# TRANSFECTION OF XC PLAQUES WITH DNA FROM MURINE LEUKEMIA VIRUS PRODUCER CELLS

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Received July 21,1975

SUMMARY

Transfection of leukemia virus resulting in virus production has been accomplished in the murine system using cellular DNA containing a vertically established leukemia virus. Virus production was detected in a bioassay by XC plaque formation and in a biochemical assay for reverse transcriptase activity. Infection by DNA was successful using the Calcium method but not with the DEAE dextran method. XC plaques were observed as early as the first subculture of transfected cells.

## INTRODUCTION

Hill and Hillova first demonstrated that proviral DNA was integrated in the cell genome by transfecting Rous sarcoma viral information from XC cells into susceptible chicken cells (1). Cooper and Temin (2) quantitated this technique and used it to determine the minimal size of infectious viral DNA in the avian system. In the mammalian system, murine sarcoma viral information from hamster and mouse non-producer cells was transfected into susceptible mouse embryo 3T3 cells; this was detected by the appearance of transformed colonies after five subcultures (six weeks) (3). Recently Scolnick and Bumgarner (4) have transfected mouse endogenous xenotropic viral information into a mink cell by assaying reverse transcriptase activity in the supernatant of the heterologous cell. This paper describes transfection, demonstrated by XC plaque formation; the infectious DNA was obtained from an exogenously infected murine leukemia virus producer cell.

## MATERIALS AND METHODS

### Cells and Virus

Moloney leukemia virus (from Dr. David Baltimore) was cloned, and then

Abbreviations used are: MLV - murine leukemia virus
oligo dG · poly rC - oligodeoxyguanylate polyribocytidylate.

used to infect NIH/3T3 cells. The cells were then cloned for virus production. Normal NIH/3T3 cells were used as recipients in the infectious DNA assay.

DNA Extraction

The extraction was a modification of the Marmur procedure (5), and essentially the same as described by Cooper and Temin (2). It differed from the latter in that after the RNase treatment only one extraction was necessary with chloroform-isoamyl alchohol (24:1) to remove denatured protein. The DNA was collected by winding on a glass rod after precipitation with two volumes of 95% ethanol,sterilized in 70% ethanol, and dissolved in 0.1X Standard Saline Citrate. The DNA concentration was determined by absorbance at 260nm ($A_{260}$). The $A_{260}$:$A_{280}$ ratio of the preparation was 1.8 to 1.9. Infectivity was not lost by storage at -70°C.

Infectious DNA Assay

NIH/3T3 cells were seeded at $10^5$ cells per 60mm petri dish one day before infection. The DNA was sheared 5X in a 1.0ml plastic pipette prior to use (6). The method of Graham and Van der Eb which consists of forming a calcium phosphate precipitate of DNA was followed exactly (7). After 20 min. adsorption at room temperature, 5ml of medium (Dulbecco's modified minimum essential medium containing 10% Calf Serum) were added without removing the DNA precipitate; the plates were incubated at 37°C for 4.5 hrs. Fluid from plates was aspirated and fresh medium was added. When cells became confluent they were passed to 100mm plates. When these became confluent they were passed at a dilution of 1:10 for the first subculture and duplicate 60mm plates were made for the XC and reverse transcriptase assays. Subcultures were made at the same dilution every four days. The XC assay was performed by adding $10^6$ XC cells on confluent UV irradiated 60mm test plates and staining for XC syncytia two days later (8).

Reverse Transcriptase Assay

Ten ml supernatant was clarified of cells by spinning in a GLC-1 centri-

TABLE 1

XC ASSAYS ON CELLS TRANSFECTED WITH DNA FROM MLV PRODUCER CELLS.

| Experiment | DNA Sample | Subcultures 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6ug, MLV Prod., $Ca^{++}$ met. | - | + | + | + | + | + | + | + | + | + |
| | 60 " " | - | - | - | - | - | - | - | - | - | - |
| 2 | 6 " " | + | + | + | + | + | + | + | + | + | + |
| | 12 " " | + | + | + | + | + | + | + | + | + | + |
| Controls a) | $Ca^{++}$ met., no DNA | - | - | - | - | - | - | - | - | - | - |
| b) | DEAE dex. met., no DNA or above concentrations | - | - | - | - | - | - | - | - | - | - |
| c) | NIH/3T3, $Ca^{++}$ met. | - | - | - | - | - | - | - | - | - | - |
| d) | DNase treated MLV Prod., $Ca^{++}$ met. | - | - | - | - | - | - | - | - | - | - |

Cells were infected with DNA using $Ca^{++}$ method (or DEAE dextran method) and XC assays were performed on subcultured cells. All samples were done in duplicate. DNase treatment consisted of treating MLV prod. DNA with 100ug/ml of DNase 1 in 0.01M $MgCl_2$, 0.01M $CaCl_2$, and 0.01M Tris-HCl, pH 7.4 for 60 min. at 37°C.

MLV Prod.: DNA from murine leukemia virus producer cell.

fuge at 1,650xg for 3 min. Supernatant was then centrifuged for virus in a type 65 rotor in a L2-65B ultracentrifuge at 160,000xg for 60 min. in the cold. Virus pellet was suspended in 0.5ml of 0.01M Tris-HCl, pH 7.5 and sonicated for 40 seconds. Twenty microliters were used in a 0.1ml reaction mixture as described (9) using the synthetic DNA-RNA hybrid oligo dG · poly rC (Collaborative Research Inc., Waltham, Ma.) as template. Trichloroacetic acid precipitable counts indicating [$^3$H] - GMP incorporation was measured in a Beckman LS-330 liquid scintillation counter.

## RESULTS AND DISCUSSION

Although transfection with proviral DNA of the RNA tumor viruses has been described in the mammalian system (3, 4), transfection of murine

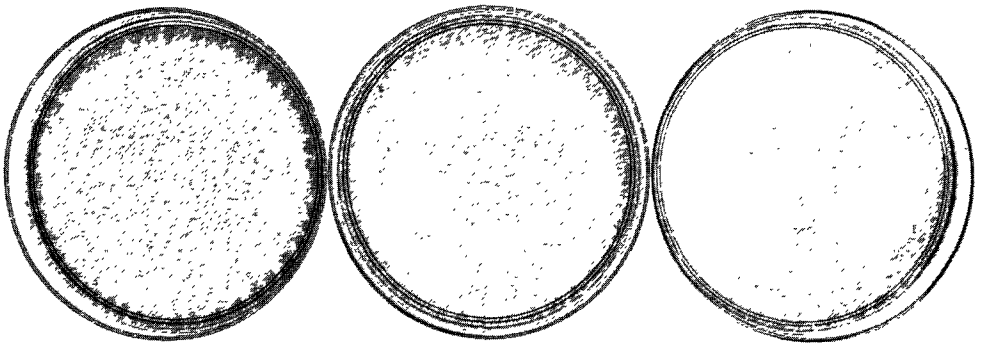

FIGURE 1

APPEARANCE OF XC PLAQUES AFTER TRANSFECTION WITH DNA FROM MLV PRODUCER CELLS

(left) Negative control. A subculture of cells mock infected with a $CaPO_4$ ppt., no DNA.
(middle) Second subculture of cells infected with 6ug of MLV Producer DNA.
(right) Third subculture of cells infected with 6ug of MLV Producer DNA.
XC cells were added to confluent plates which were stained after 2 days with Giemsa.

leukemia proviral DNA to produce XC plaques has not previously been accomplished. Table 1 shows that as early as the first subculture XC plaques were observed in NIH/3T3 cells treated with DNA (6 or 12 micrograms) extracted from NIH/3T3 cells which were previously infected with Moloney leukemia virus. A high concentration of DNA (60 micrograms) was negative as was use of the DEAE dextran method (2) instead of the calcium method (7). Control experiments with normal NIH/3T3 DNA, as well as DNase-treated DNA from NIH/3T3 cells infected with murine leukemia virus, gave negative reults. Figure 1 (middle) shows a plate with XC plaques which appeared on the second subculture. Plates from subsequent subcultures continued to demonstrate confluent XC syncytia (third subculture shown in Figure 1 right) presumably due to viral spread, for at least ten subcultures. Table 2 demonstrates that supernatants of XC positive subcultures had reverse transcriptase activity.

TABLE 2

REVERSE TRANSCRIPTASE ACTIVITY IN SUPERNATANTS OF SUBCULTURES OF TRANSFECTED CELLS

| Cell Sample | CPM [$^3$H] - GMP Inc. |
|---|---|
| MLV Producer | 5800 |
| negative control | 498 |
| 6 ug DNA | 6333 |
| 12 ug DNA | 7051 |
| 60 ug DNA | 720 |

Supernatants from cells from third subculture after DNA infection were processed and tested for reverse transcriptase activity by incorporation of [$^3$H] - GMP using oligo dG · poly rC synthetic template. Positive control used supernatant from a leukemia virus producer cell. Negative control came from subcultured cells which were previously mock infected with a $CaPO_4$ ppt, no DNA. Other samples came from subcultured cells which were infected with amounts of DNA shown.

The high concentration of DNA (60ug) was negative as expected since no XC plaques had been observed with this sample (table 1).

The precedent has been established for use of sub-genomic fractions in transformation studies using transfection in the DNA virus system (Adenovirus and SV-40; (10) ) and Avian RNA virus system (Rous sarcoma virus; (2) ). This communication establishes the ability to transfect murine leukemia virus information, which, along with murine sarcoma virus transfection (3), might allow for isolation and study of interaction between replication (leukemia) and transformation (sarcoma) function in the murine virus system.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Cancer Institute (1-RO1-CA16252-01 VR). MB is a Special Fellow of the Leukemia Society of America. I would like to thank Dr. Tom Benjamin for helpful discussions.

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