyzed by centrifugation in a 5-30% alkaline sucrose gradient, made up in buffer (0.9M NaCl, 0.1M NaOH, 0.001M EDTA, pH 12.7) in the SW-50 rotor at 40,000 rpm for 18 h at 20°C. The sedimentation coefficient was determined from the nomogram [4]. The specificity of the resulting cDNA was tested by hybridization with an excess of virion RNA isolated from RV propagated in embryonic hamster kidney cells (BHK 21/13S). The composition of the hybridization mixture and the conditions of DNA–DNA and RNA–DNA hybridization were identical with those described previously [2]. A commercial preparation of thymus DNA (from Serva) was used as the negative control. During transfection the BHK 21/13S cells were treated with

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TABLE 1. Concentration of Rabies Virus by Ultrafiltration

Parameter	Experiment 1	Experiment 2
Original volume/final volume	13 liters/ 150 ml	16 liters/ 500 ml
Original titer*	4,25	4,75
Titer in concentrate	6,25	6,5
Titer in filtrate	<0,5	0

*Titer of virus in log LD_{50}/0.03 ml.

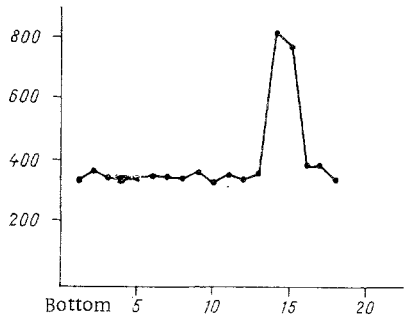

Fig. 1. Sedimentation of cDNA-^{3}H in alkaline sucrose gradient. Abscissa, Nos. of fractions; ordinate, cpm. Direction of sedimentation from right to left.

TABLE 2. Hybridization (in %) of cDNA-^{3}H and RNA-^{3}H of Rabies Virus with DNA from Cultures Persistently Infected with RV

Source of nonradioactive DNA	Concentration of DNA, moles/liter (C_0t)*	Hybridization: with cDNA-^{3}H	Hybridization: with RNA-^{3}H	Hybridization of cDNA-^{3}H with var. RNA added to reaction mixture 48 h after beginning of reaction
BHK-RV	274	5,7		
	547		7,2	
	1094	5,7		
	1642		7,3	
	3283			62,2
	6567	6,3	5,0	
HEp-2-RV	274	5,2	5,7	
	1094		4,4	
	1642	6,4		
	3283		6,3	58,5
	6567	5,1	3,9	
Thymus DNA	137	6,5		
	274		7,1	
	1094	5,5		—
	1642		6,4	
	6567	5,9	5,1	

*C_0T) DNA concentration in moles/liter per duration of hybridization in sec.

DEAE-dextran (50 μg/ml in 0.15 M NaCl for 10 min), the DNA dissolved in 0.15 M NaCl was then adsorbed for 15 min, after which the growth medium was added.

EXPERIMENTAL RESULTS

The results of two experiments to concentrate the RV by ultrafiltration are given in Table 1. They show that concentration of the virus took place proportionally to volume with minimal losses of infectivity. From

58 liters of virus-containing culture fluid 5×10^{10} LD_{50} of purified RV was obtained, from which 1.6 mg RNA was isolated. The character of the circular dichroism spectrum, from which the degree of purity of the nucleic acid could be estimated [6], showed the virtually complete absence of protein in the RV RNA preparation. Half of the RNA preparation in 0.1 × SSC buffer (0.15 M NaCl, 0.015 M sodium citrate) with 0.1% sodium dodecyl sulfate (SDS) was applied to the walls of a special flask, lyophilized, and titrated by the method described previously [5]. Radioactive RNA was dissolved in 0.1 × SSC buffer with 0.1% SDS, dialyzed against 25 volumes of the same buffer, and concentrated in an "Amicon-MMC" system through a R-10 filter, after which it was dissolved in hybridization buffer [2]. The specific activity of the preparation was 9×10^5 cpm/μg. The other half of the RNA was used as template for obtaining the DNA transcript [2]. The synthesized cDNA was sedimented in an alkaline sucrose gradient in a narrow zone with a coefficient of about 9S (Fig. 1) and it hybridized to the extent of 80-90% with virion RNA. Preparations of cellular DNA isolated by the method described in [1] were comparable in optical density ratio ($A_{260:280}$), namely 1.8, with the preparation of commercial thymus DNA.

The results of hybridization, illustrated in Table 2, show that as regards the level of hybridization (under 10%) with cDNA and RNA-^{3}H, RV DNA from persistently infected cultures does not differ from control thymus DNA. Nor was any kinetics of hybridization observed. The cDNA present in the reaction mixture hybridized to the extent of 58-62% with excess of virion RNA added to the reaction mixture for 24 h, 48 h after the beginning of hybridization.

In repeated experiments on transfection of BHK 21/13S cells with DNA preparations isolated from persistently infected cultures, the formation of virus antigen could not be detected by the immunofluorescence method, nor could infectious virus be isolated in the course of 3-5 passages of the transfected cultures.

It can thus be concluded from these results that integration of the virus and cellular genomes evidently does not take place during persistent infection with rabies virus in vitro.

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