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SHORT COMMUNICATION

THE PRESENCE OF HERPES SIMPLEX VIRUS DNA IN HAMSTER EMBRYO CELLS TRANSFORMED BY A TEMPERATURE SENSITIVE MUTANT, TS-4

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Hamster embryo cells transformed with a temperature sensitive mutant of HSV-2, ts-4T, were examined for the presence of viral genomes. DNA-DNA reassociation kinetics showed that only a small portion ($11 \pm 4\%$) of HSV-2 was present in the transformed cells.

The transforming potentials of herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) have been demonstrated in several laboratories (Duff and Rapp, 1971; Munyon et al., 1971; Rapp et al., 1973; Garfinkle and McAuslan, 1974; Darai and Munk, 1973; Takahashi and Yamanishi, 1974; Kucera and Gusdon, 1976). Studies on DNA-RNA hybridization and DNA-DNA reassociation kinetics have shown that only a portion of the viral DNA sequences are present or transcribed in the transformed cells (Collard et al., 1973; Frenkel et al., 1976; Davis and Kingsbury, 1976).

In the previous study (Takahashi and Yamanishi, 1974), the continued presence of the HSV-2 genome in hamster embryo (HaE) cells transformed by a temperature sensitive (ts) mutant of HSV-2 was suggested by indirect immunofluorescent antibody staining using anti-HSV rabbit serum and antirabbit goat serum. To confirm this result, we tried to detect HSV-2 DNA in the transformed cells by DNA-DNA reassociation kinetics.

HaE cells transformed by a ts mutant of HSV-2, ts-4T (at 14th passage in vitro of cultured cells from tumors produced by injection of transformed cells with ts 4 which had passaged 22 times in vitro) were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum. Before extracting the cellular DNA from the cells for DNA-DNA reassociation kinetics, the presence of HSV specific antigens in the transformed cells was examined by the anti-complement immunofluorescence (ACIF) test. The cells on

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coverslips were fixed with acetone for 5 to 10 min at room temperature and then incubated successively at 37 C for 30 min period with HSV human immune serum, with 4 U of human complement, and then with fluorescein isothiocyanate conjugated anti-human C3 goat serum (1: 40 dilution, Hyland). Then they were examined under a fluorescence microscope. As shown in Fig. 1, relatively strong fluorescence was observed in the cytoplasm of ts-4T cells.

DNA was extracted from ts-4T cells as described previously (Hirai, 1979 in press), and examined for the presence of HSV DNA sequences by DNA-DNA reassociation kinetics. HSV-2 DNA, used as a probe DNA was labeled with ^{3}H-TTP in vitro as described previously (Hirai, 1979 in press). The radiospecific activity of HSV ^{3}H-DNA was 4.1×10^6 cpm/μg.

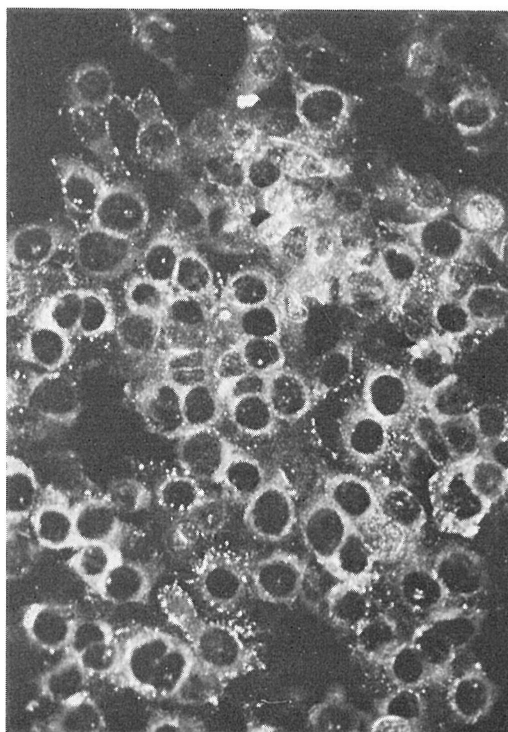

FIGURE 1. Immunofluorescence photomicrograph ts-4T cells.

Ts-4T cells were stained by the anti-complement immunofluorescence test as described in the text.

A mixture of 0.0256 μg of ^{3}H-labeled HSV DNA and 1 mg of cell DNA in 0.5 ml of 0.3 N NaOH was boiled for 15 min and then neuturalized with HCl. Then 5 M NaCl was added to a final concentration of 2 M to 1 ml volumes of the mixture and the preparations were incubated at 70 C. At various times during incubation, samples of 0.1 ml were with drawn and stored at -20 C. Later these samples were digested with single stranded specific nuclease (nuclease S1, Seikagaku Kogyo Co., Tokyo) to determine the amount of reassociated ^{3}H-labeled HSV DNA. The percentage of labeled doublestranded DNA was determined from the amounts of TCA-precipitable label in control and enzyme-digested samples as previously described (Hirai and Defendi, 1974). The reassociation kinetics of randomly sheared DNAs follows modified second order rate equation (Gubbins et al., 1977); $C/Co=(1+kCot)^{-0.42}$ (1), or $(Co/C)^{1/0.42}=1+kCot$ (2), where C and Co are the concentrations of single stranded HSV ^{3}H DNA at times t and t=0, and k is the reassociation constant. The value 0.42 is empirically determined constant used when reassociation is assayed with single-strand specific nuclease S1 (Britten and Davidson, 1976).

If all the different sequences in a hybridization mixture are present in equal concentration, a plot of $(Co/C)^{1/0.42}$ versus Cot should give a straight line with a slope k. As shown in Fig. 2a, reassociation of HSV ^{3}H-DNA can be accelerated in reconstruction experiments with HSV ^{3}H-DNA and unlabeled viral DNA and the experimental points fell on a straight line. The rate of reassociation of HSV ^{3}H-DNA in the presence of normal hamster DNA was similar to that in the presence of salmon sperm DNA, and so, salmon sperm DNA was used as control DNA in the present experiments. The addition of ts-4T DNA, instead of control DNA, to the hybridization mixture increased the rate of reassociation of HSV ^{3}H-DNA. The initial rate of reassociation was

higher than that in the presence of the same concentration of control DNA (Fig. 2b). In the presence of ts-4T DNA, however, the experimental points for association of HSV ^{3}H-DNA did not give a straight line, but a biphasic curve. This result indicates that only a restricted portion of HSV-2 DNA was present in the transformed cells, as found in other HSV-transformed cells (Frenkel et al., 1976; Davis and Kingsbury, 1976).

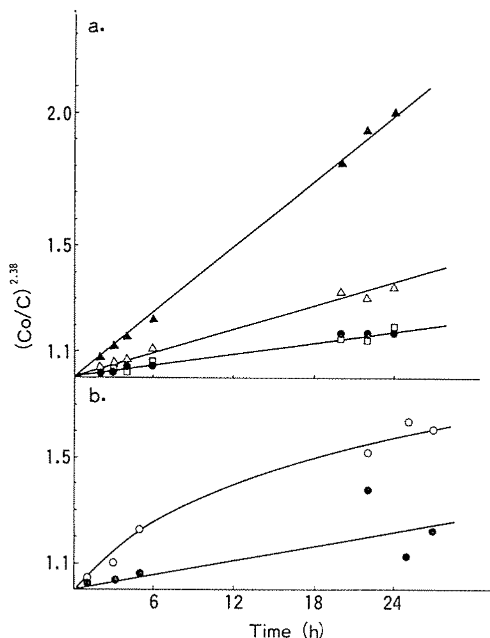

FIGURE 2. Kinetics of reassociation of HSV ^{3}H-DNA with ts-4T DNA.

One copy per cell of ^{3}H-HSV-2 DNA (0.0256 μg, 4.1×10^6 cpm/μg), prepared in vitro by nick translation of HSV-2 DNA, was reassociated in the presence of the following DNAs: in Fig. 1a, 1) 1 mg of normal hamster DNA (□), 2) 1 mg of salmon sperm DNA (●), 30 1 mg of salmon sperm DNA and 0.0256 μg of HSV-2 DNA (one copy per cell) (△), 4) 1 mg of salmon sperm DNA and 0.105 μg of HSV-2 DNA (four copies per cell) (▲), in Fig. 1b, 1) 1 mg of salmon sperm DNA (●), 2) 1 mg of ts-4T DNA (○).

DNA-DNA reassociation kinetics were examined as described in the text.

In order to estimate the amount of the total viral DNA present in transformed cells, the fraction of the HSV ^{3}H-DNA reassociated after completion of the initial reaction was corrected for the amount of self-reassociation of HSV ^{3}H-DNA at the same time. It was calculated from the experimental points in three replica experiments that ts-4T contained $11 \pm 4\%$ of the HSV-2 genome.

Li et al. (1975) reported that HSV specific antigens become undetectable during repeated passage of HSV transformed cells. But in the present study, we examined ts-4T cells for the presence of HSV genomes every 10th passage and found no alternation in the expression of HSV antigens or of viral genomes. Therefore, ts-4T cells seem suitable for use in further studies on viral genomes and antigens.

Recently, one of the DNA fragments generated by cleavage of HSV-1 DNA with restriction endonuclease Xba 1 was found to induce transformation of hamster embryo cells (Camacho and Spear, 1978). If a specific DNA fragment is required for maintenance of the transformed state, all cells transformed with HSV-1 should contain the sequences in the specific DNA fragment and HSV gene products required for transformation. It is, therefore, necessary to characterize the viral DNA sequences and gene products in ts-4T cells and to compare them with those in other cells transformed with HSV-2.

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