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Heterokaryon Formation of Simian Virus 40-transformed Cells in the Presence of Ultraviolet-irradiated Sendai Virus

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Most simian virus 40-transformed mouse kidney lines form heterokaryons with CV-1 cells in the presence of ultraviolet-irradiated Sendai. However, two nonyielder lines, mKS-U2 and mKS-U20, fuse poorly.

The entire simian virus 40 (SV40) genome can be rescued from some SV40-transformed cell lines which do not spontaneously produce this virus (1, 4, 6, 10, 11). Experiments have indicated that direct contact between the transformed cell and the susceptible cell is essential for induction of SV40 synthesis (5). Indeed, the activation of SV40 synthesis is facilitated by treating mixtures of transformed and susceptible green monkey kidney cells with ultraviolet (UV)-irradiated Sendai virus (UV-Sendai), which causes cell fusion and heterokaryon formation (3, 5, 9, 12).

Over 50 lines of mouse kidney and 3T3 cells, transformed by SV40 in our laboratory, readily yielded SV40 when co-cultivated with green monkey kidney cells, either with or without prior treatment with UV-Sendai. In contrast, of 83 mouse kidney lines (mKS-U) transformed by UV-irradiated SV40, 5 lines have yielded SV40 only rarely and 48 lines have never yielded virus by any technique thus far used, including treatment of cell mixtures with UV-Sendai (3).

Failure to rescue SV40 from nonyielder lines could be due to: (i) defectiveness of viral genes essential for detachment from the integrated state, for replication of viral components, or for viral maturation; (ii) production by transformed cells of substances incompatible with SV40 replication, e.g., repressors, interferon; or (iii) failure of transformed cells to fuse with permissive monkey kidney cells. If transformed cell lines fail to fuse or fuse poorly with the permissive monkey kidney cells, infectious virus might not be rescued, even when viral genes for detachment from the integrated state, for replication, and for maturation were unimpaired. This study was designed to learn whether nonyielder mKS-U lines were indeed defective in their capacity to fuse with CV-1 cells (7), an established line of green monkey kidney, in the presence of UV-Sendai.

The mKS-U cells were prelabeled by growth in medium containing ^{3}H-thymidine (^{3}H-dT; 1 μc and 0.5 μg/ml) for 20 hr at 37 C. We labeled 93 to 100% of the mKS-U nuclei by this procedure. The ^{3}H-dT-labeled mKS-U cells were mixed with unlabeled CV-1 cells and were treated with UV-Sendai (8,000 hemagglutinating units per 5×10^6 transformed cells) as previously described (3). Large Leighton tubes containing microscope slides [1 by 3 inches (2.54 by 7.62 cm)] were seeded with UV-Sendai-treated cell mixtures and incubated at 37 C overnight. After fixation of the cells in Formalin-acetic acid-ethyl alcohol [5:5:90 (v/v)], the slides were used for radioautography (8). The percentage of ^{3}H-dT-labeled nuclei appearing in heterokaryons with nonlabeled CV-1 nuclei was scored with phase-contrast microscopy and a 100× oil immersion objective. A total of 26 mKS-U cell lines, both yielder and nonyielder lines, were studied. In each experiment, seven or eight mKS-U lines, including two or three average or good virus yielders (see footnote *b*, Table 1), were tested, and the frequency of induction was determined for each UV-Sendai-treated cell mixture by plating samples with 10^6 freshly trypsinized CV-1 cells in 60-mm petri dishes. The same Sendai pool was used for all experiments cited here.

The mKS-U lines which are average or good virus yielders fused very well with CV-1 cells in the presence of UV-Sendai (Table 1). Of the ^{3}H-dT-labeled nuclei (transformed), 52 to 70% were found in heterokaryons with CV-1 cells. The four rare yielders and the two poor yielders, mKS-U3 and mKS-U1, also fused fairly well with CV-1 cells. As expected, SV40 virus was recovered from all UV-Sendai-treated mixtures of CV-1 cells

and good or average yielders. SV40 virus was also recovered from the two poor yielders, mKS-U1 and mKS-U3, and from one of the rare yielders (mKS-U16) in the experiments cited here.

Of the 14 mKS-U nonyielders tested, 12 also fused well with CV-1 cells in the presence of UV-Sendai, more than one-third of the transformed nuclei appearing in heterokaryons (Table 2). Indeed, with mKS-U22, over 70% of the transformed nuclei were found in heterokaryons; yet this strain has failed repeatedly to yield SV40.

Many of the heterokaryons formed with these 12 nonyielder strains were large, containing 5 to 20 nuclei, similar to those obtained with good and average yielders. Moreover, these large heterokaryons usually contained about equal ratios of transformed and CV-1 nuclei.

Two of the nonyielders, mKS-U2 and mKS-U20, fused poorly with CV-1 cells (Table 2). The capacity of mKS-U2 to form heterokaryons with CV-1 cells was compared with mKS-U13 in three separate experiments. Six to 10% of the mKS-U2 nuclei were found in heterokaryons as compared with 65 to 71% of mKS-U13, a good yielder. Moreover, heterokaryons with mKS-U2 rarely contained more than 1 CV-1 nucleus, but frequently contained 5 to 10 mKS-U2 nuclei. Thus, failure to rescue SV40 from mKS-U2 may be substantially due to lack of sufficient permissive protoplasm to permit viral replication. Failure to fuse with CV-1 cells is not the explanation for failure to rescue SV40 from many nonyielder lines, such as mKS-U5, mKS-U9, mKS-U22, and mKS-U25, in which over 50% of the cells form heterokaryons with CV-1 cells. It is possible that monkey cell lines may yet be found which do fuse with mKS-U2 and mKS-U20 or that are more permissive for the SV40 genome integrated in the various nonyielder mKS-U lines. Some of the nonyielder lines might then be classified as conditional yielders.

TABLE 1. *Heterokaryon formation with transformed cell lines which yield SV40 in UV-Sendai-treated mixtures with CV-1 cells*[a]

Transformed cell line	Classification[b]	^{3}H-dT-labeled nuclei (transformed) appearing in heterokaryons
		%
mKS-U12	Rare yielder	38
mKS-U16		49
mKS-U18		55
mKS-U23		61
mKS-U1	Poor yielder	33
mKS-U3		65
mKS-U4	Average yielder	69, 71
mKS-U6		71
mKS-U42		66, 64
mKS-U10	Good yielder	52
mKS-U13		68, 65, 71
mKS-U24		59

[a] In the experiments cited here, SV40 was recovered from all transformed lines except three rare yielders, mKS-U12, mKS-U18, and mKS-U23.

[b] mKS-U cell lines were classified on the basis of (i) frequency of induction of SV40, (ii) size of SV40 yields, and (iii) the incidence of positive trials when the transformed cells were mixed with CV-1 cells in the presence of UV-irradiated Sendai virus. SV40 was recovered from rare yielders only on one or two occasions in six or more trials, and in small amounts from poor yielders about 50% of the time. Good and average yielders always yielded SV40 and the frequencies of induction were $>10^{-4}$ and $>10^{-5}$, respectively (3).

TABLE 2. *Heterokaryon formation of nonyielder*[a] *transformed cell lines with CV-1 cells in the presence of UV-Sendai*

Transformed cell line	^{3}H-dT-labeled nuclei (transformed) appearing in heterokaryons
	%
mKS-U2	1–10
mKS-U20	11–20
mKS-U17, U21, U41	31–40
mKS-U7, U14, U19, U32A, U36	41–50
mKS-U5, U9	51–60
mKS-U25	61–70
mKS-U22	>70

[a] SV40 has never been recovered from any of these strains and was not recovered in the fusion experiments cited here.

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