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EUCARYOTIC GENE REGULATION

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TRANSFORMATION OF MAMMALIAN CELLS WITH PROKARYOTIC AND EUKARYOTIC GENES[1]

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ABSTRACT Cellular genes coding for selectable biochemical functions can be stably introduced into cultured mammalian cells by DNA-mediated gene transfer (transformation). Biochemical transformants are readily identified by the stable expression of a gene coding for a selectable marker. These transformants represent a subpopulation of the competent cells which integrate other physically unlinked genes for which no selective criteria exist. In this manner, we have used a viral thymidine kinase gene as a selectable marker to isolate mouse cell lines which we have stably transformed with the tk gene along with bacteriophage ΦX 174, plasmid pBR 322, or the cloned chromosomal rabbit β-globin gene sequences. ΦX co-transformants were studied in greatest detail. The frequency of co-transformation is high, 15 of 16 tk^+ transformants contain the ΦX sequences. Further, from one to more than fifty ΦX sequences are stably integrated into high molecular weight nuclear DNA isolated from independent clones. The introduction of cloned eukaryotic genes now provides an in vivo system to study the functional significance of various features of DNA sequence organization. We have analyzed the ability of the mouse fibroblast transformant to transcribe and process the heterologous rabbit β-globin gene. Hybridization experiments indicate that in at least one transformant, rabbit β-globin sequences are expressed in the cytoplasm as a discrete 9S species, suggesting that mouse fibroblast may contain the enzymes necessary to transcribe and correctly process a rabbit gene whose expression is usually restricted to erythroid cells. These studies demonstrate the potential value of co-transformation systems in the analysis of eukaryotic gene expression.

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INTRODUCTION

Specific genes can be stably introduced into cultured mammalian cells by DNA-mediated gene transfer. The process of transformation results in a change in the genotype of the recipient cell and provides a unique opportunity to study the function and physical state of exogenous genes in the transformed host. In our laboratories, we have developed transformation systems which may allow the introduction of virtually any defined gene into cultured cells. We have therefore performed a series of transformation experiments with a variety of different eukaryotic and prokaryotic genes: 1) to develop in vivo systems to study the functional significance of various features of DNA sequence organization; 2) as a means for gene purification where now classical routes involving recombinant DNA technology and molecular hybridization are inapplicable; 3) to examine the fluidity and promiscuity of the eukaryotic chromosome.

In initial studies, we developed a transformation system for the thymidine kinase (tk) gene of herpes simplex virus (HSV-1). Through a series of electrophoretic fractionations in concert with transformation assays, we isolated a unique 3.4 kb fragment of viral DNA which is capable of efficiently transferring tk activity to mutant Ltk$^-$ cells (Wigler et al., 1977). Extension of these studies to unique cellular genes has resulted in the stable transfer of genes coding for thymidine kinase, adenine-phosphoribosyl transferase and a methotrexate resistant mutant of dihydrofolate reductase to mouse fibroblasts (Wigler et al., 1978, 1979a).

The methods we have used to transfer these genes can, in principle, be applied to any gene for which conditional selection criteria are available. The isolation of cells transformed with genes which do not code for selectable markers, however, is problematic, since current transformation procedures are highly inefficient. We have recently demonstrated the feasibility of co-transforming cells with two physically unlinked genes (Wigler et al., 1979b). Co-transformed cells can be identified and isolated when one of these genes codes for a selectable marker. We have used the viral tk gene as a selectable marker to isolate mouse cell lines which contain the tk gene along with either bacteriophage ΦX 174, plasmid pBR 322, or the cloned rabbit β-globin gene sequences stably integrated into cellular DNA. We have further demonstrated that the gene coding for the rabbit β-globin in transformed mouse fibroblasts is properly recognized by the transcriptional and processing enzymes of the mouse cell to generate RNA indistinguishable from the mature globin mRNA of the rabbit erythroblast (Wold et al., 1979).

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These studies demonstrate the value of co-transformation systems in the analysis of eukaryotic gene expression.

RESULTS

Co-Transformation of Mouse Cells with ΦX-174 DNA. The addition of the purified thymidine kinase gene from herpes simplex virus to mutant mouse cells lacking tk results in the appearance of stable transformants expressing the viral gene which can be selected by their ability to grow in HAT (Maitland and McDougall, 1977; Wigler et al., 1977). To obtain co-transformants, cultures are exposed to the tk gene in the presence of vast excess of a well-defined DNA sequence for which hybridization probes are available. Tk^+ transformants are isolated and scored for the co-transfer of unselectable DNA sequences by molecular hybridization.

We initially used ΦX DNA in co-transformation experiments with the tk gene as the selectable marker. ΦX replicative form DNA was cleaved with Pst I, which recognizes a single site in the circular genome (Fig. 1) (Sanger et al., 1977). Purified tk gene (500 pg) was mixed with 1-10 μg of Pst-cleaved ΦX replicative form DNA. This DNA was then added to mouse Ltk^- cells using the transformation conditions previously described (Wigler et al., 1979a). After two weeks in selective medium (HAT), tk^+ transformants were observed at a frequency of one colony per 10^6 cells per 20 pg of purified gene. Clones were picked and grown into mass culture.

We then asked whether tk^+ transformants contained ΦX DNA sequences. High molecular weight DNA from the transformants was cleaved with the restriction endonuclease Eco RI, which recognizes no sites in the ΦX genome. The DNA was fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters, and these filters were then annealed with nick-translated ^{32}P-ΦX DNA (blot hybridization) (Southern, 1975; Botchan et al., 1976; Pellicer et al., 1978).

These annealing experiments indicate that 15 of 16 transformants acquired bacteriophage sequences. Results with two representative clones, ΦX 4 and ΦX 5 are shown in Figure 2. Since the ΦX genome is not cut with the enzyme Eco RI, the number of bands observed reflects the minimum number of eukaryotic DNA fragments containing information homologous to ΦX. The clones contain variable amounts of ΦX sequences: 4 of the 15 positive clones reveal only a single annealing fragment while others reveal at least fifty ΦX-specific fragments.

It should be noted that none of 15 clones picked at random from neutral medium, following exposure to tk and ΦX DNA,

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