Somatic Hybrid Cell Formation by Viral Genome-Free Envelopes Reassembled from Solubilized HVJ (Sendai Virus)

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An envelope fraction free of nucleocapsids was reassembled from HVJ (hemagglutinating virus of Japan, Sendai virus) virions solubilized with Nonidet P40 (4) or alkali-emasol (16). The procedure proved to be useful in place of ultraviolet-inactivated HVJ virions for fusion of cells in the formation of somatic hybrid cells.

MATERIALS AND METHODS

Reassembling of solubilized HVJ envelopes was carried out by the methods of (I) Hosaka and Shimizu (4) or (II) Shimizu et al. (16). HVJ virions were propagated in embryonated eggs partially purified by two cycles of differential centrifugation at 2,500 rpm for 20 min and 20,000 rpm for 30 min in a Beckman #30 rotor.

Method I. To the virions 0.25 to 0.5% Nonidet P40 (NP40) was added at room temperature; the viral envelopes were solubilized and the viral nucleocapsids became free from the envelopes by this treatment. The nucleocapsid fraction was precipitated at 40,000 rpm for 45 min in a Beckman #40 rotor. The resulting supernatant free of the nucleocapsid, was dialyzed in Neflex tubing against phosphate buffered saline for 3 days, changing the buffer every day for reassembling of solubilized envelopes. The preparation was then centrifuged at 27,000 rpm for 30 min. The precipitate contained reassembled envelopes similar in size to the envelopes of HVJ virions and no inner core (nucleocapsids). This precipitate was suspended in balanced salt solution (BSS, 14) containing 2 mM CaCl_2 with or without 10% calf serum.

Method II. A suspension of HVJ virions in 0.02 M bicarbonate buffer (pH 10) with 0.5% emasol was incubated for 2 h at room temperature and then centrifuged at 40,000 rpm for one h. The precipitate was found to be a mixture of partially disrupted virions with nucleocapsids and empty envelopes without nucleocapsids. The precipitate was resuspended in 0.0025 M phosphate buffer (pH 7.0) and 5 ml of the suspension were mixed with 1.6 g CsCl , then centrifuged at 38,000 rpm for 40 h in a Beckman SW-50 rotor. Seven bands appeared. The top band ($\rho = 1.15$) was harvested while the buoyant density of HVJ virions was $\rho = 1.18$. The fraction was dialyzed against phosphate buffer and re-banded in CsCl solution. The band appearing in the same position again was harvested and dialyzed against phosphate buffer. Morphologically, the fraction consisted of envelopes without nucleocapsids and showed cell fusion activity, hemagglutinating activity, neuraminidase activity and hemolytic activity.

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The parent cells used for hybridization were an azaguanine resistant mutant (E_{AG}) from the hyper-diploid line of Ehrlich ascites tumor cells prepared by Okada and a 5-bromodeoxyuridine resistant mutant (L_{BrdU}) of L cells prepared by Murayama and Okada (6).

RESULTS AND DISCUSSION

Firstly, the preparation of reassembled envelopes from Method I (see Materials and Methods) was tested. Five drops of a mixture of parental cells (1.8×10^6 cells/ml each) and equal volumes of various concentrations of the reassembled envelope fraction were mixed in an ice bath and left to stand for 5 min. During this period cell agglutination occurred. Then, the mixture was incubated at $37^\circ C$ for 20 min in a water bath. After incubation, cells were washed and plated at a concentration of 1.2×10^5 cells/plate (calculated on the basis of the original number of cells before fusion). The cells were cultured in HAT medium (5) for selection of hybrid cells alone.

When the cell fusion process was performed on BSS-Ca medium with 10% calf serum, the cells fused and did not degenerate and hybrid colonies appeared on the culture in HAT medium, as shown in Table 1. Twenty colonies were harvested from

TABLE 1. FUSION OF CELLS^a AND HYBRID CELL FORMATION^a WITH AN ENVELOPE FRACTION REASSEMBLED FROM HVJ VIRIONS SOLUBILIZED WITH NP40

HA activity of the envelope fraction added (HAU/0.5 ml)	Fusion index ^b	Hybrid colonies per plate (mean $\pm$ S.D.)
0	0.0	0
128	0.1	104 ± 20
256	0.2	99 ± 13
512	0.4	87 ± 17
1,024	0.3	76 ± 12

^a A mixture of equal numbers of L_{BrdU} and E_{AG} cells was used for fusion of cells. 1.2×10^5 cells, as the original number before fusion, were introduced in a plate after the cell fusion and cultured in HAT medium.

^b Fusion index = $\frac{\text{No. of cells in control without envelope fraction}}{\text{No. of cells in sample treated with envelopes}} - 1.0$

the culture. Karyologically these were hybrids of L_{BrdU} and E_{AG} . When injected into the abdomen of C3H mice, 17 of these colonies formed solid type tumors. The characteristics of the colonies were the same as those of hybrids formed using UV-inactivated HVJ reported previously (7, 8). Three of the colonies formed both solid and ascites type tumors when injected into the abdomen of C3H mice.

When BSS-Ca medium without serum was used during the course of cell fusion, cells lysed after incubation with the envelope fraction at all dilutions tested, and no plated cells or hybrid colonies appeared on cultures in the HAT medium. This highly cytolytic character of the envelopes was different from that of native virions and may resemble the characteristics of HVJ virions sonicated or freeze-thawed (15). It shows that the reassembled envelopes had cell fusion activity and that they were satisfactory for hybrid cell formation. This suggests that the structural arrangement of the re-

assembled envelopes may not be the same as the arrangement of the native virions. The cell fusion activity of the reassembled envelopes was not as stable as that of HVJ virions and was inactivated during storage at 5° to 10°C for 2 weeks or more.

The empty envelopes prepared from Method II (see Materials and Methods) were also effective for hybrid cell formation. The efficiency was nearly the same as that by the former envelope fraction. In the present case, fusion of cells was observed in BSS-Ca medium with or without serum: the cell lytic activity of the envelopes was less than that of the NP40-envelopes and was rather stronger than that of native virions. It seems that the alkali-emasol treatment was milder than NP40 treatment.

Thus, the present results show that these two envelope fractions free of nucleocapsids can be used effectively to obtain hybrid cells. The use of such fractions obviates the possibility that the viral genome may penetrate into the cells during the course of cell fusion for hybrid cell formation. Somatic hybrid cells have been used in studies on the genetics of somatic cells. During initial studies on their genetics, Barski *et al.* (1) obtained hybrids without the aid of exogenously added virus. Later it was found that HVJ induced fusion of cells in suspension (9, 11) followed by heterokaryon formation by the virus (10, 3, 13) and hybrid cell formation from the heterokaryons (18). Use of HVJ to induce fusion has proved effective because of the surprisingly higher efficiency of fusion achieved and the wide distribution of cells with HVJ-receptors in animal tissues (12). Thus, this technique seems useful in studies on genetics of animal cells. However, during the course of cell fusion, the virus penetrates into the cells, resulting in infection and growth of the virus. To minimize virus influence during hybrid cell formation, it was essential to inactivate the viral genome by UV-irradiation (11) or exposure to β -propiolactone (2). However, it is better if hybrid cells could be formed under conditions where there is no penetration of viral genome into the cells. By the present procedure, hybrid cells were obtained in fairly high frequency from heterokaryons formed by envelope fractions of HVJ, free of nucleocapsids (viral genome).

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