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# The transcriptional activity of the simian virus 40 enhancer and promoter in GH3 cells

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**ABSTRACT**

A series of experiments was designed to investigate the activity of the SV40 early promoter and enhancer in rat pituitary tumor cells in culture but still yet not clear so, a series of enhancer promoter-reporter plasmids were constructed to study the SV40 activity in the rat pituitary tumor cell lines (GH3 and GC) then sub-cloned into pSV2(enhancer-minus)-CAT, then purified in gel and ligated into pRSV(enhancer-minus)-CAT (Sph1 restricted) then transformed into E.Coli then make restriction analysis. From the results we observed that SV40 early promoter can be activated by heterogeneous enhancers in pituitary tumor cells.



## INTRODUCTION

SV40 or simian virus is one of the most intensely studied animal viruses since its potential to infect human and also induce tumors in experimental animals [1]. It is the most well characterized member of the *Polyomaviridae* family of small DNA tumor viruses that infects humans through contaminated polio vaccine [2, 3].

The virus is dormant and is asymptomatic in Rhesus monkeys. In other species, particularly hamsters, SV40 causes a variety of tumors, generally sarcomas. In rats, the oncogenic SV40 Large T-antigen was used to establish a brain tumor model for PNETs and medulloblastomas [4].

The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research [3]. Several different methods have been used to detect SV40 in a variety of human cancers, although how reliable these detection methods are, and whether SV40 has any role in causing these tumors, remains unclear [5].

SV40 is believed to suppress the transcriptional properties of the tumor-suppressing p53 in humans through the SV40 Large T-antigen and SV40 Small T-antigen. p53 is responsible for initiating regulated cell death ("apoptosis"), or cell cycle arrest when a cell is damaged. SV40 may act as a co-carcinogen with crocidolite asbestos to cause both Peritoneal and Pleural Mesothelioma [6].

A series of experiments were designed to investigate the activity of SV40 early promoter and enhancer in rat pituitary tumor cells in culture. Earlier reports suggested that the SV40 early enhancer/ promoter are non-functional in GH3 cells [7, 8].

The early promoter for SV40 contains three elements A, B and C **(Fig 1A)**. The TATA box is located approximately 20 base-pairs upstream from the transcriptional start site. The 21 base-pair repeats contain six GC boxes and are the site that determines the direction of transcription.

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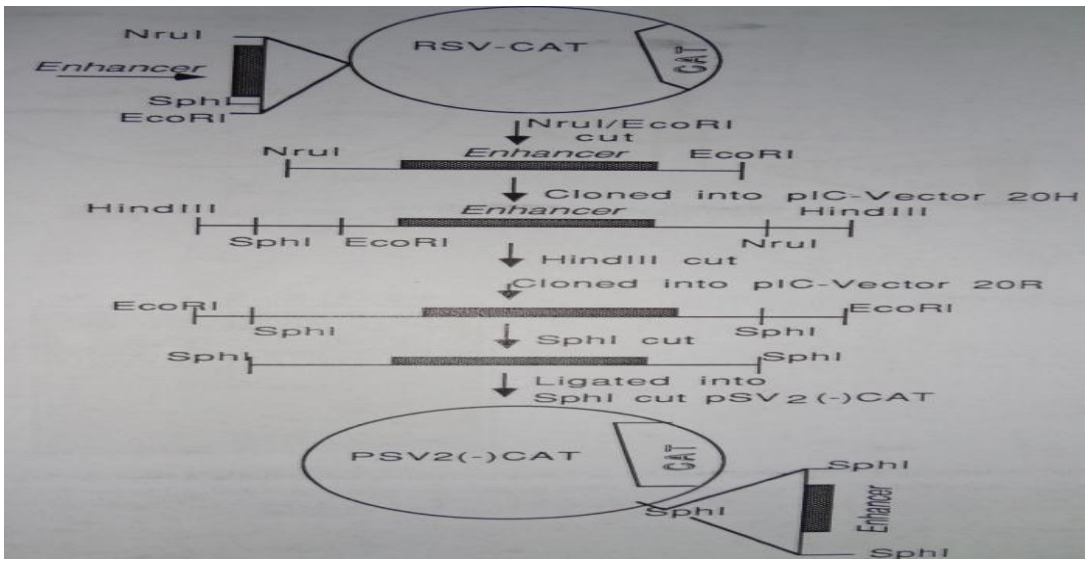


Also, the 72 base-pair repeats are transcriptional enhancers. When the SP1 protein interacts with the 21 bp repeats it binds either the first or the last three GC boxes. Binding of the first three initiates early expression and binding of the last three initiates late expression. The function of the 72 bp repeats is to enhance the amount of stable RNA and increase the rate of synthesis [9].

Our study is to follow up the activity of the SV40 early promoter and enhancer in rat pituitary tumor cells.

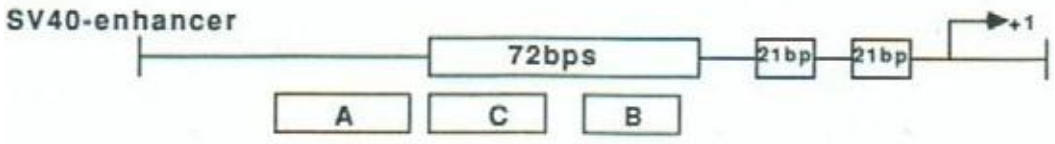


**Fig: 1 (A):** *Shows the SV40-enhancer along with position of A, B and C enhancer elements*.

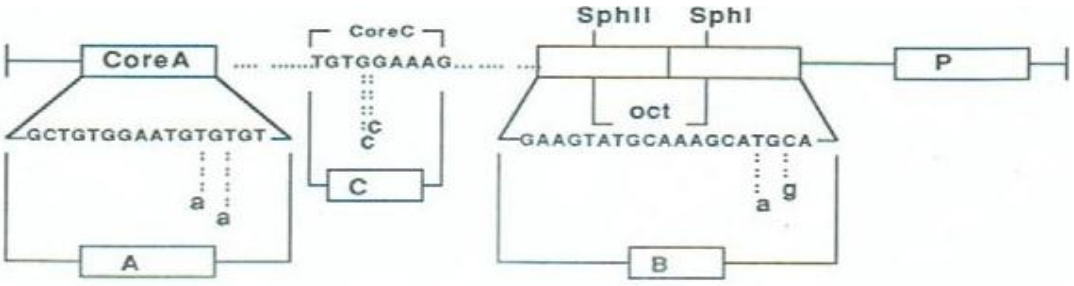


**Fig: 1 (B):** *Shows the structure within SV40-enhancer elements A, B and C and the point mutation with in the SV40-enhancer, generated by* ***Ondek,et al.,*** [9].

## Material and Methods

In order to study SV40 enhancer activity in the rat pituitary tumor cell lines (GH3 & GC) a series of enhancer promoter- reporter plasmids were constructed. An RSV (Rous sarcoma virus) enhancer fragment was sub-cloned into pSv2 (enhancer-minus)-CAT as shown in (Fig. 2).

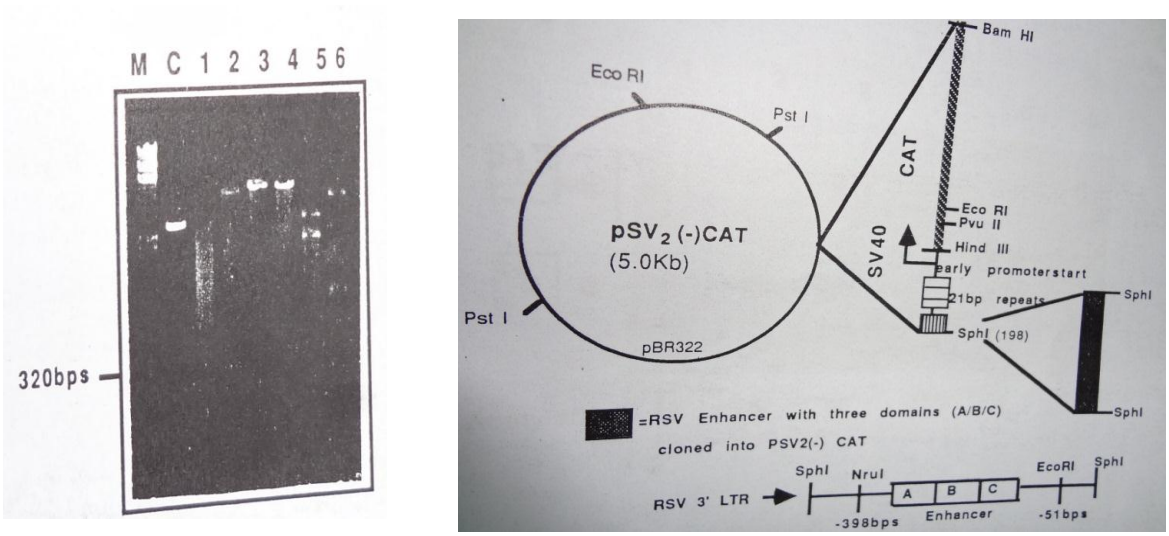


**Fig 2:** *Sub-cloning strategy used to insert the RSV-Enhancer into pSV2(enhancer-minus) Sph1 CAT construct.*

An SV40-enhancer fragment was isolated by restricting pSV2-CAT Sph1. It was gel purified and ligated into pRSV (enhancer-minus)-CAT (Sph1 restricted), transformed into E.Coli (strain HB101) the correct selected plasmid pR(-)-SV40-CAT as shown in (Fig. 3). The structure of pR(-)-SV40-CAT and its restriction analysis with a range of different restriction enzymes as shown in (Fig. 4).

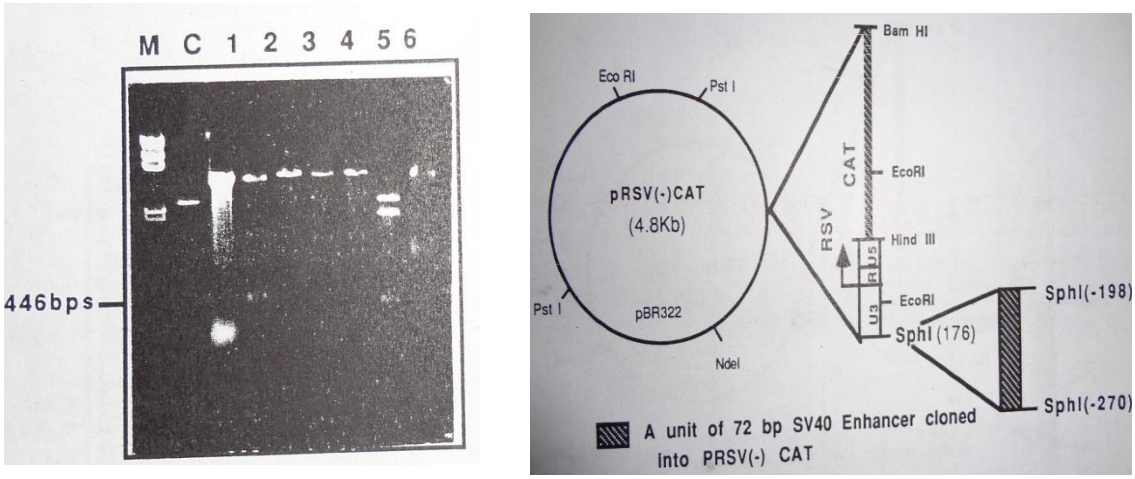


**Fig 3:** *Plasmid map and restriction analysis of pRSV(enhancer-minus)/SV40-enhancer)CAT. Analysis shows:* ***Lane 1****: pRSV(enhancer-minus) CAT, cut Sph1 (4.803bps)* ***Lane 2:*** *pRSV(SV40-E) CAT, cut HindIII/NdeI, (4.429;44bps),****Lane 3****: Hind III cut (4.875),* ***Lane 4****: BamH1 cut (4.875bps),* ***Lane 5:*** *EcoR1 cut (2.331;2.100;339bps),* ***Lane 6:*** *Pst 1 cut(3.917,958bps),* ***Lane C:*** *unrestricted plasmid,* ***M:*** *corresponds to hind III cut phag λ DNA.*

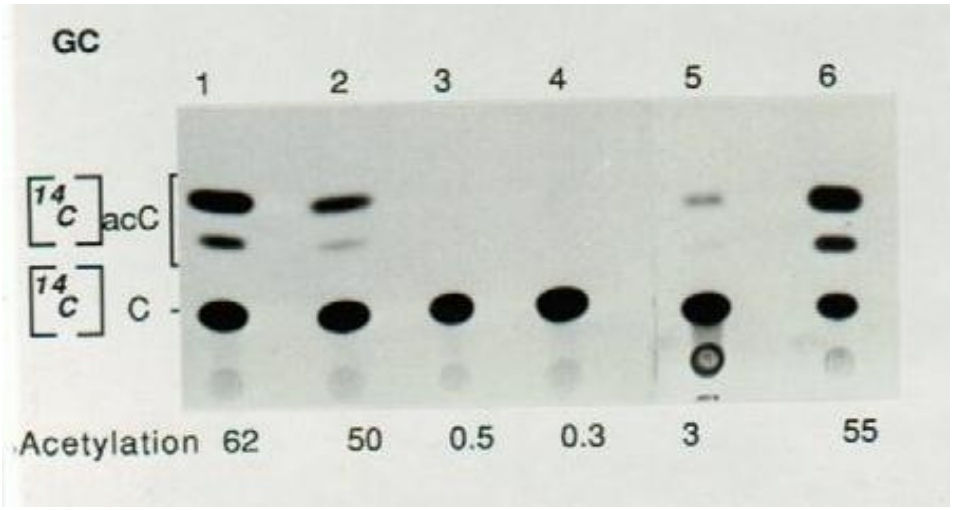


**Fig 4:** *Plasmid map and restriction analysis of pSV2(enhancer-minus)/RSV-enhancer)CAT.Analysis shows:* ***Lane 1****: pSV2 (enhancer-minus) CAT, Sph1cut (4.696bps)* ***Lane 2:*** *pSV2 (RSV-E) CAT, Sph1cut (4.696; 320bps),* ***Lane 3****: Hind III cut (5.016bps),* ***Lane 4****: BamH1 cut (5.016bps),* ***Lane 5:*** *EcoR1 cut (2.916; 2100bps),* ***Lane 6:*** *Pst 1 cut (4.115, 958bps),* ***Lane C:*** *unrestricted plasmid,* ***M:*** *corresponds to hind III cut phag λ DNA.*

**Results and discussion**

The enhancer-swap plasmids pR(-)-CAT and pS(-) RSV-CAT were used to test in particular, weather the SV40 promoter was functional when transfected into pituitary tumor cells. Therefore, the parent plasmids: pRSV-CAT and pSV40-CAT, the enhancer deleted plasmids: pR(-)-CAT and pS(-)-CAT and enhancer swap plasmids pS(-)RSV-CAT and pR(-) SV40-CAT were transfected into non-pituitary tumor cells (C6 and GC cells). Representative CAT analysis is presented in **Fig. 5**.

In both C6 and GC cells, pRSV-CAT **(lanes 1)** shows abundant activity and removal of the RSV enhancer sequences reduced the CAT activity by more than 90% **(lane 2)**. Substitution of the SV40 Sph1 enhancer fragment into pR(-)CAT **(lane 6)** at least restored enhancer promoter activity to that seen with pRSV-CAT in C6 cells. Over a series of experiments it was found not to affect pR(-)CAT activity significantly in GC cells. In C6 cells pSV2-CAT (lanes 3) had equivalent activity to pRSV-CAT and removal of the Sph1 enhancer fragment reduce its activity considerably **(lane 4)**. The RSV enhancer fragment did not function very effectively to restore activity to pS(-)-CAT in C6 cells **(lane 5).** In GC cells both pSV-CAT and pS(-)-CAT showed no inherent transcriptional activity **(lanes 3,4).** This suggests that SV40 early promoter can be activated by heterogeneous enhancers in pituitary tumor cells.

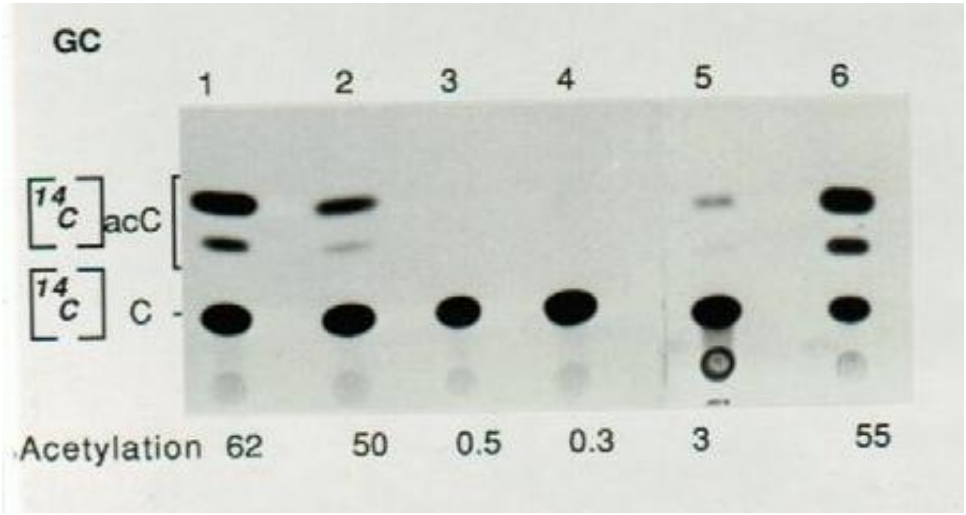


**Fig. 5**: *Tissue-specific expression of the SV40 enhancer in pituitary and non-pituitary cells as shown in the T.L.C analysis of [$^{14}$c]-chloramphenicol acetylation after 2h incubation with extract prepared 48h after cell transfection with:* ***lane 1****: pRSV-CAT,* ***lane 2:*** *pRSV(-)-CAT,* ***lane 3:*** *pSV40-CAT,* ***lane 4:*** *pSV$_2$(-)-CAT,* ***lane 5:*** *pSV$_2$(-) /RSV$_E$-CAT and* ***lane 6:*** *pRSV(-)/SV40 $_E$-CAT.*

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