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Long-term preservation of transfecting activity of avian sarcoma proviral DNA.

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Abstract

The integrated proviral DNA of avian sarcoma virus (ASV) in host cell chromosomes has been isolated and stored in saline sodium citrate (SSC) solution or in 70% ethanol at 4 degrees C in a refrigerator over 4 years. This DNA was assayed by transfection of chick embryo cells(CEC). The biological activity of cellular transformation by the stored DNA was compared with that of a fresh isolate of the proviral DNA. The efficiency of the transfection by each DNA was almost the same.

KEYWORDS: avian sarcoma proviral DNA, saline sodium citrate, transfection.

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—— BRIEF NOTE ——

LONG-TERM PRESERVATION OF TRANSFECTING ACTIVITY OF AVIAN SARCOMA PROVIRAL DNA

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Abstract. The integrated proviral DNA of avian sarcoma virus (ASV) in host cell chromosomes has been isolated and stored in saline sodium citrate (SSC) solution or in 70% ethanol at 4℃ in a refrigerator over 4 years. This DNA was assayed by transfection of chick embryo cells(CEC). The biological activity of cellular transformation by the stored DNA was compared with that of a fresh isolate of the proviral DNA. The efficiency of the transfection by each DNA was almost the same.

Key words.: avian sarcoma proviral DNA, saline sodium citrate, transfection.

Transfection studies on avian retrovirus DNA have been performed by us (1-3). It was necessary to know how stable the isolated DNA is in order to perform further study. To examine the effect of long-term preservation of ASV proviral DNA for transfection studies, the present work has been undertaken.

The DNA sources for the study were CEC transformed by the Prague strain of ASV, Balb 3T3 mouse cells transformed by the Bratislava strain of ASV, rat XC cells transformed by the Prague strain of ASV and D17 mouse cells transformed by the Schmidt-Ruppin strain of ASV.

The isolation of DNA from these culture cells was done by Marmur's method (4) slightly modified (5) as follows. Each of the monolayer culture cells was lysed overnight in a physiological saline solution containing 0.1M ethylenediaminetetraacetate (pH 8.0), 0.5% sodium dodecyl sulfate and 50 μg/ml of proteinase K (Serva, W. Germany). Isolation of DNA from the lysate was described previously (3).

The purified DNA was dissolved in a 0.1 x SSC solution at a concentration of 500 to 1000 μg/ml followed by the adjustment of salt concentration to 1 x SSC by adding 10 x SSC solution. A part of the DNA was precipitated in 70% ethanol. They were stored in a refrigerator at 4℃ for 4.5 years.

As a control, freshly isolated DNA from CEC transformed by the Prague-strain of ASV was used for the transfection study. Trasfection by the calcium method was done as described previously (3).

The *chf* negative C/O phenotype CEC as transfection recipient cells were

obtained from the Research Institute for Microbial Disease of Osaka University, Kanonji, Japan.

TABLE 1. TRANSFECTION OF CEC WITH AVIAN SARCOMA PROVIRAL DNA

DNA Source	Storage condition	No. of transfection positive dishes / No. of total dishes
CEC transformed by PR-ASV	1 x SSC	4/6
CEC transformed by PR-ASV	70% ethanol	5/7
CEC transformed by PR-ASV	fresh isolate	6/7
3T3 transformed by B77-ASV	1 x SSC	10/13
D17 transformed by SR-ASV	1 x SSC	8/14
XC transformed by PR-ASV	1 x SSC	5/9

DNAs were stored in 1 x SSC solution at a concentration of 500 to 1000 μg/ml or in 70% ethanol for 4.5 years. Ten μg of DNA after calcium phosphate precipitation was added to the C/O phenotype CEC grown in a 6 cm petri dish according to the method described previously (3).

As shown in Table 1, DNA isolated from CEC transformed by the PR strain of ASV were still infectious and transformed normal CEC even after 4.5 years of storage in 1 x SSC at 4℃ as well as in 70% ethanol. The efficiency of transfection of the stored DNA in 1 x SSC and in 70% ethanol was almost the same. Furthermore, the efficiency of transfection of the stored and the freshly isolated DNA was also almost the same. The DNAs isolated from the mouse cells (3T3 and D17) and the rat cells (XC) transformed by different kind of ASV were also infectious, although these cells produce no ASV.

It has been reported that chromosomal DNA containing avian sarcoma proviral DNA was biologically active when stored in 0.1 x PBS containing 10% glycerol at −70℃ for 7 months (6) and when stored in 0.1 x SSC at 4℃ for 9 months (7).

It can be concluded that proviral DNA is biologically stable for a long time when stored appropriately. It is especially stable in 1 x SSC at 4℃ over 4 years. This means that the retroviruses can be safely stocked in a proviral form in a refregirator.

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