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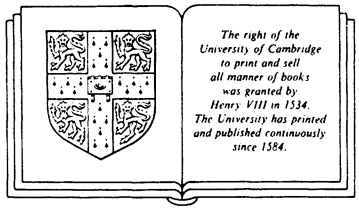

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THE SV40 "ENHANCER TRAP"

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INTRODUCTION

Transcriptional enhancers are short DNA segments that activate the transcription of linked genes, in either orientation and over distances of many kilobase pairs (kb), which were originally discovered in viral genomes (Banerji et al., 1981; de Villiers and Schaffner, 1981; Moreau et al., 1981; for recent reviews see Khoury and Gruss, 1983, and also Gluzman and Shenk, 1983). Tissue-specific cellular enhancers have recently been found in immunoglobulin genes (Banerji et al., 1983; Gillies et al., 1983; Neuberger, 1983; Queen and Baltimore, 1983; Picard and Schaffner, 1983).

We reasoned that it is possible to generally select for functional enhancers by assaying for the resurrection of enhancerless SV40, after cotransfecting it with short random DNA fragments into monkey cells. In this way we could select for the growth of recombinant viruses which had incorporated DNA with enhancer function. This technique offers clear advantages over the direct and indirect approaches used previously to identify enhancers (Banerji et al., 1981; Lusky et al., 1983; Fried et al., 1983; Folger et al., 1983).

RESULTS

We have transfected monkey CV-1 cells with non-infectious, linear SV40 DNA, lacking the 72 bp repeated enhancer region. Infectious virus was recovered from this

"enhancer trap" upon co-transfection with enhancer DNA segments from various viruses such as SV40, polyoma, cytomegalovirus and Rous sarcoma virus. The enhancer DNA segments apparently became integrated into the enhancerless SV40 DNA by intracellular resection/ligation/repair processes. A truncated polyoma "semi-enhancer" (BclI to PvuII fragment; de Villiers and Schaffner, 1981) was incorporated as a dimer and retains the host cell preference (de Villiers et al., 1982) of the complete polyoma enhancer. Co-transfection of the "enhancer trap" with fragmented DNA of mouse, monkey or human origin, yielded no recombinant virus with integrated cellular sequences, with one possible exception. This indicates that there are no more than a few hundred segments per mammalian genome with the strong enhancer activity demanded by our assay. In some transfection experiments without added viral enhancer DNA, SV40 variants were generated which have a segment of their flanking "late" DNA duplicated to substitute for the deleted 72 bp repeat. In one of these variants (SV7.2, see Figure 1) an 88 bp duplication creates a strong enhancer from this previously inactive DNA region. Both the polyoma enhancer fragment and the spontaneously created enhancers lack the physical sequence features which have been associated with enhancer elements, namely the "GTGG(A/T)-box" (Khoury and Gruss, 1983), the "CACA-box" (Lusky et al., 1983) or stretches of alternating purines-pyrimidines (Nordheim and Rich, 1983). It therefore appears that these sequence motifs are not ubiquitously associated with, and may even be redundant for, the function of transcriptional enhancers.

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LEGEND TO FIGURE 1

SVΔ50: This variant virus has a 54 bp deletion around the KpnI site. It was obtained from a cotransfection of enhancer trap DNA with the enhancer bearing restriction fragment and an excess of sonicated carrier DNA.

SVP2M2: This variant was obtained from an experiment using the mixed-in sonicated plasmid pPySVk100- where the SV40 enhancer was provided flanked by polyoma virus sequences (de Villiers et al., 1982).

SV15-: This is a virus containing the SV40 enhancer in the opposite orientation, obtained from an experiment with the same transfection protocol as in the case of SVΔ50.

SVFE2 and **SV7.2** grew out of cells transfected with SV40 enhancer trap and carrier DNA without enhancer added. The transfected SV40 DNA was circularized in vivo. 77 bp and 88 bp of the viral "late" region around the HpaII site were duplicated in SVFE2 and SV7.2, respectively, thereby giving rise to variants which grow slowly (SVFE2) or intermediately fast (SV7.2).

FIGURE 1: Maps of SV40-derived enhancers